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THE INFLUENCE OF SEED INOCULATION AND/OR INITIAL NITROGEN DOSE ON YIELD AND CHEMICAL COMPOSITION OF FABA BEAN*

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Abstract

Faba bean belongs to the most important legumes in Poland. This results from the high yield potential, significant role in crop rotation, and the possibility of using this crop in animal nutrition. The aim of this study was to evaluate the effect of seed inoculation (*Rhizobium leguminosarum* bv. *viciae*) and initial nitrogen dose on the faba bean yield quantity and quality. A controlled field experiment was carried out in the years 2019-2021, on a field of the Podkarpackie Agricultural Advisory Centre (PODR) in Boguchwała. The faba bean variety Fernando was cultivated. A one-factor experiment was set up with four replicates in a randomized block design. Moisture and thermal conditions varied in the study years and influenced the faba bean seed yield. It was shown that inoculation (B), initial nitrogen fertilization (C), and both treatments applied together (B+C) increased seed yield by 0.17, 0.14, and 0.22 t ha⁻¹, respectively, compared to the control (A). Of the yield components, the number of pods per plant and the thousand-seed weight (TSW) were significantly modified. The combined use of the inoculant and initial nitrogen dose (B+C) increased the protein content and decreased the fiber content in relation to control seeds (A). Nitrogen fertilization applied alone or in combination with the inoculant increased the content of P, K, and Fe in seeds as compared with the control (A) and the treatment with the inoculant (B). The highest content of Mn was determined in control seeds (A), while its content after the combined use of inoculation and nitrogen fertilization (B+C) was significantly lower.

Keywords: *Vicia faba* L. var. *minor* Harz., symbiotic bacteria, nitrogen fixation, fertilization, nitrogen, yield, chemical composition.

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INTRODUCTION

Legumes have many advantages as well as disadvantages. In Poland, both edible and fodder species are grown, but still on a small area (Kapusta 2012). ARIMR (<https://rejestrupraw.arimr.gov.pl/#>) data show that in recent years the interest in the cultivation of legumes has increased. One of the most frequently cultivated large-seeded species is faba bean. The Polish National Register of Varieties distinguishes three groups of varieties of this species: indeterminate varieties with a significant tannin content in seeds, indeterminate low-tannin varieties, and determinate varieties with a significant tannin content (Strażyński, Mrówczyński 2016). Szukała (2012) reported that legume habitat requirements varied depending on a species, variety, and cultivation methods. Faba bean and soybean have higher soil requirements, narrow-leaved lupin is less demanding, and European yellow lupin has the lowest soil requirements. Additionally, faba bean is characterized by high water requirements compared to other large-seeded legumes (Dudek et al. 2013), and has a high demand for nutrients (Rokosz, Podsiadło 2015, Bilski, Kajdan-Zysnarska 2019). However, fertilization of faba bean is not high. This is due to the symbiosis with rhizobia and the ability to self-supply atmospheric nitrogen. What is more, the root system takes some nutrients from compounds that are hardly available (Strażyński, Mrówczyński 2016). Many authors (Martyniuk 2012, Strażyński, Mrówczyński 2016, Bilski, Kajdan-Zysnarska 2019, Hanoon et al. 2020, Mowafy et al. 2022) believe that immediately before sowing faba bean, its seeds should be inoculated with appropriate bacterial vaccines. This is to increase nodulation and assimilation of nitrogen from the air, especially in fields where faba bean has not been cultivated yet. Lv et al. (2020) and El-Sersawy et al. (2021) showed that inoculation of faba bean seeds minimized plant infection with disease and wilting. Mansour et al. (2021) demonstrated that inoculation of faba bean seeds increased nitrogen availability to plants and mitigated the effects of drought. In the study by Mowafy et al. (2022), the number of root nodules increased fourfold compared to the control after inoculating faba bean seeds. This resulted in a significant increase in seed yield and quality. According to Martyniuk (2012), if faba bean is frequently grown in a field, the occurrence of symbiotic bacteria in the soil is high. In this case, seed inoculation may not have the expected effect (Yin et al. 2022). Maluk et al. (2022) reported that faba bean took up about 80% of its nitrogen from biological nitrogen fixation (BNF), regardless of the variety and year of the experiment. Hence, they recommended inoculating seeds to improve nodulation as required. Those authors also showed that much of nitrogen-containing crop residues remains after faba bean harvest. Klippenstein et al. (2021) confirmed that faba bean (*Vicia faba* L.) is characterized by very efficient atmospheric nitrogen fixation, one of the highest among legumes. In this respect, Jarecki and Bobrecka-Jamro (2015) showed no effect of nitrogen fertilization on faba

