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Selenium Agronomic Biofortification of Durum Wheat Fertilized with Organic Products: Se Content and Speciation in Grain

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Abstract: The biofortification of cereals is a potential solution for increasing Se levels in the human diet. Here we evaluated whether applying selenate and organic N and S sources via foliar alters Se content and species in durum wheat grain. Field trials were conducted in a Mediterranean environment in 2017–2018 and 2018–2019 on old (Cappelli and old Saragolla) and modern (Marco Aurelio and Nadif) durum wheat varieties. Four organic fertilization strategies were evaluated: the control (dry blood meal at sowing), the application of foliar S (Bio-sulphur at flag leaf) and N (liquid blood meal at the beginning of heading), and their combined use in interaction with a foliar application of selenate at the booting stage. The Se content in grain was determined using ICP-MS and its speciation throughout HPLC-ICP-MS and HPLC-ESI-MS/MS. In 2018, the lower rainfall in the ten days before the Se foliar application caused a higher Se accumulation in the grain (1.63 vs. 1.44 mg kg^{-1} in 2017–2018 and 2018–2019, respectively). A negative effect of organic foliar S on Se content in grain was observed (−24% with respect to the control). The modern Nadif showed the highest Se content (1.87 mg kg^{-1}), followed by the old variety Cappelli (1.57 mg kg^{-1}). Finally, the Se speciation analysis showed the presence of SeMet and SeMetSeCys. The organic fertilization affected only SeMet, with lower values under organic S fertilization and higher values under organic N fertilization.

Keywords: sodium selenate; nitrogen; sulphur; *Triticum durum*; SeMetSeCys; SeMet; foliar fertilization; ICP-MS; HPLC-ICP-MS; HPLC-ESI-MS/MS

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1. Introduction

Plants make up 80% of our food [1] and provide both macronutrients for energy and growth and essential micronutrients that protect us from chronic diseases, making substantial contributions to our health [2]. However, micronutrient deficiencies, known as "hidden hunger", affect the health of the world's people in many ways [3]. In developing countries, diet depends on a few staple crops, which are calorie-rich but nutritionally deficient, while in developed countries, the pervasiveness of the fast-food industries and the rising costs of fruit and vegetables have contributed to a reduction of the nutritional value of Western diets [2].

On the global scale, selenium (Se) deficiency has progressively increased over recent decades with a negative impact on health [4]. One billion people have an inadequate Se dietary intake [5,6], and, generally, this scanty Se is associated with areas where its concentration in the soil is low [7]. Today, selenium-deficient soils are common in many

regions [7], and in the future, this scenario is likely to worsen due to the ongoing climate changes that will cause a reduction of soil Se content, principally in cultivated areas [8].

The biofortification of cereals and other staple crops is a potential solution for increasing Se levels in the human diet. Biofortification generally stimulates the accumulation of microelements in the edible parts of the plant consumed by humans [9]. Durum wheat (*Triticum turgidum* var. *durum* Desf.) is the primary cereal grown in several countries of the Mediterranean basin and is mainly used for bread, couscous, and pasta production [10,11], which are foods consumed daily by many people [12].

Research investigating Se biofortification in durum wheat has been traditionally focused on defining the optimal Se fertilizer source, timing, and rate related to the accumulation in grain and its derived products [13–15]. Far less research has, however, explored whether the supply of macronutrients, such as nitrogen (N) and sulfur (S), influences the Se biofortification [16].

