Legume Research, 42(5) 2019: 688-693
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com

Influence of Seed Inoculation with Commercial Bacterial Inoculants (*Bradyrhizobium Japonicum*) on Growth and Yield of Soybean

W. Jarecki* and D. Bobrecka-Jamro

Department of Crop Production, Faculty of Biology and Agriculture,
University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, Poland.
Received: 18-03-2019 Accepted: 21-08-2019 DOI: 10.18805/LR-485

ABSTRACT

A field experiment was carried out with three bacterial inoculants: HiStick® Soy, Nitragina, Nitrazon and the control. Number and dry weight of nodules were significantly higher with inoculation of bacterial inoculants compared to the control. In 2017, the most pods per plant were obtained after the use of Nitrazon or HiStick® Soy, while in 2018, after the use of HiStick® Soy or Nitragina. In 2018, the highest 1000 seed weight was obtained after the use of Nitragina. In 2017, the seed yield was the most favourably affected by the inoculants HiStick®Soy (3.31 t ha^{-1}) and Nitrazon (3.37 t ha^{-1}) and in 2018, by HiStick® Soy (3.92 t ha^{-1}) and Nitragina (3.87 t ha^{-1}). High Soil Plant Analysis Development (SPAD) and Leaf Area Index (LAI) readings, were observed with HiStick® Soy.

Key words: *Glycine max* (L.) Merr., Leaf area index, Soil plant analysis development, Yield, Yield components.

INTRODUCTION

In 2017, the soybean cultivation area in Poland amounted to 9.3 thousand ha and the harvest was 20.3 thousand tonnes (FAOSTAT). The obtained soybean yields are variable in years and lower than the global ones.

A beneficial feature of soybean is its ability to associate with rhizobia bacteria in its root nodules to fix atmospheric nitrogen through symbiotic nitrogen (N) fixation (Thilakarathna and Raizada, 2017). Therefore, an important procedure in soybean cultivation is the inoculation of seed with symbiotic bacteria *Bradyrhizobium japonicum* (Jarecki *et al*., 2016; Martyniuk *et al*., 2018). As a result of this treatment, a high seed yield can be obtained with no or limited nitrogen fertilization of soybean plants (Zuffo *et al*., 2018). The bacteria *Bradyrhizobium japonicum* are rare in Polish soils (Narożna *et al*., 2015). The inoculation of seed with preparations containing symbiotic bacteria is therefore justified, especially if no soybean was previously grown in a given field (Tewari *et al*., 2003). The effectiveness of symbiosis of soybean plants with rhizobia depends on many factors, including cultivation technology, soil and climate (Thilakarathna and Raizada, 2017). Many studies (Kozieł *et al*., 2013; Hungria *et al*., 2015; Jarecki *et al*., 2016; Martyniuk *et al*., 2013) shows that inoculation of soybean seed with symbiotic bacteria results in a significant increase in seed yield. This is usually obtained when there are no natural *Bradyrhizobium japonicum* in the soil. Rampim *et al*. (2015), in turn, did not show the effect of seed inoculation with symbiotic bacteria on 1000 seed weight and the size of soybean yield. Thuita *et al*. (2012) and Muleta *et al*. (2017) obtained different efficacy of the bacterial inoculants used. In addition, the effect of the bacterial inoculants used was dependent on the type of soil. Ronner *et al*. (2016) proved the beneficial effect of soybean seed inoculation in cooperation with phosphate fertilization. The effect of this was increased uptake of macroelements by soybean plants. The aim of the experiment was to determine the response of soybean for the inoculation of seed with commercial bacterial inoculants.

MATERIALS AND METHODS

Field experiment was conducted during 2017 and 2018. It was located on a private farm in Makowisko (50°3'N 22°47'E), Podkarpackie voivodeship, Poland. Soybean was not been grown in the experimental field before. It was a one-factor experiment in four replications. The experimental factor was three bacterial inoculants for soybean seeds: HiStick® Soy (the company BASF, Germany), Nitragina (the company Institute of Soil Science and Plant Cultivation – State Research Institute, Poland), Nitrazon (the company Farma Žiro, LLC, Czech Republic) and the control. These preparations contain *Bradyrhizobium japonicum*, symbiotic bacteria for soybean seeds. The study was conducted on the cultivar Mavka (Scientific Research Center of Soya Development"AgeSoya" Sp. z o.o., Poland). The cultivar Mavka is a high-yielding variety recommended for cultivation in the area of the study. Bacterial inoculants were used immediately before sowing the seeds.

