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EFFECT OF INOCULATION ON THE CONTENT OF BIOGENIC ELEMENTS IN THE WHITE LUPINE AND GRASS PEA

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ABSTRACT
The aim of this work was to determine the influence of the inoculant on the content of biogenic elements in ten foreign varieties of white lupine (*Lupinus albus*) and three varieties of grass pea (*Lathyrus sativus* L.) of Slovak origin. Rizobine was used as the inoculum before sowing. Dried and homogenised seed samples were mineralised using concentrated HNO^3 using the MARS X – Press 5 instrument. Analytical determination of macro- and microelements in all samples was performed using ARIAN DUO 240FS/240Z atomic absorption spectrometer. The determined values of biogenic elements content were expressed as $mg.kg^{-1}$ of dry matter. The average content of Cu was lower for both crops in variant A compared to variant B. The addition of the inoculant increased the content of Cu in both crops in lupine by 3.7% and grass pea by 10.94%. The Zn content of variant A in lupine was 19.14% higher than that of the grass pea. Grass pea seeds contained 97.76% less Mn than white lupine seeds in both variants. The Cr content of white lupine was 67.74% higher in variant A than in grass pea. The inoculant also increased the content of Cr in lupine by 25.0%. Lupine contained 30.02% less Fe in variant A and 41.27% less Fe in variant B than the grass pea. The results we have obtained show that Ca, K, and P are the predominant elements in the seeds of grass pea in both variants. By comparing selected types of legumes we found that the grass pea features a higher content of Cu, Fe, K, and P. The analysed seeds of white lupine had a higher content of Zn, Mn, Cr, Ni, Co, Na, Ca, and Mg. In conclusion, inoculation does not significantly affect the content of biogenic elements of selected legume species.

Keywords: lupine; grass pea; inoculation; biogenic elements; analysis

INTRODUCTION
Legumes are the mature, dry seeds of annual and perennial plants of the *Fabaceae* family. Even though legumes have been in our diet for more than a thousand years, studies in recent years have pointed to their unique composition. They are a source of plant proteins, containing a complex of saccharides, soluble and insoluble fibre, essential vitamins, minerals, and polyphenols. The content of these substances advises them among functional foods with a beneficial effect on human health. In combination with cereals, they provide the body with full-value proteins.

Legumes are largely consumed by humans worldwide. Human diets are mostly insufficient in mineral elements like zinc (Zn), iron (Fe), iodine (I) and they may also be deficient in selenium (Se), copper (Cu), calcium (Ca), and magnesium (Mg) mostly in less developed countries **(Welch and Graham, 2002; White and Broadley, 2005)**. Minerals are present in greater quantities in legumes than in cereals. The most represented macroelements are potassium, phosphorus, and calcium. Legumes are rich in iron, zinc, manganese, copper, nickel, cobalt, and molybdenum **(Tichá and Vyzínová, 2006; Velíšek, 1999)**.

Essential elements, including the main elements and a number of trace elements, make up electrolytes, enzymes, vitamins and hormones **(Gruchow, Sobucinski and Barboriak, 1988; Kirbaşlar et al., 2012)**. The importance of mineral composition is due to their nutritional properties and beneficial health effects, as well as their meeting dietary guidelines required for a healthy diet **(Welna, Klimpel and Zyrnicki, 2008; Kirbaşlar et al., 2012)**.

The demand of the ever-growing world population for protein foods is no longer sustainable through animal products alone. To compensate for this deficiency, soya bean has become the prevalent source of plant protein for food and feed.

During recent years, human consumption of lupine seeds has increased worldwide as lupine seeds are a good source of nutrients, not only of proteins but also lipids, dietary fibre, minerals, and vitamins. There are variations in the protein content between species, cultivars, the growing conditions, and soil types **(Martínez-Villaluenga, Friás and Vidal-Valverde, 2006)**. Although lupine belongs to the legumes and is not described as an oilseed crop, it has a considerable amount of oil in its seeds **(Uzun et al., 2007)**. Lupine seeds are a good source of macro- and microelements **(Ehsan et al., 2015)**. Some *Lupinus* species are able to accumulate Zn, Cd, Mg, Al, Hg **(Esteban et al., 2008)**, Pb, and Cr **(Ximénez-Embún et al., 2001)**; therefore new species of lupine should be examined before its application for food.

