

Article

Seed Mineral Composition and Protein Content of Faba Beans (*Vicia faba* L.) with Contrasting Tannin Contents

Hamid Khazaei *  and Albert Vandenberg

Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada; bert.vandenberg@usask.ca

* Correspondence: hamid.khazaei@usask.ca; Tel.: +1-306-966-5859

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Abstract: Two-thirds of the world's population are at risk of deficiency in one or more essential mineral elements. The high concentrations of essential mineral elements in pulse seeds are fundamentally important to human and animal nutrition. In this study, seeds of 25 genotypes of faba bean (12 low-tannin and 13 normal-tannin genotypes) were evaluated for mineral nutrients and protein content in three locations in Western Canada during 2016–2017. Seed mineral concentrations were examined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and the protein content was determined by Near-Infrared (NIR) spectroscopy. Location and year (site-year) effects were significant for all studied minerals, with less effect for calcium (Ca) and protein content. Genotype by environment interactions were found to be small for magnesium (Mg), cobalt (Co), Ca, zinc (Zn), and protein content. Higher seed concentrations of Ca, manganese (Mn), Mg, and cadmium (Cd) were observed for low-tannin genotypes compared to tannin-containing genotypes. The protein content was 1.9% higher in low-tannin compared to tannin-containing genotypes. The high estimated heritability for concentrations of seed Mg, Ca, Mn, potassium (K), sulphur (S), and protein content in this species suggests that genetic improvement is possible for mineral elements.

Keywords: faba bean; biofortification; genotype by environment; heritability; white flower

1. Introduction

Seeds of faba bean (*Vicia faba* L.) are a good source of protein, energy, and fiber, and the crop is widely grown for food and feed [1]. The protein content of faba bean ranges from 24% to 35% of the seed dry matter [2], with an average of 29% [3], making it the most protein-rich major pulse crop [4]. It is relatively high in lysine, which is an essential amino acid in human and monogastric diets [5]. In addition to being an excellent source of protein and starch, it contains valuable mineral micronutrients [2]. Faba bean is used as a source of protein in human diets, as fodder and a forage crop for animals, and for its excellent ability to fix atmospheric nitrogen. In many countries, mature faba bean seeds are cooked into a stew or paste for human consumption, or fed directly to livestock. According to FAOSTAT [6], faba bean ranked sixth in terms of world production, with 4.5 Mt from 2.5 Mha. In terms of global production, common bean (*Phaseolus vulgaris* L.) is the largest pulse crop, followed by pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* L. Walp.), and lentil (*Lens culinaris* Medik.). China is the major producer of faba bean, followed by Ethiopia and Australia.

Plants are known to be good sources of required dietary mineral elements for humans [7]. Mineral malnutrition affects millions of people globally, and is one of the most serious challenges to humankind (WHO, <https://www.who.int/news-room/fact-sheets/detail/malnutrition>). To address the mineral deficiencies in human populations, genetic biofortification through plant breeding has become an

option as a sustainable, cost-effective, and long-term approach for improving the amount of essential mineral elements in agricultural products [8–10]. This technique involves screening and developing micronutrient-rich germplasm, conducting genetic studies (including magnitude of stability through genotype \times environment studies, trait heritability, and associations with important agronomic traits), and developing molecular markers to facilitate breeding [11].

Pulses are a rich source of many micronutrients often found lacking in human diets [4,12]. Seeds of pulse crops generally have higher concentrations of minerals (e.g., Fe, Zn, Ca, and Mg) than cereal grains (reviewed in [12]). A handful of studies have investigated the diversity in mineral nutrients in pulse crop species (e.g., [11,13–17]). However, little information is available regarding the micronutrient composition of faba bean, and if available, only a few mineral elements have been studied [18–20].

