Original scientific paper
Journal of Central European Agriculture, 2022, 23(1), p.40-53
DOI: /10.5513/JCEA01/23.1.3397

Effectiveness of endophytic-rhizobial seed inoculation of soybean (*Glycine max* (L.) Merr.) cultivated in irrigated soil

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Received: August 26, 2021; accepted: January 20, 2022

ABSTRACT

Application of seed inoculation with active nodule bacteria strains increases crop productivity of legumes and reduces the impact of negative environmental factors. The effectiveness of complex inoculation has not been studied thoroughly. The purpose of the study was to determine the impact of complex seed inoculation with nodule and endophytic bacteria on plant growth and development, rhizosphere microbiota, and productivity of soybean in irrigated soil in the Southern Steppe of Ukraine. In field research, soybean seeds of the ultra-early maturing cultivar Diona and the medium-maturing cultivar Aratta were inoculated with the bioformulation Ryzobin^K (based on the association of *Bradyrhizobium japonicum* UCM B-6018, B-6023 and B-6035), and with its combination with endophytic bacteria. During the growing season, the number of phosphorus-mobilizing, pedotrophic, prototrophic, oligonitrotrophic, and nitrogen-fixing bacteria increased in the rhizosphere of soybean inoculated with Ryzobin^K, and combined with one of the endophytes – *Bacillus* sp. 4, *Brevibacillus* sp. 5 or *Pseudomonas* sp. 6. Under unfavorable conditions of high temperatures and droughts, endophytic-rhizobial inoculation contributed to an increase in the productivity of the soybean. On average in 2018-2020, under complex seed inoculation with Ryzobin^K combined with *Bacillus* sp. 4, the highest yields of soybean cultivars Diona and Aratta of 2.66 and 2.90 t/ha were obtained, that exceeded the yields of the bacteria-free treatment variants by almost 30% and 40% respectively, with a simultaneous increase in protein and fat content in the seeds.

Keywords: soybean, nodule bacteria, endophytic bacteria, rhizosphere, irrigation, yield

INTRODUCTION

Soybean is one of the oldest legume crops grown in many countries of the world for food, industrial, forage and medical purposes. Currently, it is the main source of plant protein and oil in the world (Sobko et al., 2020). Due to soybean capability of atmospheric nitrogen fixation in symbiosis with rhizobium bacteria, it is one of the best pre-crops for grain, vegetable, and other crops (Izumi et al., 2004). Among cultivated legume crops, soybean has occupied one of the leading positions in the structure of sown areas in Ukraine over the past few years. According to the data of the State Service of Statistics of Ukraine (SSSU), the total sown area under soybean in Ukraine in 2020 was 1340.5 thousand hectares in comparison with 93.0 thousand hectares in 1990 (SSSU. 2020).

Implementation of energy-saving technologies of soybean production under current economic conditions allows involving additional sources of mineral plant nutrition and obtaining high and steady crop yields. These technologies are based on the use of bioformulations containing live microorganisms (Di Benedetto et al., 2017). Specificity of interactions between microorganisms and plants have been studied thoroughly, an exceptionally important role of rhizosphere microbiota in providing crops with necessary nutritious elements has been determined. Microorganisms transform compounds unavailable for plants into mobile, optimal for metabolism (Nihorimbere et al., 2011). Therefore, plants provided with a full-fledged complex of microorganisms can obtain sufficient nutrition and, as a result, realizing their potential in terms of the formation of high yields.

The main factors contributing to steady and high soybean yields are creating and implementing highly productive cultivars of a new generation into agricultural production and improving the technology of crop production. One of the effective means contributing to an increase in soybean seed productivity is the application of environmentally friendly inoculants based on nodule bacteria.

Symbiotic microorganisms play an important role in soybean plant development, promote their mineral nutrition, protect against pathogens, and adapt to different stresses (Pedrozo et al., 2018). In symbiosis with rhizobium bacteria, soybean can fix atmospheric nitrogen more than other legume crops. According to the results of scientific research conducted in different climate zones of Europe (Zimmer et al., 2016) and Ukraine (Novytska et al., 2020), inoculation of soybean seeds with rhizobium bacteria increases symbiotic fixation of molecular atmospheric nitrogen, and, as a result, crop productivity. Over the past years, researchers have become more interested in endophytic bacteria. Endophytes are microorganisms isolated from surface-sterilized explants or from plant tissues, living asymptomatically during a part of life cycle inside host plants. They are capable of coexisting with a plant organism without damaging it and can even be useful to some extent (Stone et al., 2000; Sturz et al., 2010). Endophytic bacteria, like rhizobia, synthesize biologically active metabolites with antimicrobial effect on phytopathogens, or they are inductors of systemic plant resistance preventing disease development (Strobel et al., 2004). Most endophytic bacteria improve plant growth, boost their development, and have a protective effect to unfavorable factors of the environment, some of them can fix molecular atmospheric nitrogen that improves nitrogen nutrition of plants (Brovko et al., 2015; Lu et al., 2017). Many recent studies show that endophytes are normal microbiota of internal plant tissues.

Bioregulation of microbial-plant systems is related to the ability of microorganisms introduced in phytosphere to improve mineral nutrition, promote growth, strengthen immunity, and plant resistance to unfavorable environmental conditions (Glick, 2015; Vacheron et al., 2015; O'Callaghan, 2016; Omena et al., 2019). Among modern biotechnological products for crop production, bioformulations based on endophytic bacteria are the least examined. Endophytes are capable of producing many biologically active secondary metabolites – antibiotics, antioxidants, quinones, phenols, steroids, phytohormone, etc. (Cohen et al., 2009; Ludwig-Müller, 2015). There is information about synthesis of antibiotics by endophytes: oxacilline, ampicillin, catechin, gallic acid, cefalexin, triterpenoid and helvellic acid (Gouda et al., 2016). A host plant is protected against different harmful impacts of the environment due to bio-synthetic activity of endophytes (Strobel et al., 2004). Stajković et al. (2011) suggested direct and indirect mechanisms of promoting plant growth by endophytes, in particular, the ability to produce siderophores, solve organic and non-organic phosphates and synthesize indoleacetic acid, that was discovered in *Pseudomonas* sp. LG, isolated from bean nodules.

However, the issue of effective compatibility of endophytic bacteria with nodule bacteria has not been examined thoroughly, though the combination of nitrogen-fixing and growth-regulating functions of endophytic bacteria community is very valuable in terms of economic efficiency. The most commonly endophytes isolated from various plant parts are *Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Burkholderia*, *Clavibacter* (Hallmann et al., 1997). This work aimed to investigate the influence of seed inoculation with endophytic bacteria of *Paenibacillus*, *Bacillus*, *Brevibacillus*, or *Pseudomonas* genera in a complex with *Bradyrhizobium japonicum* on rhizospheric microbiota and soybean cultivars differing in maturity groups productivity grown in irrigated soil of the Southern Steppe of Ukraine.

