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Selenium Biofortification of Wheat as a Strategy to Improve Human Nutrition

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Abstract: This paper analyses the effects of soil and foliar fertilization with sodium selenate (VI) on the selenium content in spring wheat grain. The research was carried out at the Departmental Experimental Station of the Institute of Agriculture WULS in Skierniewice in 2018 and 2019. The dose of selenium used was 5.00 g Se·ha^{-1} in various development stages of spring wheat. The results showed that selenium fertilisation did not affect the size of the grain yield, but both soil and foliar fertilisation significantly increased the content of selenium in wheat grain compared to the control group. The highest Se content was obtained with the method of soil fertilisation combined with the foliar application with a total dose of 10.00 g·ha^{-1} Se in the stem elongation phase (S + F2), and in the tillering and stem elongation phase (S + F1 + F2), which resulted in the values of 0.615 and 0.719 mg·kg^{-1} Se in grain, respectively. On this basis, it was concluded that the best time to carry out foliar fertilisation treatment is in the stem elongation phase (BBCH 30–39). The results show that the greatest increase in selenium content in the grain is achieved with soil and foliar fertilisation combined.

Keywords: selenium; wheat; biofortification; grain; fertilisation

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1. Introduction

Selenium is a necessary nutrient important for human health. Like the majority of trace elements, it is bimodal. Its positive effect occurs in a particular, narrow content range below which it cannot fulfil its basic functions in the organism, and in excessive amounts, it becomes toxic [1,2]. The toxic dose is 400 µg·day^{-1} of Se [3], while recommended daily intake of selenium is approximately 53–60 µg·day^{-1} [4], although it is much lower in many countries. Daily intake of selenium in Poland, as well as in Germany, is less than half of the recommended dose [5]. Based on these data, medical associations recommend selenium supplementation [6–8].

Selenium deficiency is a cause for concern worldwide because leads to the occurrence of higher than average indicators of thyroid disorders, serious viral diseases, circulatory diseases, inflammations, as well as cancer. Such disorders are related to unfavourable changes in the immune system caused by selenium deficit [9], such as immune suppression to viral and bacterial infections, as well as reduction of the activity of lymphocytes and macrophages, responsible for immune processes [10]. Although much less common, excess Se consumption can also be harmful to human health [11]. Selenium poisoning can lead to cirrhosis of the liver, pulmonary oedema and diabetes [12].

The occurrence of selenium in the soils of the world is variable. The average selenium content in the surface horizon of soils around the globe is in a range from less than 0.1 mg·kg^{-1} to 100 mg·kg^{-1}. The majority of them, however, contain from approximately 0.1 to 0.6 mg·kg^{-1} of selenium. The average global selenium content in soil is approximately 0.33 mg·kg^{-1} Se [13,14]. Its greatest amounts occur in the USA, Canada, and Australia. A major part of the globe covers areas with a deficit of the element [15]. Poland is included in countries with low selenium content in the soil. According to Dudka [16], Se content in the territory of Poland was in an approximate range of 0.070–0.410 mg·kg^{-1}.

Food is the primary source of selenium in the human body [17]. Selenium deficiency in the diet is currently a very big problem all over the world, especially in Europe [18]. To this end, various actions are taken to counteract this phenomenon. In this case, agriculture is the main and basic sector that can effectively contribute to reducing this negative phenomenon, which is the low supply of micronutrients in food. In countries with selenium deficit, an increase in its content in foods of plant origin through the implementation of appropriate methods of introducing selenium could increase daily intake of foods rich in Se, and therefore alleviate the negative effects of the element's deficit. It can be done through biofortification, defined as the process of increasing the bioavailable content of elements necessary for the organism in edible parts of crops among others through dedicated agrotechnical treatments [19]. For example, in 1984 in Finland, due to the low intake of selenium, an official order was issued to enrich compound fertilisers with selenium with sodium selenate. This measure contributed to an increase in the selenium content in food, and thus a greater consumption of selenium by citizens. It was the first nationwide case of biofortification by supplementing fertilisers with a deficient element, which effectively and safely increased the consumption of selenium in the entire population [20]. Biofortification based on many years of research in recent years is considered one of the cheapest, fastest and most natural methods that contribute to maintaining high-quality food. Biofortification has been recognised as an excellent method of producing the so-called functional food, whose task is to provide the necessary amount of micronutrients. This has global importance in the fight against latent starvation (supplying too little nutrients) [11,21]. The impact of selenium deficiency and the lack of optimization in global conditions are difficult to assess, but due to the high incidence of this phenomenon, it will result in various cancers, cardiovascular diseases and viral diseases. Therefore, it is immeasurably important that countries begin addressing this major public health problem and devising effective, sustainable ways to increase selenium consumption [9,10].

Selenium in food usually occurs in amounts lower than the content necessary for the proper functioning of the organism, resulting in its deficits in approximately a billion people around the globe [14]. Its content in food of plant origin usually reflects the content of the element in the soil in areas that were under cultivation. Food shows variable selenium contents because the content of the element in the soil is uneven in terms of its distribution and availability for plants. It is predicted that in the future selenium deficiency will increase due to the forecasted climate changes, which will contribute to the reduction of Se in soil, mainly in agricultural areas [22].

In foods of plant origin, cereals are the primary source of selenium in the majority of countries around the globe. Next to rice, wheat dominates global cereal production and is the basic source of selenium in the human diet in most countries of the world. For these reasons, it is the best plant to be used for the biofortification process. Selenium content in the grain of native wheat, the primary cereal for the production of baked goods with the highest consumption in Poland [23], is 17.0–112 µg·kg^{-1} Se [24]. In many countries around the world, selenium content in wheat grain is at a level similar to that in Poland, e.g., in Algeria, it averages approximately 52 µg·kg^{-1} [25], in Slovakia it is in a range of 15–39 µg·kg^{-1} [26], and in Great Britain in a range of 25–33 µg·kg^{-1} [27]. Countries with the highest selenium content in grain include among others the USA and Canada, where the average selenium content in wheat grain is in a range of 206–707 µg·kg^{-1} [24].

The purpose of the research was to evaluate two ways of fertilisation on the content of selenium in spring wheat grain to identify an effective way to increase the selenium content of the highest-consuming plant food products.