Faba bean has a bright future as a protein crop that provides additional crop rotation benefits for many areas of the world. The expansion of the use of faba bean as a nutritious plant-based protein food ingredient requires chemical and genetic investigation into its seed mineral elements. Previous research on faba bean has mainly focused on improving agronomic traits and reducing anti-nutritional factors, with limited research on mineral nutritional improvement of the crop [4], particularly genotype by environment interaction studies. Therefore, the main aim of this study was to investigate the variation of seed mineral components and protein content of faba bean with contrasting tannin contents across environments and years.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Twenty-five faba bean genotypes (Table 1) were grown at three locations in Manitoba and Saskatchewan, Canada, during 2016–2017. Two locations were in Manitoba: Morden (49.12° N, 98.10° W) and Roblin (51.23° N, 101.35° W). The third location was at Rosthern, SK (52.66° N, 106.33° W). The combination of location/year was considered as an environment. The trials were sown in May and harvested in September to November. The plants were grown in plot sizes of 1.2 m \times 5 m in a randomized complete block design with three replicates. Plots were harvested with combine harvesters. The soil at these locations was brown, dark brown, and black Chernozem, respectively. The soil properties in the 0–15 cm soil profile at site locations are presented in Supplementary Materials Table S1.

Table 1. Overview of the flower color and origin of faba bean genotypes used in this study.

Genotype	Flower Color	Breeder/Origin
Snowbird	White	Limagrain, The Netherlands
CDC Snowdrop	White	Crop Development Centre (CDC), Canada
219-16	White	Crop Development Centre (CDC), Canada
667-5	White	Crop Development Centre (CDC), Canada
795-2	White	Crop Development Centre (CDC), Canada
826-18	White	Crop Development Centre (CDC), Canada
707-1-1	White	Crop Development Centre (CDC), Canada
751-2	White	Crop Development Centre (CDC), Canada
656/657-3	White	Crop Development Centre (CDC), Canada
NPZ 14.7310	White	Norddeutsche Pflanzenzucht (NPZ), Germany
NPZ 14.7330	White	Norddeutsche Pflanzenzucht (NPZ), Germany
NPZ 14.7340	White	Norddeutsche Pflanzenzucht (NPZ), Germany
CDC Fatima	Spotted	Crop Development Centre (CDC), Canada
Fabelle	Spotted	Norddeutsche Pflanzenzucht (NPZ), Germany
186-4	Spotted	Crop Development Centre (CDC), Canada
551-4	Spotted	Crop Development Centre (CDC), Canada
688-8	Spotted	Crop Development Centre (CDC), Canada
1007-1	Spotted	Crop Development Centre (CDC), Canada
700-19	Spotted	Crop Development Centre (CDC), Canada
766-3	Spotted	Crop Development Centre (CDC), Canada
Boxer	Spotted	Lantmännen SW Seed Hadmersleben, Sweden
Laura	Spotted	Lantmännen SW Seed Hadmersleben, Sweden
Trumpet	Spotted	Norddeutsche Pflanzenzucht (NPZ), Germany
Tiffany	Spotted	Norddeutsche Pflanzenzucht (NPZ), Germany
RLS 57301	Spotted	Norddeutsche Pflanzenzucht (NPZ), Germany

2.2. Phenotyping

Plot samples were threshed, and seed samples (~5 g) were carefully washed with distilled water prior to micronutrient analysis. Seeds were ground to a fine powder (1.0 mm) using a Cyclone Sample mill (UDY Corporation, Model 3010-030, Fort Collins, Colorado, USA).

2.2.1. Analysis of Micronutrients with Microwave Digest and ICP-MS Analysis

The seed samples were analysed by weighing 0.25 g of sample; adding 2.25 mL HNO₃ + 0.5 mL HCl + 2.25 mL water; and then placing the samples in a microwave oven, increasing the temperature to 200 °C for 20 min, and holding the samples at 200 °C for 15 min, according to the CEM procedure, Microwave Digestion of Feed Grains [21].

After digestion, samples were analyzed using an ICP-MS (model: ICAP-RQ, S/N ICAPRQ00250, Thermo Fisher Scientific (Bremen)—GmbH, Hanna-kunath-Str. 11, 28199 Bremen, Germany). We used the Ked (Kinetic Energy Discrimination) cell mode for all analyses. The one exception to this was the Se analysis, for which the He gas was replaced with H gas in the cell for greater interference cancelation. The analysis was conducted after digestion using a solution of 2.25% Nitric acid and 0.5% Hydrochloric acid. We measured Lithium (⁷Li), Boron (¹¹B), Sodium (²³Na), Magnesium (²⁴Mg), Aluminum (²⁷Al), Phosphorus (³¹P), Sulphur (³⁴S), Potassium (³⁹K), Calcium (⁴⁴Ca), Chromium (⁵³Cr), Manganese (⁵⁵Mn), Iron (⁵⁶Fe), Cobalt (⁵⁹Co), Nickel (⁶¹Ni), Copper (⁶⁵Cu), Zinc (⁶⁶Zn), Arsenic (⁷⁵As), Selenium (⁷⁸Se), Molybdenum (⁹⁸Mo), Cadmium (¹¹⁴Cd), Barium (¹³⁷Ba), Lanthanum (¹³⁹La), Mercury (²⁰²Hg), and Lead (²⁰⁸Pb).

