

## Response of soybean (*Glycine max* (L.) Merr.) to bacterial soil inoculants and foliar fertilization

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### ABSTRACT

Soybean yields can be considerably improved by inoculation with selected *Bradyrhizobium japonicum* strains and foliar fertilization. An exact field experiment was carried out in 2012–2014 at the Experimental Station of Cultivar Assessment in Przecław, Poland. The test plant was soybean cv. Aldana. The experimental factors were: bacterial inoculant Nitragina (*Bradyrhizobium japonicum*); foliar fertilization with Mikrokomplex; combined applications Nitragina + Mikrokomplex and the control treatment. Significant effect of Nitragina on an increase in the number of plants prior to harvest, plant height and the number of pods per plant was indicated. Fertilization with Mikrokomplex caused an increase in the number of pods per plant and thousand seed weight. Nitragina + Mikrokomplex increased the number of plants prior to harvest, plant height, the number of pods per plant and thousand seed weight. Seed yield was significantly higher in all the treatments compared to the control (2.31 t/ha). Higher soil plant analysis development values were found after the application of Nitragina + Mikrokomplex, and in the stage of pod development, also after foliar fertilization with Mikrokomplex. Application of Nitragina and Nitragina + Mikrokomplex resulted in an increase in leaf area index and mean tip angle and total protein in seeds. Fe content in seeds was the lowest in the control (69.2 mg/kg) and significantly higher in the other treatments (Nitragina, Nitragina + Mikrokomplex), and Mg content significantly increased after the application of Mikrokomplex and Nitragina + Mikrokomplex.

**Keywords:** legumes; nutrients; chlorophyll; canopy; chemical composition of seeds

Legumes are important crops for food or feed worldwide (Sato et al. 2012, Vollmann et al. 2015). In the system of sustainable agriculture, studies on the increase in the amount of nitrogen symbiotically fixed by legumes are of particular importance. Thanks to the symbiosis with nodule bacteria fixing atmospheric nitrogen from the air these plants are not fertilized with nitrogen or with a starting rate only (Lošák 2007, Chianu et al. 2011, Portes et al. 2012). In soils where appropriate symbiotic bacteria do not occur at all or are very few, it is recommended to inoculate soybean sowing seeds with a suitable strain of the bacterium *Bradyrhizobium japonicum* (Abbasi et al. 2008, Afzal et al. 2010). The effect of seed inoculation with nodule bacteria is dependent on many factors, including the environmental conditions, cultivars or the applied bacterial strain (Pan et al. 2002, Rodríguez-Navarro et al. 2011).

In soybean cultivation, it is also recommended to use the starting rate of nitrogen (Fecák et al. 2010), which has a favourable effect on seed yield. Also other macro- and microelements, which can be applied in the form of soil fertilization or foliar fertilization (Freeborn et al. 2001, Seidel et al. 2015), play an important role in plant nutrition (Afzal et al. 2010, Fecák et al. 2010). The previous studies have indicated that foliar fertilization of soybean is justified and the effects of the fertilizer application depend on many factors, including cultivation technology and the weather conditions during the growth period (Odeleye et al. 2007, Kobraee and Shamsi 2013). The aim of this study was to estimate the response of soybean to inoculation of seeds with Nitragina, foliar fertilization with Mikrokomplex and combined applications of Nitragina + Mikrokomplex. In the research hypothesis it was assumed that inoculation of

Table 1. Total monthly precipitation (mm)

Year	April	May	June	July	August	September	Total
2012	21.7	66.7	66.9	65.6	61.8	55.0	337.7
2013	39.4	111.7	192.4	58.3	21.2	68.6	491.6
2014	34.8	108.9	71.7	146.8	101.8	49.7	513.7
1956–2011	48.1	39.2	79.3	101.6	71.3	54.7	394.2

seeds with bacteria *Bradyrhizobium japonicum* and foliar fertilization of plants would cause a positive change in tested quantitative and qualitative characteristics of soya. It was assumed that soil plant analysis development (SPAD) measurements would be useful for assessment of the state of plant nourishment during their growth. The results of leaf area index (LAI) and mean tip angle (MTA) measurements, in turn, would allow noticing changes in the canopy architecture determining the seed yield.