2. Materials and Methods

2.1. Study Area

The research was conducted in the period 2018–2019 at the Experimental Station of the Institute of Agriculture of Warsaw University of Life Sciences in Skierniewice (51°57′535″ N, 20°9′254″ E). According to the Köppen–Geiger climatic classification [28], the research area is located in the climate zone of a humid continental climate with mild summers and rainfall all year round. The average annual temperature in 2018 was 9.9 °C, with an annual rainfall of 545.90 mm. However, in 2019 the average annual temperature was 10.2 °C and the annual rainfall was 470.70 mm. Monthly precipitation totals from the climate station in Skierniewice from 2018 to 2019 are shown in Figure 1.

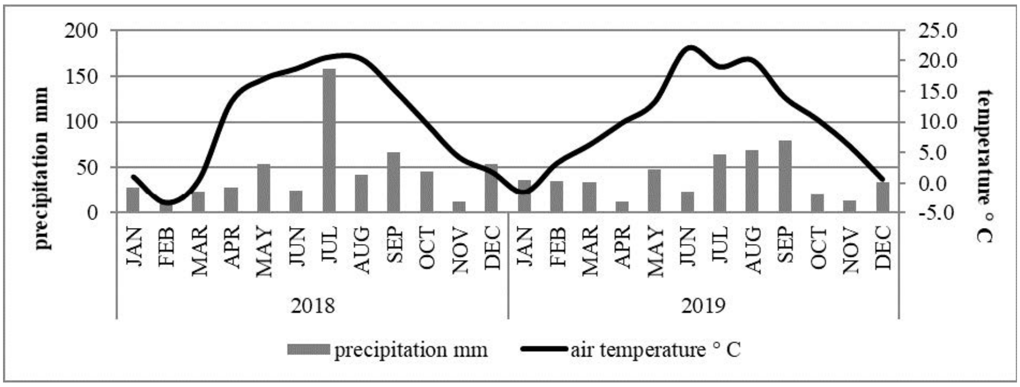

Figure 1. Monthly precipitation totals and temperature in 2018 and 2019.

2.2. Experimental Design and Procedure

The research was carried out on Luvisol soil experimental plots with an area of 16.5 m^2 [29], the agrochemical properties of which are presented in Table 1. The pH of the soil on which the tests were carried out was 4.97 and it was acidic. It is a light, sandy soil. It is recommended that the soils be limed very lightly to a pH of 5.0–5.5. Currently, about half of the soil in Poland is acidic or slightly acidic [30]. The study aimed to conduct agrotechnical biofortification in conditions typical for the area of Poland.

Table 1. Agrochemical properties of the soil.

pH_{KCl}	N_{tot}, $g \cdot kg^{-1}$	C_{tot}, $g \cdot kg^{-1}$	S_{tot}, $g \cdot kg^{-1}$	$P_{ER\text{-}DL}$, $mg \cdot kg^{-1}$	$K_{ER\text{-}DL}$, $mg \cdot kg^{-1}$	Se_{tot}, $mg \cdot kg^{-1}$
4.97	0.76	8.40	0.33	45.57	150.00	0.128

The plant used for this research was spring wheat (*Triticum aestivum* L.), the cv. Mandaryna. The sowing was done with the Poznaniak seeder. The sowing density was 5 million grains per ha. Mineral fertilisation was applied on all plots at the following doses: 120 kg N·ha^{-1} ($CO(NH_2)_2$), 35 kg P·ha^{-1} ($Ca(H_2PO_4)_2$), and 100 kg K·ha^{-1} (KCl). The research was conducted in three replications. Comprehensive chemical protection was used–seed dressing Oxafun T 75 DS/WS, herbicides Chwastox Trio 540 SL and Puma Uniwersal 069 EW (1.2 $dm^3 \cdot ha^{-1}$–BBCH 24–25), Alert 375 SC fungicide (1.0 $dm^3 \cdot ha^{-1}$–BBCH 41–49) and insecticide Decis 2.5 EC (250 $cm^3 \cdot ha^{-1}$– BBCH 61–69).

Selenium was provided in the form of sodium selenate (Na_2SeO_4). Two types of selenium fertilisation were applied: soil fertilisation (S) and foliar fertilisation (F) (Table 2). Soil fertilisation (5.00 g Se·ha^{-1}) was applied before sowing (spraying volume per hectare: 300 L. To precisely define the stage of development, a scale was used, the abbreviation of which comes from the German Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH scale). This scale is used in the European Union countries to determine the stage of plant development. Foliar fertilisation (5.00 g Se·ha^{-1}, spraying volume per hectare: 300 l) was used in various development stages of the plant: F1—tillering (BBCH 22), F2—steam elongation (BBCH 32), F3—inflorescence emergence (BBCH 52), and F4—ripening (BBCH 85). In addition, foliar fertilisation treatments were carried out in several development stages, where the full dose (5.00 g Se·ha^{-1}) was divided into the number of applications: F1 + F2—tillering and stem elongation phase; F1 + F2 + F3—tillering, stem elongation, and inflorescence emergence phase; F1 + F2 + F3 + F4—tillering, stem elongation, inflorescence emergence and ripening phase. Another method involved soil fertilisation applied along with foliar fertilisation (S + F), where foliar treatments were applied in the same development stages of wheat as in the case of foliar fertilisation only.

Table 2. Research scheme.

Treatment		**Dose g Se·ha^{-1}**	**Total Dose g Se·ha^{-1}**
Control (C)		0.00	0.00
Soil application (S)		5.00	5.00
Foliar application (F)	F1	5.00	5.00
	F2	5.00	5.00
	F3	5.00	5.00
	F4	5.00	5.00
	F1 + F2	2.50 in each treatment	5.00
	F1 + F2 + F3	1.67 in each treatment	5.00
	F1 + F2 + F3 + F4	1.25 in each treatment	5.00
Soil and foliar application (S + F)	S + F1	5.00 soil + 5.00 foliar	10.00
	S + F2	5.00 soil + 5.00 foliar	10.00
	S + F3	5.00 soil + 5.00 foliar	10.00
	S + F4	5.00 soil + 5.00 foliar	10.00
	S + F1 + F2	5.00 soil + 2.50 foliar in each treatment	10.00
	S + F1 + F2 + F3	5.00 soil + 1.67 foliar in each treatment	10.00
	S + F1 + F2 + F3 + F4	5.00 soil + 1.25 foliar in each treatment	10.00

F1–application in tillering stage (BBCH 22), F2—application in stem elongation stage (BBCH 32), F3—application in inflorescence emergence stage (BBCH 52), F4—application in ripening stage (BBCH 85), F1 + F2—application in the tillering and stem elongation stages, F1 + F2 + F3—application in the tillering, stem elongation and inflorescence emergence stages, F1 + F2 + F3 + F4—application in the tillering, stem elongation, inflorescence emergence and ripening stages.