bean yield. However, they obtained a significant increase in yield after applying foliar fertilization. Bilski and Kajdan-Zysnarska (2019) confirmed that fertilization with nitrogen is unnecessary in soils rich in that component. In the study by Księżak and Kęsik (2017), organic-mineral fertilization of faba bean modified the seed quality. Ash content increased, while protein, fiber, and macronutrient (P and K) contents varied slightly.

The aim of the study was to demonstrate the response of the faba bean variety Fernando to seed inoculation and/or initial nitrogen dose.

MATERIAL AND METHODS

The field experiments were carried out in 2019-2021 in a field of the Podkarpackie Agricultural Advisory Centre PODR (Map 1) in Boguchwała (21°57'E, 49°59'N).



Fig. 1. Location of field experiment

The test plant was the indeterminate, low-tannin faba bean variety Fernando. A one-factor experiment was set up with four replicates in a randomized block design.

The tested factors were: A – control, B – seed inoculation, C – initial nitrogen dose (30 kg ha⁻¹), B+C – inoculation + initial nitrogen dose.

The weather conditions were given according to the readings from the Meteorological Station of the PODR in Boguchwała (49°59'05"N 21°56'43"E).

The soil was chemically analyzed (PN-R-04023:1996, PN-R-04022:1996+Az1:2002, PN-R-04020:1994+Az1:2004) at the District Chemical-Agri-

cultural Station in Rzeszów. Soil samples for testing were taken in the spring before soil cultivation. The experiment was established on sandy loam soil, Fluvic Cambisols (World Reference Base for Soil Resources 2014), of the good wheat complex, class IIIa. The soil was slightly acidic. The contents of available phosphorus and potassium were high, while the content of magnesium was medium (Table 1).

Table 1

Soil analysis under field experiment (0-30 cm)

Parameter	Unit	2019	2020	2021
pH in 1 M KCL	–	6.4	6.1	5.8
Organic carbon	(%)	0.99	1.04	0.75
N _{min}	(kg ha ⁻¹)	65	59	55
P	(mg kg ⁻¹ soil)	73.2	76.7	78.9
K		190.1	168.5	176.8
Mg		56	61	66

Faba bean was cultivated according to the methodology of the PODR in Boguchwała. The seeds were sown in the last ten days of March (2019 and 2020) or first ten days of April (2021). Inoculation (*Rhizobium leguminosarum*) was done just prior to sowing (Biofood s.c. Walcz) according to the experimental design. Row spacing was 25 cm and sowing depth was 8 cm. Fifty seeds were sown per 1 m². The area of a single plot was 15 m² for sowing and harvesting. Each year, the preceding crop was winter wheat. Plant protection treatments are presented in Table 2.

Table 2

Chemical plant protection and foliar fertilization treatments

Preparation	Dose	Content of active substances (g kg ⁻¹ or g dm ⁻³)
Boxer 800 EC (propriflufenazone)	4.0 dm ³ ha ⁻¹	800
Mospilan 20 SP (acetamiprid)	0.2 kg ha ⁻¹	200
Bulldock 025 EC (beta-cyfluthrin)	0.3 dm ³ ha ⁻¹	25

The dates and doses of the applied preparations were in accordance with the manufacturer's recommendations. Nitrogen fertilization was applied before sowing. 34% ammonium nitrate was used for nitrogen fertilization. Phosphorus and potassium fertilization was carried out prior to the autumn ploughing at the following doses: 26.2 kg ha⁻¹ P and 74.7 kg ha⁻¹ K.