2.2.2. Protein Content

The seed protein content (%) was determined with an NIR sensor (DA 7440TM, Perten, Stockholm, Sweden).

2.3. Statistical Analysis

The combination of year and location was considered as an environment (site-year). Mixed model analysis of variance (ANOVA) was performed using the “lmer” function of the “lme4” R package (R Development Core Team, [22]). Homogeneity and normality were checked before subjecting data to ANOVA. The genotype was treated as a fixed effect, while the location, year, and replications nested within site-year were considered as random effects.

The “sommer” package in R was used to determine the variance components. Genotypes, locations, years, replicates, and their interactions were random effects employed to determine the genetic variability. Phenotypic variance (σ^2p) was estimated as reported by Falconer and Mackay [23]:

$$\sigma^2p = \sigma^2g + \sigma^2gy/y + \sigma^2gl/l + \sigma^2gyl/ly + \sigma^2e/lyr \quad (1)$$

where σ^2g is the genotypic variance, σ^2gy is the genotype × year interaction, σ^2gl is the genotype × location interaction, and σ^2gyl is the genotype year × location interaction. Broad-sense heritability (H^2) of the traits was estimated as

$$H^2 = \sigma^2g/\sigma^2p \quad (2)$$

For principal component analysis (PCA), the “prcomp” function in R was used to characterize the associations among genotypes or mineral elements. The data were visualized using ggbiplot.

3. Results

Among the 24 analyzed elements, Li, Cr, As, La, Hg, and Bp had values of zero. High levels of As, La, Hg, and Bp in grain pose a potential threat to human health—they are non-essential and non-threshold carcinogens. Our results showed that faba bean seeds had generous amounts of K (11,315 ppm), P (5118 ppm), S (1903 ppm), Mg (1334 ppm), and Ca (971 ppm). The Fe and Zn

concentrations were 51 and 42 ppm, respectively. The average mineral element and protein content at three locations, along with the estimated heritability, genotype, environment (year and location), and genotype \times environment ($G \times E$), are presented in Table 2. There was genetic variation in the concentrations of all mineral elements except Al and Se.

Table 2. Concentration of faba bean seed minerals and protein content averaged \pm standard deviation (SD) across all genotypes and years along with analysis of variance.

Element (ppm DW ¹)	Site/Location			<i>H</i> ²	S.O.V. ²		
	Morden	Roblin	Rosthern		Genotype	E (Site-Year)	$G \times E$
	Mean \pm SD	Mean \pm SD	Mean \pm SD				
B	12.32 \pm 1.42	10.35 \pm 0.98	11.73 \pm 1.26	0.58	*	***	**
Na	21.85 \pm 7.38	163.74 \pm 55.41	47.33 \pm 22.54	0.51	***	***	***
Mg	1478 \pm 91	1232 \pm 67	1292 \pm 91	0.91	***	***	ns
Al	2.24 \pm 0.46	1.91 \pm 0.49	5.05 \pm 0.91	0.35	ns	***	***
P	6055 \pm 305	4961 \pm 329	4339 \pm 214	0.54	***	***	***
S	1828 \pm 136	1833 \pm 82	2047 \pm 125	0.81	***	***	***
K	12055 \pm 504	11049 \pm 507	10842 \pm 343	0.86	***	***	***
Ca	1030 \pm 175	955 \pm 125	928 \pm 157	0.90	***	**	*
Mn	13.38 \pm 0.83	13.03 \pm 1.02	15.87 \pm 1.79	0.87	***	***	**
Fe	50.87 \pm 4.09	48.57 \pm 3.59	52.54 \pm 3.15	0.77	***	***	***
Co	0.438 \pm 0.283	0.316 \pm 0.044	0.495 \pm 0.066	0.65	**	***	ns
Ni	3.55 \pm 0.45	2.79 \pm 0.26	3.85 \pm 0.30	0.71	***	***	**
Cu	9.08 \pm 0.86	8.00 \pm 0.69	7.53 \pm 0.51	0.31	**	***	***
Zn	46.13 \pm 3.16	33.77 \pm 2.53	45.50 \pm 3.09	0.52	***	***	*
Se	0.238 \pm 0.036	0.169 \pm 0.027	0.233 \pm 0.068	0.50	ns	***	***
Mo	3.04 \pm 0.72	3.70 \pm 1.03	0.60 \pm 0.22	0.48	**	***	***
Cd	0.027 \pm 0.012	0.006 \pm 0.004	0.024 \pm 0.013	0.47	***	***	***
Ba	1.18 \pm 0.27	1.16 \pm 0.28	3.04 \pm 0.56	0.39	***	***	**
Protein content (%)	28.43 \pm 0.56	29.68 \pm 0.61	28.55 \pm 0.66	0.81	***	*	*

