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ORIGINAL PAPER

RESPONSE OF SOYA BEAN TO DIFFERENT NITROGEN FERTILIZATION LEVELS

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Abstract

Soya bean, like other plants from the Fabaceae family, uses nitrogen from the air owing to its symbiosis with rhizobia. As a result, it does not require intensive fertilization with this element. A controlled field experiment was conducted over 2012-2014 at the Experimental Station of Variety Testing in Przecław. The test plant was soya, cv. Aldana. The experimental factor consisted of nitrogen doses: 0, 30, 60, 30+30 kg ha^{-1}. Nitrogen fertilization significantly diversified the seed yield of soya bean. A single application of the dose of 30 or 60 kg ha^{-1} increased the seed yield by 0.63 and 0.67 t ha^{-1}, respectively, compared with the control. The highest increase in yield, by 1.21 t ha^{-1}, was obtained after the application of nitrogen in two doses (30+30 kg ha^{-1}). The plants from the control treatment (without nitrogen fertilization) were characterized by the smallest number of pods and TGW (*Thousand Grain Weight*). The use of nitrogen in two doses effected an increase in the SPAD index at 69 and 75 BBCH stages. Also, the SPAD index was observed to vary in the years of the study. High doses of nitrogen increased the content of total protein in seeds and decreased the content of crude fat, as well as causing a significant decrease in the content of ash and fibre in seeds compared with the control. The highest content of total protein was obtained in 2012 and the crude fibre content was the highest in 2014. Fertilization with nitrogen did not affect the concentration of macroelements, whereas it increased the content of iron (Fe) and decreased the content of copper relative to the control. The highest content of manganese in grains was obtained in the control and zinc was the highest in treatments with higher nitrogen doses. The content of macroelements and microelements in seeds was significantly diversified in the years of the study.

Keywords: *Glycine max* (L.) Merr., mineral nitrogen, SPAD values, chemical composition, yield structure, seed yield.

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INTRODUCTION

Soya bean (*Glycine max* (L.) Merr.) is one of the most important species of the Fabaceae plants. It is grown for both food and animal feeds. Soya beans contain about 40% of protein and 20% of fat. Owing to the symbiosis with nodule bacteria (*Bradyrhizobium japonicum*), soya bean plants do not require intensive nitrogen fertilization. Hence, only a small starter dose of nitrogen is applied in soya bean cultivation. CROZAT et al. (1994) indicated that soya bean requires twice as much nitrogen as pea to produce 1 t of seeds. Numerous studies (RAY et al. 2006, CALISKAN et al. 2008, MEHMET 2008, SALVAGIOTTI et al. 2008) confirmed that soya responds positively to nitrogen fertilization, while high doses of nitrogen are not always optimal (OSBORNE, RIEDELL 2006, VALINEJAD et al. 2013). Excessively high levels of mineral nitrogen primarily inhibit the development of root nodules and atmospheric nitrogen fixation (HUNGRIA et al. 2006, KASCHUK et al. 2016). According to BOBRECKA-JAMRO and PIZŁO (1996), it is best if soya bean plants use both forms of nitrogen, symbiotic and mineral. It should be also noted that many researchers (FREEBORN et al. 2001, SCHMITT et al. 2001, SOGUT 2006) did not show an effect of nitrogen fertilization on soya bean seed yield. Moreover, VALINEJAD et al. (2013) and FERREIRA et al. (2016) did not obtain a significant effect of nitrogen fertilization on the chemical composition of seeds.

The aim of this study was to assess the effect of different nitrogen fertilization levels on the quantity and quality of soya seed yield.