## MATERIAL AND METHODS

**Field experiment.** An exact field experiment was carried out over 2012–2014. It was located at the Experimental Station of Cultivar Assessment in Przecław (50°11'N, 21°29'E), south-eastern Poland, in soil originated from clay loam, classified as Gleyic Fluvisol (FAO 2006). These soils are commonly used to grow soybean in this region of Poland. The experiment was established in four replications in complete randomized block design. The experiment comprised the following treatments: bacterial inoculant Nitragina (N); foliar fertilization with Mikrokomplex (M); bacterial inoculant Nitragina plus foliar fertilization with Mikrokomplex (N + M); control treatment (C). The weather conditions were recorded at the Experimental Station of Cultivar Assessment in Przecław. The lowest total precipitation from April

to September occurred in 2012. High rainfalls were recorded in May and June 2013 and in May, July and August 2014. They considerably exceeded the long-term average (Table 1). Higher air temperatures during the plant growth were recorded in 2012, and the lowest in 2014 (Table 2). Analysis of soil samples was performed at the Chemical and Agricultural Station in Rzeszów, according to the Polish Standards (accredited laboratory). Soil samples were collected by a sampling stick to a depth of 0–30 cm and 30–60 cm. The soil showed neutral reaction.  $C_{org}$  content was moderate and  $N_{min}$  was low. Soil abundance in Mg was high, in P and K was medium or high, and in S was low. The content of microelements were medium or high, except for B, which was low (Table 3). Cultivation practices were performed according to the methods of the Research Centre for Cultivar Testing (COBORU), Poland. The breeder of soybean cv. Aldana was the Institute of Plant Breeding and Acclimatization (IHAR), Radzików, Poland. The bacterial inoculant Nitragina (Biofood s.c. Wałcz) and the foliar fertilizer Mikrokomplex (Intermag sp. z.o.o.) were produced in Poland. Nitragina contained the bacterium *Bradyrhizobium japonicum*. The chemical composition of Mikrokomplex was as follows (% m/m): Mg – 9.6; S – 12.8; B – 0.05; Cu – 0.3; Mn – 0.35; Mo – 0.01; Zn – 0.2. Soybean seeds were dressed with the dressing Vitavax 200 FS (fungicide), and Nitragina was applied on the day of sowing. Nitragina was dissolved in water and then mixed with seeds. For starting fertilization

Table 2. Mean monthly air temperature (°C)

Year	April	May	June	July	August	September	Mean
2012	9.89	14.72	18.24	20.87	18.75	14.29	16.13
2013	8.84	15.00	18.52	19.36	18.59	11.43	15.29
2014	8.80	13.30	15.10	19.30	17.70	13.37	14.60
1956–2011	8.80	14.20	17.50	19.40	18.10	13.30	15.22

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Table 3. Physicochemical soil properties

	2012	2013	2014
<b>Specification</b>			
Soil reaction (1 mol/L KCl)	7.20	7.12	7.02
C <sub>org</sub> (%)	1.03	0.99	0.99
N <sub>min</sub> 0–0.6 m (kg/ha)	63.2	66.8	58.4
<b>Content of available nutrients in soil (mg/kg)</b>			
P	85	55	72
K	149	108	168
Mg	81	77	72
SO <sub>4</sub> <sup>2-</sup> -S	4.8	5.3	5.1
Fe	2504	3105	3340
Mn	463	344	474
Zn	17.7	16.9	20.7
B	2.1	1.9	2.0
Cu	7.2	8.1	7.4

with nitrogen, ammonium nitrate 34% was used, at a rate of 30 N kg/ha. Phosphorus and potassium fertilizers were applied in the amount of 30 P kg/ha and 100 K kg/ha. The plot area was 16.5 m<sup>2</sup>. The previous crop for soybean was winter wheat. The seeding rate amounted to 90 seeds per m<sup>2</sup>, row spacing 21.4 cm, and sowing depth 3–4 cm. Sowing was performed within the first ten days of May. Weeds were controlled mechanically. Foliar fertilization with Mikrokomples at a dose of 5.0 L/ha each, was performed three times at stages: leaf and shoot development (13 BBCH), end of budding (59 BBCH) and beginning of pod development (71 BBCH).

