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EFFECT OF FERTILIZATION WITH NITROGEN AND SEED INOCULATION WITH NITRAGINA ON SEED QUALITY OF SOYA BEAN (*Glycine max* (L.) MERRILL)

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Abstract. Nitrogen fertilization of soya bean is usually limited to the starter rate. This results from the ability to plant self-supply in atmospheric nitrogen, thanks to symbiosis with nodule bacteria from the genus *Bradyrhizobium japonicum*. These bacteria do not occur commonly in Polish soils. Therefore it is of great importance to inoculate seed material of soya bean with the bacterial inoculant – Nitragina. In 2011-2013 a strict field experiment was conducted located at the Experimental Station of Varietal Testing in Przecław. The test plant was soya bean of the cultivar Aldana. The following factors were taken into consideration in the one-factorial experiment: the control, Nitragina, the starter rate of nitrogen (25 kg·ha^{-1}), Nitragina with the starter rate of nitrogen (25 kg·ha^{-1}). The aim of this study was to estimate the effect of application of Nitragina and nitrogen fertilization on the chemical composition of soya bean seeds. It was found that the used starter rate of nitrogen had a significant effect on increase in the total protein content in seeds as compared with the control. The ash content increased after the application of the bacterial inoculant – Nitragina. Significant differences in the amino acid composition of soya bean seeds were found only after the combined application of nitrogen fertilization and Nitragina. The seeds then contained the most glutamic acid and methionine and less cysteine. Nitrogen fertilization and the inoculant Nitragina did not have an effect on the content of crude fat, fibre, macroelements, zinc and copper in soya bean seeds. The application of Nitragina with the starter rate of nitrogen increased the iron content as compared with the content determined in seeds of plants fertilized with nitrogen. Manganese concentration decreased after the combined application of Nitragina with the starter rate of nitrogen, as compared with seeds harvested from the control.

Key words: amino acids, bacterial inoculant, chemical composition of seeds, macroelements, microelements, soya bean, starter rate of nitrogen

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INTRODUCTION

Soya bean is one of the agricultural crops which are most important for the economy in the world. Its seeds are used both in people and animal nutrition as the valuable source of nutrients. The small cropping area of this species in Poland results mostly from the habitat determinants (sowing in the soil with a temperature of 12°C, mean daily air temperature during growth not lower than 15°C) [Florek *et al*. 2012, Jerzak *et al.* 2012]. According to Bujak and Frant [2009], another important reason is the few registered herbicides used for soya crops cultivation, hence difficulties in chemical weed control of plantations. Dobek and Dobek [2008], however, based on the conducted calculations of the economic effectiveness, are of the opinion that soya bean production in Poland can be profitable.

An important treatment in soya bean cultivation is seed material inoculation with Nitragina. The nodule bacteria species *Bradyrhizobium japonicum* does not commonly occur in Polish soils [Bury and Nawracała 2004]. Soya bean, just as the other plants from the family Fabaceae, uses nitrogen from the air by means of symbiosis with nodule bacteria fixing free atmospheric nitrogen. As a result, it requires less nitrogen fertilization [Lorenc-Kozik and Pisulewska 2003, Martyniuk 2012], which is of vast economic importance [Podleśny 2005].

The nutritional value of soya bean seeds result from their composition, and mostly from the content of protein well balanced in amino acids. Soya bean seeds are also a valuable source of fats, mineral elements, vitamins, saponins and isoflavones. The mentioned elements arouse particular interest due to their health improving properties.

The conducted study aimed at determination of the effect of the starter rate of nitrogen and the bacterial inoculant (Nitragina) on the chemical composition of soya bean seeds of the cultivar Aldana.

MATERIAL AND METHODS

The research material included soya beans seeds of the cultivar Aldana, obtained from the strict field experiment located at the Experimental Station of Varietal Testing in Przecław (50°11' N; 21°29' E). The study was carried out over 2011-2013 in the randomized block design with four replications. The experiment was established in the proper brown soil formed from loess, classified as the good wheat complex, soil quality class IIIa. This soil was characterized by neutral pH value (pH KCl 7.0-7.2), a moderate to high content of assimilable forms of phosphorus and potassium and a high or very high content of magnesium. The total content of determined microelements was moderate or high. The following treatments were taken into consideration in the one-factorial experiment: the control, Nitragina, the starter rate of nitrogen (25 kg·ha^{-1}), Nitragina with the starter rate of nitrogen (25 kg·ha^{-1}). Nitrogen fertilization was applied in the form of ammonium nitrate.