MATERIAL AND METHODS

A controlled field experiment with soya, cv. Aldana, was conducted in 2012-2014. The experiment was located at the Experimental Station of Variety Testing in Przecław (50°11′ N, 21°29′ E), on soil developed from clay loam, classified as Gleic Fluvisol (WRB 2014). The soya bean cultivar Aldana was bred at the Plant Breeding and Acclimatization Institute (IHAR), Radzików. A one-factor experiment was with four replications established in a randomized complete block design. The experimental factor consisted of different levels of fertilization with nitrogen: 0, 30, 60 and 30+30 kg ha^{-1} (Table 1). Nitrogen was applied as 34% ammonium nitrate.

The weather conditions are given after the Experimental Station of Cultivar Assessment in Przecław. In the research years, the lowest rainfalls were recorded in 2012. However, they were evenly distributed, which had a favourable effect on soya bean yields. Heavy rainfalls were noted in May and June 2013 and in May, July and August 2014 (Table 2). In 2012, monthly air temperatures were higher than the long-term mean. The lowest monthly temperatures during the growing period of soya bean were noted in 2014. (Table 2).

Table 1

Fertilization design

Factor	Application time, dose (kg ha^{-1})	
	before sowing	59 BBCH
Control	–	–
30 N	30	–
60 N	60	–
30 N + 30 N	30	30

Table 2

Weather conditions

Years	Total monthly precipitation (mm)						Total
	April	May	June	July	August	September	
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
1956–2012	48.1	39.2	79.3	101.6	71.3	54.7	–
Years	mean monthly air temperature (°C)						mean
	April	May	June	July	August	September	
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
1956–2012	8.80	14.20	17.50	19.40	18.10	13.30	–

Soil analyses were performed at the Chemical and Agricultural Station in Rzeszów, according to the Polish standards. The soil had neutral reaction. The content of humus was average and N_{min} was low. The soil abundance of Mg was high and that of P and K was average or high. The content of microelements was average or high (Table 3).

The cultivation practice adhered to the methodology of the Research Centre for Cultivar Testing (COBORU) in Słupia Wielka. Soya bean seeds were dressed with the Vitavax 200 FS seed dressing and a microbial inoculant Nitragina (Biofood s.c. Wałcz) was applied on the day of sowing. Nitragina contained the bacteria *Bradyrhizobium japonicum*. Phosphorus and potassium fertilizers were applied in doses of 30 P kg ha^{-1} (triple superphosphate) and 100 K kg ha^{-1} (potassium salt). The area of a plot for harvest equalled 16.5 m^2. The preceding crop was winter wheat. The seeding rate was 80 seeds per m^2, row spacing 21.4 cm, and sowing depth 3 cm. Sowing took place in the first ten days of May. Afalon Dyspersyjny 450 SC (linuron) was applied directly after sowing, in a dose of 1.5 dm^3 ha^{-1}, to control weeds. Pests and diseases were not controlled.

The measurement of leaf greenness using the soil plant analysis development (SPAD) index was performed with a 502 P Konica Minolta meter.

Table 3

Physicochemical soil properties

Specification	2012	2013	2014
Soil reaction (pH in KCl)	7.20	7.12	7.02
Humus (%)	1.76	1.71	1.70
N_{min} (kg ha^{-1})	63.2	66.8	58.4
Content of available nutrients in soil (mg kg^{-1})			
P	85.1	55.5	72.6
K	149.1	108.5	168.7
Mg	81.3	77.7	72.3
Fe	2504	3105	3340
Mn	463.4	344.8	474.1
Zn	17.7	16.9	20.7
Cu	7.2	8.1	7.4

SPAD readings were made on 30 soya leaves, at budding (65 BBCH), flowering (69 BBCH) and pod development (75 BBCH). After emergence and before harvest, the plant density per 1m^2 was calculated. At the stage of technical maturation, 20 plants were collected from each plot for biometric measurements. The seed yield from the plots on a 15% moisture basis was calculated per 1 ha. Fat and protein yields were calculated from the content of these components in the seed and the seed yield.