The weather conditions in 2017 differed from the long-term average. In 2018, the weather conditions were beneficial for soybean cultivation (Fig 1).

*Corresponding author's e-mail: waclaw.jarecki@wp.pl

The experiment was established in the sandy loam, Gleic Fluvisol - FLgl (Food and Agriculture Organization of the United Nations, 2015). This was the good wheat complex. The soil was slightly acidic. The content of available phosphorus, potassium and magnesium was high (Table 1). The soil samples analysis was carried out at the District Chemical-Agricultural Station in Rzeszów, according to the Polish standards.

Sowing of seeds was made on 19.05.2017 and 14.05.2018. The seeding rate was 50 germinating seeds per m^2, the row spacing 45 cm, the sowing depth 3 cm. The plot area was 18 m^2. The previous crop was winter wheat. NPK fertilization in kg ha^{-1} amounted to 30 N, 40 P_2O_5 and 60 K_2O, respectively. Afalon Dyspersyjny 450 S.C. (linuron) was used for weed control in a dose of 1.5 dm^3 ha^{-1}. No disease and pest control was used. Plant density per m^2 was calculated before harvest. The LAI index (Leaf Area Index) was measured with the apparatus AccuPAR PAR/LAI Ceptometer Model LP-80 by Meter Group, Inc. USA. Plant nutritional status SPAD (Soil Plant Analysis Development) was measured with a chlorophyll meter SPAD 502P by Konica Minolta, Inc. Japan. Plant growth stages were given according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamtund Chemische Industrie). The LAI measurement was made at stages BBCH 60 and BBCH 70. The SPAD index measurement was performed at stages BBCH 12, BBCH 51, BBCH 60, BBCH 70 and BBCH 75. At the flowering stage, root nodules were counted on 10 randomly harvested roots and then their dry weight was determined. During the technical maturity stage, 10 representative plants were collected from each plot for biometric measurements. Soybean seeds were harvested on 21.09.2017 and 1.10.2018 at full maturity. The seed yield obtained from the plots was converted into per ha yield, taking into account 15% moisture content. The obtained results were subjected to statistical analysis. An analysis of variance was made. The significance of differences between the characteristic values was found based on Tuckey's half-confidence intervals, with the significance level $\alpha = 0.05$. Calculations were made using the software Statistica 10 PL (Stat Soft, Inc., Tulsa, USA).

RESULTS AND DISCUSSION

The number of nodules and the dry weight of nodules were significantly higher after the use of bacterial inoculants compared with the control. However, it should be noted that the inoculant Nitragina was not very effective in 2017 and Nitrazon in 2018. Martyniuk *et al.* (2018), after applying seed inoculation with rhizobia, obtained an average of 4.9 nodules per root. However, after applying a bacterial inoculant to the soil, they obtained an average of 12.3 nodules per root. Many authors (Tewari *et al.*, 2003; Kozieł *et al.*, 2013; Marinković *et al.*, 2018) indicated an increase in nodulation on soybean roots after seed inoculation with *Bradyrhizobium japonicum*. Thuita *et al.* (2012) report that both the number of nodules on the roots and their weight are significantly dependent on the inoculant used. At the same