Plant density per 1 m² was counted after emergence and before harvest. Plant development stages are given according to the BBCH (Biologische

Bundesanstalt, Bundessortenamt und Chemische Industrie) scale.

Before harvest, 20 plants were collected from each plot and their yield components were determined: number of pods per plant and number of seeds per pod. TSW was determined at the constant moisture content of 14%. Harvesting (15 m²) was done in one stage in the second (2019 and 2020) or third ten days of August (2021). Seed yield was calculated per 1 ha, taking into account the 9% moisture content. Seeds for chemical analyses were obtained during harvest from each experimental combination. The chemical composition of seeds was determined using the near-infrared method on an FT-LSD MPA spectrometer (Bruker, Germany) in the laboratory of the Department of Plant Production, the University of Rzeszów.

The results were statistically compiled using analysis of variance. The significance of differences between the parameter values was tested based on the Tukey's half-confidence intervals, at a significance level of $\alpha = 0.05$. The ANALWAR-5.3.FR statistical program was used for calculations (author: prof. Franciszek Rudnicki, Bydgoszcz). Analysis of variance of the multiple, one-factor experiment data (combined error model) was used.

RESULTS AND DISCUSSION

Moisture and thermal conditions were diverse in the study years and influenced the effectiveness of the applied inoculation and nitrogen fertilization. Low rainfall was recorded in March each year and in April 2019 and 2020. The rainfall in May did not differ from the multi-annual average. In contrast, June was rainy in 2020. Little rainfall was recorded in August 2020 and in July each year. April in 2021 and May in 2020 and 2021 were cold. The temperature in August 2021 was low. The warmest were June 2019 and July 2021 (Table 3).

Table 3

Weather conditions in the years 2019-2021

Month	Sum of precipitation (mm)				Mean temperature (°C)			
	2019	2020	2021	1990-2020	2019	2020	2021	1990-2020
March	23.0	19.8	-	37.0	5.9	5.1	-	2.8
April	21.4	10.0	49.4	46.0	9.9	9.2	6.5	8.7
May	73.5	83.3	63.9	77.1	13.1	11.3	12.8	13.7
June	30.8	162.9	47.3	80.2	21.5	18.1	18.8	17.1
July	49.8	18.9	55.0	95.4	19.1	18.8	21.6	19.0
August	60.9	7.3	107.4	65.0	20.3	19.9	17.5	18.4
Data in the growing season	259.4	302.2	323.0	400.7	14.9	13.7	15.4	13.3

The use of inoculant and/or nitrogen fertilizer had no effect on the plant density after emergence and before harvest. It was shown that the number of pods per plant was significantly higher after applying seed inoculant together with the initial nitrogen dose (B+C) compared to the control (A). The difference obtained was 0.8 pods on average. The number of seeds per pod was not modified. The lowest thousand-seed weight was obtained from control, being significantly higher from treatments with inoculant and/or initial nitrogen dose (Table 4). According to Kulig et al. (2012), there are between 2 and 3 seeds in a pod and the TSF is from 466 to 528 g.

Table 4

Yield components of faba bean (mean from 2019-2021)

Factor	The plant density (pcs. m ⁻²)		The number of pods per plant	The number of seeds per pod	TSW (g)
	after emergence	before harvest			
Control	48.2	42.1	8.6	3.4	362.3
Inoculation	47.1	41.3	9.1	3.3	375.3
Starting dose of nitrogen	46.3	42.2	8.9	3.3	372.5
Inoculation + starting dose of nitrogen	47.1	40.3	9.4	3.2	388.2
LSD _{0.05}	n.s.	n.s.	0.62	n.s.	9.91
Total mean	47.2	41.5	9.0	3.3	374.6
LSD _{0.05} years	n.s.	n.s.	0.42	n.s.	7.53

n.s. – non-significant differences

The seed yield was considerably diversified after seed inoculation and/or nitrogen fertilization as compared with the control (A). The yield increase was 0.17 t ha⁻¹ after inoculation (B), 0.14 t ha⁻¹ after initial nitrogen dose (C), and 0.22 t ha⁻¹ after the combined application of both treatments (B+C) – Figure 2. Klippenstein et al. (2021) obtained an average faba bean yield of 5.3 t ha⁻¹ in a field experiment, which was influenced by the proper nodu-