Analytical methods

The chemical composition of seeds (total protein, crude fat, ash, fibre, nitrogen-free extract) was determined with the near infrared method on a Bruker Spectrometer FT NIR MPA (Billerica, USA). Macroelements and microelements were assayed at the Laboratory of the Faculty of Biology and Agriculture, the University of Rzeszow. To determine macroelements and microelements, plant samples were mineralized in a 20:5:1 mixture of concentrated acids HNO_3:$HClO_4$:HS_2O_4. Determinations were carried out in an open system, in a Tecator heating block. The content of Ca, K, Mg, Zn, Mn, Cu, Fe in the mineralisates was determined with atomic absorption spectroscopy (FAAS), using a Hitachi Z-2000 apparatus (Tokyo, Japan), whereas P was determined with colorimetry, using a UV-VIS Shimadzu spectrophotometer (Kyoto, Japan), with the vanadium-molybdenum method.

Statistical analyses

The results were statistically evaluated by the ANOVA method of analysis of variance. Differences between mean values were evaluated by the Tukey's (*HSD*) test at the level of significance $P = 0.05$. The computations were aided by Statistica 8.0 (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Nitrogen fertilization did not have an effect on the plant density. However, it was observed that the plant density was significantly diversified in the years of the study, both after emergence and prior to harvest. Fertilization with nitrogen in two doses had the most favourable effect on the number of pods per plant. The thousand seed weight was higher after the application of each used nitrogen dose than in the control (Table 4). Lorenc-Kozik and

Table 4

Plant density and yield components

Fertilization (N kg ha^{-1})	Number of plants (pcs. m^{-2})		Number of pods per plant	Number of seeds per pod	Thousand grain weight (g)
	after emergence	before harvest			
0 – control	80.52^a	77.47^b	11.37^d	2.13	156.6^b
30	81.11^a	78.25^b	14.20^c	2.03	159.3^a
60	79.57^a	77.83^b	14.93^b	1.97	161.4^a
30 + 30	81.69^a	78.54^b	15.38^a	2.18	162.3^a
Mean for years					
2012	85.25^a	81.46^b	14.85^a	1.86^b	167.3^a
2013	79.64^b	77.69^b	13.64^b	2.16^a	157.6^b
2014	77.28^b	74.91^b	13.42^b	2.21^a	154.9^b

Average values for each factor marked with different letters in the same column differ significantly ($P < 0.05$).

Pisulewska (2003) prove that higher nitrogen fertilization significantly increases the number of pods per plant. In the studies by Caliskan et al. (2008), Mehmet (2008) and Chafi et al. (2012), it has been demonstrated that both the number of pods per plant and the TGW (*Thousand Grain Weight*) increase under the influence of nitrogen fertilization.

The lowest SPAD index values were obtained from the control plants. After the application of nitrogen in two doses, the highest SPAD index value was recorded both at 69 BBCH and 75 BBCH stages. It was also shown that the SPAD index varied in the years of the study (Table 5). Jarecki et al. (2016) suggest that the SPAD readings are useful in an assessment of a plant's nutritional status during the growing period. Krivosudska and Filova (2013) demonstrated the usefulness of the SPAD readings for comparison of soya bean genotypes and their response to water stress.

The highest doses of nitrogen significantly increased the content of total protein in the seeds and decreased the content of crude fat. Moreover, higher nitrogen doses effected a significant decrease in the content of fibre and ash in the seeds as compared with the control. The basic chemical composition of seeds varied in the years of the study. The highest content of total protein

Table 5

Measurements of SPAD index

Fertilization (N kg ha^{-1})	65 BBCH	69 BBCH	75 BBCH
0 – control	41.33^{c}	42.37^{c}	40.26^{c}
30	42.65^{b}	42.98^{b}	41.77^{b}
60	42.84^{ab}	43.04^{b}	41.82^{b}
30 + 30	43.29^{a}	43.65^{a}	43.05^{a}
Mean for years			
2012	40.54^{c}	41.17^{c}	40.22^{c}
2013	45.18^{a}	45.57^{a}	43.58^{a}
2014	41.86^{b}	42.29^{b}	41.37^{b}