MATERIALS AND METHODS

Experimental design

The field experiments were conducted under conditions of the Southern Steppe of Ukraine at Askaniia Agricultural Research Station of the Institute of Irrigated Agriculture of the National Academy of Agrarian Sciences of Ukraine (Kherson region) in 2018 - 2020. The soil is dark-chestnut medium-loamy, with the depth of a humus layer of 45-50 cm. The content of humus (by Tiurin) in the arable layer is 2.15%; that of slightly hydrolyzed nitrogen (by Cornfield) is 50.0 mg per kilogram of soil; mobile phosphorus (by Machyhin) is 24.0; that of exchangeable potassium is 400 mg/kg of soil. The least soil water capacity of the layer of 0-50 cm is 22.6%; that of 0-70 cm is 22.0%; that of 0-100 cm is 21.3%; the withering point is 9.8%; 9.7% and 9.5%, respectively, in relation to the weight of completely dry soil. The soil analyzes were performed in Agrochemical Laboratory of the Institute of Irrigated Agriculture of the National Academy of Agrarian Sciences of Ukraine.

The two-factor field experiment was carried out by the method of split plots where the main plots (the plots of the first order, Factor A) were soybean cultivars: the ultra-early ripening cultivar Diona and the medium-early cultivar Aratta. The plots of the second order (the subplots, factor B) were variants of pre-sowing seed treatment with water or different bioformulations including strains of rhizobium and endophytic bacteria. The bioformulation Ryzobin^K based on association of 3 strains of rhyzobium bacteria *Bradyrhizobium japonicum* UCM B-6018, B-6023 and B-6035 in ratio 1:1:1. These strains have been selected by authors of this work from Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The Ryzobin^K is a cultural liquid of *Bradyrhizobium japonicum*, includes living bacterial cells and a natural complex of biologically active substances produced by rhizobium (B vitamins; phytohormones with stimulating action – auxins, cytokinins, gibberellins; enzymes; amino acids; organic acids; polysaccharides; fatty acids, etc.). This bioformulation is an effective modern multi-strain inoculant for pre-sowing treatment of soybeans seeds.

In this work, we used a preparation that was obtained under laboratory conditions by cultivation of *B. japonicum* UCM B-6018, B-6023 and B-6035 in bean liquid medium in flasks on a circular shaker at the temperature of 28 °C for 4 days. The titre of symbiotic nitrogen-fixing rhizobia in the inoculant is not less than $3\text{-}5 \cdot 10^9$ cells/ ml. Treatment was carried out by spraying the seeds, the consumption rate of the Ryzobin^K was 0.5-1.0 liters per 1 ton of seeds.

The Ryzobin^K was used as an inoculant separately or in combination with one of the endophytes, isolated from soybean: *Paenibacillus* sp.1, *Bacillus* sp.4, *Brevibacillus* sp.5, *Pseudomonas* sp. 6. The seed treatment with the fungicidal dressing Maxim XL 035 FS (25 g/l fluidioxonil 10 g/l + mefonoxam, Syngenta, USA) was performed 5 days before sowing at the rate of 1 l/t of seeds, and complex inoculation of 1 l/t of seeds – on the day of sowing. In the control variant 1 the seeds were not treated and not inoculated, and in the control variant 2 – the seeds were soaked by water and not inoculated. In order to protect the crops against pests only the contact and systemic insecticide Galil (Imidacloprid: al 22.26% + Bifentrina: al 4.45%. Adama Ltd, Argentina) was applied in 0.2 l/ha.

The sown area was 60.0 m^2, the registered area was 17 m^2, and the experiment was replicated four times. The forecrop was corn (*Zea mays* L. ssp. *mays*). The soybean cultivars were sown in the third decade of April with the seeder «Klen» with the row spacing of 45 cm at a depth of 3-4 cm. The seeding norm for the cultivar Diona is 800000 and that for the cultivar Aratta 600000 germinated seeds per hectare. The crops were irrigated with the machine "Reinke" (Reinke Manufacturing Company, Inc USA) with the irrigation norm of 350-400 m^3/ha.

Evaporation rates, moisture deficit and the coefficient of moisture were determined according to Ivanov (1962). The recording of yields of variants was done at 100% seed maturity. The crops were harvested with the combine harvester "Sampo-130" (Sampo Rosenlew Ltd, Finland). The soybean yield and its components were estimated by the generally accepted methods of a field experiment (Finney, 1956), and modern recommendations were also taken into account (Lindsey, 2020). The content of protein in soybean seeds was determined according to Kjeldahl method (State Standard of Ukraine 13496.4-93) and fat content according to Rushkovsky (State Standard of Ukraine 13496).

Measurement

The number of microorganisms of the main ecological-trophic groups in the soybean rhizosphere was examined in the period between the stages R2 flowering and R3 pod development. The number of microorganisms was determined in fresh samples of rhizosphere soil with the method of sowing soil suspension, prepared with the method of Whittles (2009), on the corresponding agar nutrition media: pedotrophic – on soil agar, oligonitrotrophic and nitrogen-fixing – on Ashby medium, prototrophic – on Krasilnikov medium, phosphorus-mobilizing – on Muromtsev medium with sodium phenolphthalein phosphate (Aseeva et al., 1991). The number of microorganisms in the soil was determined as the number of colony-forming units (CFU) in 1 g of completely dry soil. The microbiological examination was replicated three times.

The fractional content of nitrogen in the soil layer of 0-40 cm under soybean crops was determined in the field experiment in 2020 by Shkonde and Koroleva (1964) method.

Statistical analysis

Analysis of the data on the number of microorganisms was performed using the software Microsoft Excel 2017. The field experiment was designed, and analysis of soybean productivity was performed according to Finney (1956).

RESULTS

Agroclimatic conditions of the subzone of the Southern Steppe, where the research was conducted, are characterized by moderately hot and very dry climate. The impact of weather and climate conditions at the time of production processes and yield formation of soybean cultivars differing in maturity groups was determined by measuring evaporation rates, moisture deficit and the coefficient of moisture (K) calculated by the average daily temperature indexes and relative air humidity and the amount of rainfall by Ivanov (1962). The total indexes of weather conditions were obtained based on the materials of the meteorological station located in Askaniia-Nova, at a distance of 8.0 km from the place of the field experiment.

The following classification is established for the climate zones of Ukraine:

with K = 1.00-1.33 and more – a highly wet zone;
K = 0.77-1.00 – a half-wet zone;
K = 0.55-0.77– a semi-arid zone;
K = 0.44-0.55 – an arid zone;
K = 0.33-0.44 – an extremely arid zone;
K = 0.22-0.33 – a semi-dry zone;
K = 0.12-0.22 – a semi-desert,
K = 0.12 and less – a desert.

Over the years of research, the southern part of the steppe zone of Ukraine can be attributed to different climatic zones, because weather conditions and, above all, average temperature, and relative humidity, as well as rainfall, evaporation and moisture deficit were different (Fig. 1).

The peculiarities of the semi-dry rainfall of the growing period (April-September) in 2018 were high average daily temperature (20.3 °C), and lack of rainfall (88.9 mm). Under such weather conditions, the moisture deficit in general during the growing period reached 919.5 mm. Compared to the average multi-year indicators for 1945-2010 the moisture deficit was higher by 88.6%. On average, during the growing season, the moisture coefficient in 2018 was 0.09, which indicated that the southern part of the Steppe zone during April - September 2018 belonged to the desert.

