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Effect of inoculation of soybean seeds with *Bradyrhizobium japonicum* on plant development, nodulation and seed yield under conditions of Central North Bulgaria

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Abstract

Radkova, M., Iantcheva, A., Naydenova, G. & Todorova, R. (2023). Effect of inoculation of soybean seeds with *Bradyrhizobium japonicum* on plant development, nodulation and seed yield under conditions of Central North Bulgaria. *Bulg. J. Agric. Sci., 29 (3)*, 464–474

Soybean is a crop with a great importance, as a source of vegetable protein and oils. In a period of three consecutive years 2018-2020, the plant growth, nodulation capacity and yield parameters of ten selected soybean cultivars were monitored under the field conditions of Central North Bulgaria, the region of town Pavlikeni. The widely established agrotechnology of cropping soybean in Bulgaria was applied in combination, with additional inoculation of seeds with *Bradyrhizobium japonicum* before sowing. Selected elite Bulgarian cultivars from different maturity groups with high income for the Bulgarian agriculture were tested together, with three foreign cultivars, which are part of the recent soybean breeding program. The aim is to study the respond of morphological parameters related to the plant growth, nodulation and yield to additional *Rhizobium* inoculation for each cultivar. The results demonstrated that cultivars Rosa and Srebrina expressed better capacity to produce seeds (respectively, 189 and 205 seeds per plant after inoculation, vs 135 and 150 from control plants), as a result of better development of branches, fruiting nods and pods in the condition of additional inoculation. Better nodulation was demonstrated by foreign cultivars Romantica, Atlanta and Saikai in comparison with the Bulgarian cultivars adapted to the soil microbiota. The cultivars Avigeya, Daniela and Pavlikeni seed inoculation before sowing supports the overcoming critical climatic conditions. These results provide a base to promote these cultivars to Bulgarian farmers, intended to produced soybean especially in a regions without soybean history.

Keywords: soybean; nodulation; *Bradirhizobium japonicum;* cultivars; seed yield

Introduction

Soybean is a crop with huge value in human nutrition. The soybean is one of main source for vegetable oil and critical for meeting of global demand of plant protein (Joglekar et al., 2020). The soy production raised from 0.26 million ha to 56 million ha in South America, from the period from 1961 to 2014, resulted from the increased consumption of meat and soy-based animal feed (Voora et al., 2020). It was published that in 2018, 125 million ha were planted with soybean worldwide with production of 348,7 million tones (FAO, 2020). The soybean present around of 84.5% of the world grain legumes trade (Agoyi et al., 2018).

Soybean is not only great source for food and feed, its inclusion in crop rotation improve the soil fertility by symbiosis with nitrogen, fixing bacteria and provide high amount of accumulated nitrogen for the next crop (Iantcheva & Naydenova, 2020; Kavadia et al., 2020). In the other hand, soybean needs a large amount of nitrogen during its development. According to Hungria & Mendes (2015), cited by Moretti et al. (2018), for the production of 1000 kg grains 80 kg of nitrogen are needed. This nitrogen is supplied mostly from biological nitrogen fixation (BNF) by soybean bradyrhizobia (*Bradyrhizobium* spp. (*Bradyrhizobium japonicum*, *B. diazoefficiens*, and *B. elkanii*), which fix atmospheric nitrogen, within the root nodules of the soybean plant. The interaction between the soybean plant and rhizobial bacteria in the root and subsequent symbiotic nitrogen fixation is one of the most important physiological processes, which occurs in the growth and development of the soybean plant (Schulz et al., 2005). The biological nitrogen fixation is one of the main ecologically friendly way to provide the nitrogen needed for proper plant growth, as an alternative of chemical N fertilizers. The overusing of chemical fertilizers cause several negative environmental problems like water eutrophication, global warming, increase of greenhouse emissions (Kavadia et al., 2020).

Many researchers pointed that inoculation of the soybean seeds before sowing with effective *Bradyrhizobium japonicum* strain contribute to the enhanced nodule formation. The bacterial inoculation stimulates the plant growth, increase the adaptability and resistance of the crop, and finally the plant are able to express its genotype potential for productivity and quality (Solomon et al., 2012; Leggett et al., 2017; Kavadia et al., 2020).

Soybean seed inoculation by *Bradyrhizobium japonicum* bacteria has long been established, as an important and required practice for improvement soybean yield on fields that have not been used for soybean production (Takas et al., 2018). Yield increase of 40% have been observed in soybean inoculated by *B. Japonicum*, compared to non-inoculated in fields new for soybean production. (Schulz et al., 2005). In other studies it was provided evidences for successful effect from inoculation with *B. japonicim* in areas with high density of natural Rhizobia population (Leggett et al., 2017). This contrary results open the perspectives to study the inoculation effect in diverse geographical regions (Carciochi et al., 2019). In Bulgaria, the soybean crop production is conducted without inoculation with bradyrhizobia, although it is recommended, as an important element in crop technology. One of the reason for this disadvantage is the interruption of the production of Bulgarian product Nitragin containing adapted strains of *Bradyrhizobium japonicum* successfully used up to year 2000. The other reason is the scarcity of scientific studies, related to the reaction/response of the present Bulgarian and introduced soybean cultivars to inoculation with available on the market strains and bio-fertilaizers. Production of soybean in Bulgaria is conducted without irrigation. This fact require to use the regions with better moisture supply during the summer period. In the other hand, such arrears are characterized with low soil fertility especially with low level of phosphorus and high acidity. In relation with this, application of biofertilization (including Rhizobium) will allow to grow soybean crop in such areas, which will increase the regions for soybean growing. In Bulgaria, the area occupied with soybean was decreased from 90 000 ha during 70-80 years of 20 century, with maximum level of yield of 225kg/dka, to 15-20 000 ha in 2014 (Georgiev et al., 2015). As a result the density of natural local strains of nitrogen fixing bacteria is very low. The missing scientific data for the effect of inoculation on the crop productivity by enlargement of areas with Bulgarian cultivars is a gap, which has to be fixed in order to enlarge the soybean arears in a regions with low level of soil fertility.