Average values for each factor marked with different letters in the same column differ significantly ($P < 0.05$).

was obtained in 2012, and that of crude fat – in 2014 (Table 6). In the earlier study, Jarecki and Bobrecka-Jamro (2015) proved that nitrogen fertilization increased the protein content in soya bean seeds, but it did not diversify the content of fat. In contrast, Valinejad et al. (2013) and Ferreira et al. (2016) did not confirm a significant effect of fertilization with nitrogen on the protein and fat content in soya beans. Popovic et al. (2016) report that the weather conditions have a strong impact on the content of protein and fat in soya beans.

The seed yield was diverse in the years of the study and it increased significantly after the application of the nitrogen fertilizer (Table 7). The most favourable yield-forming effect was obtained using fertilization with nitrogen at two equal doses. The yield rose by 1.21 t ha^{-1}, i.e. 42.5%, compared with the control. Many authors (Pisulewska et al. 1999, Ray et al.

Table 6

Basic chemical composition of grains (% DM)

Fertilization (N kg ha^{-1})	Total protein	Crude fat	Nitrogen-free extract	Crude fiber	Ash
0 – control	35.42^{c}	20.32^{a}	31.93^{a}	5.81^{a}	6.68^{a}
30	36.31^{b}	20.58^{a}	31.28^{a}	5.60^{ab}	6.44^{ab}
60	36.96^{a}	19.91^{b}	31.67^{a}	5.49^{bc}	6.25^{bc}
30 + 30	37.43^{a}	19.87^{b}	31.82^{a}	5.12^{c}	5.96^{c}
Mean for years					
2012	37.28^{a}	19.80^{b}	31.00^{b}	5.72^{a}	6.36^{b}
2013	36.66^{b}	19.28^{b}	32.60^{a}	5.87^{a}	5.84^{c}
2014	35.66^{c}	21.43^{a}	31.40^{b}	4.91^{b}	6.79^{a}

Average values for each factor marked with different letters in the same column differ significantly ($P < 0.05$).

Table 7

Yields of oilseed rape

Fertilization (N kg ha^{-1})	Seed yield (t ha^{-1})	Total protein yield (kg ha^{-1})	The yield of crude fat (kg ha^{-1})
0 – control	2.85^{c}	1000.9^{c}	578.6^{c}
30	3.48^{b}	1263.2^{b}	713.4^{b}
60	3.52^{b}	1298.9^{b}	700.5^{b}
30 + 30	4.06^{a}	1518.4^{a}	803.9^{a}
Mean for years			
2012	3.61^{a}	1342.9^{a}	714.8^{a}
2013	3.42^{b}	1251.7^{b}	656.6^{b}
2014	3.40^{b}	1210.4^{b}	727.6^{a}

Average values for each factor marked with different letters in the same column differ significantly (P < 0.05).

2006, Caliskan et al. 2008, Mehmet 2008, Salvagiotti et al. 2008, Chafi et al. 2012, Valinejad et al. 2013) report that soya bean responds to fertilization with nitrogen by an increase in yield. Lorenc-Kozik and Pisulewska (2003) also claim that an optimal nitrogen dose for the cultivar Aldana is 30 kg ha^{-1}, whereas in dry years a dose of 60 N kg ha^{-1} is more favourable. According to Śliwa et al. (2015) and Popovic et al. (2016), the site conditions, primarily the amount and distribution of rainfall, substantially modify the quantity and quality of soya bean seed yield. Hungria et al. (2006) and Kaschuk et al. (2016) conclude that excessively high doses of mineral nitrogen are not recommended in soya bean fertilization, since they inhibit the development of nodules and atmospheric nitrogen fixation.