In the growing season 2019, there was a shortfall of 244.3 mm of precipitation. The moisture deficit reached 570.3 mm and, in comparison with the average indexes for many years was higher by 17.0 %. On the average in the growing season, the moisture coefficient was 0.30. This indicates that the Southern Steppe of Ukraine during April - September 2019 belonged to the semi-dry zone.

During the growing season in the semi-dry 2020 there was 163.6 mm of rainfall, the evaporation rate was 947.5 mm. The moisture deficit reached 783.9 mm and, in comparison with the average indexes for many years, was higher by 60.8 %. The moisture coefficient was 0.17, which indicated that the Southern Steppe of Ukraine during April-September 2020 belonged to the semi-desert.

Thus, the negative impact of regional climate change on the productivity of agrocenoses in recent years indicates a significant increase in the Southern Steppe of Ukraine of evaporation rate and lack of moisture, which significantly affects the formation of crops. High productivity of soybean cultivars of different earliness (in the range of 2.81-2.98 t / ha) is achieved through the use of irrigation, selected cultivars of new generation, adapted to the climatic conditions of the zone, and a complex endophytic-rhizobial seed inoculation.

Only timely irrigation contributed to a significant lowering of the temperature regime, which prevented the negative impact on the growth and development of soybean cultivars.

The level of pre-irrigation humidity in the soil layer 0-50 cm before flowering of both soybean cultivars was maintained at the level of 70-75% of MMHC (minimum moisture holding capacity), respectively, 75-80% of MMHC in the interphase period of flowering - bean formation and 60-70% of MMHC in the period of seed filling - ripening beans. During the growing season, 13 irrigations were carried out on the ultra-early maturing cultivar Diona with an irrigation rate of 3900 m^3 / ha, and on the medium-maturing cultivar Aratta with 14 irrigations with an irrigation rate of 4200 m^3 / ha.

Microbiological research of the soybean rhizosphere conducted in 2018-2020 proves a considerable impact of microbial inoculants application on the number of microorganisms participating in transformation of nitrogen, phosphorus, humus compounds and cellulose-containing plant residues.

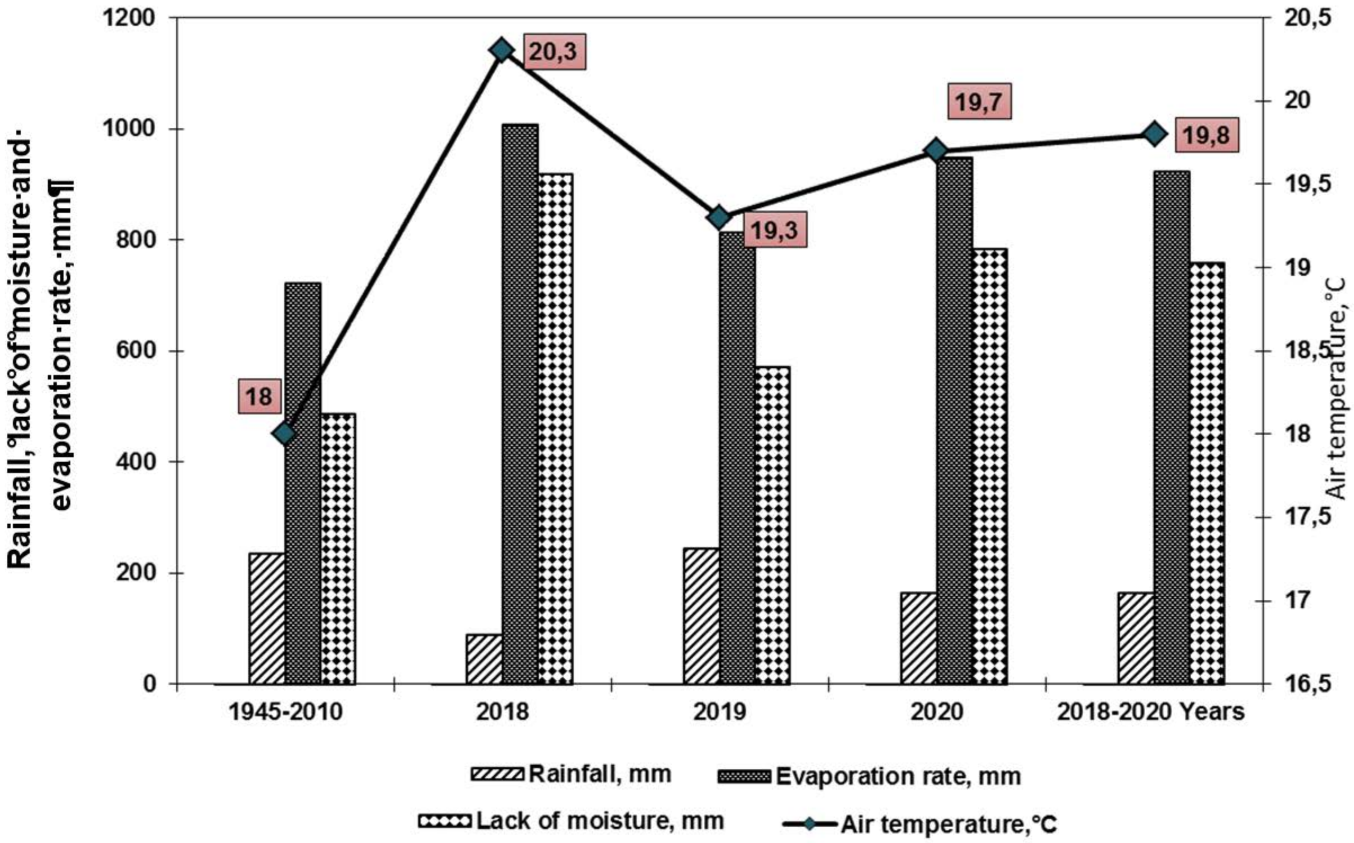

Figure 1. Indicators of weather and climatic conditions of the Southern Steppe of Ukraine

In the rhizosphere of the ultra-early ripening cultivar Diona, the number of oligonitrotrophic and nitrogen-fixing bacteria in the soil increased 2.8 and 2.6 times, respectively, in comparison with the control variant 2, under complex inoculation with Ryzobin^K in combination with one of the endophytes *Paenibacillus* sp.1 or *Bacillus* sp. 4 (Table 1).

Phosphorus-mobilizing bacteria transforming unavailable mineral-and organophosphates into available forms that are assimilated by plants, play an important role in phosphorus plant nutrition. The number of phosphorus-mobilizing bacteria when using microbial inoculation was higher by 15% in comparison with the Control 2 variant under application Ryzobin^K and by 25-35% with rhizobium and endophytic bacteria *Paenibacillus* sp.1, *Bacillus* sp.4 or *Pseudomonas* sp.6.