¹ Dry weight. ² Source of variance. Asterisks indicate significance at * $P \leq 0.05$, ** $P \leq 0.01$, or *** $P \leq 0.001$. ns, non-significant.

The environment was highly significant for all elements ($P \leq 0.001$). The $G \times E$ interaction effect was significant for most elements except Mg and Co. The protein, Zn, and Ca content showed very weak significance levels ($P \leq 0.05$) for $G \times E$. The heritability was high for Mg, Ca, Mn, K, S, protein content, and Fe; moderate for Cd, Se, Na, Zn, P, B, and Co; and low for Cu, Al, Ba, and Mo (see Table 2). Seeds harvested from Morden were higher in Mg and P compared to the other locations, and seeds from Roblin had a markedly high concentration of Na and low concentration of Cd. Seeds harvested from Rosthern were rich in Al and Ba and poor in Mo.

Figure 1a–s presents the mean of elements at each location on the basis of the tannin content (white-flowered low-tannin genotypes vs. spotted-flowered high-tannin genotypes). The Ca, Mn, Mg, Cd, and Bd levels were higher (24.8%, 13.7%, 7.1%, 70.0%, and 27.7%, respectively—across years and locations) in low-tannin genotypes. For the other mineral elements, no consistent trend was found across locations between low-tannin and tannin-containing genotypes. Additionally, low-tannin faba beans had a 1.9% higher protein content on average (Figure 1s), compared to tannin-containing genotypes (29.14% and 28.59% for low-tannin and tannin-containing genotypes, respectively).

The PCA analysis was employed to illustrate relationships between mineral elements and genotypes, and showed that the genotypes were, for the most part, arranged into two groups: the first one includes low-tannin faba beans and the second one includes normal-tannin genotypes (Figure 2). PCA projection demonstrated that the PC1 (principal component) and PC2 explained an overall variability of 51%. According to the vector direction of each mineral element, those that are close together are highly correlated, while vectors that are orthogonal are poorly correlated. The protein content, Ca, Mg, Mn, Na, and Co were positively correlated and found to be higher in low-tannin faba bean genotypes, while tannin-containing faba bean genotypes had higher B, Ni, and Al (Figure 2).

The Fe and Cu vectors distinguished two different sets of germplasm equality along PC1 and PC2. Genotype NPZ 14.7340 was characterized by high concentrations of seed Zn, S, P, K, and Mo.