2.3. Sampling and Analysis

After the harvest, the grain was weighed, and then the samples of material were dried at 50 °C with forced air circulation, then at 105 °C to constant weight [31] and ground using a mill (Retsh, Katowice, Poland) at 5000 rpm. The content of selenium in the grain was determined after mineralisation in the mixture of HNO_3 and $HClO_4$, through the method of atomic absorption spectrometry (AAS), using the Thermo Elemental SOLAAR M6 apparatus (Thermo Scientific, Wilmington, NC, USA).

2.4. Statistical Data Analysis

Statistical analyses were performed with the Statgraphics 5.1 software (The Plains, VA, USA). The results were subjected to the one-way analysis of variance (ANOVA) with a p-value of 0.05, as well as Student's t-test and Pearson's linear correlation analysis.

3. Results

3.1. Average Yield of Wheat Grain

The article presents the average results of the yield of spring wheat for two years. Annual precipitation totals in 2018 and 2019 were lower than the average annual precipitation totals for many years (1951–2010). In 2018, even though rainfall was greater than in 2019, an unfavourable distribution in terms of plant vegetation was found. As a result, wheat accelerated its development, entered the heading stage faster, which contributed to a reduction in the grain yield. Spring varieties give about 30% lower yields compared to winter varieties. With such unfavourable weather conditions, there was an additional reduction in the crop yield [32,33].

The yield of wheat grain in the control group (C) ranged from 1.86 to 2.35 t·ha^{-1}, while the average yield was 2.11 t·ha^{-1}. In the groups fertilised with selenium, the grain yield ranged from 1.45 to 3.18 t·ha^{-1}. No significant effect of selenium fertilisation on the grain yield was observed. Only in the case of soil fertilisation combined with foliar application in the tillering phase (S + F1) was grain yield significantly higher compared to other fertilisation combinations (Table 3). Compared to the control group, the average yield for this combination was about 27.73% higher. The lowest yield among combined methods of fertilisation with selenium was observed for foliar fertilisation in the tillering phase (F1), where the yield was lower by 13.18% (compared to the control group). No correlation was found between the yield and the content of selenium in wheat grain.

Table 3. Yield depending on fertilisation.

Treatment		Grain Yield (t·ha^{-1})	SD
Control (C)		2.11 ^{a} (1.86–2.35) *	0.249
Soil application (S)		2.20 ^{a} (2.15–2.24) *	0.046
Foliar application (F)	F1	1.91 ^{a} (1.80–2.02) *	0.109
	F2	2.07 ^{a} (1.81–2.33) *	0.264
	F3	2.15 ^{a} (2.00–2.33) *	0.169
	F4	2.23 ^{a} (2.09–2.42) *	0.172
	F1 + F2	2.27 ^{a} (2.18–2.45) *	0.157
	F1 + F2 + F3	1.96 ^{a} (1.76–2.06) *	0.175
	F1 + F2 + F3 + F4	2.14 ^{a} (2.21–2.15) *	0.017
Soil and foliar application (S + F)	S + F1	2.81 ^{b} (2.55–3,18) *	0.332
	S + F2	2.07 ^{a} (1.82–2.32) *	0.250
	S + F3	2.38 ^{a,b} (2.24–2.61) *	0.195
	S + F4	2.35 ^{a,b} (2.09–2.58) *	0.245
	S + F1 + F2	2.04 ^{a} (1.82–2.18) *	0.195
	S + F1 + F2 + F3	2.19 ^{a} (2.03–2.36) *	0.167
	S + F1 + F2 + F3 + F4	1.99 ^{a} (1.64–2.34) *	0.351

a,b—Means followed by the same letter are not significantly different from one another based on Tukey's test at $p \leq 0.05$. *—The range of variability of the obtained results was given.

3.2. Selenium Content in Grain

In the conducted research, although no effect of selenium fertilisation on the wheat yield was observed, significant differences were found in the selenium content in grain (Table 4). These differences depended both on the method of fertilisation, as well as the development phase in which the element was introduced. The average content of selenium in wheat grain in the control group (C) ranged from 0.155 to 0.162 mg·kg^{-1}, while the average content was 0.155 mg·kg^{-1}. In the fertilised groups, the selenium content in the grain ranged from 0.104 to 0.795 mg·kg^{-1}.

Table 4. Se content in grain depending on fertilisation treatment.

Treatment		Se (mg·kg^{-1})	SD
Control (C)		0.155 ^{a} (0.150–0.162) *	0.006
Soil application (S)		0.368 ^{b,c} (0.381–0.391) *	0.032
Foliar application (F)	F1	0.253 ^{a,b} (0.178–0.362) *	0.096
	F2	0.294 ^{a,b} (0.233–0.344) *	0.057
	F3	0.267 ^{a,b} (0.201–0.390) *	0.107
	F4	0.234 ^{a,b} (0.181–0.313) *	0.070
	F1 + F2	0.335 ^{b,c} (0.207–0.472) *	0.132
	F1 + F2 + F3	0.395 ^{b,c} (0.256–0.564) *	0.156
	F1 + F2 + F3 + F4	0.274 ^{a,b} (0.129–0.357) *	0.126
Soil and foliar application (S + F)	S + F1	0.279 ^{a,b} (0.262–0.302) *	0.020
	S + F2	0.615 ^{d,e} (0.572–0.691)*	0.066
	S + F3	0.347 ^{b,c} (0.335–0.362) *	0.014
	S + F4	0.279 ^{a,b} (0.255–0.298) *	0.022
	S + F1 + F2	0.719 ^{e} (0.560–0.795) *	0.137
	S + F1 + F2 + F3	0.474 ^{c,d} (0.297–0.620) *	0.164
	S + F1 + F2 + F3 + F4	0.249 ^{a,b} (0.104–0.495) *	0.214

a,b,c,d,e—Means followed by the same letter are not significantly different from one another based on Tukey's test at $p \leq 0.05$. *-The range of variability of the obtained results was given.