The seeds for sowing were derived from the company Hodowla Roślin Strzelce, sp. z o.o. IHAR Group. The seeds were dressed with the Sarfun T 450 FS dressing (250 ml per 100 kg of seeds). Nitragina was obtained from the Biofood Wałcz company. Agricultural practices were performed according to the rules of soya bean cultivation and in accordance with the methodology of COBORU. The previous crop was winter wheat. Each year in autumn the same phosphorus and potassium fertilization was

applied. Phosphorus was introduced at a rate of 30.8 P kg·ha^{-1} in the form of granulated triple superphosphate and potassium at a rate of 99.6 K kg·ha^{-1} in the form of potash salt. Seed harvest was performed at the full maturity stage, without prior application of plant desiccation. The results of chemical analyses of seeds were given as the mean from the years of the study, taking into account the standard deviation.

The chemical composition of seeds was determined using the apparatus SPEKTROMETR FT; NIR MPA made by Bruker. Amino acids were determined with the method of ion exchange chromatography at the Central Agroecological Laboratory of the University of Life Sciences in Lublin using the amino acid analyser AAA 400 by Ingos (Czech Republic, Prague). Macroelements and microelements were determined at the Laboratory of the Faculty of Biology and Agriculture, the University of Rzeszów. The prepared weighed sample (0.3 g of soya bean seeds) was placed in a Teflon dish for mineralization, and then 10 cm^3 of nitric acid (V) and 2 cm^3 30% perhydrol were added. The poured samples were left for 10 minutes. At the same time he blind sample was prepared. Then the dishes were closed and placed in the microwave mineralizer Berghof Speedwave – Four. The temperature program: from room temperature to 180°C, increase during 10 minutes; then a temperature of 180^{o}C kept for 10 minutes; from temperature 180 to 200^{o}C, increase during 5 minutes; keeping a temperature of 200^{o}C for 7 minutes. After cooling, mineralized samples were transferred to measuring flasks of 25 cm^3. Determinations of the contents of individual elements were performed using the flame absorption spectrophotometer Hitachi Z-2000. For determination of Ca, Mg and K an addition of lanthanum was used (to a concentration of 0.1% in solution).

The obtained results were subjected to the statistical analysis using the univariate analysis of variance. The significance of differences between the mean values were tested based on Tukey's confidence half-intervals, at the significance level P = 0.05. Statistical calculations were made with the software FR-ANALWAR-5FR. Standard deviation was calculated using the calculation sheet Excel 2007.

RESULTS AND DISCUSSION

The applied starter fertilization with nitrogen significantly increased the total protein content in soya bean seeds as compared with the seeds harvested from the plots of the control (Table 1). There was also observed a favourable effect of the addition of bacterial inoculant and of the combined application of Nitragina and mineral nitrogen, but these differences were not statistically proved. On average in the years of the study, the total protein content in soya bean seeds amounted to 36.73%, at distinct values of standard deviation (Table 1). Pasternakiewicz and Dżugan [2009] give the mean protein content at a level of 37.34 % for seeds of the cultivar Aldana. Michałek and Borowski [2006] as well as Kozak *et al*. [2008] proved that the total protein content in soya bean seeds is distinctly differentiated between cultivars.

Based on the conducted study, there was proved a tendency to accumulate higher amount of crude fat in seeds collected from the control, as compared with the seeds harvested from treatments where nitrogen fertilization and inoculation with Nitragina was applied. The mean content of crude fat in soya bean seeds amounted to 19.3% and was similar to the upper value given by Pasternakiewicz and Dżugan [2009]. According to these authors, soya bean seeds can contain from 18.19 to 19.71% of fat in dry matter. Janda *et al*. [2013] found small varietal differences in fat content in soya bean seeds.

However, Michałek and Borowski [2006] as well as Kozak *et al*. [2008] observed that soya bean seeds of the cultivar Aldana were characterized by a higher content of crude fat than in other cultivars.