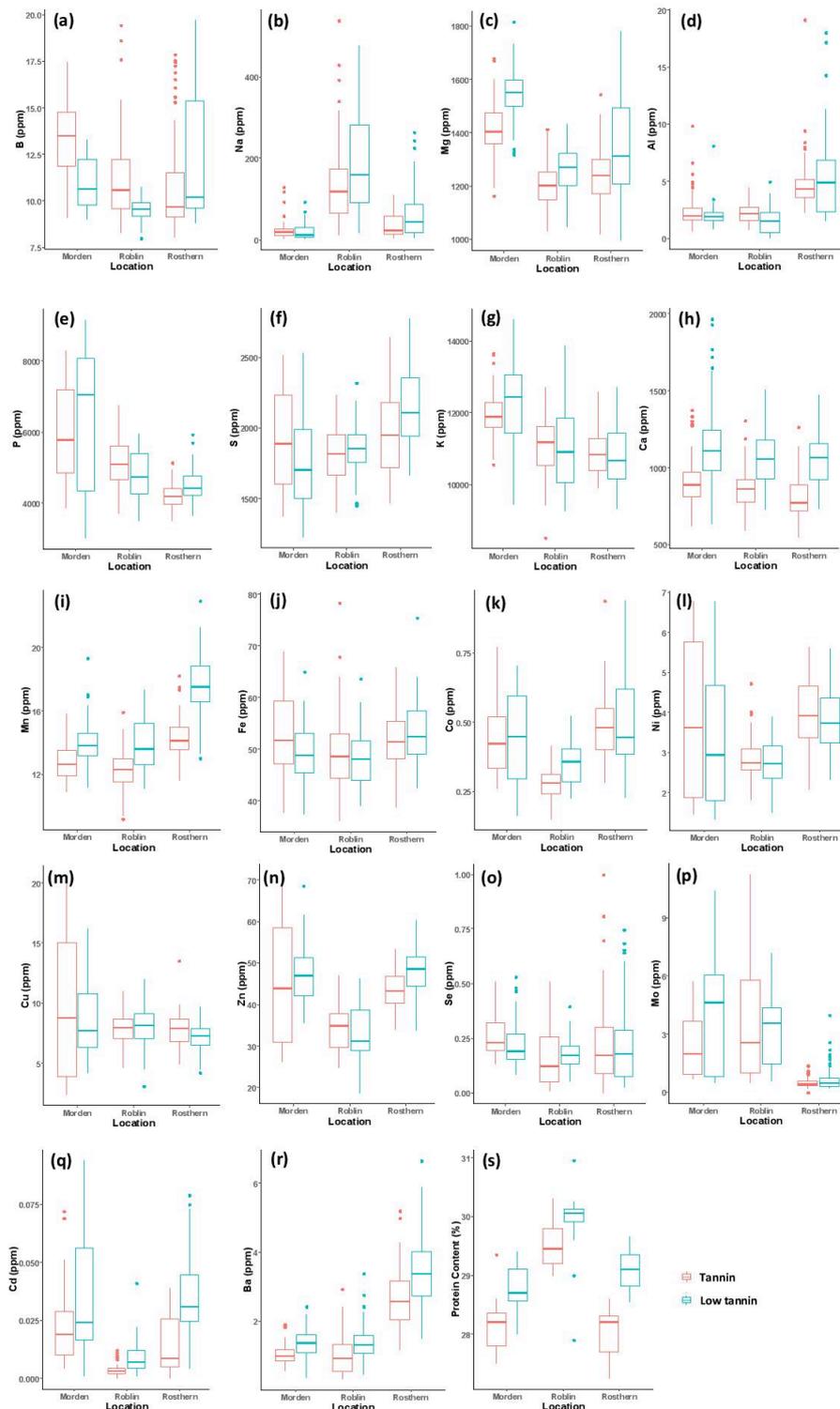


Figure 1. Boxplots of the seed mineral concentrations and protein content averaged over locations in ppm dry weight. Red bars are the mean of spotted-flowered high-tannin and blue bars are mean of white-flowered zero-tannin genotypes. (a) boron; (b) sodium; (c) magnesium; (d) aluminum; (e) phosphorus; (f) sulphur; (g) potassium; (h) calcium; (i) manganese; (j) iron; (k) cobalt; (l) nickel; (m) copper; (n) zinc; (o) selenium; (p) molybdenum; (q) cadmium; (r) barium; (s) protein content.