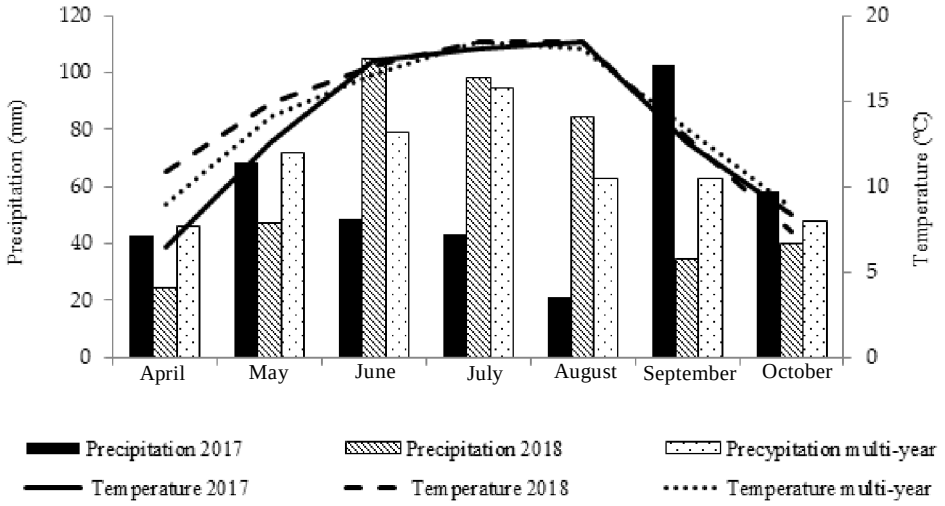

Fig 1: Weather conditions in 2017-2018.

Table 1: Properties of the soil profile.

Year	Soil reaction (1 mol/L KCl)	Humus %	N_{min} kg ha^{-1} DM	P_2O_5	K_2O	Mg
				mg $^{-1}$ 100 g $^{-1}$ soil		
2017	6.24	1.82	60.2	28.4	34.6	4.4
2018	5.80	1.66	54.5	22.5	22.5	2.5

time, the obtained results were also heavily influenced by the cultivar. Bekere *et al*. (2013) showed that the number and dry weight of nodules on soybean roots was greater when lime fertilization was used in addition to seed inoculation with symbiotic bacteria. This was due to the low acidity of the soil in the experiment.

The plant density before harvest was low, which resulted from the accepted seeding rate. Bacterial inoculants did not influence the discussed parameter. The plant density was similar in 2017 and 2018. The number of pods per plant was the highest after the use of Nitrazon and HiStick® Soy. The plants on the control developed the least pods per plant. In 2018, the most pods per plant were obtained after the use of HiStick® Soy and Nitragina. Kozieł *et al.* (2013) and Martyniuk *et al.* (2018) report that a significant increase in the number of pods on the soybean plant is obtained under the influence of inoculation of seeds with symbiotic bacteria. In contrast, Htwe *et al.* (2018) did not show the effect of bacterial inoculants on the number of pods per plant.

The number of seeds per pod was not modified by the bacterial inoculants used. In 2017, the number of seeds per pod was higher compared with 2018. Martyniuk *et al*. (2018) also did not confirm the effect of inoculation with symbiotic bacteria on the number of seeds in the pod. In 2017, 1000 seed weight was not modified by the bacterial inoculants used. In 2018, the use of Nitragina significantly increased 1000 seed weight compared with the other inoculants and the control. Rampim (2015) did not show the effect of soybean seed inoculation on 1000 seed weight. In 2017, the seed yield was significantly higher after the use of the inoculants HiStick® Soy and Nitrazon as compared with Nitragina. The plants on the control gave the lowest yield. In 2018, the plants gave high yields after applying HiStick® Soy and Nitragina. Significantly lower yield was obtained after using Nitrazon and the lowest on the control (Table 2). On average, in the years of the study, HiStick® Soy had the most favourable effect on soybean yield. The obtained increase in seed yield was 23% compared with the control. The study by Martyniuk *et al.* (2018) shows that inoculation of seed with symbiotic bacteria results in a significant increase in soybean yield. The obtained yield gain was higher when the rhizobia were applied to the soil. Hungria *et al.* (2015) report that seed inoculation allows for significant reductions in nitrogen fertilization of soybean plants. This is of great economic and environmental importance. Căpăţnă *et al.* (2017) after seed inoculation with nitrogen-fixing bacteria, obtained an increase in seed yield by 3.76% compared with the control. Bekere *et al.* (2013) report that the effectiveness of seed inoculation on acidic soils can be increased by means of lime fertilization. Nyoki and Ndakidemi (2016), in turn, showed a beneficial effect of soybean seed inoculation along with fertilization with phosphorus and potassium. In a study by Rampim *et al*. (2015), no increase in soybean seed yield was obtained under the influence of seed inoculation with symbiotic bacteria.