Table 1. Basic chemical composition of seeds in % of dry matter (mean from years)
Tabela 1. Podstawowy skład chemiczny nasion w % suchej masy (średnia z lat)

Treatments Obiekty doświadczalne	Statistical measure Miara statystyczna	Total protein Białko ogólne	Crude fat Tłuszcz surowy	Ash Popiół	Fibre Włókno
Obiekt kontrolny Control	mean – średnia	35.94	19.45	4.66	8.65
	wahania	34.00-37.88	18.79-20.12	4.55-4.78	8.45-8.86
	s	3.89	1.33	0.23	0.41
Nitragina	mean – średnia	36.61	19.33	4.61	8.83
	wahania	35.03-38.19	18.55-20.11	4.48-4.74	8.68-8.98
	s	3.16	1.56	0.26	0.30
Starter rate of nitrogen Dawka startowa azotu	mean – średnia	37.79	19.09	4.81	8.43
	wahania	36.06-39.52	18.34-19.84	4.61-5.01	8.32-8.55
	s	3.46	1.50	0.40	0.23
Nitragina and starter rate of nitrogen Nitragina i dawka startowa azotu	mean – średnia	36.58	19.33	4.64	8.67
	wahania	34.78-38.38	18.58-20.09	4.48-4.81	8.46-8.88
	s	3.60	1.51	0.33	0.42
$LSD_{0.05}$ – $NIR_{0,05}$		1.519	ns – ni	0.187	ns – ni
Total mean – Średnia ogólna		36.73	19.30	4.68	8.65
Wahania dla lat		34.97-38.49	18.57-20.04	4.53-4.84	8.48-8.82

s – standard deviation – odchylenie standardowe
ns – ni– non-significant difference – różnica nieistotna

When comparing the ash content in soya bean seeds (Table 1), it may be found that the seeds of plants fertilized with the starter rate of nitrogen contained the most of it, but this was the difference statistically proved only in comparison with the content determined after the application of the bacterial inoculum. The mean content of ash in soya bean seeds amounted to 4.68%. Pasternakiewicz and Dżugan [2009] determined 6.72%, and Kozak *et al*. [2008] for seeds of the cultivar Aldana give the value of 6.4% of ash.

The applied nitrogen fertilization and Nitragina did not have an effect on the fibre content in soya bean seeds (Table 1). Its content ranged from 8.43 to 8.83% D.M. and it was higher than the results obtained by Kozak *et al*. [2008]. These authors gives the content at a level of 5.5% for seeds of the cultivar Aldana. They also found that the chemical composition of soya bean seeds is to the largest degree dependent on the course of weather conditions, and then on the varietal and agricultural factors.

The content of individual amino acids (Table 2) in soya bean protein was slightly modified by the applied nitrogen fertilization and Nitragina. Significant differences were obtained only for glutamic acid, cysteine and methionine. After the combined application of Nitragina with the starter rate of nitrogen the content of glutamic acid (Glu), as well as methionine (Met) was by 16% higher than that determined for protein of seeds harvested from the control. As compared with the cysteine content in protein of soya bean seeds harvested from the control, the application of the starter rate of nitrogen did not affect the amount of this amino acid, whereas an addition of Nitragina had

a limited effect. Its lowest content was found after combined application of Nitragina and nitrogen. This was a significantly lower value than that determined in seeds harvested from the soil fertilized with the starter rate of nitrogen (Table 2). According to Friedman and Brandon [2001] and Hanczakowska and Księżak [2012], legume protein is poor in sulphur amino acids (e.g. methionine), whereas it contains relatively much lysine. Therefore legume seeds are a good additive to fodders based on cereals and rapeseed cake. The study indicated that increasing the content of methionine (Met) in soya bean seeds can be obtained in conditions of combined application of the inoculum and the starter rate of nitrogen. This, however, will contribute to a decrease in the content of cysteine (Cys). Li *et al*. [2005] report that an increase in content of sulphur amino acids in soya bean protein can be obtained by breeding transgenic cultivars.