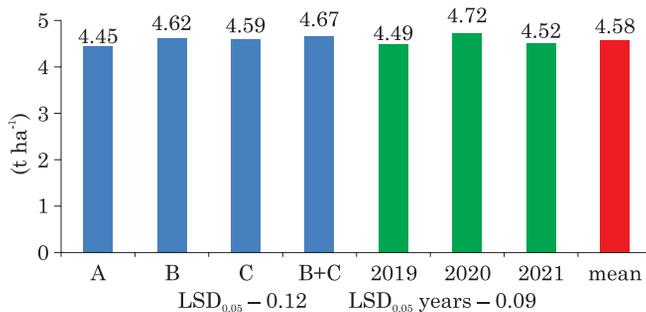


Fig. 2. Seed yield of faba bean

lation. Rasul et al. (2018) proved that nitrogen and phosphorus fertilizers did not modify most of the studied parameters and the faba bean yield. Dudek et al. (2013) reported that high doses of nitrogen did not increase the yield of faba bean. They showed that the seed yield was at a similar level in the control and in samples with the initial nitrogen dose. Allito et al. (2021) proved that inoculation of faba bean seeds significantly increased nodulation in both greenhouse and field experiment. As a result, after *Rhizobium* application, the shoot and root dry weights increased with respect to the control. Also, Uçar (2021) determined that inoculation of faba bean seeds considerably increased the yield, especially when combined with vermicompost. Youseif et al. (2021) reported that the inoculation efficiency largely depends on soil properties and nitrogen availability. They showed that in all locations, seed inoculation had a positive effect on the faba bean yield, while mineral fertilization with nitrogen was less effective.

Seed inoculation combined with initial nitrogen fertilization (B+C) significantly increased the protein content of faba bean seeds. An opposite relationship was obtained for fiber. The contents of fat and ash were not modified by the experimental factor (Table 5). In the study of Mowafy et al. (2022), faba bean seed inoculation significantly improved seed quality owing to an increase in the protein content. Sánchez-Navarro et al. (2020) stated that the use of symbiotic bacteria or fungi for mycorrhiza is a well-known agricultural practice. These treatments increase the nutrient and water uptake and tolerance to biotic and abiotic stresses. Additionally, inoculation usually raises the protein content of seeds.

Table 5
Chemical composition of faba bean seeds (% DM), mean from 2019-2021

Factor	Protein	Fat	Ash	Fiber
Control	29.6	1.11	3.09	6.86
Inoculation	29.8	1.06	3.11	6.79
Starting dose of nitrogen	29.7	1.08	3.05	6.82
Inoculation + starting dose of nitrogen	30.2	1.05	3.08	6.61
LSD _{0.05}	0.52	n.s.	n.s.	0.23
Total mean	29.8	1.08	3.08	6.77
LSD _{0.05} years	0.33	n.s.	n.s.	0.18

n.s. – non-significant differences

The macronutrient content in faba bean seeds was partially modified by the experimental factor (Table 6). It was shown that the application of nitrogen fertilization or inoculation together with nitrogen fertilization increased the content of P and K in seeds. Considerably lower contents of these elements were determined in seeds collected from the control and the inoculant treatment. Concentrations of Ca and Mg in seeds were statistically similar.