The study aimed to assess the effect of seed inoculation with standard, available on the market strain of *Bradyrhizobium japonicum* before sowing on the plant development and productivity of soybean cultivars highly distributed for seed production in the region of Central North Bulgaria, as well as cultivars used in recent Bulgarian breeding program.

Materials and Methods

Plant material and growing conditions

The field experiments in the period 2018-2020 were based on the method of long plots and organized on non-irrigated area in the region of Pavlikeni, Soybean Experimental station, with the long term tradition of soybean cultivation. The region belongs to the temperate continental climate zone with continental rainfall regime – with a maximum in May-June and a minimum in August-September.

The soil type in region of Pavlikeni is leached chernozem, with humus horizon of 40-50 cm and good water holding capacity. The soil porosity and quench humidity are 47% and 15.2%, respectively. The soil reaction (pH) is neutral with humus content of 3-4%. (Naydenova & Vasileva, 2019).

The established soybean cultivation technology for our country was applied. The effect of seed inoculation with fresh culture of *B. japonicum* was established on 10 soybean cultivars belong to different maturity groups (MG00-MGII). Some of the cultivars are significant part from the soybean production and distribution in Bulgaria – Avigeya (MG I), Isidor (MG I), Srebrina (MG I), Richi (MG I), Rosa (MG II), Daniela (MG I) and Pavlikeni (MG I). The others – Romantika (MG 00, Ukrainian origin), Atlanta (MG 00, Ukrainian origin) and Saikai 20 (MG II, Japanese origin) are the main foreign genotypes, used in the establishment of a new hybrid gene pool in the Bulgarian soybean breeding program.

Chemical fertilization was not applied. Sowing was organized in rows, with a row spacing of 70 cm and a sowing rate of 400 000 seeds/ha. It was performed in the second ten days of April during the three experimental years 2018-2020.

Inoculation procedure and investigated parameters

The inoculation was conducted immediately before sowing by incubation of 100 seeds per row in 30ml fresh suspension for 10 min avoiding the direct sun light. The bacterial culture was prepared in YEB medium and incubated for 2 days at 28°C. The optical density was measured at 595nm and the values from 0.8 to 1.0 was accepted as optimal. The *B. japonicum* strain with collection number 10324, purchased from the American Type of Culture Collection (www.atcc.org.) was used.

Ten plants per cultivar grown from inoculated seeds and control seeds were monitored for the following morphological parameters: plant height (cm); number of branches; fresh plant mass (g) at R3 phase (reproductive phase); number of fertile nodes on the main stem/ fruiting nodes**;** fresh root mass (g); number of nodules; dry mass of nodules; number of pods per plant; number of seeds per plant; seed weight (g) per plant. The measurements were conducted during the three consecutive years 2018-2020.

Statistical analyses

The inoculation effect on the monitored parameters by cultivars and years was assessed by application of ANOVA: Single Factor (unfactored disperses analysis) and comparison of means was made with Least Significant Difference at 5%, 1% and 0.1% level of probability (LSD at $P<0.05$; $P<0.01$; $P<0.001$). The differences between mean values (from three-years period) of control and inoculated plants per cultivar were assessed by application of ANOVA: Two Factor with Replication (two factored disperses analysis). By this analysis the significance of the inoculation is monitored both way: separately (factor Inoc) from the environmental/year conditions (Y), and in combination with environmental/year conditions (Inoc X Year interaction).

Meteorological conditions during the investigated period

The investigations includes years with different rainfall during the vegetative and reproductive phases of crop development (Table 1). The first two experimental years had more favorable conditions for the flowering period, with corresponding monthly precipitation for June, of 134.6 mm in 2018, and 149.0 mm in 2019, respectively, and the distribution of precipitation over ten days was also even. Pods development in 2020 year takes place under conditions of severe drought – the precipitation amounts for July was 6.4 mm, and for August – 36.4 mm, respectively. For comparison in 2018, the precipitation amount for the same period is significantly higher – 143.2 mm.

Results and Discussion

Influence of* B. japonicum *inoculation on plant growth parameters

The parameters plant height (cm), number of branches, number of fertile nodes and fresh plant mass in R3, were monitored and data presented in Tables 2-5 and Figures 1-4. In general all evaluated parameters demonstrated that the effect of the additional inoculation of seeds with *B. japonicum* strongly depends by the genotype.

Data collected by the four parameters related to the plant growth show, that the number of branches and number of fertile nodes were significantly positively influenced by inoculation in comparison with the parameters plant height and fresh plant mass.