Grass pea (*Lathyrus sativus* L.) is an annual plant of the *Fabaceae* family, commonly used for feed or feed components. Grass pea is high in protein (26 – 32%), highly adaptable to extreme conditions, resistant to disease, and has a low moisture requirement for growing. *Lathyrus sativus* L. is cultivated worldwide as a resistant leguminous plant showing tolerance to various biotic and abiotic factors **(Vaz Pato et al., 2006; Nagati et al., 2015)**.

One of the most beneficial characteristics of legumes is their ability to fix atmospheric nitrogen in symbiosis with soil bacteria called rhizobia which form several species **(Peix et al., 2015)**. These bacteria induce nodules in legume roots or stems where the nitrogen fixation takes place after the infection process. This process requires several steps.

After nodule formation, the rhizobial cells are released into the plant cells, and they are transformed into bacteroids which are able to fix atmospheric nitrogen. This process involves plant and bacterial proteins such as leghemoglobins and nitrogenases **(Peix et al., 2010)**. Therefore, the inoculation of a legume with rhizobia induces metabolic changes in the plant. The most studied changes have been the increase in nitrogen and protein contents, which have been exploited in agriculture to improve the crop yield of several legumes **(Morel, Braña and Castro-Sowinski, 2012)**. Also, in the last decades, the increase of other plant components, such as phosphorous, has been studied after the inoculation of phosphate solubilising rhizobia **(Dahale, Prashanthi and Krishnaraj, 2016)** and currently, the increase of potassium by using K-solubilising bacteria is starting to be analysed **(Kumar et al., 2016)**.

It is apparent that the studied legumes are meaningful sources of all the necessary elements in the human diet. The aim of this work was to determine the influence of inoculant on the content of biogenic elements in selected varieties of white lupine (*Lupinus albus*) and grass pea (*Lathyrus sativus* L.).

Scientific hypothesis

We hypothesize that the content of inoculant will have an effect on the content of macro and microelements in white lupine and grass pea.

MATERIAL AND METHODOLOGY

The analysed plant material was sown in field trial plots (GPS coordinates 48.5917973 and 7.827155) at the National Agricultural and Food Center – Research Institute of Plant Production in Piešťany (Figure 1, Figure 2, Figure 3, Figure 4). Piešťany belongs to the corn production area, which is located at an altitude of 162 m.n.m. In this area, the average annual temperature is around 9.2 °C, and long-term precipitation average is 625 mm. The climate is typically lowland, slightly dry, and slightly windy. The average number of sunny days is 265 per year.

The evaluated material consisted of two types of legumes: white lupine (*Lupinus albus*) and grass pea (*Lathyrus sativus* L.). From the selected types of legumes, we analysed 11 genotypes of foreign origin *(Lupinus albus)* and three genotypes of Slovak origin (*Lathyrus sativus* L.) (Table 1), which belong to the gene pool of legumes in the Gene Bank of the Slovak Republic. The weight of each legume average sample was 200 g.

Two variants were sown from each genotype control variant (A) and the variant with inoculant (B). The experimental plot had dimensions of 5.2 x 1.5 m. Rizobin was used to inoculate the legume seeds. It has a high content of live bacteria (5*109). An organic polymer was used as a binder in the formulation. Seed inoculation was performed by simple mixing directly in the seed drill. The advantage of seed inoculation is an increase of 13 – 25% of the crop, but also an increase of some qualitative indicators, such as the content of crude protein and oil. Samples were taken at full maturity, dried, and then cleaned.