Measurements LAI and MTA were made with the apparatus LAI 2000 (LI-COR, Lincoln, USA). Measurements SPAD were made with the apparatus SPAD-502 P Konica Minolta (Tokyo, Japan). Chlorophyll meter is a hand-held tool that objectively indicates leaf colour. SPAD measurements were made on 30 soybean leaves, at the stages of beginning of flowering (61 BBCH) and pod development (72 BBCH). At technical maturity stage, 20 plants were collected from each plot for biometric measurements and yield structure components. Plant height (cm) was measured from the root crown to the top of the shoot. Prior to harvest, the plant density per 1 m<sup>2</sup> was counted.

Seed yield from the plots per 1 ha was calculated, taking into consideration 15% humidity.

**Analytical methods.** The seed chemical composition (total protein) was determined with the near infrared method using the apparatus Spectrometer FT NIR MPA (Bruker, Billerica, USA). Macroelements and microelements were determined at the Laboratory of the Faculty of Biology and Agriculture, the University of Rzeszow. The prepared weighed sample (0.3 g of ground soybean bean seeds) was placed in a Teflon dish for mineralization, and then 10 cm<sup>3</sup> of nitric acid (HNO<sub>3</sub>) and 2 cm<sup>3</sup> 30% perhydrol were added. The samples were left for 10 min. At the same time a blind sample was prepared. Then the dishes were closed and placed in the microwave mineralizer Berghof Speedwave-Four (Eningen, Germany). After cooling, mineralized samples were transferred to measuring flasks of 25 cm<sup>3</sup>. Determinations of the contents of individual elements (P, K, Ca, Mg, Fe, Cu, Mn, Zn) were performed using the flame absorption spectrophotometer Hitachi Z-2000 (Tokyo, Japan). For determination of Ca, Mg and K an addition of lanthanum was used (to a concentration of 0.1% in solution).

**Statistical analyses.** The obtained results were statistically evaluated by analysis of variance. Differences between mean values were evaluated by the Tukey's (*LSD*) test assuming significance at *P* = 0.05. The computations were done using the Statistica 8.0 programme (StatSoft, Tulsa, USA).

## RESULTS AND DISCUSSION

**Biometric measurements and yield components.** The application of Nitragina and N + Mikrokomples resulted in a significant increase in plant density before harvest and plant height as compared with the control treatment (Table 4). De Luca and Hungría (2014) conclude that high density of soya plants does not result in an increase in seed yield.

Shahid et al. (2009) and Afzal et al. (2010) in a field experiment proved the effect of a bacterial inoculant and applied phosphorus on soybean plant height. In the study by Meschede et al. (2004) the use of foliar fertilizers (Bas-Citrus + Fetrilon) increased the plant height, but also lodging of soybean. The first pod was set on average at a height of 11.8 cm, and experimental factors did

Table 4. Plant density, selected morphological features and yield (2012–2014)

Factor	Number of plants before harvest (pcs./m <sup>2</sup> )	Plant height (cm)	Height of first pod (cm)	Number of pods per plant	Number of seeds per pod	1000 seed weight (g)	Seed yield (t/ha)
Control	68 <sup>b</sup>	80.5 <sup>b</sup>	11.9 <sup>a</sup>	10.5 <sup>b</sup>	2.05 <sup>a</sup>	159.0 <sup>b</sup>	2.31 <sup>c</sup>
Bacterial inoculant Nitragina (N)	72 <sup>a</sup>	83.7 <sup>a</sup>	11.6 <sup>a</sup>	12.2 <sup>a</sup>	2.13 <sup>a</sup>	162.3 <sup>ab</sup>	3.01 <sup>a</sup>
Foliar fertilization with Mikrokomplex (M)	69 <sup>ab</sup>	80.9 <sup>ab</sup>	11.8 <sup>a</sup>	11.8 <sup>a</sup>	2.08 <sup>a</sup>	165.4 <sup>a</sup>	2.75 <sup>b</sup>
N + M	71 <sup>a</sup>	84.0 <sup>a</sup>	11.9 <sup>a</sup>	12.6 <sup>a</sup>	2.14 <sup>a</sup>	165.8 <sup>a</sup>	3.12 <sup>a</sup>