The data, presented on Figure 1, show a significant positive effect of inoculation to plant height only for cultivar Pavlikeni for growing season 2020 ($p<0.01$) (Table 2). For the parameter number of branches it was found that cultivar Srebrina was able to form significantly more branches from plants developed after inoculation ($p<0.01$). This effect was positive for the three growing season monitored, especially in 2020, when the perception was extremely low (Table 1). According to recent studies for the Avigeya cultivar, it was demonstrated that number of branches passes a high relative part to the total yield per plant (Georgiev et al. 2019). In this aspect is important to underline that this particular parameter show positive answer to inoculation for cultivar Avigeya during the growing season of 2020.

Table 1. Monthly precipitation (mm) for the vegetation period (April-October) during the experimental years (mm)

Experimental years	IV	V	VI	VII	VIII	IX	X	Rainfall amount April – October
2018	10.3	44.3	134.6	126.6	12.6	49.2	17.6	395.2
2019	94.4	41.8	149.0	77.8	8.6	2.4	13.0	387.0
2020	20.2	47.3	99.1	6.4	36.4	22.4	69.9	295.7

The Srebrina cultivar together with cultivars Pavlikeni and Rosa show significant elevation of number of fertile nodes, developed by treated plants: p<0.05; p<0.01; p<0.001 respectively, (Figure 3 and Table 4).

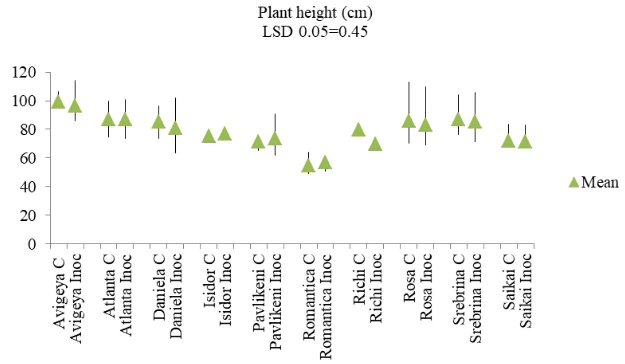

Fig. 1. Plant height (cm) of soybean cultivars presented as mean of three consecutive years

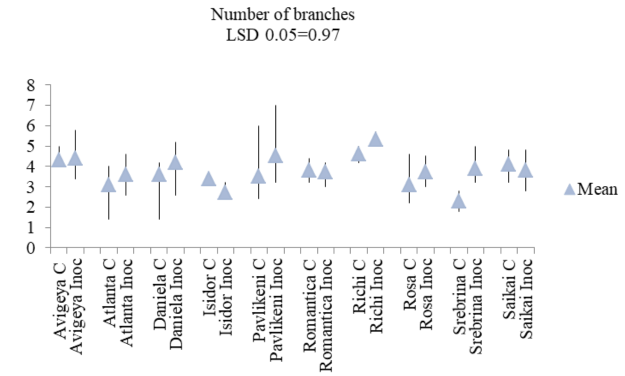

Fig. 2. Number of branches of soybean cultivars presented as mean of three consecutive years

The fresh plant mass was positively influenced (p<0.05) by seeds inoculation only for plants of cultivar Saikai (Figure 4). On the base of collected results we may conclude that the complex parameters like fresh plant mass were not affected by the additional inoculation with *B. japonicum*. According to Moretti et al. 2018, the additional inoculation do not caused a positive effect on plant biomass. The authors reported for an increase of biomass produced from the second crop grown under the crop rotation.

Influence of B. japonicum inoculation on nodulation potential

The nodulation potential of the cultivars was assessed by monitoring the parameters: fresh root mass, number of nodules and dry weight of nodules (Tables 6-8, Figures 5-7). The collected data demonstrates tendency, where the Bulgarian cultivars Pavlikeni, Rosa, Richi, Srebrina, Daniela and Avigeya, which are highly adapted to the soil conditions, after inoculation plants showed low values for the parameter fresh root mass in phase R3, as the deviation from the control plant was not significant (with exception of cultivar Richi) (Table 6). In the same time for foreign genotypes Atlanta, Romatica and Saikai, the fresh root mass is positively influenced (p<0.05 for Saikai). We assume that the introduced bacterial strain affects the root growth by disturbing the balanced interactions in the root microbiota, to which the Bulgarian cultivars are already adapted.

Additionally, our monitoring show that plants from foreign early cultivar Romantica were able to form more nodules after inoculation with *B. japonicim* (Table 7, Figure 6), in comparison with the other genotypes. The mean value of this parameter was significantly (p<0.01) positively affected during the three years observation.

Table 2. Plant height (cm) of soybean cultivars during three consecutive years

Experimental years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	95.0	89.4	85.3	na	85.6**	63.7	72.4	73.6	72.6	62.0	49.2	60.2	81.4**	66.7	70.4	69.3	80.0	78.2	72.2	71.5
2019	106.4	114.0	100.0	101.0	96.2	102.2	76.4	78.0	65.3	67.3	64.0*	59.8	na	na	113.2	110.0	104.0	105.6	83.8	83.2
2020	94.8	85.6	74.6	73.4	73.6	69.2	76.8	78.6	75.6	**90.8****	51.2	50.6	77.6	72.4	74.2	70.4	76.6	71.6	71.8	71.2
Mean	99.3	96.3	86.8	87.2	85.1*	80.6	75.2	76.7	71.1	73.4	54.8	56.9	79.5**	69.6	85.9	83.2	86.9	85.1	71.9	71.3
Y	P<0.001		P<0.001		P<0.001		P<0.05		P<0.001		P<0.001		P<0.001		P<0.001		P<0.001		ns	
Inoc	ns		ns		P<0.05		ns		ns		ns		P<0.01		ns		ns		ns	
Y*Inoc	ns		ns		P<0.05		ns		P<0.001		P<0.05		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc – inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant.