For all groups fertilised with selenium, this value ranged from 0.234 to 0.719 mg·kg^{-1}, so the content of this element in grain was 1.5 to more than 4 times higher than the control group. In the case of soil fertilisation (S) an increase of 137.42% was observed, for fertilisation in the tillering and stem elongation phase (F1 + F2) there was an increase by 116.13%, while for foliar fertilisation in the tillering, stem elongation, and inflorescence emergence phase (F1 + F2 + F3) increased by 154.84%. The least effective method involved fertilisation with selenium in the ripening phase (F4), whereby, compared to the control group, only a 50.97% increase in selenium content was achieved. Regression analysis showed no significant correlation between the selenium content in grain and the yield (Table 5).

Table 5. Linear regression for selenium content in grain (y) and yield (x).

Fertilization Method	Equation	r^2	*p*-Value
Soil (S)	Y = 0.34 + 0.01x	+0.13	0.917
Foliar (F)	Y = 0.52 − 0.11x	−0.22	0.336
Soil and foliar (S + F)	Y = 0.81 − 0.17x	−0.35	0.113
All experiment	Y = 0.56 − 0.10x	−0.19	0.186

The highest selenium content in grain was found in the groups for which soil fertilisation was combined with foliar application in the tillering and stem elongation phase (S + F1 + F2), as well as soil fertilisation combined with foliar application in the stem elongation phase (S + F2) was used (Table 4). In these groups, selenium content increased by approx. 363.87% and 205.81% respectively. In the case of soil fertilisation combined with the foliar application (S + F), the least effective method turned out to be the one in which soil fertilisation was combined with foliar application in the tillering, stem elongation, inflorescence emergence, and ripening phase (S + F1 +F2 + F3 + F4). In this case, selenium content increased only by 60.65% compared to the control group.

In the conducted research, soil fertilisation combined with foliar application in predefined phases (S + F) turned out to be the most effective. Soil fertilisation and foliar application, when carried out separately, did not result in an increase in selenium content in wheat grain as compared to the combination of these two methods. This is partly due to the double dose of selenium introduced (5.00 g Se·ha^{-1} into the soil and 5.00 g Se·ha^{-1} in foliar application), which in total gives 10 g Se·ha^{-1}. Comparative analysis of the doses of selenium fertilization (Table 6) showed that in the case of higher doses, the content of selenium in grain increased significantly for soil fertilisation combined with the foliar application during the tillering and stem elongation phase (S + F1 + F2), as well as soil fertilisation combined with foliar application in the stem elongation phase (S + F2). This allows for the conclusion that it is not the amount of selenium in the fertiliser that determines the final selenium content in grain, but rather the development phase in which the element is introduced.

Table 6. Selenium content in wheat grain depending on fertilisation method.

Combination	Dose 5 F	Dose 10 F + S	*p*-Value
F1	0.253	0.279	0.676
F1 + F2	0.335	0.719	0.025
F1 + F2 + F3	0.395	0.474	0.579
F1 + F2 + F3 + F4	0.274	0.249	0.869
F2	0.294	0.615	0.003
F3	0.267	0.347	0.265
F4	0.234	0.279	0.344

Based on the conducted research, it was found that the best time to carry out the foliar application of selenium for spring wheat is the stem elongation phase (BBCH 30–39). This phase of the plant has an increased demand for nutrients, which in turn, increases selenium absorption in this period.

4. Discussion

Due to the element's properties, selenium deficit raises controversies, and attempts at biofortification with selenium are being undertaken around the globe. Research is conducted both on vegetable and cereal crops, for example in Italy, France, Mexico, Brazil, Malawi, part of China [1,34–38]. Considering the possibilities of selenium accumulation in plant tissues, wheat shows a substantial ability for the uptake of the element in higher than average quantities with no negative effect on the plant's development [39].

Many studies point to the positive effect of fertilisation with selenium on crop yields, potentially constituting a positive side effect of agrotechnical biofortification with this element. Hajiboland [40] fertilising rape with selenium obtained a significantly higher yield of vegetative parts, as well as grains and inflorescences in comparison to control. Studies conducted in the United States [41] on lentils also provided positive results in terms of an increase in crop yield under fertilisation with selenium. Similar conclusions were drawn in the case of wheat. According to Nawaz [42], foliar application of selenium contributes to an increase in grain yield in the conditions of water deficit. The positive effect of foliar application of Se on grain yield of other crops such as buckwheat [43] or rice [44] has also been reported. Studies by Lyons et al. [45] and Broadley et al. [46] showed that neither soil nor the foliar application of selenium contributes to an increase in crop yield, but only to the content of the element in wheat grain. The conducted experiment also suggests that fertilisation with selenium does not affect wheat yield. Neither soil (S), foliar (F), nor combined soil and foliar application (S + F) at particular stages caused significant differences in the crop yield (apart from soil fertilization with foliar application in tillering stage (BBCH 22) (S + F1). Lack of effect of application time on the yield of wheat grain is also confirmed by Ducsay and Ložek [47]. Notice applies in the case of soil application combined with foliar application (S + F), where a dose of selenium twice as high as in the remaining types of fertilisation was applied, and the final yield still showed no difference towards control or the remaining methods of introduction of the element. Therefore, biofortification with selenium can be conducted with no risk of a negative effect on the yield of the analysed crop.

In the experiment, the content of the analysed element for the control sample averaged 0.155 $mg \cdot kg^{-1}$. It is approximate to Se content obtained in studies by Zhao et al. [48], where the content of the element was in a broad range, from 0.010 to 0.115 $mg \cdot kg^{-1}$. In another study, the contents do not exceed 0.044 $mg \cdot kg^{-1}$ [49]. Wheat cultivated in Great Britain contains only 0.028 $mg \cdot kg^{-1}$ Se [50]. In Belgium, the value was twice higher, but still relatively low, namely 0.054 $mg \cdot kg^{-1}$ Se [51]. It is related to the low supply of selenium in the region of European soils. Selenium contents in wheat grain are considerably lower in comparison to regions characterised by a higher content of this element in soil. For example, in South Dakota, the average selenium content in wheat grain was 0.63 $mg \cdot kg^{-1}$ [52]. According to Sharma et al. [53], selenium content in the cultivated plants in India is considerably higher (115 $mg \cdot kg^{-1}$ Se) in comparison to global data concerning this element in crops. The level of selenium observed in plant products (15–670 $mg \cdot kg^{-1}$) in this region is also considerably higher than global data concerning Se in food crops.