Analytical methods used

For the analysis, we used dry seeds of selected types of legumes, 1 g from each genotype. Samples were analysed in two phases. In the first phase, the material was decomposed in a wet way with the addition of 10 cm^3 of oxidised HNO_3.

Mineralisation of the samples was performed by microwave digestion in the Mars X-Press 5 (CEM Corp., USA). In the second phase, after the mineralisation, the residue was filtered and filled with distilled water in a 50 cc volumetric flask to the exact volume. The analytical endpoint of the determination of the heavy metal content in the plant material was atomic absorption spectrometry using the VARIAN DUO 240FS/240Z (Varian. Australia) instrument. All risk metal content values were expressed as $mg.kg^{-1}$ of dry matter in order to assess the safety of the monitored plant food raw materials as objectively as possible. Eachl analysis was done in 4 repetitions.

Statistical analysis

The measured data were statistically evaluated using the statistical package STATGRAPHICS Centurion XVI.II. The t-test was used to compare means, F-test to compare standard deviations, Mann-Whitney (Wilcoxon) W-test to compare medians, and Kolmogorov-Smirnov test to compare the distributions of the two samples.

RESULTS AND DISCUSSION

Legume seeds are known to contain important nutrients like Mn, Cu, P, Zn, Mg, Ca, and K **(Glew et al., 1997; Tharanathan and Mahadevamma, 2003)**. The average values for the content of select macro- and microelements of lupine and pea in the control variant (A) and in the variant with inoculum addition (B) are given in Table 2 and Table 3.

We found that the average content of Zn, Mn, Cr, Ni, and Co was higher in lupine in both variants (Table 2). On the other hand, the content of Cu and Fe were higher in grass peas in both variants (Table 2). Zinc plays a central role in many biochemical and immunological functions **(Fell and**

Lyon, 1994; O´Dell and Sunde, 1997). The Zn content of variant A in lupine was 19.14% higher than that of the grass pea. The addition of the inoculant reduced the Zn content by 16.40% in grass pea. White lupine seeds contained Zn in variant A by 2.87% more than in variant B. Many metabolic processes require magnesium, iron, and potassium.

Similarly, the content of microminerals in the evaluated leguminous plant seeds was dependent on variety too, and the greatest differences were noted for manganese. Noteworthy, the white lupine seeds accumulated significant amounts of manganese **(Page, Weisskopf and Feller, 2006)**. The content of Mn in lupine seeds strongly depends on the field conditions and the species. **Hung et al. (1987)** examined 33 samples of seeds from two species of lupin and found that *L. angustifolius* had a much lower Mn content (61 $\mu g.g^{-1}$) than *Lupinus albus* (1316 $\mu g.g^{-1}$). The average Mn content was higher in white lupine in variant A (571.63 $mg.kg^{-1}$) and also in variant B (588.20 $mg.kg^{-1}$) in comparison to the results of other types of lupine seeds. Generally, the Mn content of *Lupinus albus* L. 2277.16 $mg.kg^{-1}$) was found to be higher than other legume seeds **(Özcan, Dursun and Al Juhaimi, 2013)**. Grass pea seeds contained 97.76% less Mn than white lupine seeds in both variants. The Mn content in variant A was 8.11% lower than in variant B. According to **Trumbo et al. (2001)** the greatest levels of this element were noted in the white lupine seeds (especially in the Butan cultivar – 370 $mg.kg^{-1}$ DM), compared with only 13 – 20 $mg.kg^{-1}$ DM in the lentil, grass pea, common bean, and broad bean seeds (p <0.05) (Table 2). In these investigations, the average content of manganese was 252 $g.kg^{-1}$ DM, while the adequate intake (AI) of this element is 1.8 mg per day^{-1}.