In 2017, the LAI index measured at stage BBCH 70 was the highest after the use of Nitrazon in relation to

Table 2: The effect of bacterial inoculations on the parameters tested.

Parameter tested	Year	Control	HiStick® Soy	Nitragina	Nitrazon
The number of nodules	2017	0.55±0.05^{c}	17.1±0.27^{a}	5.86±0.09^{b}	16.3±0.28^{a}
	2018	1.80±0.11^{c}	26.8±0.32^{a}	27.2±0.37^{a}	6.05±0.09^{b}
	Average	1.18±0.08^{c}	22.0±0.30^{a}	16.5±0.23^{b}	11.2±0.19^{b}
The dry weight of nodules (g)	2017	0.04±0.01^{c}	0.67±0.06^{a}	0.23±0.03^{b}	0.65±0.05^{a}
	2018	0.08±0.02^{c}	0.83±0.09^{a}	0.85±0.08^{a}	0.32±0.03^{b}
	Average	0.06±0.02^{c}	0.75±0.08^{a}	0.54±0.06^{b}	0.49±0.04^{b}
The plant density before harvest (pcs. m^{-2})	2017	31.3±2.35	30.2±2.46	32.9±2.21	28.9±2.57
	2018	31.5±1.57	30.5±1.31	30.3±1.28	30.7±1.69
	Average	31.4±1.96	30.4±1.89	31.6±1.75	29.8±2.13
The number of pods per plant	2017	30.9±3.18^{c}	39.3±2.34^{a}	33.9±2.75^{b}	41.9±2.91^{a}
	2018	32.8±2.14^{c}	38.5±3.54^{a}	38.7±3.66^{a}	35.8±2.75^{b}
	Average	31.9±2.66^{c}	38.9±2.94^{a}	36.3±3.21^{b}	38.9±2.83^{a}
The number of seeds per pod	2017	1.98±0.03	2.00±0.02	1.98±0.03	2.01±0.02
	2018	1.82±0.02	1.96±0.03	1.88±0.03	1.94±0.02
	Average	1.90±0.03	1.98±0.03	1.93±0.03	1.98±0.02
Thousand grains weight (g)	2017	141.1±1.72	143.3±1.67	141.9±1.53	142.7±1.70
	2018	175.8±1.91^{b}	178.5±1.82^{b}	185.0±1.86^{a}	177.2±1.90^{b}
	Average	158.5±1.82^{b}	160.9±1.75^{ab}	163.5±1.70^{a}	160.0±1.80^{ab}
Yield (t ha^{-1})	2017	2.65±0.11^{c}	3.31±0.21^{a}	3.09±0.18^{b}	3.37±0.23^{a}
	2018	3.22±0.26^{c}	3.92±0.33^{a}	3.87±0.28^{a}	3.50±0.15^{b}
	Average	2.94±0.19^{c}	3.62±0.27^{a}	3.48±0.23^{b}	3.44±0.19^{b}

Values with the same letters are not significantly different at P< 0.05 level.

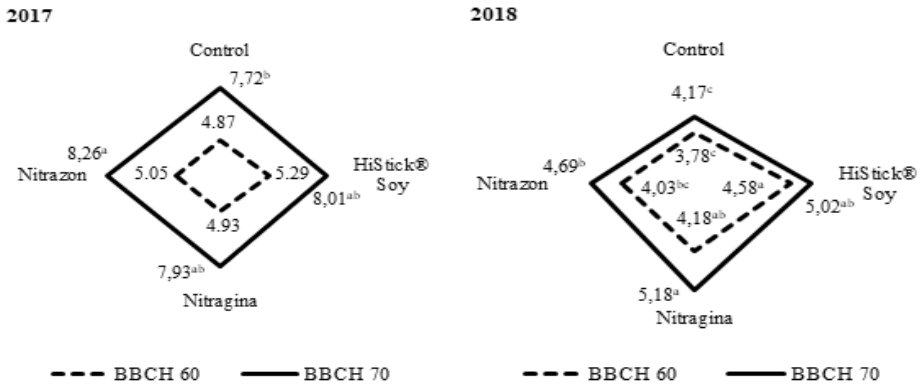

Fig 2: Leaf area index (LAI) in 2017 and 2018. Values with the same letters are not significantly different at P< 0.05 level.