In the study, despite the general no effect of fertilisation with selenium on wheat crop yield, considerable differences were found in selenium content in grain. They were evident both for the application method and for the development stage at which the element was introduced. This suggests that soil (S), foliar (F), and combined soil and foliar (S + F) selenium application affects its content in wheat grain. The positive effect of soil application was also presented in the study by Keskinen et al. [54] where increasing selenium doses were accompanied by an increase in its content in the grain of spring wheat. Boldrin et al. [55] and Ducsay [56] also reported a positive effect of foliar application on an increase in selenium content in wheat grain. Manojlivic et al. [57] evidenced that both soil and foliar application contributed to an increase in selenium content in grain of spring wheat, whereas foliar application was more efficient. Higher efficiency of foliar application of Se in spring wheat in comparison to soil application was also evidenced in the study by Mao et al. [58]. Similar results were obtained by Grant et al. [59] who introduced selenium in the form of seed coating, granulated fertiliser, and spraying. The most efficient method of increasing selenium content in grain was foliar application, and soil application provided results comparable to seed coating. In the study by Poblaciones et al. [60], irrespective of the dose, foliar application of selenium also contributed to an increase in its content in wheat grain.

In the study, combined soil and foliar application at particular stages (S + F) proved the most efficient. Neither soil nor foliar application performed separately resulted in contents of the element in grain as high as in the case of combining of these two methods of fertilisation. It is among others related to the double dose of selenium supplied to wheat (5.00 $g \cdot ha^{-1}$ Se in soil application and 5.00 $g \cdot ha^{-1}$ Se in foliar application). The quantity of assimilated selenium, however, is not a simple sum of the introduced element. The comparison of the obtained results shows that in some cases, despite the provision of selenium in soil and foliar application at total dose 10.00 $g \cdot ha^{-1}$ Se (5.00 $g \cdot ha^{-1}$ Se in soil application and 5.00 $g \cdot ha^{-1}$ Se in foliar application), the selenium content did not increase in the same way in each variant. The highest selenium content was obtained in a combination of soil and foliar application at the stage of tillering, stem elongation (S + F1 + F2), and at the stage of stem elongation (S + F2), where selenium content was approximately 4.64 and 3.97 times higher than in control. In the remaining cases, the content of the element was considerably lower. This suggests that it is not the volume of application that determines the final selenium content in grain, but the development stage at which the element is introduced. The study showed that the best term for foliar application of selenium in the cultivation of spring wheat is the stage of stem elongation (BBCH 30–39).

The study by Davydenko and Mayurnikova [61] also showed that enriching crops in selenium by means of the foliar application provides for more significant effect when performed at particular stages when the plant assimilates the highest quantities of microelements. According to Chu et al. [62], the introduction of selenium to wheat in the form of spraying is more efficient at the stage of flowering. The study by Wang et al. [63] showed that the average selenium content in grain in the case of foliar application at the pre-filling stage was 55% higher than in the case of application at the pre-flowering stage using selenite, and 4% higher using selenate. In a study concerning two wheat cultivars, Galinha et al. [64] observed that in the case of Marialva cultivar, the best term for selenium application was the grain filling stage, and for Jordão cultivar the booting stage. The study results are in accordance with an experiment conducted in Spain [65], where the foliar application was performed for wheat at the following growth stages: BBCH-31, BBCH-35, BBCH-45, and BBCH-51. The highest level of selenium accumulation in grain was obtained at stages BBCH-35, BBCH-45, with consideration of recommendations for humid regions in a temperate climate such as Central and North Europe.

Selenium is usually used in amounts (10.00–20.00 $g \cdot ha^{-1}$ Se) to achieve the goals of biofortification. [20,66–69]. Low doses of 5.00 and 10.00 g ha^{-1} Se were used in the studies, which did not contribute to excessive toxic accumulation of selenium in the grain in comparison with the above studies, in which, despite the higher dose, no toxic effects were found. In the USA and part of China, in areas rich in selenium, the content of this element in plant products is close to the highest values obtained in the study [70,71]. At these amounts of selenium, no toxic effect was found, while the local population had the desired level of selenium in the blood. Similar conclusions were obtained in Finland, where the addition of selenium to fertilisers is mandatory [20]. Most plants with low Se levels have a content of about 0.025 $mg \cdot kg^{-1}$ Se and rarely exceed 0.100 $mg \cdot kg^{-1}$ Se. In countries with high selenium content in plants without the toxic effect, this content ranges from 0.34 to 0.92 $mg \cdot kg^{-1}$ Se [72]. The increased amounts of selenium in grain obtained in the study, where its highest content was 0.719 $mg \cdot kg^{-1}$ Se, prove the efficiently conducted agrotechnical biofortification, which may contribute to ensuring foods rich in selenium and reduce its deficiency, that is foods of appropriate quality, especially from the perspective of food safety and consumer health.

5. Conclusions

The study evidenced that an efficient method of increasing selenium content in grain of spring wheat can be the introduction of this element through soil application combined with the foliar application at the stage of stem elongation (BBCH 30–39) with a total dose of 10.00 g·ha^{-1} Se. Moreover, fertilisation with selenium the general did not affect the yield of wheat grain. Therefore, biofortification with selenium at moderate doses can be performed with no risk of a negative effect on the yield of the analysed crops. Agrotechnical biofortification is an efficient way of increasing the nutritional value of wheat with respect to selenium. This is an extremely important aspect of the strategy of increasing food quality. The use of biofortified wheat for food purposes will contribute to higher consumption of selenium by humans, and thus to reduce its deficiencies in the diet.