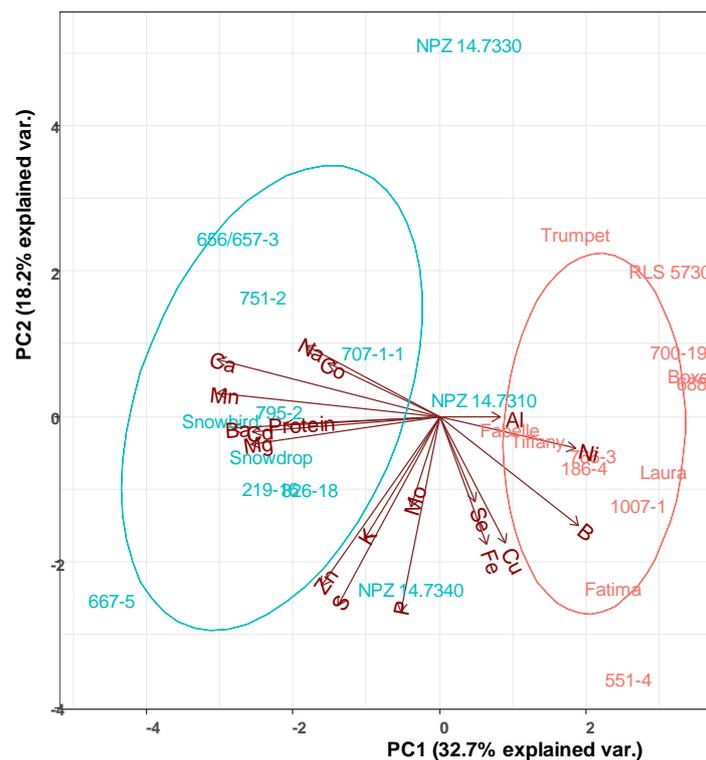


Figure 2. The biplot illustrating the principal components (PC) analysis (PC1 and PC2) for the 25 genotypes with 18 mineral elements and protein content as vectors. Vectors that are close together are correlated in terms of the observed minerals for each crop over years and locations. Genotypes shown in a red color are tannin-containing genotypes, and those shown in a blue color represent low-tannin genotypes. var.: variation.

4. Discussion

The genetic variation for faba bean mineral elements reported here indicates that it may be feasible to breed for specific mineral profiles in faba bean seeds. Our results revealed that the seed mineral concentrations in faba bean are affected by numerous factors, such as environmental variation, $G \times E$ interaction, and the tannin profile. Our observations are consistent with results for pulses grown in Saskatchewan, Canada [16], where the location, year, and their interaction effects were the predominant sources of variance for several minerals. We have shown that low-tannin white-flowered faba beans were rich in Ca, Mg, Fe, and Zn, which are minerals often lacking in the human diet globally. They also had a higher protein content when compared to tannin-containing faba beans.

Seed mineral element values reported in the literature for crop species should be used with caution—when interpreting/comparing these data, one must consider differences in the soil data, sampling, milling, analytical protocols, and experimental screening design used. The mineral concentrations observed for faba bean in this study can be compared to concentrations reported for several pulse crops based on a field evaluation in Western Canada [16]. For most minerals, the content in faba bean was generally similar to that in other pulse crops examined previously from similar landscapes [16]. Notably, the Ca concentration was 2–3 fold higher than previously reported for chickpea and lentil. The Mg seed concentration was 14% and 30% higher in faba bean than pea and lentil, respectively. Furthermore, the K concentration in faba bean seeds was 9% and 18% higher than pea and lentil, respectively. Additionally, the Cu concentration in faba bean was 34% higher compared to pea and similar to lentil. The Fe concentration of faba bean seeds, on average, ranged from 45 to 55 ppm, similar to that observed for other pulses, but less than that reported for lentil (53–93 ppm) grown in Western Canada [24]. In our study, the average Se concentration (0.213 ppm) was lower compared to other pulse species (0.443, 0.732, 0.470, and 1.179 ppm for common bean, chickpea, pea,

and lentil, respectively—Ray et al. [16]). This might be due to the low Se concentration in soil at the three locations employed in this study. The values for Fe are significantly higher than other seed or grain crops (e.g., 7 ppm Fe in white wheat flour) and vegetables such as broccoli (10 ppm Fe) and spinach (16 ppm Fe) [25]. In our study, the seed Zn concentration was 38–47 ppm, which is higher than that reported for other pulse species [16,24,26].