Table 3. Number of branches of soybean cultivars during three consecutive years

Experimenta lyears	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	5.0**	4.0	3.7	na	4.2	5.0	3.4	3.2	3.0	7.0	4.4	4.2	5.0	5.7	4.6	4.5	2.8	5.0**	4.8	3.9
2019	4.0	3.4	1.4	2.6**	1.4	2.6	3.2	2.4	6.0	5.5	3.2	3.0	na	na	2.2	3.0	1.8	3.2**	3.2	2.8
2020	4.2	**5.8***	4.0	4.6	4.2	5.2	3.5	2.6	2.4	3.2	3.8	4.0	4.2	5.0	2.4	3.6	2.4	3.6*	4.5	4.8
Mean	4.3	**4.4**	3.1	**3.6**	3.6	4.2	3.4	2.7	3.5	4.5	3.8	3.7	4.6	5.3	3.1	3.7	2.3	**3.9****	4.1	3.8
Y	P<0.05		P<0.05		P<0.01		ns		P<0.001		P<0.05		ns		P<0.05		ns		ns	
Inoc	ns		ns		ns		ns		ns		ns		ns		ns		P<0.01		ns	
Y*Inoc	P<0.05		ns		ns		ns		ns		ns		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor A – Year; Factor B – inoculation; Interaction A*B; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant

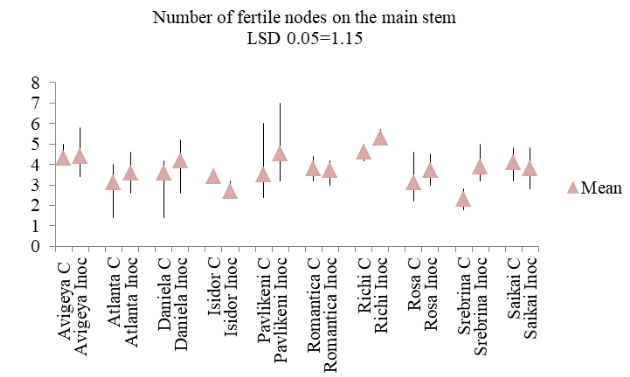

Fig. 3. Number of fertile nodes on the main stem of soybean cultivars presented as a mean of three consecutive years

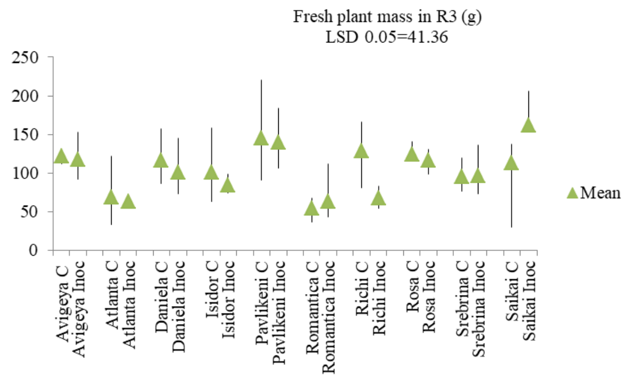

Fig. 4. Fresh plant mass in R3 (g) of soybean cultivars presented as a mean of three consecutive years

Table 4. Number of fertile nodes on the main stem of soybean cultivars during three consecutive years

Experimental years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	18.3	17.4	15.0	na	15.8	14.0	15.8	15.6	14.8	15.0	11.0	12.2	16.4	17.3	14.0	16.0*	15.6	18.2	16.4	16.2
2019	16.4	18.6	12.4	14.8*	13.4	13.8	16.2	15.4	15.3	18.3*	12.8	12.0	na	na	18.2	20.0	17.8	20.2*	15.4	16.1
2020	14.4	16.2	14.0	13.4	14.4	14.4	15.8	15.8	15.2	16.4	11.8	12.0	16.0	15.6	13.2	15.6*	14.8	14.2	14.8	17.0
Mean	16.1	17.4	13.9	**14.1**	14.5	14.1	15.9	15.6	15.1	**17.0****	11.9	12.1	16.2	16.3	15.1	**17.1****	16.1	**17.5***	15.6	16.5
Y	ns		P<0.05		ns		ns		P<0.05		ns		ns		P<0.001		P<0.001		ns	
Inoc	ns		ns		ns		ns		P<0.01		ns		ns		P<0.01		P<0.05		ns	
Y*Inoc	ns		P<0.05		ns		ns		P<0.05		ns		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc- inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na- not available; ns – not significant.