Values followed by the same letter are not significantly different at the 5% level of probability

not significantly modify this character. Meschede et al. (2004) also did not find a significant effect of foliar fertilization on the height of the first pod setting. A significant increase in the number of pods per plant was observed in the treatments with Nitragina, Mikrokomplex and N + M as compared with treatment C. Correlation between the number of pods per plant and the seed yield amounted to  $r = 0.71$ . Shahid et al. (2009) proved that inoculation of soybean seeds increases the number of pods per plant and the number of seeds per pod. Afzal et al. (2010) under the influence of a bacterial inoculant obtained an increase in the number of pods per plant only. In the study by Heidarian et al. (2011) foliar fertilization with Zn and Fe increased the number of pods per plant and thousand seed weight, which was dependent on the application time of foliar fertilizer. Kumar et al. (2013) after foliar fertilization obtained an increase in the number of pods per plant and the number of seeds per pod. Seidel et al. (2015) did not indicate a significant effect of foliar fertilizer (B + Ca) on the soybean yield structure components. Mikrokomplex and N + M caused a significant increase in the thou-

sand seed weight (by 6.4 g and 6.8 g) as compared with the control. Pan et al. (2002) and Afzal et al. (2010) did not show a significant effect of a bacterial inoculant, and Kobraee and Shamsi (2013) of foliar fertilization, on the thousand seed weight. Odeleye et al. (2007) obtained an increase in the thousand seed weight under the influence of foliar fertilization, but with macroelements.

**Seed yield.** Significant increase in seed yield was obtained after the application of Nitragina (3.01 t/ha) and N + M (3.12 t/ha) as compared with M and C treatments. Abbasi et al. (2008) proved that the use of a mixture of two symbiotic bacteria strains in soybean results in an increase in yield by 21.0% as compared with the control. By contrast, Pan et al. (2002) did not indicate the effect of the bacterial inoculant on soybean yield. Fertilization with Mikrokomplex caused a significant increase (by 19.0%) in soybean yield as compared with C treatment (Table 4). Meschede et al. (2004) confirmed an increase in soybean yield (by 20.0%) applying foliar Mo + Co. Heidarian et al. (2011) proved a significant effect of the foliar application of Zn + Fe on soybean yield, which was not confirmed by Freeborn et al. (2001).

Table 5. Measurements of soil plant analysis development (SPAD), leaf area index (LAI) and mean tip angle (MTA) indicator (2012–2014)

Factor	SPAD		LAI		MTA	
	I	II	I	II	I	II
Control	38.2 <sup>b</sup>	43.8 <sup>b</sup>	5.05 <sup>b</sup>	4.91 <sup>b</sup>	32.5 <sup>b</sup>	37.5 <sup>b</sup>
Bacterial inoculant Nitragina (N)	38.6 <sup>ab</sup>	43.9 <sup>b</sup>	5.32 <sup>a</sup>	4.98 <sup>a</sup>	35.3 <sup>a</sup>	38.1 <sup>a</sup>
Foliar fertilization with Mikrokomplex (M)	38.9 <sup>ab</sup>	44.5 <sup>a</sup>	5.08 <sup>b</sup>	4.91 <sup>b</sup>	32.8 <sup>b</sup>	37.6 <sup>b</sup>
N + M	40.1 <sup>a</sup>	44.7 <sup>a</sup>	5.33 <sup>a</sup>	5.02 <sup>a</sup>	35.2 <sup>a</sup>	38.3 <sup>a</sup>

I – 61 BBCH; II – 72 BBCH; Values followed by the same letter are not significantly different at the 5% level of probability

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Table 6. Total protein content in dry matter of the soybean seeds (%; 2012–2014)

Factor	Total protein
Control	35.2 <sup>b</sup>
Bacterial inoculant Nitragina (N)	36.4 <sup>a</sup>
Foliar fertilization with Mikrokomplex (M)	35.1 <sup>b</sup>
N + M	36.3 <sup>a</sup>

Values followed by the same letter are not significantly different at the 5% level of probability