The yields of total protein and crude fat were significantly higher after the application of nitrogen fertilization than in the control. Also differences were shown in the yields of total protein and crude fat between the years of the study. Pisulewska et al. (1999) proved that cv. Aldana gave stable yields in years, and that nitrogen fertilization increased the fat yield. Bobrecka-Jamro and Pizło (1996) claim that fertilization with nitrogen has a positive effect on the seed and protein yields obtained in the soil and climatic conditions of Poland.

Nitrogen fertilization did not have an effect on the content of the macroelements determined in seeds. Differences were only shown in their content between the years of the study. In 2014, the highest content of phosphorus and potassium, and the lowest content of calcium and magnesium were found in seeds (Table 8). Jarecki and Bobrecka-Jamro (2015) did not verify a significant effect of nitrogen fertilization on the content of macroelements in soya bean seeds.

The application of nitrogen at two equal doses increased the content of

Table 8

Macroelement content in grain (g kg^{-1} DM)

Fertilization (N kg ha^{-1})	P	K	Ca	Mg
0 – control	6.02^{a}	18.15^{a}	1.66^{a}	2.43^{a}
30	6.23^{a}	18.56^{a}	1.77^{a}	2.36^{a}
60	6.10^{a}	18.38^{a}	1.92^{a}	2.47^{a}
30 + 30	6.29^{a}	18.24^{a}	1.81^{a}	2.52^{a}
Mean for years				
2012	6.00^{b}	17.19^{b}	2.08^{a}	2.51^{a}
2013	5.69^{b}	17.55^{b}	1.90^{a}	2.68^{a}
2014	6.80^{a}	20.27^{a}	1.41^{b}	2.15^{b}

Average values for each factor marked with different letters in the same column differ significantly ($P < 0.05$).

iron in seeds but reduced the content of copper. The highest content of manganese in seeds was obtained in the control plots, and the zinc content was the highest in plants from the plots fertilized with higher nitrogen doses. The content of microelements determined in seeds was significantly diverse in the years of the study (Table 9). Jarecki and Bobrecka-Jamro (2015, 2016), who used a starter dose of nitrogen, obtained slight differentiation in the content of microelements in soya bean seeds compared with the control.

Table 9

Microelement content in grain (mg kg^{-1} DM)

Fertilization (N kg ha^{-1})	Fe	Mn	Zn	Cu
0 – control	70.01^{b}	20.22^{a}	33.38^{b}	16.33^{a}
30	77.33^{a}	19.28^{b}	33.61^{b}	15.30^{b}
60	75.30^{a}	18.33^{c}	34.66^{a}	15.84^{b}
30 + 30	77.52^{a}	19.05^{b}	34.87^{a}	15.25^{b}
Mean for years				
2012	74.11^{b}	19.49^{a}	33.48^{b}	15.54^{b}
2013	73.80^{b}	19.61^{a}	33.25^{b}	16.82^{a}
2014	77.22^{a}	18.56^{b}	35.66^{a}	14.69^{c}

Average values for each factor marked with different letters in the same column differ significantly ($P < 0.05$).

CONCLUSIONS

1. Nitrogen fertilization significantly increased the seed yield as compared with the control. The highest increase in yield was achieved after the application of nitrogen in two doses (30+30 kg ha^{-1}). The number of pods and TGW were significantly higher after fertilization with nitrogen than in the control.

2. Measurement of the SPAD index at 69 and 75 BBCH development stages showed that the application of nitrogen in two doses had the most favourable effect on the plant nutritional status.

3. The highest doses of nitrogen significantly increased the content of total protein in seeds, but they reduced the content of crude fat, in addition to which they effected a significant decrease in the content of fibre and ash in seeds as compared with the control.

4. The content of macroelements in seeds was not significantly diversified. Nitrogen fertilization decreased the content of copper in seeds and increased the content of iron. The highest amount of manganese was determined in seeds from the control and the zinc content was the highest after the use of high doses of nitrogen.

5. The quantitative and qualitative traits of seeds determined were significantly diversified in the years of the study.

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