Table 5. Fresh plant mass in R3 (g) of soybean cultivars during three consecutive years

Expermen-tal years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	130.3	153.4	43.4	52.0	157.7	130.6	89.9	85.9	125.3	137.2	67.5	112.4	166.3*	68.5	141.0	130.3	119.8	136.0	77.1	85.6
2019	123.3	109.9	50.9	60.4	84.8*	66.7	65.6	72.0	220.6	168.2	40.2	38.8	139.7	78.1	114.1	102.8	75.5	61.7	147.7	130.9
2020	112.3	91.6	122.4	67.0	111.0	106.0	158.8	99.0	91.0	106.0	60.7	49.2	81.0	54.0	126.0	120.0	100.0	105.0	115.0	206.0
Mean	122.1	117.7	**68.3***	63.2	115.8	100.8	100.8	83.9	145.6	139.9	54.3	63.5	128.0**	67.5	124.6	115.9	95.7	96.3	113.2	**162.2***
Y	ns		P<0.05		P<0.05		P<0.05		P<0.05		P<0.01		P<0.001		P<0.001		P<0.05		P<0.01	
Inoc	ns		ns		ns		ns		ns		ns		P<0.01		ns		ns		P<0.05	
Y*Inoc	ns		P<0.05		ns		ns		ns		ns		P<0.05		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc – inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant.

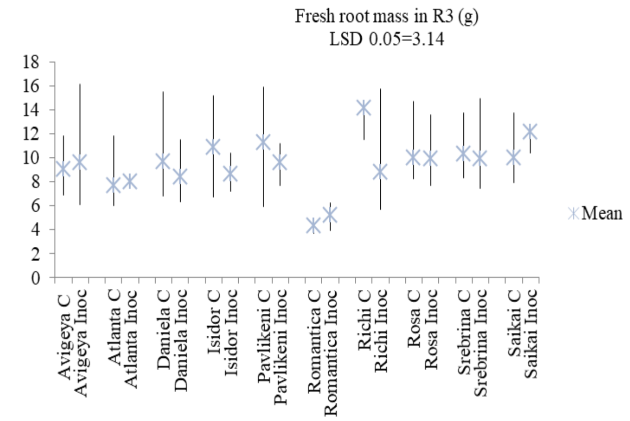

Fig. 5. Fresh root mass in R3 (g) of soybean cultivars presented as mean of three consecutive years

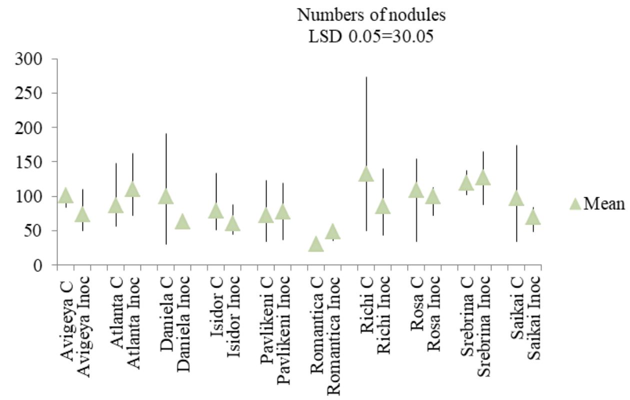

Fig. 6. Numbers of nodules of soybean cultivars presented as mean of three consecutive years

Table 6. Fresh root mass in R3 (g) of soybean cultivars during three consecutive years

Experimental years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	11.8	16.1	6.0	7.8	15.5	11.5	14.2	10.4	12.1	10.8	3.7	6.2	26.0*	15.7	14.7	13.6	13.7	14.9	8.0	10.4
2019	8.4	7.1	6.4	8.2	6.8	6.3	6.7	7.2	15.9	11.2	4.4	5.0	11.5	6.9	8.5	7.7	9.2	7.4	13.7	11.5
2020	6.9	6.1	11.8	7.9	7.4	7.2	15.2	9.1	5.9	7.7	4.7	3.9	8.2*	5.7	8.2	9.4	8.3	8.4	7.9	11.6
Mean	9.0	9.6	7.7	8.0	9.7	8.4	10.9	8.6	11.3	9.6	4.3	5.2	14.1*	8.8	10.0	9.9	10.3	9.9	10.0	12.1
Y	ns		P<0.05		P<0.05		P<0.05		P<0.05		P<0.01		P<0.05		P<0.05		P<0.01		ns	
Inoc	ns		ns		ns		ns		ns		ns		P<0.05		ns		ns		ns	
Y*Inoc	ns		P<0.05		ns		ns		ns		ns		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc – inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant.

Table 7. Numbers of nodules of soybean cultivars during three consecutive years

Experimental years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	108.2	110.6	69.0	NA	191.0*	66.3	133.5	88.0	60.2	75.6	37.4	56.4	273.8	140.0	155.0	110.0	137.4	164.4	34.0	NA
2019	108.0*	50.0	56.1	71.9	81.3*	55.8	62.4	55.4	123.0	119.3	27.6	52.0***	109.1	84.3	135.6	112.3	102.0	88.4	174.7*	84.1
2020	83.4	63.6	148.6	163.0	30.8	**69.0***	51.0	44.6	33.8	36.6	25.4	35.2	49.8	43.8	34.4	71.8	126.2	142.4	78.8	48.6
Mean	100.4	73.2	86.1	109.8	99.8*	63.4	**78.3***	60.2	72.3	77.2	29.8	**48.4****	**131.8****	85.7	108.8	99.7	119.5	126.6	96.8	69.3
Y	ns		P<0.001		P<0.01		P<0.001		P<0.001		P<0.05		P<0.001		P<0.001		P<0.05		P<0.01	
Inoc	ns		ns		P<0.05		P<0.05		ns		P<0.01		P<0.01		ns		ns		P<0.05	
Y*Inoc	ns		ns		P<0.01		ns		ns		ns		P<0.05		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc- inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na- not available; ns – not significant.