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References

1. Wu, Z.; Bañuelos, G.S.; Lin, Z.Q.; Liu, Y.; Yuan, L.; Yin, X.; Li, M. Biofortification and phytoremediation of selenium in China. *Front. Plant Sci.* **2015**, *6*, 136. [CrossRef]
2. Sperotto, R.A.; Ricachenevsky, F.K.; Williams, L.E.; Vasconcelos, M.W.; Menguer, P.K. From Soil to Seed: Micronutrient Movement into and within the Plant. *Front. Plant Sci.* **2014**, *5*, 438. [CrossRef]
3. Vinceti, M.; Filippini, T.; Wise, L.A. Environmental Selenium and Human Health: An Update. *Curr. Environ. Health Rep.* **2018**, *5*, 464–485. [CrossRef]
4. Rayman, M.P. The use of high-selenium yeast to raise selenium status: How does it measure up? *Br. J. Nutr.* **2004**, *92*, 557–573. [CrossRef] [PubMed]
5. SCF 2000. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Selenium, SCF/CS/NUT/UPPLEV /25 Final 1–18. Available online: www.ec.europa.eu/food/fs/sc/scf/out80g_en.pdf (accessed on 18 December 2020).
6. Ashton, K.; Hooper, L.; Harvey, L.; Hurst, R.; Casgrain, A.; Fairweather-Tait, S.J. Methods of assessment of selenium status in humans: A systematic review. *Am. J. Clin. Nutr.* **2009**, *89*, 2025–2039. [CrossRef]
7. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on dietary reference values for selenium. *EFSA J.* **2014**, *12*, 3846. [CrossRef]
8. Hurst, R.; Collings, R.; Harvey, L.J.; King, M.; Hooper, L.; Bouwman, J.; Gurinovic, M.; Fairweather-Tait, S.J. EURRECA—Estimating selenium requirements for deriving dietary reference values. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 1077–1096. [CrossRef] [PubMed]
9. Rayman, M.P. Selenium and Human Health. *Lancet* **2012**, *379*, 1256–1268. [CrossRef]
10. Zagrodzki, P. Selenium and the immune system. *Adv. Hig. Med. Exp.* **2004**, *58*, 140–149. (In Polish)
11. Schiavon, M.; Nardi, S.; Vecchia, F.D.; Ertani, A. Selenium biofortification in the 21 st century: Status and challenges for healthy human nutrition. *Plant Soil.* **2020**, 1–26. [CrossRef]
12. Klecha, B.; Bukowska, B. Selenium in the human body-characteristics of the element and potential therapeutic application. *Bromatol. Chem. Toxicol.* **2016**, *4*, 818–829. (In Polish)
13. Kabata-Pendias, A.; Pendias, H. *Trace Elements in the Biological Environment*; Geological Publishers: Warsaw, Poland, 1979; pp. 298–299. (In Polish)
14. Fordyce, F.M. Selenium deficiency and toxicity in the environment. In *Essentials of Medical Geology*; Springer: Dordrecht, The Netherlands, 2013; pp. 375–416.
15. Hartikainen, H. Biogeochemistry of selenium and its impact on food chain quality and human health. *J. Trace Elem. Med. Biol.* **2005**, *18*, 309–318. [CrossRef] [PubMed]
16. Dudka, S. Assessment of Total Content of Root and Trace Elements in the Surface Layer of Polish Soils. Ph.D. Thesis, Institute of Soil Cultivation, Fertilization and Soil Science in Puławy, Puławy, Poland, 1992. (In Polish)
17. Navarro-Alarcon, M.; Cabrera-Vique, C. Selenium in food and the human body: A review. *Sci. Total Environ.* **2008**, *400*, 115–141. [CrossRef] [PubMed]
18. Kipp, A.P.; Strohm, D.; Brigelius-Flohé, R.; Schomburg, L.; Bechthold, A.; Leschik-Bonnet, E.; Heseker, H. Revised reference values for selenium intake. *J. Trace Elem. Med. Biol.* **2015**, *32*, 195–199. [CrossRef]