In humans and animals, Cr is an essential nutrient that plays a significant role in the metabolism of glucose, fat, and protein through potentiation of insulin action. There is limited data on which to base tolerable daily intakes for chromium. The Cr content of white lupine was 67.74% higher in the variant A than in grass pea. The addition of the inoculant also decrease the content of Cr in lupine by 25.0%. We also noticed significant differences in Cr content by comparing both variants in lupine. Variant A contained 52.69% more Cr than variant B. It is the highest difference observed in the comparison of variants A and B for all monitored elements. The average content of Cu was higher for both crops in the A variant compared to the B variant (Table 2). The addition of inoculant increased the content of Cu in both crops of lupine by 3.7% and grass pea by 10.94%. Iron is compulsory for haemoglobin and myoglobin synthesis **(Saleh-E-In et al., 2008)**. The Cu content was increased by 27.1% for grasspea in variant A and by 17.47% in variant B. We found that the content of Fe was higher in the seeds of the grass pea in both variants. Lupine contained 30.02% less Fe in variant A and 41.27% less Fe in variant B than the grass pea. The difference in Fe content of variants A and B was 1.64% for white lupine.

Nickel promotes iron absorption and protects against anemia. Nickel is considered synergistic to Fe by promoting its intestinal absorption. The highest content of Ni was found in seeds of white lupine for the A variant (3.99 $mg.kg^{-1}$). The addition of the inoculant reduced the content of Ni in white lupine by 6.02%. Compared to grass pea, the content of Ni was lowered by 56.64% in the variant A and 58.13% in variant B.

Macroelements such as Na, Mg, K, and Ca are essential for a wide variety of metabolic and physiological processes in the human body; therefore they should be included in daily diets to prevent chronic diseases **(Williams, 2006; Alsafwah et al., 2007)**. Sodium and potassium influence acid-base balance and nerve transmittance. Calcium and phosphorus are necessary for the development and appropriate functioning of bones muscles and teeth **(Saleh-E-In et al., 2008)**. Sodium, potassium, and calcium also impact heart functions. Calcium increases heart shrinkage **(Rajurkar and Damame, 1997)**.

The results we have obtained show that K and P are the predominant elements in the seeds of grass pea in both variants. The higher content of P was determined for the variant B in grass pea (Table 3). The content of K in lupin seeds is 11% lower than that in grass pea in both variants with the addition of an inoculant.

Özcan, Dursun and Al Juhaimi (2013) reported a P content in seeds (*Lupinus albus* L.) of 2.719 $mg.kg^{-1}$ and a Ca content from 1.309 $mg.kg^{-1}$ (*C. arietinum* L.) to 2.781 $mg.kg^{-1}$ *(Lupinus albus* L.). According to our results, the P content of lupine is 1999.04 $mg.kg^{-1}$ for variant A, and the addition of the inoculant increased it to 2596.87 $mg.kg^{-1}$.

The results of our analyses show that the Ca content in the white lupine is 2822.60 $mg.kg^{-1}$ for variant A and 3053.51 $mg.kg^{-1}$ for B variant. By comparing selected types of legumes, we found that the grass pea features higher content of K and P. According to **Grela et al. (2017)**, the seeds of the grass pea contain 8.75 – 9.23% K, 1.14 – 1.24% Mg, 0.97 – 1.03% Ca, 4.68 – 5.13% P, 6.98 – 7.95% Cu, 43.52 $mg.kg^{-1}$ Fe, 29.57 – 31.24 $mg.kg^{-1}$ Zn, and 13.29 – 15.31 $mg.kg^{-1}$ Mn. This data suggests that seeds of the grass pea can be a good source of minerals due to the high content of calcium and magnesium.

The analysed white lupin seeds had a higher content of Na, Ca, and Mg (Table 3). The content of Na in variant A was 37.88% higher in the seeds of white lupine compared to the grasspea seed. Variant B (white lupine) contained 68.41% more Na than variant B (grasspea). There was a significant difference between of Ca content of the crops. The lupine contained 90.56% more Ca than the grasspea in variant A. Variant B contained 120.22% more Ca in the lupine compared to the grasspea. By comparing these two crops the Mg values were not significantly changed.