The number of pedotrophic bacteria participating in transformation of water-soluble humus compounds under complex endophytic-rhizobial inoculation was higher by 17-43.9% in comparison with the variant where the seeds were soaked by water. Under inoculation with Ryzobin^K there was no difference in comparison with the control. Plant growth promoting bacteria are very important for the formation of effective microbial plant systems. Prototrophic bacteria are capable of synthesizing biologically active compounds, necessary for the growth of both bacteria and plants participating in mutualistic relationships. The number of prototrophic bacteria in the rhizosphere of soybean whose seeds were inoculated with microbial formulations, was higher in comparison with the Control 2. When applying Ryzobin^K or in combination with *Paenibacillus* sp. 1, the number of

Table 1. The number of microorganisms of ecological-trophic groups in the soybean rhizosphere (the average in 2018-2020)

Variant of treatment (B)	Oligonitrotrophic microorganisms, CFU•106/g	Phosphorus-mobilizing microorganisms, CFU •106/g	Pedotrophic microorganisms, CFU •106/g	Prototrophic microorganisms, CFU •106/g	Cellulose-decomposing microorganisms, CFU •103/g
			Cultivar Diona (A_1)		
Control 1	29.2	3.4	10.7	18.2	25.2
Control 2	21.5	4.0	22.3	14.8	44.7
Ryzobin^K	20.0	4.6	22.2	23.3	33.9
Ryzobin^K + *Paenibacillus* sp.1	59,3	5.4	32.1	19.6	43.3
Ryzobin^K + *Bacillus* sp. 4	56.7	5.3	31.8	36.3	35.7
Ryzobin^K + *Brevibacillus* sp.5	77.3	4.7	27.8	32.0	42.2
Ryzobin^K + *Pseudomonas* sp. 6	21.3	5.0	26.1	37.2	27.3
			Cultivar Aratta (A_2)		
Control 1	98.4	11.8	77.7	15.8	27.4
Control 2	81.9	8.8	93.3	14.5	44.8
Ryzobin^K	173.1	14.5	117.8	24.5	104.2
Ryzobin^K + *Paenibacillus* sp.1	123.1	17.9	107.8	18.8	56.4
Ryzobin^K + *Bacillus* sp. 4	168.6	23.8	171.0	25.1	59.9
Ryzobin^K + *Brevibacillus* sp.5	178.9	16.8	120.5	29.0	80.7
Ryzobin^K + *Pseudomonas* sp. 6	327.1	16.0	145.3	23.9	58.4
LSD_{05} of the cultivar Diona	17.5	0.5	5.9	7.3	7.3
LSD_{05} of the cultivar Aratta	82.3	3.6	24.0	6.8	19.4

prototrophs exceeded the control indexes by 57.4 and 32.4 %, respectively, and under complex inoculation with other endophytes it was 2.2-2.5 times higher.

In the rhizosphere of the medium-early cultivar Aratta in 2018-2020 there was more active development of the studied microorganisms ecological-trophic groups, especially in the variants with endophytic-rhizobial inoculation. The complex inoculant on the basis of Ryzobin^K and *Bacillus* sp.4 had the greatest impact on the number of rhizosphere phosphorus-mobilizing and pedotrophic microorganisms. On the average for three years of the research, the corresponding indexes increased in comparison with the Control 2 variant 2.7 and 1.8 times. The highest number of prototrophic bacteria was recorded in the variants with seed inoculation by the combinations of Ryzobin^K and *Bacillus* sp. 4 or *Brevibacillus* sp. 5: by 1.7 and 2.0 times higher, respectively, in comparison with the control variant 2. It should be mentioned that in all the variants under inoculation with rhizobia and co-inoculation with endophytic bacteria the number of cellulose- decomposing microorganisms in the rhizosphere of the cultivar Aratta increased by 1.3-2.3 times in comparison with the variant of water seed treatment. Application of Ryzobin^K and its combinations with *Bacillus* sp. 4, *Brevibacillus* sp. 5 and *Pseudomonas* sp. 6 contributed to a considerable increase in the representatives of the oligonitrotrophic and nitrogen-fixing microorganisms in the rhizosphere, where their numbers increased 2.1-4.0 times.

Considering a special contribution of nitrogen-fixing microorganisms into enriching the pool of nitrogen compounds it was possible to predict agronomically beneficial changes in the fractional composition of soil nitrogen. In the last year of research, we have studied fractional content of nitrogen in the soil under soybean crops (Table 2). Complex microbial formulations had a positive after-effect on the soil, enriching it with nutrients available to plants. The main reserve of available nitrogen is easily hydrolyzed nitrogen. In the soil under plants inoculated with rhizobium and endophytic bacteria, there was an increase in the content of mineral nitrogen and easily hydrolyzed nitrogen.

Table 2. The fractional content of nitrogen in the dark-chestnut soil under soybean crops, the soil layer of 0-40 cm (2020 y.)

Variant of treatment (B)	Mineral nitrogen, mg/kg	Easily hydrolyzed, mg/kg	Poorly hydrolyzed, mg/kg	Nonhydro-lyzed, mg/kg	Total nitrogen, mg/kg	Share of available to зtotal, %
Cultivar Diona (A_1)						
Control 1	39.21	77.82	192.08	1000.75	1309.87	8.93
Control 2	37.66	78.24	197.77	989.36	1303.30	8.82
Ryzobin^K	43.24	91.65	197.51	1031,89	1364.36	9,89
Ryzobin^K + *Bacillus* sp. 4	40.64	98.29	212.30	1075.34	1426.58	9.74
Ryzobin^K + *Brevibacillus* sp.5	41.62	101.94	227.36	1040.28	1411.13	10.17
Cultivar Aratta (A_2)						
Control 1	36.15	80.36	200.59	983.23	1300.33	8.96
Control 2	36.99	86.64	213.96	965.61	1303.20	9.49
Ryzobin^K	38.52	93.99	202.55	959.79	1294.84	10.23
Ryzobin^K + *Bacillus* sp. 4	45.99	96.83	212.59	1011.84	1367.24	10.45
Ryzobin^K + *Brevibacillus* sp.5	37.50	95.69	234.24	1052.04	1419.47	9.38
LSD_{05} of the cultivar Diona	2.71	11.45	14.73	34.89	57.81	0.62
LSD_{05} of the cultivar Aratta	5.14	7.18	13.69	38.92	56.05	0.63

When growing the soybean cultivar Diona, the content of mineral nitrogen under bacterial inoculation, in comparison with the control variants, increased by 3.9-14.8%, and that of easily hydrolyzed nitrogen – by 17.1-31%.

As a result, the share of nitrogen available for plants in the total nitrogen increased from 8.82-8.93% in the control variants to 9.74-10.17% under application of microbial formulations.

When growing the soybean cultivar Aratta, the content of mineral nitrogen and easily hydrolyzed nitrogen in the fractional composition of the soil in the variants with inoculation increased by 1.4-27.2% and 8.5-20.5%, respectively, in comparison with the control indexes. The share of mineral and easily hydrolyzed nitrogen in the total amount of nitrogen increased from 8.96-9.49% (in the control variants) to 9.38-10.45% (Table 2).

Pre-sowing seed inoculation with rhizobium and endophytic bacteria considerably promoted growth and development of plants and contributed to a higher position of lower pods and the formation of a larger number of nodes and pods per plant and seeds per pod that resulted in a considerable increase in the yield and its quality (Table 3).