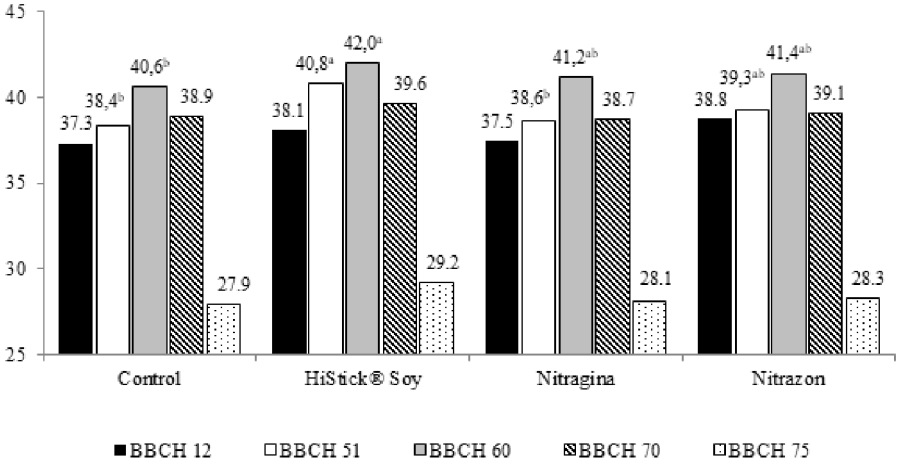

Fig 3: Soil Plant Analysis Development (SPAD) in 2017. Values with the same letters are not significantly different at P< 0.05 level.

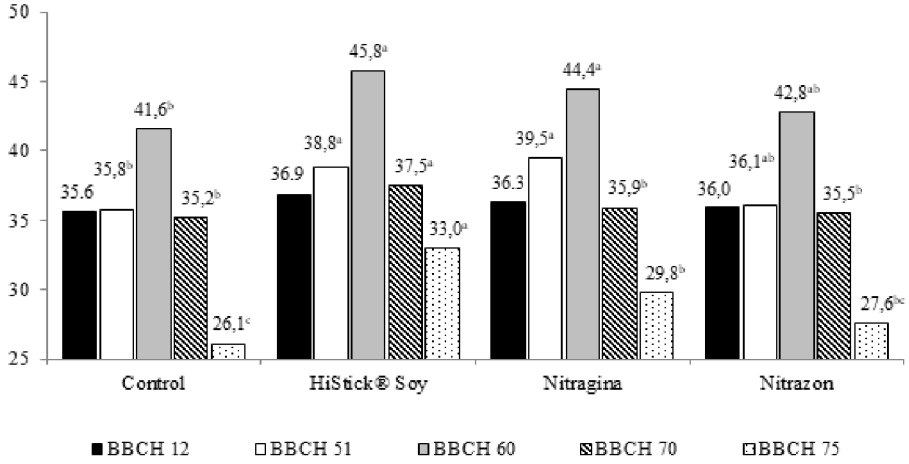

Fig 4: Soil Plant Analysis Development (SPAD) in 2018. Values with the same letters are not significantly different at P< 0.05 level.

the control. The resulting difference amounted to 0.54 $m^2 \cdot m^2$. The LAI measurement at stage BBCH 60 was not varied. In 2018, the LAI index was significantly modified at both stage BBCH 60 and BBCH 70. The lowest LAI values were obtained on the control. In the discussed year, the LAI index at stage BBCH 70 was more than 5 $m^2 \cdot m^2$ after the use of Nitragina or HiStick® Soy (Fig 2). Mondani *et al*. (2019) after using the soil microorganisms Bacillus obtained an increase in the LAI index by 13.2%.