The measured data were statistically evaluated using the statistical package STATGRAPHICS Centurion XVI.II (Table 4 and Table 5).

A statistically significant difference between lupine variant A and lupine variant B was detected for chromium, sodium, and calcium. A statistically significant difference between grass pea variant A and grass pea variant B was not detected for any element.

Table 1 List of genotypes white lupine and grass pea.

Genotypes	Country of origin
White lupine	
Alban	FRA
Astra	CHL
R-933	POL
Satmarean	ROM
Nelly	HUN
POP I	POL
Los Palacios	ESP
Primorskij	RUS
Solnecnyj	USSR
Weibit	DEU
WTD	POL
Grass pea	
Arida	SVK
Krajova z Kralovej	SVK
Cachticky cicer	SVK

Table 2 Average values of the content of selected microelements in lupine (*Lupinus albus*) and grass pea (*Lathyrus sativus* L.) in control variant and variant with addition of inoculant.

Crop	Content of microelements $mg.k^{-1}$					
White lupine	**Cu**	**Zn**	**Mn**	**Fe**	**Cr**	**Ni**	**Co**
variant (A)	6.48	20.90	571.63	36.61	0.93	3.99	0.30
variant (B)	6.24	20.30	588.20	36.01	0.44	3.75	0.27
Grass pea							
variant (A)	8.23	16.90	12.83	47.60	0.30	1.73	0.27
variant (B)	7.33	16.97	13.87	50.87	0.33	1.57	0.27

Table 3 Average values of the content of selected macroelements in lupine (Lupinus albus) and grass pea (*Lathyrus sativus* L.) in control variant and variant with addition of inoculant.

Crop	Content of macroelements $mg.kg^{-1}$				
White lupine	**K**	**Na**	**Ca**	**Mg**	**P**
variant (A)	7946.83	163.49	2822.60	1106.46	1999.04
variant (B)	7779.35	223.86	3053.51	1090.66	2127.55
Grass pea					
variant (A)	8998.17	118.57	1481.20	991.30	2596.87
variant (B)	8781.23	132.90	1386.60	986.00	3538.00

Table 4 The amounts of individual elements in lupine plants without the addition of inoculum (lupine A) and with the addition of inoculum (lupine B) with *p*-values derived from t-test, F-test, W-test, and K-S Test.

Element	Lupine A (average ± SD)	Lupine B (average ± SD)	t-test[1] (*p*-value)	F-test[2] (*p*-value)	W-test[3] (*p*-value)	K-S Test[4] (*p*-value)
Cu	6.482 ±0.525	6.236 ±0.631	0.3333	0.5706	0.3560	0.4700
Zn	20.9 ±2.086	20.3 ±1.891	0.4878	0.7615	0.5531	0.8079
Mn	571.627 ±38.968	588.2 ±34.153	0.3014	0.6846	0.2372	0.4699
Fe	36.609 ±2.221	36.009 ±2.007	0.5139	0.7546	0.4693	0.8079
Cr	0.927 ±0.350	0.436 ±0.201	0.0006	0.0966	0.0011	0.0013
Ni	3.991 ±0.727	3.755 ±0.754	0.4630	0.9109	0.4491	0.8079
Co	0.3 ±0.118	0.273 ±0.079	0.5315	0.2134	0.5584	0.2062
K	7946.83 ±579.885	7779.35 ±577.145	0.5050	0.9883	0.5994	0.8079
Na	163.491 ±31.664	223.864 ±41.463	0.0010	0.4084	0.0013	0.0059
Ca	2822.6 ±239.052	3053.51 ±136.617	0.0115	0.0921	0.0215	0.0758
Mg	1106.46 ±67.687	1090.66 ±46.555	0.5308	0.2536	0.5994	0.8079
P	1999.04 ±409.325	2127.55 ±188.249	0.3554	0.0219	0.0878	0.0758

Note: [1]t-test to compare means; [2]F-test to compare standard deviations; [3]Mann-Whitney (Wilcoxon) W-test to compare medians; [4]Kolmogorov-Smirnov test to compare the distributions of the two samples.