Content of macroelements (g kg⁻¹) of faba bean (mean from 2019-2021)

Factor	Phosphorus	Potassium	Calcium	Magnesium
Control	6.39	10.68	0.79	1.04
Inoculation	6.32	10.85	0.80	1.06
Starting dose of nitrogen	6.82	12.51	0.76	1.20
Inoculation + starting dose of nitrogen	6.91	12.42	0.78	1.16
LSD _{0.05}	0.411	1.523	n.s.	n.s.
Total mean	6.61	11.62	0.78	1.12
LSD _{0.05} years	0.359	1.241	n.s.	0.11

n.s. – non-significant differences

Księżak and Kęsik (2017) revealed that faba bean seeds fertilized with various doses of NPK contained slightly more P (by 0.10 g kg⁻¹) and K (by 0.38 g kg⁻¹) than when only organic fertilization with straw or the intercrop is applied. Podleśna (2015) noted an increase in K content in faba bean seeds from 1.0 to 1.3% after applying increased potassium doses. Farmaha et al. (2011) reported that excessive drying of the soil, especially during ripening, can reduce the P and K uptake and their further content in legume seeds. In our study, the experimental factor did not vary the average Ca and Mg contents, which were 0.78 and 1.12 g kg⁻¹, i.e. close to the values given by Lombardo et al. (2016). Etemadi et al. (2018) obtained a significantly lower content of these elements for the tested faba bean varieties, ranging from 0.16 to 0.24 g kg⁻¹ Ca and from 0.19 to 0.23 g kg⁻¹ Mg. Zandvakili et al. (2017) showed higher Ca and Mg concentrations in faba bean leaves, which may indicate minimal translocation of these elements from vegetative parts of plants to seeds and pods.

The initial nitrogen dose (C) and the inoculation combined with the nitrogen dose (B+C) significantly increased the Fe content in faba bean seeds compared to that determined in the seeds of the control (A) and the inoculant treatment (B). Compared to the control, the Mn content in faba bean seeds considerably decreased due to the applied inoculation combined with nitrogen fertilization (Table 7). By using the initial N dose with Nitragin, Jarecki and Bobrecka-Jamro (2016) also obtained a significant increase in Fe in soybean seeds as compared with the control. Lombardo et al. (2016) showed that Fe concentration in faba bean seeds varies depending on the environmental conditions and can range from 23.1 to 94.5 mg kg⁻¹. The determined average contents of Cu (12.98 mg kg⁻¹) and Zn (31.44 mg kg⁻¹) in faba bean seeds were within the range given by Baloch et al. (2014) and Karaköy et al. (2018). On the other hand, the average content of Mn (18.54 mg kg⁻¹) was higher than the content obtained by Lombardo et al. (2016) in faba bean seeds grown under coastal climate conditions. As reported by Etemadi et al. (2018), faba bean accumulates most Zn and Mn in seeds, while Cu in leaves and pods.

Table 7

Content of microelements (g kg⁻¹) of faba bean (mean from 2019-2021)

Factor	Iron	Copper	Manganese	Zinc
Control	40.97	12.08	19.22	30.73
Inoculation	40.46	12.79	18.64	31.78
Starting dose of nitrogen	47.15	14.04	18.57	31.43
Inoculation + starting dose of nitrogen	48.11	13.02	17.71	31.83
LSD _{0.05}	6.062	n.s.	1.124	n.s.
Total mean	44.17	12.98	18.54	31.44
LSD _{0.05} years	4.351	1.423	0.953	0.649

n.s. – non-significant differences

CONCLUSIONS

The number of pods per plant was significantly higher after the combined use of seed inoculation and the initial nitrogen dose (B+C) compared to the control (A). The lowest thousand-seed weight was determined for the control (A). Moisture and thermal conditions varied in the study years and influenced the faba bean seed yield. The increase in seed yield was 0.17 t ha⁻¹ after inoculation (B), 0.14 t ha⁻¹ after nitrogen fertilization (C), and 0.22 t ha⁻¹ after the combined use of inoculation and nitrogen fertilization (B+C). Seed inoculation applied together with initial nitrogen fertilization (B+C) considerably increased the protein content in seeds. The opposite relationship was obtained for fiber. It was shown that the initial nitrogen dose (C) and inoculation combined with nitrogen fertilization (B+C) increased the content of P, K, and Fe in seeds as compared with the control (A) and the treatment with the inoculant (B). The content of Mn in seeds significantly decreased due to the combined use of inoculation and nitrogen fertilization (B + C). The highest Mn content was determined in control seeds (A).

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