Table 2. Amino acid content in soya bean seeds in $mg \cdot g^{-1}$ (mean from years)
Tabela 2. Zawartość aminokwasów w nasionach soi w $mg \cdot g^{-1}$ (średnia z lat)

Amino acid Aminokwas	Control Obiekt kontrolny	Nitragina	Starter rate of nitrogen Dawka startowa azotu	Nitragina and starter rate of nitrogen Nitragina i dawka startowa azotu	Mean Średnia	$LSD_{0.05}$ $NIR_{0,05}$
Asp	39.1	40.3	38.5	39.9	39.45	ns – ni
Thr	14.4	14.9	14.3	15.0	14.65	ns – ni
Ser	17.7	18.6	17.5	18.0	17.95	ns – ni
Glu	69.1	72.6	68.2	79.9	72.45	9.258
Pro	18.3	19.2	18.2	18.8	18.63	ns – ni
Gly	13.8	14.2	13.5	13.5	13.75	ns – ni
Ala	13.2	13.1	13.0	13.2	13.13	ns – ni
Cys	1.33	1.25	1.37	1.12	1.27	0.239
Val	13.6	13.2	13.1	13.4	13.33	ns – ni
Met	2.85	3.12	3.15	3.30	3.11	0.356
Ile	12.4	12.2	11.4	11.7	11.93	ns – ni
Leu	22.5	22.8	22.2	22.5	22.50	ns – ni
Tyr	9.74	9.88	9.76	9.95	9.83	ns – ni
Phe	14.2	14.4	13.8	14.2	14.15	ns – ni
His	9.19	9.34	8.95	9.06	9.14	ns – ni
Lys	19.7	20.3	19.7	19.8	19.88	ns – ni
Arg	20.2	20.8	19.6	20.3	20.23	ns – ni

ns – ni – non-significant difference – różnica nieistotna

The content of macroelements in soya bean seeds was slightly varied. No significant effect of nitrogen fertilization and the inoculant on changes in the contents of phosphorus, potassium, calcium and magnesium was found. The obtained differences between the studied treatments stayed within the limits of statistical error. The calculated mean content of macroelements in soya bean seeds (Table 3) was ta the level similar to that reported by Kozak *et al*. [2008]. Czapla *et al*. [2003] proved that higher transfer of phosphorus and nitrogen to soya bean seeds can be obtained by spraying plants with synthetic auxins. This, however, may lead to a decrease in potassium content (by about 9%) and a slight decrease in calcium and magnesium contents.

The mean content of microelements in soya bean seeds was presented in Table 4. Nitragina applied together with the starter rate of nitrogen significantly affected an

increase in iron content in soya bean seeds as compared with the seeds harvested from the treatment fertilized only with nitrogen. The conditions of conducting the experiment did not have an effect on the content of copper and zinc in the seeds of cultivated soya bean. The manganese content in soya bean seeds decreased significantly under the influence of applied Nitragina and nitrogen fertilization, as compared with the control. Vasconcelos et al. [2014] focus attention to the important role of iron in people and animal nutrition. They also found that in soya bean plants iron performs an important function in distribution and accumulation of other microelements.

Table 3. Macroelement content in $g \cdot kg^{-1}$ (mean from years)
Tabela 3. Zawartość makroelementów w $g \cdot kg^{-1}$ (średnia z lat)

Treatments Obiekty doświadczalne	Statistical measure Miara statystyczna	Phosphorus Fosfor	Potassium Potas	Calcium Wapń	Magnesium Magnez
Control Obiekt kontrolny	mean – średnia	5.69	18.68	1.58	2.04
	s	0.45	2.16	0.29	0.30
Nitragina	mean – średnia	5.79	18.55	1.60	2.06
	s	0.63	2.00	0.27	0.32
Starter rate of nitrogen Dawka startowa azotu	mean – średnia	5.84	18.33	1.52	2.06
	s	0.55	1.92	0.24	0.27
Nitragina and starter rate of nitrogen Nitragina i dawka startowa azotu	mean – średnia	5.91	18.51	1.56	2.06
	s	0.51	1.97	0.23	0.33
$LSD_{0.05} – NIR_{0,05}$		ns – ni	ns – ni	ns – ni	ns – ni
Total mean – Średnia ogólna		5.81	18.52	1.56	2.05

s – standard deviation – odchylenie standardowe
ns – ni – non-significant difference – różnica nieistotna

Table 4. Microelement content in $mg \cdot kg^{-1}$ D.M. (mean from years)
Tabela 4. Zawartość mikroelementów w $mg \cdot kg^{-1}$ s.m. (średnia z lat)