**Measurements of indexes.** The highest SPAD (40.1) values were obtained at the beginning of flowering in N + M treatment (Table 5). Also at pod development, the SPAD index was high in N + M and M treatments (44.7) (44.5). The results of SPAD measurement showed that the state of plant nourishment was determined by their comprehensive supply in macro- and microelements. Better nourished plants (higher SPAD) gave a higher yield, which was confirmed by strong correlation ( $r = 0.83$ ). Therefore measurements of SPAD may be useful for predicting soya seed yield. Zhang et al. (2013) indicated differentiation of SPAD measurements depending on the developmental stage of soybean and nitrogen rate. Krivosudská and Filová (2013) suggest the practical application of SPAD measurements on soybean plants for evaluation of their response to site and cultivation factors. The LAI and MTA values both at the beginning of flowering and pod development were significantly higher in treatments with Nitragina (N and N + M) as compared with M and C treatments. These results indicate that proper supply of plants (first of all in nitrogen) increases the area of assimilation organs and affects more horizontal position of leaves. However, the results of the study did not

show a relationship between the measurements of LAI and MAT indices and the seed yield. Heidarian et al. (2011) recorded the highest LAI applying foliar Fe, whereas the other microelements differentiated LAI to a lesser extent.

**Chemical composition of seeds.** Protein content in the seeds was significantly higher in Nitragina (36.4% dry matter) and N + M (36.3% dry matter) treatments as compared with M treatment and control (Table 6). Using a bacterial inoculant, Shahid et al. (2009) and Afzal et al. (2010) obtained an increase in protein content, and Jarecki and Bobrecka-Jamro (2015) an increase in ash content, in soybean seeds. Vratarić et al. (2006) reported that foliar fertilization differentiates the chemical composition of soybean seeds, affecting an increase in the contents of protein and fat. Liu et al. (2005), using Mo + B applied to soil, achieved an increase in protein content, and after fertilization with Mo, B or Mo + B, a decrease in fat content in soybean seeds. The content of Fe in seeds increased in N and N + M treatments as compared with control, which was not observed for Mg in N treatment (Table 7). Jarecki and Bobrecka-Jamro (2015) using a bacterial inoculant combined with the starting nitrogen rate obtained an increase in Fe content and a decrease in Mn concentration in soybean seeds. Bellaloui et al. (2010) indicated that foliar application of B increased the content of this component in seeds.

In conclusion, Nitragina, Mikrokomplex and Nitragina + Mikrokomplex resulted in a significant increase in yield and a change in the chemical composition of seeds. SPAD reached the highest values after the application of Nitragina + Mikrokomplex, and at the stage of pod development, also after foliar fertilization with Mikrokomplex. Higher values of LAI and MTA were obtained after the application of the bacterial inoculant Nitragina and Nitragina + Mikrokomplex.

Table 7. Macroelement and microelement content (2012–2014)

Factor	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	(g/kg DM)				(mg/kg DM)			
Control	5.7 <sup>a</sup>	18.3 <sup>a</sup>	1.4 <sup>a</sup>	1.9 <sup>b</sup>	69.2 <sup>b</sup>	14.9 <sup>a</sup>	20.1 <sup>a</sup>	33.5 <sup>a</sup>
Bacterial inoculant Nitragina (N)	5.9 <sup>a</sup>	18.2 <sup>a</sup>	1.5 <sup>a</sup>	2.0 <sup>ab</sup>	75.1 <sup>a</sup>	15.5 <sup>a</sup>	20.1 <sup>a</sup>	34.8 <sup>a</sup>
Foliar fertilization with Mikrokomplex (M)	5.7 <sup>a</sup>	18.4 <sup>a</sup>	1.6 <sup>a</sup>	2.1 <sup>a</sup>	74.2 <sup>ab</sup>	15.3 <sup>a</sup>	20.4 <sup>a</sup>	33.4 <sup>a</sup>
N + M	5.9 <sup>a</sup>	18.3 <sup>a</sup>	1.6 <sup>a</sup>	2.1 <sup>a</sup>	74.8 <sup>a</sup>	15.5 <sup>a</sup>	20.4 <sup>a</sup>	33.7 <sup>a</sup>

Values followed by the same letter are not significantly different at the 5% level of probability. DM – dry matter



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