In 2017, the SPAD index was significantly different at stages BBCH 51 and BBCH 60. At stage BBCH 51, the inoculant HiStick®Soy had the most favourable effect on the SPAD index and significantly lower SPAD readings were obtained after the inoculant Nitragina and on the control. At

stage BBCH 60, the highest SPAD was obtained after the application of HiStick® Soy and significantly lower in the control plants. The difference obtained was 1.4 units. The use of bacterial inoculants did not affect SPAD measurements at stages BBCH 12, BBCH 70 and BBCH 75 (Fig 3).

In 2018, the use of bacterial inoculants significantly modified the SPAD index. Only at stage BBCH 12, the obtained differences were insignificant. At BBCH 51 and BBCH 60, high SPAD values were obtained after the use of the inoculants HiStick® Soy and Nitragina and significantly lower on the control. At stages BBCH 70 and BBCH 75, the significantly highest SPAD index was obtained after using HiStick® Soy (Fig 4). In a study by Kühling *et al*. (2018), it was shown that the SPAD value was significantly higher after the inoculation of soybean seed with symbiotic bacteria, but only at the seed filling stage. At earlier development stages, they did not obtain any differences in the SPAD index.

CONCLUSION

The bacterial inoculants used had a favourable effect on the number and dry weight of nodules. The number of pods per plant was most significant under the influence of bacterial inoculants, while 1000 seed weight was less affected. The inoculant HiStick® Soy had the most favourable effect on seed yield. High seed yields were also obtained after the inoculant Nitrazon in 2017 and Nitragina in 2018.

ACKNOWLEDGMENT

This work was supported by the National Centre for Research and Development, within the framework of the strategic R&D programme “Environment, agriculture and forestry” - BIOSTRATEG. No. BIOSTRATEG3/346390/4/ NCBR/2017. Duration of the project 2017-2020. Title “Development of innovative biodegradable soybean seed coating based on biopolymers from renewable raw materials for better tolerance of plants to adverse environmental conditions” (acronym: BIOSOYCOAT).

REFERENCES

Bekere, W., Kebede, T., Dawud, J. (2013). Growth and nodulation response of soybean (*Glycine max* L.) to lime, *Bradyrhizobium japonicum* and nitrogen fertilizer in acid soil at Melko, south western Ethiopia. *Int. J. Soil Sci.* **8**: 25-31.

Căpăţnă, N., Bolohan, C., Marin, D.I. (2017). Research regarding the influence of mineral fertilization along with *Bradyrhizobium japonicum* on soybean grain yield (*Glycine max* (L.) Merrill), under the conditions of south - east Romania. *Sci. Papers. Agron*. **60**: 207-214.

Food and Agriculture Organization of the United Nations (2015). World reference base for soil resources - 2014. FAO, Rome, Italy. *World Soil Resources Reports*. **106**: 1-192.

Htwe, A.Z., Moh, S.M., Moe, K., Yamakawa, T. (2018). Effects of co-inoculation of *Bradyrhizobium japonicum* SAY3-7 and *Streptomyces griseoflavus* P4 on plant growth, nodulation, nitrogen fixation, nutrient uptake and yield of soybean in a field condition. *Soil Sci. Plant Nutr*. **64**: 222-229.

Hungria, M., Nogueira, M.A., Araujo, R.S. (2015). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. *Am. J. Plant Sci*. **6**: 811-817.

Jarecki, W., Buczek, J., Bobrecka-Jamro, D. (2016). Response of soybean (*Glycine max* (L.) Merr.) to bacterial soil inoculants and foliar fertilization. *Plant Soil Environ*. **62**: 422-427.

Kozieł, M., Gêbala, B., Martyniuk, S. (2013). Response of soybean to seed inoculation with *Bradyrhizobium japonicum* and with mixed inoculants of *B. japonicum* and *Azotobacter chroococcum*. *Pol. J. Microbiol*. **62**: 457-460.

Kühling, I., Hüsing, B., Bome, N., Trautz, D. (2018). Soybeans in high latitudes: effects of *Bradyrhizobium* inoculation in northwest Germany and southern west Siberia. *Organic Agri*. **8**: 159-171.