Treatments Obiekty doświadczalne	Statistical measure Miara statystyczna	Iron Żelazo	Copper Miedź	Manganese Mangan	Zinc Cynk
Control Obiekt kontrolny	mean – średnia	71.97	15.08	21.22	34.73
	s	9.66	2.01	1.20	7.01
Nitragina	mean – średnia	77.15	15.79	20.64	35.78
	s	13.06	2.56	0.93	6.23
Starter rate of nitrogen Dawka startowa azotu	mean – średnia	70.46	16.04	20.57	34.43
	s	7.19	2.28	0.79	6.38
Nitragina and starter rate of nitrogen Nitragina i dawka startowa azotu	mean – średnia	78.11	15.02	19.71	34.83
	s	9.06	1.59	1.57	5.02
$LSD_{0.05} – NIR_{0,05}$		7.062	ns – ni	1.423	ns – ni
Total mean – Średnia ogólna		74.42	15.48	20.53	34.94

s – standard deviation – odchylenie standardowe
ns – ni – non-significant difference – różnica nieistotna

CONCLUSIONS

1. The starting rate of nitrogen affected an increase in the total protein content in soya bean seeds as compared with the control and in the ash content as compared with the bacterial inoculant. The content of crude fat and fibre did not depend on nitrogen fertilization.

2. The content of amino acids in soya bean protein was slightly differentiated in seeds harvested from soils fertilized by the starter rate of nitrogen and in seeds harvested form the control. The combined application of Nitragina with the starter rate of nitrogen resulted in a significant increase in contents of glutamic acid and methionine as compared with the amounts determined in seeds harvested from the control. The combined application of Nitragina with the starter rate of nitrogen significantly decreased the content of cysteine in seeds in relations to the control.

3. The content of macroelements (phosphorus, potassium, calcium and magnesium) in soya bean seeds was not subjected to significant differentiation as affected by the used Nitragina and nitrogen fertilization.

4. The combined application of Nitragina with the starter rate of nitrogen resulted in an increase in iron content in seeds as compared with the plants harvested from the treatment fertilized with the starter rate of nitrogen and in a decrease in manganese content in relations to the control.

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WPŁYW NAWOŻENIA AZOTEM I SZCZEPIENIA NITRAGINĄ NA JAKOŚĆ NASION SOI ZWYCZAJNEJ (*Glycine max* (L.) MERRILL)

Streszczenie. Nawożenie azotowe soi ograniczone jest zwykle do dawki startowej. Wynika to z możliwości samozaopatrzenia się roślin w azot atmosferyczny dzięki symbiozie z bakteriami brodawkowymi z rodzaju *Bradyrhizobium japonicum*. Bakterie te nie występują powszechnie w polskich glebach, dlatego ważnym zabiegiem jest inokulacja nasion siewnych soi szczepionką bakteryjną – Nitraginą. W latach 2011-2013 przeprowadzono ścisłe doświadczenie polowe, zlokalizowane w Stacji Doświadczalnej Oceny Odmian w Przecławiu. Rośliną testową była soja zwyczajna odmiany Aldana. W doświadczeniu jednoczynnikowym uwzględniono: obiekt kontrolny, Nitraginę, dawkę startową azotu (25 kg·ha^{-1}), Nitraginę z dawką startową azotu (25 kg·ha^{-1}). Celem badań było określenie wpływu stosowania Nitraginy i/lub nawożenia azotem na skład chemiczny nasion soi. Stwierdzono, że zastosowana dawka startowa azotu istotnie wpłynęła na zwiększenie zawartości białka ogólnego w nasionach w porównaniu z obiektem kontrolnym. Zawartość popiołu zwiększyła się po zastosowaniu szczepionki bakteryjnej – Nitraginy. Istotne różnice w składzie aminokwasowym nasion soi stwierdzono jedynie po zastosowaniu łącznie nawożenia azotem i Nitraginy. Nasiona zawierały wtedy najwięcej kwasu glutaminowego i metioniny, a mniej cysteiny. Nawożenie azotem i szczepionka Nitragina nie miały wpływu na zawartość tłuszczu surowego, włókna, makroelementów,

cynku i miedzi w nasionach soi. Zastosowanie Nitraginy z dawką startową azotu zwiększyło natomiast zawartość żelaza w porównaniu z zawartością oznaczoną w nasionach roślin nawożonych azotem. Koncentracja manganu zmniejszyła się po zastosowaniu Nitraginy łącznie z dawką startową azotu w porównaniu z nasionami zebranymi z obiektu kontrolnego.

Słowa kluczowe: aminokwasy, dawka startowa azotu, makroelementy, mikroelementy, skład chemiczny nasion, soja, szczepionka bakteryjna

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