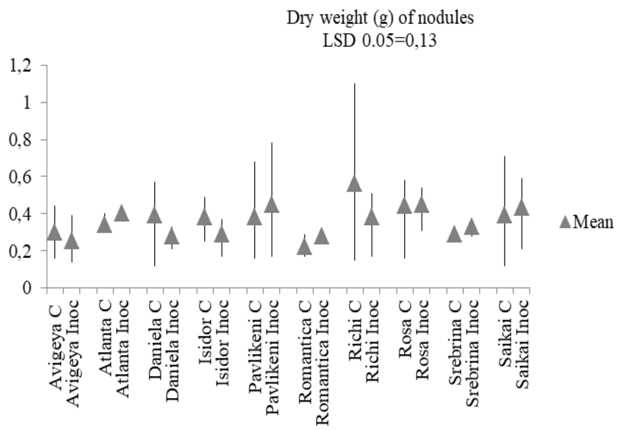

Fig. 7. Dry weight (g) of nodules of soybean cultivars presented as mean of three consecutive years

The data from dry mass of nodules shows that the Saikai cultivar was able to compensate the reduction in the nodules number by their higher dry mass (Table 8). The mean value from the three years observation a significant (p<0.01) positive influence of inoculation on dry mass of Romantica nodules was obtained (Table 8).

It is important to note that the cultivars Avigeya, Rosa and Srebrina, which possess specific adaptation to the ecological condition of North Bulgaria region, respond to the additional inoculation with the smallest factorial variance in terms of number and weight of nodules per plant. These results confirm the ecological stability of these genotypes, which are one of the widely used cultivars for soybean production in Bulgaria.

The successful nitrogen fixation depends by the interaction between the symbiotic bacteria and the other microorganisms of the natural microbiota. Additionally, the complex between legume plant and symbiotic bacteria is with the close connection with the microorganisms from the rizosphere zone of the plant (Donkova et al., 2014; Kavadia et al., 2020). The successful biological nitrogen fixation (BNF) relies on the legumes genotype and on the rhizobial strains. Differences between genotypes capacity for BNF have been reported for a variety of legumes (Dwivedi et al., 2015), cited by Vanlauwea et al. (2019). Taskas et al. (2018), also reported for selective response of the different soybean cultivars to *B. japonicum* strains.

Autoregulation of nodulation (AON) is other phenomena, which should to be discussed here. AON is related with the ability of plants to control the number of nodules, which process is started within 4 days after inoculation of soybean plant with symbiont (Kosslak & Bohlool, 1984), cited by Moretti et al. (2018) and suppress the further formation of nodules. The obtained results in this study show that the different soybean genotypes possess different capacity to overcome this process. In our study cultivar Romantica demonstrated best positive answer to additional inoculation with *B. japonicum* concerning nodulation capacity evaluated for all three parameters: fresh root mass, number of nodules and dry weight of nodules.

Influence of B. japonicum inoculation on yield parameters

The yield of all studied cultivars was assessed by the monitoring of number of pods per plant; number and weight of seeds per plant (Figures 8 -11). The mean value of data from three years of investigation showed highest capacity (p<0.05) to form pods under inoculation with *B. japonicum* for cultivar Pavlikeni (Figures 8, 9). Similar to Pavlikeni, the cultivars Rosa and Srebrina also demonstrated significant elevation in number of pods per plant, formed by inoculated

Table 8. Dry weight (g) of nodules of soybean cultivars during three consecutive years

Experimen-ta years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	0.27	0.39	0.35	NA	0.57	0.33	0.49**	0.31	0.31	0.41	0.17	0.25	1.10	0.51	0.55	0.45	0.29	0.35	0.12	NA
2019	0.44	0.22	0.30	0.38	0.46*	0.29	0.39	0.37	0.68	0.78	0.29	0.31	0.55	0.46	0.58	0.54	0.30	0.35	0.71	0.59
2020	0.16	0.14	0.40	0.43	0.12	0.21	0.25	0.17	0.16	0.17	0.18	0.27	0.15	0.17	0.16	0.31	0.29	0.28	0.31	0.21
Mean	0.30	0.25	0.34	0.40	0.39	0.28	**0.38***	0.29	0.38	0.45	0.22	**0.28***	**0.56***	0.38	0.44	0.45	0.29	0.33	0.39	**0.43**
Y	ns		ns		P<0.01		P<0.001		P<0.001		P<0.05		P<0.001		P<0.001		ns		P<0.01	
Inoc	ns		ns		Ns		P<0.05		ns		P<0.05		P<0.05		ns		ns		ns	
Y*Inoc	ns		ns		ns		ns		ns		ns		P<0.05		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc – inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant.

plants regardless of the conditions of the experimental years for cultivar Rosa (p<0.05) (Figure 9, Table 9). These results could be related to the proven significant positive effect of inoculation on the number of fertile nodes formed by these genotypes.

For the parameters number of seeds per plant and seeds weight per plant a positive effect (not significant) was found for the cultivars Rosa and Srebrina. This positive tendency was observed during the all three years (Figures 10-11) and could be related to the above-mentioned significant increase in the values of indicators number of pods, number of fruiting nods and number of branches for these cultivars due to the inoculation.