Table 5 The amounts of individual elements in grass pea plants without addition of inoculum (grass pea A) and with addition of inoculum (grass pea B) with P-values derived from t-test, F-test, W-test, and K-S Test.

Element	Grass pea A (average ± SD)	Grass pea B (average ± SD)	t-test[1] (*p*-value)	F-test[2] (*p*-value)	W-test[3] (*p*-value)	K-S Test[4] (*p*-value)
Cu	8.233 ±0.737	7.333 ±1.779	0.4635	0.2932	0.6625	0.9963
Zn	16.9 ±0.361	16.967 ±1.617	0.9478	0.0948	1.0000	0.9963
Mn	12.833 ±1.210	13.867 ±2.743	0.5826	0.3257	0.8248	0.9963
Fe	47.6 ±10.892	50.867 ±1.850	0.6355	0.0561	0.6625	0.5320
Cr	0.3 ±0.1	0.333 ±0.231	0.8298	0.3158	1.0000	0.5320
Ni	1.733 ±0.252	1.567 ±0.321	0.5185	0.7600	0.8248	0.9963
Co	0.267 ±0.115	0.267 ±0.153	1.0000	0.7273	0.8222	0.9963
K	8998.17 ±241.531	8781.23 ±327.498	0.4081	0.7046	0.6625	0.9963
Na	118.567 ±13.83	132.9 ±22.324	0.3980	0.5547	0.3827	0.5320
Ca	1481.2 ±222.769	1386.6 ±144.05	0.5702	0.5897	0.6625	0.9963
Mg	991.3 ±52.747	986.0 ±50.854	0.9063	0.9635	1.0000	0.9963
P	2596.87 ±233.377	3538.0 ±665.986	0.0820	0.2187	0.0809	0.0996

Note: [1]t-test to compare means; [2]F-test to compare standard deviations; [3]Mann-Whitney (Wilcoxon) W-test to compare medians; [4]Kolmogorov-Smirnov test to compare the distributions of the two samples.

Figure 1 Field collection of white lupine in our Gene bank.

Figure 2 Genetic resources of Grass pea in field collection in our Gene bank.

Figure 3 Genetic resources of *Lupinus albus* in field collection in our Gene bank.

Figures 4 Grass pea – our regional variety.

Cabrera et al. (2003) and **Özcan, Dursun and Al Juhaimi (2013)** reported that the levels of macroelements in *Fabaceae* were present in the following ranges: 7426 – 16.558 mg.g^{-1} K; 269.75 – 445.81 mg.kg^{-1} Na; 2719 – 5556 mg.kg^{-1} P; 1309 – 2781 mg.kg^{-1} Ca; and 2083 – 2900 mg.kg^{-1} Mg. According to our analyzes, the values of macroelements were lower for Ca, Mg, P in both crops. The pea had a lower Na content and a higher K content than the stated values as stated **Cabrera et al. (2003)** and **Özcan, Dursun and Al Juhaimi (2013)**. White lupine contained more Na. The levels of microelements were as follows: 2.1 – 22.0 mg.kg^{-1} Cu; 22.5 – 152.80 mg.kg^{-1} Fe; 31 – 109 mg.kg^{-1} Zn; and 17.53 – 2277.16 mg.kg^{-1} Mn **(Cabrera et al., 2003; Özcan, Dursun and Al Juhaimi, 2013)** which is consistent with the values obtained in this study.

CONCLUSION

Despite the fact that the inoculation with bacteria of the Rhizobium did not significantly affect the content of biogenic elements in the selected leguminous species, it is a significant part of legume cultivation. Biologically fixed nitrogen positively affects the harvest as well as improving the nutritional status of the next crop. The effectiveness of seed inoculation is dependent on the common interaction of Rhizobii and the soil moisture, temperature, and the nitrate content of the soil.

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