19. Borrill, P.; Connorton, J.M.; Balk, J.; Miller, A.J.; Sanders, D.; Uauy, C. Biofortification of wheat grain with iron and zinc: Integrating novel genomic resources and knowledge from model crops. *Front. Plant Sci.* **2014**, *5*, 53. [CrossRef]
20. Alfthan, G.; Eurola, M.; Ekholm, P.; Venäläinen, E.R.; Root, T.; Kork-alainen, K.; Hartikainen, H.; Salminen, P.; Hietaniemi, V.; Aspila, A.; et al. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. *J. Trace Elem. Med. Biol.* **2015**, *31*, 142–147. [CrossRef]
21. Ros, G.H.; Van Rotterdam, A.M.D.; Bussink, D.W.; Bindraban, P.S. Selenium fertilization strategies for bio-fortification of food: An agro-ecosystem approach. *Plant Soil.* **2016**, *404*, 99–112. [CrossRef]
22. Jones, G.D.; Droz, B.; Greve, P.; Gottschalk, P.; Poffet, D.; McGrath, S.P.; Seneviratne, S.I.; Smith, P.; Winkel, L.H. Selenium deficiency risk predicted to increase under future climate change. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 2848–2853. [CrossRef]
23. Contemporary Trends in Bakery production-the Use of Oats and Barley as Non-Bread Cereals. Available online: http://agro.icm.edu.pl/agro/element/bwmeta1.element.dl-catalog-4bc89867-e8de-49d1-8200-dac730d3425d (accessed on 12 December 2020).
24. Cereal Products as a Source of Selenium in Domestic Food Rations. Available online: http://wydawnictwa.pzh.gov.pl/roczniki_pzh/produkty-zbozowe-jako-zrodlo-selenu-w-krajowych-racjach-pokarmowych?lang=pl (accessed on 20 December 2020).
25. Beladel, B.; Nedjimi, B.; Mansouri, A.; Tahtat, D.; Belamri, M.; Tchanchane, A.; Khelfaoui, F.; Benamar, M.E.A. Selenium content in wheat and estimation of the selenium daily intake in different regions of Algeria. *Appl. Radiat. Isot.* **2013**, *71*, 7–10. [CrossRef]
26. Ducsay, L.; Ložek, O.; Varga, L.; Lošák, T. Effects of winter wheat supplementation with selenium. *Ecol. Chem. Eng.* **2007**, *14*, 3–4.
27. Adams, M.L.; Lombi, E.; Zhao, F.J.; McGrath, S.P. Evidence of low selenium concentrations in UK bread-making wheat grain. *J. Sci. Food Agric.* **2002**, *82*, 1160–1165. [CrossRef]
28. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World map of the Köppen-Geiger climate classification updated. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef]
29. Working Group World Reference Base for Soil Resources. The International Union of Soil Sciences. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. Available online: http://www.fao.org/soils-portal/data-hub/soil-classification/world-reference-base/en/ (accessed on 12 December 2020).
30. Central Statistical Office. *Environment 2020*; Statistical Publishing House: Warsaw, Poland, 2020; p. 41.
31. Norm, P. *PN-R-04013: 1988. Chemical and Agricultural Analysis of Plants. Determination of Air-Dry and Dry Mass*; Polish Committee for Standardization: Warsaw, Poland, 1988.
32. Meng, T.; Carew, R.; Florkowski, W.J.; Klepacka, A.M. Analyzing temperature and precipitation influences on yield distributions of canola and spring wheat in Saskatchewan. *J. Appl. Meteorol. Climatol.* **2017**, *56*, 897–913. [CrossRef]
33. Dmowski, Z.; Dzieżyc, H. Rainfall needs of spring wheat on a good wheat and very good rye complex of soil in north-east Poland. *Acta Agrophys.* **2009**, *13*, 39–48.
34. De Vita, P.; Platani, C.; Fragasso, M.; Ficco, D.B.M.; Colecchia, S.A.; Del Nobile, M.A.; Padalino, L.; Di Gennaro, S.; Petrozza, A. Selenium-enriched durum wheat improves the nutritional profile of pasta without altering its organoleptic properties. *Food Chem.* **2017**, *214*, 374–382. [CrossRef]
35. Longchamp, M.; Castrec-Rouelle, M.; Biron, P.; Bariac, T. Variations in the accumulation, localization and rate of metabolization of selenium in mature Zea mays plants supplied with selenite or selenate. *Food Chem.* **2015**, *182*, 128–135. [CrossRef]
36. Ortiz-Monasterio, I.; Cárdenas, M.E.; Lyons, G.H. Biofortification of irrigated wheat with Se fertilizer: Timing, rate, method and type of wheat. In *Global Advances in Selenium Research from Theory to Application, Proceedings of the 4th International Conference on Selenium in the Environment and Human Health, Sao Paulo, Brazil, 17–20 September 2015*; CRC Press: Boca Raton, FL, USA, 2015; p. 167.
37. Dos Reis, A.R.; El-Ramady, H.; Santos, E.F.; Gratão, P.L.; Schomburg, L. Overview of selenium deficiency and toxicity worldwide: Affected areas, selenium-related health issues, and case studies. In *Selenium in Plants*; Springer: Cham, Switzerland, 2017; pp. 209–230.
38. Joy, E.J.; Kalimbira, A.A.; Gashu, D.; Ferguson, E.L.; Sturgess, J.; Dangour, A.D.; Banda, L.; Chiutsi-Phiri, G.; Bailey, E.H.; Langley-Evans, S.C.; et al. Can selenium deficiency in Malawi be alleviated through consumption of agro-biofortified maize flour? Study protocol for a randomised, double-blind, controlled trial. *Trials* **2019**, *20*, 795. [CrossRef]
39. Lazo-Vélez, M.A.; Chávez-Santoscoy, A.; Serna-Saldivar, S.O. Selenium-enriched breads and their benefits in human nutrition and health as affected by agronomic, milling, and baking factors. *Cereal Chem.* **2015**, *92*, 134–144. [CrossRef]
40. Hajiboland, R. Selenium supplementation stimulates vegetative and reproductive growth in canola (*Brassica napus* L.) plants. *Acta Agric. Slov.* **2012**, *99*, 13. [CrossRef]
41. Ekanayake, L.J.; Tharavarajah, D.; Vial, E.; Schatz, B.; McGee, R.; Thavarajah, P. Selenium fertilization on lentil (*Lens culinaris* Medikus) grain yield, seed selenium concentration, and antioxidant activity. *Field Crops Res.* **2015**, *177*, 9–14. [CrossRef]
42. Nawaz, F.; Ashraf, M.Y.; Ahmad, R.; Waraich, E.A.; Shabbir, R.N.; Bukhari, M.A. Supplemental selenium improves wheat grain yield and quality through alterations in biochemical processes under normal and water deficit conditions. *Food Chem.* **2015**, *175*, 350–357. [CrossRef]
43. Jiang, C.Q.; Shen, J.; Zu, C.L. Selenium uptake and transport of rice under different Se-enriched natural soils. *Chin. J. Appl. Ecol.* **2015**, *26*, 809–817. (In Chinese)
44. Boldrin, P.F.; Faquin, V.; Ramos, S.J.; Boldrin, K.V.F.; Ávila, F.W.; Guilherme, L.R.G. Soil and foliar application of selenium in rice biofortification. *J. Food Compos. Anal.* **2013**, *31*, 238–244. [CrossRef]