In the variants with complex seed inoculation of the cultivar Diona were taller by 5.0-9.3 cm, and those of the cultivar Aratta by 3.0-10.2 cm in comparison with the control variant 2. And in comparison with the control variant 1, they were taller by 7.7-12.0 and 10.7-17.9 cm, respectively. The tallest plants of the cultivar Diona were in the variants Ryzobin^K + *Bacillus* sp.4 – 84.6 cm, and those of the cultivar Aratta in the variants Ryzobin^K + *Bacillus* sp.4 and Ryzobin^K + *Brevibacillus* sp.5, i.e. 132.7 cm.

Overall, the formation of pods on the plants started at a lower part of the main stem. The pods on the Aratta cultivar were formed considerably higher than those on the Diona cultivar. The pods number and seed mass per one plant are important indexes of soybean productivity. On average in 2018–2020, the yield components were essentially dependent on the biological characteristics of cultivars and on complex pre-sowing seed inoculation. The least number of pods in both cultivars formed in the control variants, which did not exceed 24 pcs/plant in the Diona cultivar, and 36 pcs/plant in the Aratta cultivar. Under complex endophytic-rhizobial inoculation with the combination of Ryzobin^K and *Bacillus* sp. 4, the number of pods increased by 11 pcs/plant in the Diona cultivar and by 14-17 pcs/plant in the Aratta cultivar.

In the research variants, in comparison with the Control 2, an increase in the number of pods per one plant of Diona and Aratta cultivars was: Ryzobin^K – 10 and 26 pcs/plant, Ryzobin^K + *Paenibacillus* sp. 1 – 19 and 18; Ryzobin^K + *Bacillus* sp. 4 – 24 and 36; Ryzobin^K + *Brevibacillus* sp. 5 – 14 and 10; Ryzobin^K + *Pseudomonas* sp. 6 – 6 and 17 pcs/plant.

The seed mass per one plant under combined seed inoculation with rhizobium and endophytic bacteria, in comparison with the control variants, was also considerably higher. Thus, an increase of the cultivars Diona and Aratta under inoculation with the bacterial bioformulation Ryzobin^K, regardless of the cultivars, was 1.8-6.1 g; with the combination of Ryzobin^K + *Paenibacillus* sp. 1 it was 3.5-4.7 g; with Ryzobin^K + *Bacillus* sp. 4 it was 4.7-7.6 g; and with Ryzobin^K + *Brevibacillus* sp. 5 – 1.5-3.9 g and Ryzobin^K + *Pseudomonas* sp. 6 it was 1.1-4.5 g, respectively.

The formation of 1000 seeds' mass under irrigated conditions was also dependent on the cultivar and complex seed inoculation with rhizobium and endophytic bacteria. Whereas 1000 seeds mass in the control variants of the cultivar Diona on the average for three years (2018-2020) was 130.7-132.7 g and that of the cultivar Aratta it was 122.0-126.7 g. In the variants with inoculations, it increased to 138.0-141.0 g under application of Ryzobin^K and rose to 134.3–147.3 g under complex endophytic-rhizobial inoculation.

Under seed inoculation with rhizobium and endophytic bacteria an increase in the productivity of the soybean cultivar Diona in different variants, in comparison with Control 1, was 0.31-0.45 t/ha (Table 4). The productivity reached the highest level under inoculation with Ryzobin^K

Table 3. Influence of rhizobium and endophytic bacteria seed inoculation on soybean yield attributes (the average in 2018-2020)

Variants (B)	Height, cm		The number per plant, pcs.			Seed mass, g	
	of plants	of the first pod position	productive nodes	pods	seeds	per plant	1000 seeds
Cultivar Diona (A_1)							
1.	72.6	11	12	24	54	7.5	130.7
2.	75.3	11	13	24	63	8.0	132.7
3.	81.7	12	13	30	73	9.8	141.0
4.	82.7	12	14	31	82	11.5	140.3
5.	84.6	11	16	35	87	12.7	147.3
6.	80.3	12	14	31	77	11.4	144.3
7.	82.5	12	13	27	69	9.1	143.0
Cultivar Aratta (A_2)							
1.	114.8	18	14	33	73	10.2	122.0
2.	122.5	19	15	36	76	10.9	126.7
3.	128.8	19	16	42	102	16.3	138.0
4.	130.6	18	15	45	94	14.9	134.3
5.	132.7	17	17	50	112	17.8	136.3
6.	132.7	19	15	41	86	12.4	137.0
7.	125.5	16	16	43	93	14.7	140.3
$LSD_{05}(A_1)$	3.4	0.4	1.0	3.0	8.5	1.5	4.7
$LSD_{05}(A_2)$	4.9	0.9	0.7	4.3	10.7	2.2	5.1

Notes: 1 – Control 1 (without seed treatment); 2 – Control 2 (water seed treatment); 3 – Ryzobin^K (association of 3 strains *Bradyrhizobium japonicum*: UCM B-6018, UCM B-6023, UCM B-6035); 4 – Ryzobin^K + *Paenibacillus* sp.1; 5 – Ryzobin^K + *Bacillus* sp. 4; 6 – Ryzobin^K + *Brevibacillus* sp.5; 7 – Ryzobin^K +*Pseudomonas* sp. 6

+ *Bacillus* sp. 4 (0.61 t/ha). An increase in the productivity of the medium-maturing cultivar Aratta reached 0.26-0.42 t/ha, it was the highest 0.80 t/ha in the variant under inoculation with Ryzobin^K + *Bacillus* sp. 4.

Pre-sowing complex seed inoculation with different strains of rhizobium and endophytic bacteria also had a considerable impact on qualitative indexes of the yield and, primarily, on fat and protein content in the seeds. For instance, the protein content in the seeds of the control variants of the cultivar Diona was 36.39 and 37.08%, and that of the cultivar Aratta it was 36.75 and 36.91% in relation to total dry matter (Table 5).

The highest protein content in the seeds of the cultivar Diona (at the level of 38.62% and 38.78%) was in the variants in which pre-sowing seed inoculation was performed with the bacterial bioformulation Ryzobin^K + *Bacillus* sp. 4 and Ryzobin^K + *Pseudomonas* sp. 6. The highest protein content in the seeds of the cultivar Aratta was in the variant in which pre-sowing seed inoculation was performed with the bacterial bioformulation Ryzobin^K + *Bacillus* sp. 4 – 39.16%.

Under seed inoculation with Ryzobin^K + *Bacillus* sp. 4 the protein content in the seeds of the cultivar Diona, in comparison with Control 1, increased by 2.2%, and in the cultivar Aratta by 2.4%. The fat content by the variants in the seeds of cultivar Diona reached 15.62-17.05% and in the seeds of the cultivar Aratta it reached 15.17-18.13% in relation to total dry matter. The highest protein yield, at the level of 1027 kg/ha, and the highest fat yield, at the level of 451 kg/ha, when growing the cultivar Diona, were obtained under seed inoculation with Ryzobin^K + *Bacillus* sp. 4, and, when growing the cultivar Aratta, i.e.1136 and 502 kg/ha.