Marinković, J., Bjelić, D., Tintor, B., Miladinović, J., Đukić, V., Đorđević, V. (2018). Effects of soybean co-inoculation with plant growth promoting rhizobacteria in field trial. *Rom. Biotech. Lett*. **23**: 13401-13408.

Martyniuk, S., Kozieł, M., Stalega, J. (2013). Effect of various strains of symbiotic bacteria on yields and nodulation of lupine and soybean. *J. Res. Appl. Agric. Eng*. **58**: 67-70.

Martyniuk, S., Kozieł, M., Gałązka, A. (2018). Response of pulses to seed or soil application of rhizobial inoculants. *Ecol. Chem. Eng. S*. **25**: 323-329.

Mondani, F., Khani, K., Honarmand, S.J., Saeidi, M. (2019). Evaluating effects of plant growth-promoting rhizobacteria on the radiation use efficiency and yield of soybean (*Glycine max*) under water deficit stress condition. *Agric. Water Manage*. **213**: 707-713.

Muleta, D., Ryder, M.H., Denton, M.D. (2017). The potential for rhizobial inoculation to increase soybean grain yields on acid soils in Ethiopia. *Soil Sci. Plant Nutr*. **63**: 441-451.

Narożna, D., Pudełko, K., Króliczek, J., Goliñska, B., Sugawara, M., Mądrzak, C.J., Sadowsky, M.J. (2015). Survival and competitiveness of *Bradyrhizobium japonicum* strains 20 years after introduction into field locations in Poland. *Appl. Environ. Microbiol*. **81**: 5552-5559.

Nyoki, D., Ndakidemi, P.A. (2016). Intercropping system, rhizobia inoculation, phosphorus and potassium fertilization: a strategy of soil replenishment for improved crop yield. *Int. J. Current Microbiol. Appl. Sci*. **5**: 504-522.

Rampim, L., Lana, M.D.C., Lima, P.R., Rosset, J.S., Klein, J., Richart, A., Guimaraes, V.F., Sarto, M.V.M., Frandoloso, J.F., Mattei, D., Molin, P.V.D., Koppo, J., Diel, P.S., de Souza, A.K.P. (2015). Inoculation of Bradyrhizobium with cellular additives and micronutrients in soybean seeds cultivated in Oxisol under no-tillage system. *Afr. J. Microbiol. Res*. **9**: 1613-1621.

Ronner, E., Franke, A.C., Vanlauwe, B., Dianda, M., Edeh, E., Ukem, B., Bala, A., van Heerwaarden, J., Giller, K.E. (2016). Understanding variability in soybean yield and response to P-fertilizer and rhizobium inoculants on farmers' fields in northern Nigeria. *Field Crop. Res.* **186**: 133-145.

Tewari, K., Minagawa, R., Suganuma, T., Fujikake, H., Ohtake, N., Sueyoshi, K., Takahashi, Y., Tsuchida, T., Ohyama, T. (2003). Effect of deep placement of slow release nitrogen fertilizers and inoculation of Bradyrhizobia on the first cropping of soybean in the field dressed with mountain soil. *Soil Sci. Plant Nutr.* **74**: 183-189.

Thilakarathna, M.S., Raizada, M.N. (2017). A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions. *Soil Biol. Biochem.* **105**: 177-196.

Thuita, M., Pypers, P., Herrmann, L., Okalebo, R.J., Othieno, C., Muema, E., Lesueur, D. (2012). Commercial rhizobial inoculants significantly enhance growth and nitrogen fixation of a promiscuous soybean variety in Kenyan soils. *Biol. Fertil. Soils.* **48**: 87-96.

Zuffo, A.M., Steiner, F., Busch, A., Zuffo Júnior, J.M., Mendes, A.E.S., de Oliveira, N.T., Zambiazzi, E.V. (2018). Quality of soybean seeds in response to nitrogen fertilization and inoculation with *Bradyrhizobium japonicum*1. *Pesqui. Agropecu. Trop. Goiânia*. **48**: 261-270.