45. Lyons, G.; Stangoulis, J.; Graham, R. High-Selenium Wheat: Biofortification for Better Health. *Nutr. Res. Rev.* **2003**, *16*, 45–60. [CrossRef]
46. Broadley, M.R.; Alcock, J.; Alford, J.; Cartwright, P.; Foot, I.; Fairweather-Tait, S.J.; Hart, D.J.; Hurst, R.; Knott, P.; McGrath, S.P.; et al. Selenium biofortification of high-yielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilisation. *Plant Soil.* **2010**, *332*, 5–18. [CrossRef]
47. Ducsay, L.; Ložek, O. Effect of selenium foliar application on its content in winter wheat grain. *Plant Soil Environ.* **2006**, *52*, 78–82. [CrossRef]
48. Zhao, F.J.; Lopez-Bellido, F.J.; Gray, C.W.; Whalley, W.R.; Clark, L.J.; McGrath, S.P. Effects of soil compaction and irrigation on the concentrations of selenium and arsenic in wheat grains. *Sci. Total Environ.* **2007**, *372*, 433–439. [CrossRef] [PubMed]
49. Lyons, G. Selenium in cereals: Improving the efficiency of agronomic biofortification in the UK. *Plant Soil.* **2010**, *332*, 1–4. [CrossRef]
50. Fan, M.S.; Zhao, F.J.; Poulton, P.R.; McGrath, S.P. Historical changes in the concentrations of selenium in soil and wheat grain from the Broadbalk experiment over the last 160 years. *Sci. Total Environ.* **2008**, *389*, 532–538. [CrossRef] [PubMed]
51. De Temmerman, L.; Waegeneers, N.; Thiry, C.; Du Laing, G.; Tack, F.; Ruttens, A. Selenium content of Belgian cultivated soils and its uptake by field crops and vegetables. *Sci. Total Environ.* **2014**, *468*, 77–82. [CrossRef] [PubMed]
52. Lee, S.; Woodard, H.J.; Doolittle, J.J. Selenium uptake response among selected wheat (*Triticum aestivum*) varieties and relationship with soil selenium fractions. *Soil Sci. Plant Nutr.* **2011**, *57*, 823–832. [CrossRef]
53. Sharma, N.; Prakash, R.; Srivastava, A.; Sadana, U.S.; Acharya, R.; Prakash, N.T.; Reddy, A.R. Profile of selenium in soil and crops in seleniferous area of Punjab, India by neutron activation analysis. *J. Radioanal. Nucl. Chem.* **2009**, *281*, 59–62. [CrossRef]
54. Keskinen, R.; Yli-Halla, M.; Hartikainen, H. Retention and uptake by plants of added selenium in peat soils. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 3465–3482. [CrossRef]
55. Boldrin, P.F.; de Figueiredo, M.A.; Yang, Y.; Luo, H.; Giri, S.; Hart, J.J.; Faquin, V.; Guilherme, L.R.G.; Thannhauser, T.W.; Li, L. Selenium promotes sulfur accumulation and plant growth in wheat (*Triticum aestivum*). *Physiol. Plant.* **2016**, *158*, 80–91. [CrossRef]
56. Ducsay, L.; Ložek, O.; Marček, M.; Varényiová, M.; Hozlár, P.; Lošák, T. Possibility of selenium biofortification of winter wheat grain. *Plant Soil Environ.* **2016**, *62*, 379–383. [CrossRef]
57. Manojlović, M.S.; Lončarić, Z.; Cabilovski, R.R.; Popović, B.; Karalić, K.; Ivezić, V.; Ademi, A.; Singh, B.R. Biofortification of wheat cultivars with selenium. *Acta Agric. Scand. B Soil Plant Sci.* **2019**, *69*, 715–724. [CrossRef]
58. Mao, H.; Wang, J.; Wang, Z.; Zan, Y.; Lyons, G.; Zou, C. Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China. *J. Soil Sci. Plant Nutr.* **2014**, *14*, 459–470. [CrossRef]
59. Grant, C.A.; Buckley, W.T.; Wu, R. Effect of selenium fertilizer source and rate on grain yield and selenium and cadmium concentration of durum wheat. *Can. J. Plant Sci.* **2007**, *87*, 703–708. [CrossRef]
60. Poblaciones, M.J.; Rodrigo, S.; Santamaría, O.; Chen, Y.; McGrath, S.P. Agronomic selenium biofortification in Triticum durum under Mediterranean conditions: From grain to cooked pasta. *Food Chem.* **2014**, *146*, 378–384. [CrossRef]
61. Davydenko, N.I.; Mayurnikova, L.A. On the possibility to grow high-selenium wheat in the Kuznetsk Basin. *Foods Raw Mater.* **2014**, *2*, 11. [CrossRef]
62. Chu, J.; Yao, X.; Yue, Z.; Li, J.; Zhao, J. The effects of selenium on physiological traits, grain selenium content and yield of winter wheat at different development stages. *Biol. Trace Elem. Res.* **2013**, *151*, 434–440. [CrossRef]
63. Wang, M.; Ali, F.; Wang, M.; Dinh, Q.T.; Zhou, F.; Bañuelos, G.S.; Liang, D. Understanding boosting selenium accumulation in Wheat (*Triticum aestivum* L.) following foliar selenium application at different stages, forms, and doses. *Environ. Sci. Pollut. Res.* **2020**, *27*, 717–728. [CrossRef] [PubMed]
64. Galinha, C.; do Freitas, M.C.; Pacheco, A.M.; Coutinho, J.; Maçãs, B.; Almeida, A.S. Selenium supplementation of Portuguese wheat cultivars through foliar treatment in actual field conditions. *J. Radioanal. Nucl. Chem.* **2013**, *297*, 227–231. [CrossRef]
65. Rodrigo, S.; Santamaria, O.; Poblaciones, M.J. Selenium application timing: Influence in wheat grain and flour selenium accumulation under mediterranean conditions. *J. Agric. Sci.* **2014**, *6*, 23. [CrossRef]
66. Garousi, F. Toxicity of selenium, application of selenium in fertilizers, selenium treatment of seeds, and selenium in edible parts of plants. *Acta Univ. Sapientiae Aliment.* **2017**, *10*, 61–74. [CrossRef]
67. Ngigi, P.B.; Lachat, C.; Masinde, P.W.; Du Laing, G. Agronomic biofortification of maize and beans in Kenya through selenium fertilization. *Environ. Geochem. Health* **2019**, *41*, 2577–2591. [CrossRef]
68. Xia, Q.; Yang, Z.P.; Xue, N.W.; Dai, X.J.; Zhang, X.; Gao, Z.Q. Effect of foliar application of selenium on nutrient concentration and yield of colored-grain wheat in China. *Appl. Ecol. Environ. Res.* **2019**, *17*, 2187–2202. [CrossRef]
69. Lara, T.S.; de Lessa, J.H.L.; de Souza, K.R.D.; Corguinha, A.P.B.; Martins, F.A.D.; Lopes, G.; Guilherme, L.R.G. Selenium biofortification of wheat grain via foliar application and its effect on plant metabolism. *Food Compost. Anal.* **2019**, *81*, 10–18. [CrossRef]
70. Bañuelos, G.S.; Freeman, J.; Arroyo, I. Accumulation and speciation of selenium in biofortified vegetables grown under high boron and saline field conditions. *Food Chem. X* **2020**, *5*, 100073. [CrossRef]
71. Xing, K.; Zhou, S.; Wu, X.; Zhu, Y.; Kong, J.; Shao, T.; Tao, X. Concentrations and characteristics of selenium in soil samples from Dashan Region, a selenium-enriched area in China. *Soil Sci. Plant Nutr.* **2015**, *61*, 889–897. [CrossRef]
72. Ramkissoon, C.; Degryse, F.; da Silva, R.C.; Baird, R.; Young, S.D.; Bailey, E.H.; McLaughlin, M.J. Improving the efficacy of selenium fertilizers for wheat biofortification. *Sci. Rep.* **2019**, *9*, 19520. [CrossRef]