Table 4. Soybean productivity under endophytic-rhizobial seed inoculation in irrigated conditions of the Southern Steppe of Ukraine (the average in 2018-2020; t/ha)

Cultivar (A)	Variant of treatment (B)	Years of the research				Increase compared to Control 1	
		2018	2019	2020	average	t/ha	%
Diona	Control 1	1.89	2.42	1.84	2.05	-	-
	Control 2	1.90	2.45	1.85	2.07	-	-
	Ryzobin^K	2.13	2.79	2.23	2.38	0.33	16.1
	Ryzobin^K + *Paenibacillus* sp.1	2.19	3.15	2.29	2.54	0.45	21.9
	Ryzobin^K + *Bacillus* sp. 4	2.29	3.33	2.35	2.66	0.61	29.8
	Ryzobin^K + *Brevibacillus* sp.5	2.05	2.90	2.20	2.38	0.33	16.1
	Ryzobin^K + *Pseudomonas* sp. 6	2.03	2.89	2.16	2.36	0.31	15.1
Aratta	Control 1	1.90	2.54	1.85	2.10	-	-
	Control 2	1.92	2.54	1.86	2.11	-	-
	Ryzobin^K	2.03	2.72	2.32	2.36	0.26	12.4
	Ryzobin^K + *Paenibacillus* sp.1	2.08	2.95	2.06	2.36	0.26	12.4
	Ryzobin^K + *Bacillus* sp. 4	2.55	3.16	2.98	2.90	0.80	38.1
	Ryzobin^K + *Brevibacillus* sp.5	2.27	2.96	2.29	2.51	0.41	19.5
	Ryzobin^K + *Pseudomonas* sp. 6	2.34	2.92	2.30	2.52	0.42	20.0

A. Evaluation of the significance of fractional differences: LSD_{05} (A) – 0.16 t/ha; LSD_{05} (B) – 0.11 t/ha
B. Evaluation of the significance of medium (main) effects: LSD_{05} (A) – 0.06 t/ha; LSD_{05} (B) – 0.08 t/ha

Table 5. The impact of endophytic-rhizobial inoculation of seed on soybean yield, protein, and fat contents in the seeds (the average in 2018-2020)

Variant of treatment (B)	Yield, t/ha	Protein, %	Fat, %	Protein, kg/ha	Fat, kg/ha
Cultivar Diona (A_1)					
Control 1	2.05	36.39	15.62	746	320
Control 2	2.07	37.08	15.78	767	327
Ryzobin^K	2.38	38.25	16.36	910	389
Ryzobin^K + *Paenibacillus* sp.1	2.54	37.56	16.90	954	429
Ryzobin^K + *Bacillus* sp. 4	2.66	38.62	16.96	1027	451
Ryzobin^K + *Brevibacillus* sp.5	2.38	38.36	17.05	913	406
Ryzobin^K + *Pseudomonas* sp. 6	2.36	38.78	16.67	915	393
Cultivar Aratta (A_2)					
Control 1	2.10	36.75	15.17	772	319
Control 2	2.11	36.91	15.51	779	327
Ryzobin^K	2.36	38.69	16.88	913	398
Ryzobin^K + *Paenibacillus* sp.1	2.36	38.79	18.13	915	428
Ryzobin^K + *Bacillus* sp. 4	2.90	39.16	17.30	1136	502
Ryzobin^K + *Brevibacillus* sp.5	2.51	37.63	17.68	944	444
Ryzobin^K + *Pseudomonas* sp. 6	2.52	38.63	16.77	973	423
LSD_{05} of the cultivar Diona	0.17	0.77	0.44	75.1	36.7
LSD_{05} of the cultivar Aratta	0.21	0.73	0.89	93.0	49.6

DISCUSSION

The effect of combined seed inoculation with rhizobium and endophytic bacteria on legume crops is of interest to a lot of researchers. Currently, co-inoculation of soybean seeds with rhizobia together with plant growth-promoting rhizobacteria (PGPR), including endophytic rhizobacteria, is considered a promising measure to increase soybean productivity.

The obtained results showed the efficiency of applying complex endophytic-rhizobial inoculants to soybean under unfavorable growth conditions. A similar positive effect of endophytic bacteria on reducing stress due to severe soil acidification has also been demonstrated. The acidotolerant *Bradyrhizobium* Bra6 isolated from soybean nodules and various endophytic *Pseudomonas* isolates were used as co-inoculants (Tu et al., 2021). Enhanced nodulation, symbiotic nitrogen fixation, and biomass production of soybeans in strongly acidic soils were demonstrated by co-inoculation. The positive effect of co-inoculation has also been observed in the cultivation of soybeans at low root zone temperatures (Bai et al., 2003). Soybean seeds were inoculated with *Bradyrhizobium japonicum* together with one of the endophytic strains *Bacillus subtilis* NEB4 or NEB5 or *B. thuringiensis* NEB17. Under greenhouse conditions at 15 °C application of NEB17 provided the largest and most consistent increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen, and grain yield. Field experiments were conducted in the Steppe zone of Ukraine under extremely unfavorable weather and climatic conditions for the yield formation such as insufficient rainfall and high average daily temperatures. Although the research was conducted under irrigated conditions, there was moisture deficit and a low coefficient of moisture. Nevertheless, a positive effect of co-inoculation was observed. Thus, the protective function of endophytic bacteria in complex co-inoculation with rhizobia was demonstrated under the conditions of various unfavorable abiotic factors.

There has been little quantitative analysis of the effects of this technique on yield variables. A quantification of the effects of the co-inoculation of *Bradyrhizobium* and plant growth promoting rhizobacteria (PGPR) on the soybean crop using a meta-analysis approach was performed (Zeffa et al., 2020). Authors examined data from 42 articles published between 1987 and 2018. The results showed that co-inoculation of soybean with *Bradyrhizobium* + PGPR had a positive and significant effect on the number of nodules (11.4%), nodule biomass (6.5%), root biomass (12.8%), and shoot biomass (6.5%). However, there was no increase in grain yield and shoot nitrogen content. For shoot N content and grain yield, none of the differences were statistically significant. On the contrary, the results of our experiments showed an increase in the content of nitrogen (protein) in soybeans inoculated with *B. japonicum* + endophytes by 0.1 – 0.5% (cultivar Diona) and by 0.1 – 0.5% under inoculation with *B. japonicum* + *Penibacillus* sp.1 or *B. japonicum* + *Bacillus* sp. 4 (cultivar Aratta) compared to rhizobial monoinoculation.

Most experiments on the study of complex inoculation were performed in greenhouse conditions. Isolates of non-rhizobial endophytic bacteria were obtained from the surface-sterilized soybeans (*Glycine max* (L.) Merr.) (Hallmann et al., 1997). *Bacillus subtilis* NEB4 and *Bacillus thuringiensis* NEB5 increased soybean weight under conditions of combined inoculation with *B. japonicum* in comparison with the plants inoculated only with B.japonicum. Endophytes did not affect soybean growth with no rhizobia. The positive effect of co-inoculation with *B. japonicum* + endophytic bacteria belonging to *Bacillus*, *Pseudomonas*, Methylobacterium genera was confirmed by Subramanian et al. (2015).