The high estimated heritability of seed Mg, Ca, Mn, K, and S concentration and protein content in this study indicates that good gain from selection can be expected, particularly in early generations in faba bean breeding strategies aiming to enhance the micronutrient profile. The lower estimated heritability for some elements and higher site–year effect can be explained by the large impact of environmental factors on this trait (Table 2). The content of mineral elements such as Zn, Fe, and Se in seeds is affected by both permanent and variable soil environmental factors, and the larger variation due to $G \times E$ interactions when compared with the protein content, for example, indicates that the inheritance of these traits may be more complex. However, multi-environment data for several crop species, including pulses, have identified genotypes with high and stable trait expression in the presence of high $G \times E$ interactions [10]. The seed mineral concentration is shown to be a quantitative trait in seeds of legume species. In the case of the seed Fe concentration, 2–13 quantitative trait loci (QTL) for common bean, 21 QTL for lentil, 4–10 QTL for chickpea, and 3–9 QTL for pea Fe concentration have been reported. Like Fe, several QTL/genes for the Zn concentration (3–13 QTL in common bean, 5–10 in chickpea, and 4–6 QTL in pea) and for Se (3–44 QTL in mung bean) have been identified (reviewed in [17]). The reported heritability estimates for the uptake of mineral elements were of an intermediate [24,27] to high [28] magnitude. Similar to our results, heritability was found to be higher for Ca, Mg, and S and moderate for Fe and Zn in common bean [28]. The high heritability observed for this species and for other species suggests that genetic improvement is possible for mineral elements.

Although faba bean is a dietary source of several minerals and protein, their bioavailability may be affected by anti-nutritional factors, such as phytates and condensed tannins. About 65–85% of total P is stored in crop seeds as phytate commonly referred to as phytic acid (PA, [29]). High levels of PA in the diet can lead to a deficiency of K, Mg, Mn, Mg, Ca, Fe, Zn, and Ba [30,31]. PA has strong binding properties with minerals, protein, and starch, thereby causing a reduction in the digestibility and bioavailability of nutrients. Faba bean has about 1% PA of seed dry weight [32]. It has been shown that PA is markedly reduced through numerous processing methods, such as cooking, soaking, fermentation, and gamma-radiation (reviewed in [33,34]). Plant breeding efforts to reduce faba bean PA are under way at the University of Saskatchewan. Our preliminary results on different sets of faba bean genotypes showed that PA ranged from 0.98 (genotype AO1155) to 4.98 mg g dry weight (genotype 346-10) [unpublished data]. We found no clear pattern between low- and high-tannin genotypes. The PA was measured following [31]. Tannins, one of the main anti-nutritional factors that limit the bioavailability of proteins and minerals, are located in the seed testa [1]. Low-tannin faba bean genotypes are available and relatively easy to develop through breeding due to the monogenic nature of genes (*zt1* and *zt2*) controlling this characteristic. The reduction of tannins is associated with the white-flower phenotype in pulses. As our results clearly revealed, the nutritional quality and protein content of these genotypes are enhanced compared to tannin-containing genotypes (Figure 2). Similar results were also observed by Crépon et al. [2] for faba beans grown in France. This is mainly due to the reduction of condensed tannins (proanthocyanidins) in low-tannin genotypes. It is well-documented that tannins interfere with the digestion of protein in monogastric animals and they can negatively affect the absorption of several essential micronutrients [33,35,36].

One of the main current challenges for agriculture is attaining nutritional security [37], which is intended to be a guarantee that every human being will have access to a fully nutritious diet. From this perspective, research leading to enhancement of the content and availability of micronutrients of underutilized staple foods of a leguminous origin, such as faba bean, is becoming a priority. The development of nutritionally improved faba bean varieties represents a new opportunity to increase the production and use of a crop which has not been exploited to its full potential on a global scale.

The environmental impacts of the production of specific crops in specific ecosystems represent an increasingly important topic. Due to its value in extending crop rotations and its superior nitrogen fixation ability, it can be expected that there will be an expansion of production of the crop in response to the increasing demand for plant-based protein. The enhanced use of faba bean in appropriate agricultural ecosystems will provide increased environmental, ecological, and economic benefits through the mitigation of biotic stresses that occur when crop rotations are extended.

5. Conclusions

This study indicates a higher seed concentration for Ca, Mn, Mg, Cd, and protein content in low-tannin faba bean genotypes compared to tannin-containing genotypes when grown in soils of the eastern Canadian prairies. The high heritability observed for seed mineral concentrations of Mg, Ca, Mn, K, S, and protein content suggests that genetic improvement is possible for these traits, but the challenges of reducing anti-nutritional factors such as PA require consideration. The increased consumption of faba bean seeds and ingredients in the human diet can provide enhanced nutritional value, including some important mineral micronutrients.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/4/511/s1>: Table S1: Soil analysis of samples obtained from three field locations (mg kg⁻¹).

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Conflicts of Interest: The authors declare no conflicts of interest.

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