For the early cultivars Atlanta and Romantica a significant (p<0.05) positive effect of inoculation on the yield of seeds per plant was observed only for 2019, growing season. In both cultivars this result could be associated with an increased number of pods formed by the "inoculated plants", compared to the control in that particular year.

As a conclusion the number of pods was the only yield parameter that was positively affected by inoculation under the growth conditions tested.

The number of branches also could be considered as a trait sensitive to inoculation with potential effect on yield, but in this study only cultivar Srebrina has been shown to react with an increased number of branches under additional inoculation with nitrogen-fixing bacteria. The number of branches in addition to strong genotypic influence has a significant dependence by the nutrient area of the plant density and assessment of this indicator under inoculation should be organized in more precise formulation of the experiment.

Based on the analysis of variance the inoculation alone and in relation to the conditions of the year does not significantly affect the individual productivity of the plants, expressed in terms of number and weight of seeds per plant investigated under the growth conditions of Central North Bulgaria (Figures 10-11).

According to Carciochi et al. (2019), the lack of the positive effect on seed yield by additional inoculation could be explain by several factors like quantity and type of the nat-

Fig. 8. Number of pods developed by cultivar Pavlikeni, growing season 2019.
***A* – control plants, *B* – plants from inoculated seeds**

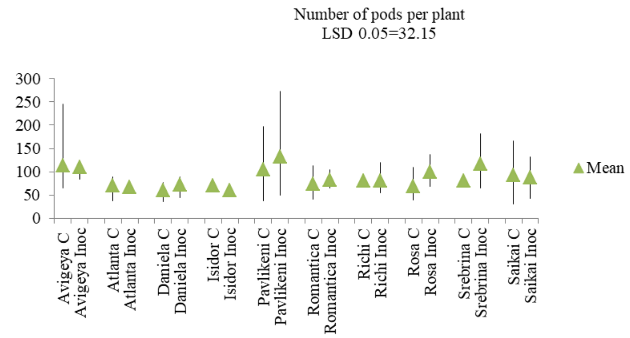

Fig. 9. Number of pods per plant of soybean cultivars presented as mean of three consecutive years

Table 9. Number of pods per plant of soybean cultivars during three consecutive years

Experimen-tal years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	244.7*	124.2	81.7	na	76.6	88.3	80.4	65.2	115.6	272.0	112.6	104.2	94.6	120.0	109.2	137.5	90.0	181.0*	167	132.4
2019	64.6	83.4	37.2	59.2*	36.0	44.6	65.8	64.2	197.0	199.8	40.6	64.2*	na	na	55.6	100.3*	77.6	104.0	80.0	84.0
2020	81.2	**120.6**	88.2	75.2	68.2	87.0	65.0	49.4	37.6	**50.0**	67.8	76.8	67.0	55.4	39.8	67.8	71.2	64.2	30.0	43.4
Mean	112.5	109.4	69.8	67.2	60.3	71.0	70.8	59.6	104.4	**132.1***	73.7	81.7	80.8	79.6	68.2	**99.2***	79.6	**116.4***	92.4	86.6
Y	P<0.001		P<0.05		P<0.05		ns		P<0.001		P<0.001		P<0.001		P<0.01		P<0.05		P<0.001	
Inoc	ns		ns		ns		ns		P<0.05		ns		ns		P<0.05		P<0.05		ns	
Y*Inoc	P<0.01		ns		ns		ns		P<0.05		ns		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc – inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant.

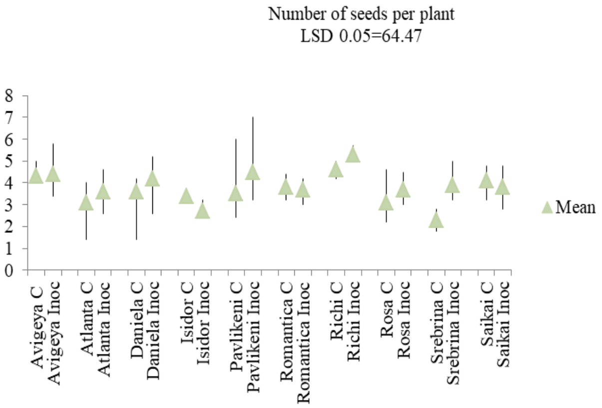

Fig. 10. Number of seeds per plant of soybean cultivars presented as mean of three consecutive years

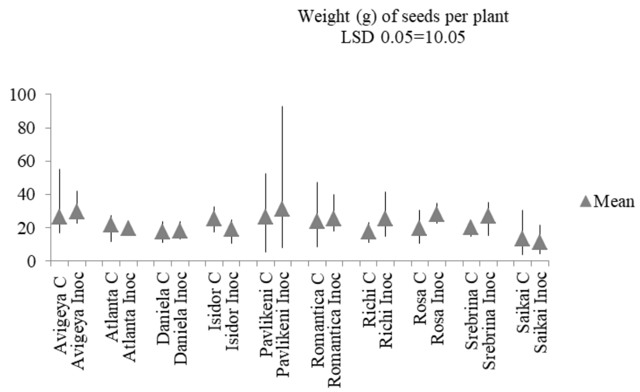

Fig. 11. Weight (g) of seeds per plant of soybean cultivars presented as mean of three consecutive years

Table 10. Number of seeds per plant of soybean cultivars during three consecutive years