The beneficial effects of inoculation on soil microbiome diversity and symbiosis can be enhanced when rhizobial inoculation is combined with plant-growth-promoting bacteria (PGPB) (Mishra et al., 2009; Tytova et al., 2013; Iutynska et al., 2017). In experiments carried out in Jensen's tube and growth pouch conditions the strain *Bacillus thuringiensis*-KR1, isolated from the nodules of *Kudzu* vine (*Pueraria thunbergiana*) was applied with *Bradyrhizobium japonicum*-SB1 for seeds co-inoculation of soybean plants. As a result, an increase in nodule number, shoot weight, root weight, root volume, and total biomass was demonstrated compared to rhizobia inoculation and the control (Masciarelli et al., 2014).

The number of field experiments on co-inoculation of seeds with *Bradyrhizobium* and endophyts is small. Their results depend on soybean growing conditions. The synergistic effect of endophyte *Pseudomonas aeruginosa* (LSE-2) strain (KX925973) with *Bradyrhizobium* sp. (LSBR-3) (KF906140) was confirmed (Kumawat et al., 2019). Single *Bradyrhizobium* inoculation improved grain yield by 4.25% over the un-inoculated control treatment, and enhancement in yield was recorded with consortium inoculant (*Bradyrhizobium* + *Pseudomonas aeruginosa*) by 3.47% over the *Bradyrhizobium* alone. The results obtained in our field experiment revealed to increase in grain yield by 12.4 – 16.1% compared untreated control due Ryzobin^{K} application. When seeds of cultivar Diona were co-inoculated with *Bradyrhizobium* + *Paenibacillus* sp. 1 or *Bradyrhizobium* + *Bacillus* sp. 4, an increase in yield was obtained respectively by 6.7 and 11.8% in comparison with rhizobia alone. Application of *Bradyrhizobium* + *Bacillus* sp. 4 on soybean seeds cultivar Aratta provided an increase in yield by 22.9% compared inoculation with rhizobia alone.

Considering the positive role of endophytic microorganisms in plant life cycles, the research on the prospects of applying them as inoculants is a topical trend in increasing the efficiency of microbial-plant systems and sustainable development of crop production.

CONCLUSIONS

The aim of our work was to study how affects the complex seeds inoculation with nodule bacteria (Ryzobin^K) and some endophytic bacteria of the *Paenibacillus*, *Bacillus*, *Brevibacillus*, or *Pseudomonas* genera on rhizospheric microbiota and soybean plants. The studies were carried out in the region of the Southern Steppe of Ukraine, in which hot and very dry climate requires the use of irrigation. The research shows that pre-sowing seed inoculation of the soybean cultivars differing in earliness with rhizobium and endophytic bacteria contributed to an increase in the productivity and improvement of the product quality. Pre-sowing seed inoculation with Ryzobin^K in combination with *Bacillus* sp. 4 or with *Brevibacillus* sp. 5 proved to be the most efficient due to the synergistic effect on soybean plants of the studied cultivars. The obtained results show the prospects for the development of new approaches to increase the productivity of legume crops based on new endophytic-rhizobial inoculants by improving the functional activity of beneficial soil microbiota and the development of agrophytocenosis under the current extreme climate change.

ACKNOWLEDGEMENTS

The program of the scientific research was determined by the state task of Askaniia State Agricultural Research Station of the Institute of Irrigated Agriculture of the NAAS, and also by the demands of agricultural production of the subzone of the Southern Steppe of Ukraine. This research was supported by grant of the National Academy of Sciences of Ukraine 5.20/2020 "Targeted comprehensive interdisciplinary research program the problems of sustainable development and rational use of nature in the context of global environmental change for 2020-2024."

REFERENCE

Aseeva, I.V., Babeva, I.P., Byzov, B.A. (1991) Metody pochvennoy mikrobiologii i biokhimii [Methods of soil microbiology and biochemistry]. Moscow: Moskovskiy gosudarstvennyy universitet [Moscow State University]. (In Russian)

Bai, Y., Zhou, X., Smith, D.L. (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Science, 43, 1774–1781. DOI: https://doi.org/10.2135/cropsci2003.1774

Di Benedetto, N.A., Corbo, M.R., Campaniello, D., Cataldi, M.P., Bevilacqua, A., Sinigaglia, M., Flagella, Z. (2017) The role of Plant Growth Promoting Bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. AIMS Microbiology, 3 (3), 413-434. DOI: https://doi:0.3934/microbiol.2017.3.413. eCollection 2017

Brovko, I.S., Tytova, L.V., Iutynska, H.O. (2015) [Influence of endophytic soybean bacteria on the rhizobium-soybean symbiosis and rhizosphere microbial community]. Microbiology and Biotechnology, 4, 36-45 (In Ukrainian). DOI: https://doi.org/10.18524/2307-4663.2015.4(32).57460

Cohen, A.C., Travaglia, C.N., Bottini, R., Piccoli, P.N. (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the all eviation of drought effects in maize. Botany, 87 (5), 455–462. DOI: http://doi.org./10.1139/B09-023

Finney, D. J. (1956) The Statistician and the Planning of Field Experiments. Journal of the Royal Statistical Society, Series A (General), 119 (1), 1-27. DOI: https://doi.org/10.2307/2342960

Glick, B.R. (2015) Beneficial Plant-Bacterial Interactions. Heidelberg: Springer. Available at: https://www.amazon.com/Beneficial-Plant-Bacterial-Interactions-Bernard-Glick/dp/3319139207 [Accessed 22 January 2015]

Gouda, S., Das, G., Sen, S.K., Shin, H.-S., Patra, J.K. (2016) Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. Frontiers in Microbiology, 7, 1-8. DOI: https://doi.org/10.3389/fmicb.2016.01538

Hallmann, J.A., Quadt-Hallmann, W.F., Mahaffee, Kloepper, J.W. (1997) Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology, 43 (10), 895-914. DOI: https://doi.org/10.1139/m97-131

Ivanov, N.N. (1962) Pokazatel' biologicheskoy effektivnosti klimata [Indicator of biological efficiency of climate]. Izvestiya Vsesoyuznogo geograficheskogo obshchestva [Bulletin of the All-Union Geographical Society], 94 (1), 65-70 (In Russian).

Iutynska, G.A., Tytova, L.V., Pinaev, A.G., Andronow, E.E., Vozniuk, S.V. (2017) [Microbiome biodiversity of soybean rhizosphere under application of fungicides and inoculation by microbial bioformulation Ecovital]. Microbiology and biotechnology, 1, 23–35. DOI: http://dx.doi.org/10.18524/2307-4663.2017.1(37).96322 (In Russian).