Experimental years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	432.3	249.0	162.7	na	161.4	151.0	183.0	124.6	258.6	607.0	219.6	208.2	169.0	252.7	218.4	227.8	169.4	257.8	269.2	213.0
2019	128.8	146.3	70.8	**124.4**	71.0	89.2	140.0	140.2	427.0	394.3	55.4	**112.0**	na	na	116.6	216.8	162.2	228.2	53.8	51.6
2020	149.8	**200.8**	177.4	151.6	132.8	160.0	119.3	78.8	62.0	50.0	102.4	116.0	91.2	115.2	71.4	136.4	121.2	130.4	48.6	73.7
Mean	206.9	204.4	138.6	138.0	121.7	130.7	149.4	114.5	221.8	243.4	125.8	145.4	130.1	166.8	135.5	**189.2**	150.9	**205.5**	123.9	112.8
Y	P<0.001		P<0.05		ns		ns		P<0.001		P<0.001		P<0.01		P<0.05		P<0.05		P<0.05	
Inoc	ns		ns		ns		ns		ns		ns		ns		ns		ns		ns	
Y*Inoc	P<0.05		ns		ns		ns		ns		ns		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc- inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na- not available; ns – not significant.

Table 11. Weight (g) of seeds per plant of soybean cultivars during three consecutive years

Experimental years	Avigeya (MG I)		Atlanta (MG 00)		Daniela (MG I)		Isidor (MG I)		Pavlikeni (MG I)		Romantica (MG 00)		Richi (MG I)		Rosa (MG II)		Srebrina (MG I)		Saikai 20 (MG II)	
	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc	Con	Inoc
2018	55.4	42.3	27.6	na	23.7	16.4	32.7	24.8	31.5	93.1	47.5	40.1	23.2	41.8	30.3	34.5	22.9	35.5	30.7	21.9
2019	17.1	22.5	11.8	**20.8***	11.1	13.5	24.3	21.1	52.5	45.0	8.6	**18.2***	na	na	17.4	28.1	22.6	29.9	4.9	4.6
2020	17.4	23.1	24.6	18.2	18.2	24.0	17.3	10.4	5.6	7.8	14.3	18.1	11.4	15.0	10.5	22.8	14.8	15.1	3.8	6.4
Mean	26.1	29.3	**21.7***	19.5	17.7	18.2	25.3	18.8	26.4	31.2	23.5	25.5	17.3	25.1	19.4	**28**	20.1	**26.9**	13.1	11.0
Y	P<0.01		ns		ns		P<0.05		P<0.001		P<0.001		P<0.01		ns		P<0.05		P<0.01	
Inoc	ns		P<0.05		ns		ns		ns		ns		ns		ns		ns		ns	
Y*Inoc	ns		ns		ns		ns		ns		ns		ns		ns		ns		ns	

Legend: con – control plants; Inoc – plants from inoculated seeds; Factor Y – Year; Factor Inoc- inoculation; Interaction Y*Inoc; * (P<0.05); **(P<0.01); ***(P<0.001); na – not available; ns – not significant.

ural population of rhizobial bacteria in the evaluated region, soil organic meter, stress conditions. Therefore, future studies focused on the natural biodiversity of soil microbiota and BNF capacity of the nitrogen fixing bacteria in this region has to be conducted.

Assessment of the interaction between the* B. japonicum *seed inoculation and climatic conditions

It was discussed already that the effect of inoculation with *B. japonicum* to some genotypes depends by the conditions of the year. The calculation of the significance of the interaction between both parameters – inoculation and year (Y*Inoc) demonstrate the positive influence of inoculation under the severe conditions of the year 2020. This year was with extremely high temperatures in combination with low precipitation during June-August (Table 1). It was observed that the inoculated plants of the cultivar Avigeya were able to form more branches (p<0.05); from cultivar Rosa – more fertile nodes (p<0.05); from cultivar Daniela – more nodules per plant (p<0.05); from cultivars Avigeya and Pavlikeni more pods (p<0.01; p<0.05) and finally more seeds per plant from cultivar Avigeya (p<0.05). These observation support additional positive effect of inoculation with nitrogen fixing bacteria, related with increase the resistance of inoculated plants to various stress conditions. Such results were summarized in details in the review of Kavadia et al. (2020). The authors describe several examples as: induction of phytohormone production by inoculated plants; regulation of the ethylene produced under various stress conditions in the plant tissue by symbiotic bacteria; improve availability of phosphorus in the soil.

Conclusion

In the period of three consecutive years (2018-2020), the parameters related the plant growth, nodulation and yield of ten soybean cultivars with high importance for soybean production were monitored under conditions of Central North Bulgaria. The cultivation technology developed for this particular region was compared with application of additional inoculation of seeds before sowing with *B. japonicim*. The collected data demonstrated strong dependence of the plant response by the genotype and by the climatic conditions of the year.

Our results give new information for cultivars with high application in Bulgarian seed production. For example, the cultivars Rosa and Srebrina demonstrated tendency for better seed production, as a result of better development of branches, fruiting nods and pods under additional inoculation. It is important to underline that the cultivars Avigeya, Daniela and Pavlikeni expressed good ability to overcome critical climatic conditions under additional inoculation with *B. japonicum.* Better nodulation performance of the foreign cultivars Romantica, Atlanta and Saikai was demonstrated after inoculation. All these results provide a significant base to promote these cultivars to Bulgarian farmers intended to produced soybean, especially in the regions without soybean history.

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