Izumi, V., Uchida, K., Iijima, M. (2004) Crop Production in Successive Wheat-Soybean Rotation with No-Tillage Practice in Relation to the Root System. Development. Plant Production Science, 7 (3), 329–336. DOI: https://doi.org/10.1626/pps.7.329

Kumawat, K.C, Sharma, P., Sirari A., Singh I., Gill, B.S., Singh, U., Saharan, K. (2019) Synergism of *Pseudomonas aeruginosa* (LSE-2) nodule endophyte with *Bradyrhizobium* sp. (LSBR-3) for improving plant growth, nutrient acquisition and soil health in soybean. World Journal of Microbiology and Biotechnology, 35 (3), 47. DOI: https://doi.org/10.1007/s11274-019-2622-0

Lindsey, L. (2020) Agronomic Crops Network: Estimating Soybean Yield [Online]. Available at: https://agcrops.osu.edu/newsletter/corn-newsletter/2015-26/estimating-soybean-yield [Accessed 28 January 2020]

Lu, J., Yang, W.S., Ma, H., Liang, J., Chen, Y. (2017) Co-existence of Rhizobia and Diverse Non-rhizobial Bacteria in the Rhizosphere and Nodules of *Dalbergia odorifera* Seedlings Inoculated with *Bradyrhizobium elkanii*, *Rhizobium multihospitium*–Like and *Burkholderia pyrrocinia*–Like Strains. Frontiers in Microbiology, 8, Article 2255. DOI: https://doi.org/10.3389/fmicb.2017.02255

Ludwig-Müller, J. (2015) Plants and endophytes: equal partners in secondary metabolite production? Biotechnology Letters, 37, 1325–1334. DOI: https://doi.org/10.1007/s10529-015-1814-4

Masciarelli. O., Lianes, A., Virginia Luna, V. (2013) A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. Microbiological Research, 169, (7-8), 609-615. DOI: https://doi.org/10.1016/j.micres.2013.10.001

Mishra, P.K., Mishra, S., Selvakumar, G., Kundu, S., Gupta, H.S. (2009) Enhanced soybean (*Glycine max* L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus thuringiensis*-KR1. Acta Agriculture Scandinavica, Section B, Soil and Plant Science, 59, 189–196. DOI: https://doi.org/10.1080/09064710802040558

Nihorimbere, V., Ongena, M., Smargiassi, M., Thonart, P. (2011) Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnologie. Agronomie, Société et Environment, 15 (2), 327-337. DOI: https://doi.agris.fao.org/agris-search/search.do?recordID=DJ2012063017

Novytska, N., Gadzovskiy, G., Mazurenko, B., Kalenska, S., Svistunova, I., Martynov, O. (2020) Effect of seed inoculation and foliar fertilizing on structure of soybean yield and yield structure in Western Polissya of Ukraine. Agronomy Research, 18 (4), 2512–2519. DOI: https://doi.org/10.15159/ar.20.203

O'Callaghan, M. (2016) Microbial inoculation of seed for improved crop performance: is¬sues and opportunities. Applied Microbiology and Biotechnology, 100 (13), 5729–5746. DOI: https://doi.org/10.1007/s00253-016-7590-9

Omena, B.O., Oluwaseyi, S.O., Olubukola, O.B. (2019) Plant Growth Promoting Rhizobacterial Mitigation of Drought Stress in Crop Plants: Implications for Sustainable Agriculture. Agronomy, 9 (11), 712. DOI: https://doi.org/10.3390/agronomy9110712

Pedrozo, A., Girelli de Oliveira, N.J, Alberton, O. (2018) Biological nitrogen fixation and agronomic features of soybean (*Glycine max* (L.) Merr.) crop under different doses of inoculant. Acta Agronómica, 67 (2), 297. DOI: https://doi.org/10.15446/acag.v67n2.56375

Shkonde, E.I., Koroleva, I.E. (1964) O prirode i podvizhnosti pochvennogo azota [On the Nature and Mobility of Soil Nitrogen]. Agrokhimiya [Agrochemistry], 10, 17-36. (In Russian).

Sobko, O., Zikeli, S., Claupein, W., Gruber, S. (2020) Seed Yield, Seed Protein, Oil Content, and Agronomic Characteristics of Soybean (*Glycine max* L. Merrill) Depending on Different Seeding Systems and Cultivars in Germany. Agronomy, 10 (7), 1020. DOI: https://doi.org/10.3390/agronomy10071020

Stajković, O., Delić, D., Jošić, D., Kuzmanović, D., Rasulić, N., Knežević-Vukcević, J. (2011) Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth promoting bacteria. Romanian Biotechnological Letters, 16 (1), 5919- 5926.

State Statistics Service of Ukraine (SSSU). Annual report (2020). Available at: http://www.ukrstat.gov.ua

Stone, J.K., Bacon, C.W., White, J.F. (2000) An overview of endophytic microbes: Endophytism defined. In: Baco, C.W., White, J.F., eds. Microbial Endophytes. New York-Basel: Marcel Dekker. 3-29.

Strobel, G., Daisy, B., Castillo, U., Harper, J. (2004) Natural Products from Endophytic Microorganisms. Journal of Natural Products, 67 (2), 257-268. DOI: https://doi.org/10.1021/np030397v

Sturz, A.V., Christie, B.R., Nowak, J. (2010) Bacterial Endophytes: Potential Role in Developing Sustainable Systems of Crop Production. Critical Reviews in Plant Sciences, 19 (1), 1-30. DOI: https://doi.org/10.1080/07352680091139169

Subramanian, P., Kim, K., Krishnamoorthy, R., Sundaram S., Sa, T. (2015) Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. Plant Growth Regulation, 76, 327–332. DOI: https://doi.org/10.1007/s10725-014-9993-x

Tu, T.-C.; Lin, S.-H.; Shen, F.-T. (2021) Enhancing Symbiotic Nitrogen Fixation and Soybean Growth through Co-Inoculation with *Bradyrhizobium* and *Pseudomonas* Isolates. Sustainability, 13, 11539. DOI: https://doi.org/10.3390/

Tytova, L.V., Brovko, I.S., Kizilova, A.K., Kravchenko, I.K., Iutynska, G.O. (2013) Effect of Complex Microbial Inoculants on the Number and Diversity of Rhizospheric Microorganisms and the Yield of Soybean. International Journal of Microbiological Research, 4 (3), 267–74.

Vacheron, J., Renoud, S., Muller, D., Babalola, O.O., Prigent-Combaret, C. (2015) Alleviation of abiotic and biotic stresses in plants by *Azospirillum*. In: Cassain, F.D., et al., eds. Handbook for *Azospirillum*, Technical issues and protocol. Basel: Springer International Publishing, Switzerland, 333-365.

Zeffa, D.M., Fantin, L.H., Koltun, A., de Oliveira, A.L.M., Nunes, M.P.B.A., Canteri, M.G., Gonçalves L.S.A (2020) Effects of plant growth-promoting rhizobacteria on co-inoculation with *Bradyrhizobium* in soybean crop: a meta-analysis of studies from 1987 to 2018. PeerJ, 8: e7905. DOI: https://doi.org/10.7717/peerj.7905

Zimmer, S., Messmer, M., Haase, T., Piepho, H.-P. (2016) Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. European Journal of Agronomy, 72, 38-46. DOI: https://doi.org/10.1016/j.eja.2015.09.008

Whittles, C.L. (2009) The determination of the number of bacteria in soil. (Preliminary communication). The Journal of Agricultural Science, 13 (1), 18-48. DOI: https://doi.org/10.1017/S0021859600003208