An essential consideration in the agronomic biofortification of Se concerns the effect of S because they compete for uptake from the soil [17–19]. S is essential to durum wheat crops for optimal yield and grain quality [20,21]. Selenium, in the form of selenate (SeO_4^{2-}), is structurally similar to sulfate (SO_4^{2-}); for this reason, this latter may influence SeO_4^{2-} uptake through two different plant physiological processes: (i) competition for the membrane transport since SeO_4^{2-} is taken up by the plants via sulfate transports [22,23]; and (ii) regulation of the expression of genes encoding sulfate transports by S status of the plant [24–26]. Additionally, the assimilation of selenate follows the sulfate pathway, including some of the same enzymes and their binding sites [27,28]. Furthermore, many Se species (e.g., selenocysteine (SeCys) and selenomethionine (SeMet)) are analogs of S-containing amino acids (e.g., cysteine, methionine) [28–30]. Thus, Se-amino acids can be integrated into proteins, compromising their function [4] and altering gluten protein aggregation levels, which is essential for durum wheat quality [31].

N represents the primary constraint in obtaining adequate yield and quality in durum wheat [32,33], especially in the organic system. Considering its importance, it is also interesting to understand the possible effect of this macronutrient on Se biofortification in durum wheat. Previous studies on *Triticum aestivum* (winter/spring wheat), carried out in a conventional system showed that mineral N availability promotes Se accumulation in grain [34,35]. The interaction between N and Se passes through the effect that N has on the increasing assimilation and metabolization of S; since Se and S use the same metabolic pathway in plants, N can also promote Se absorption and its metabolization into selenoproteins [36]. The interactions between N, S, and Se in durum wheat have been little studied, often in a controlled environment and using mineral fertilizers [16]. However, these interactions could change using organic fertilizers, which are increasingly important, due to the significant difficulties associated with the supply of mineral fertilizers worldwide [37]. To the best of our knowledge, no information is available on the possible effect of organic N and S fertilization, distributed via foliar, on Se biofortification in durum wheat grown in the open field in a Mediterranean area.

Besides evaluating the total Se content in grain, another critical issue is assessing the individuation and concentration of Se species to provide information about how Se in grain is bioavailable to end-users and its possible toxicological effects. Indeed, the inorganic Se species, such as selenate (SeVI) and selenite (SeIV), are generally considered to be the most toxic [38], while the organic Se species are considered less toxic [38]. However, among the studies investigating the Se species in wheat grain, no one assesses the influence of organic fertilizer management on the production of Se organic species in durum wheat.

This research focused on whether the application of selenate and organic N and S sources via foliar affects the content of total Se in grains of old and modern durum wheat varieties under Mediterranean climate conditions. In addition, a possible alteration in the production of Se species in the grains of durum wheat cultivated under organic fertilization management was evaluated.

2. Materials and Methods

2.1. Field Experiment

The detailed procedures of field experiments conducted in the 2017–2018 and 2018–2019 growing seasons (namely 2018 and 2019, respectively) have already been described [39]. Briefly, the field experiments were arranged in a split–split plot design in the growing seasons with three replicates. The main plots consisted of different varieties: Cappelli, old Saragolla, Marco Aurelio, and Nadif. Plots consisted of four organic fertilization treatments: (1) control (CTR) fertilized with dry blood meal in a single application at seeding; (2) CTR plus foliar S application with bio-sulfur (CTR + S) at flag leaf sheath opening stage (BBCH stage 47); (3) CTR plus N foliar application with liquid blood meal (CTR + N) at the beginning of heading (BBCH stage 51); and (4) CTR plus the combination of N and S foliar applications at flag leaf sheath opening stage and the beginning of the heading, respectively (CTR + NS). Sub-plots (10.0 m^2; 2.0 m × 5.0 m), consisting of the Se application, were evaluated by comparing a control without selenium (Se0) and one foliar application of sodium selenate (Se60) (Na_2SeO_4; BioXtra), at the rate of 60 g ha^{-1} [15] at the booting stage (BBCH stage 41). A weather station close to the experimental field recorded daily precipitation and temperature. Total rainfall in 2018 and 2019 was 401 mm and 299 mm, respectively. During the two growing seasons, rainfall was well distributed, even if in 2019, during the grain filling period (from the end of April to the middle of June), there was 104 mm less rainfall than in 2018 (Figure 1).

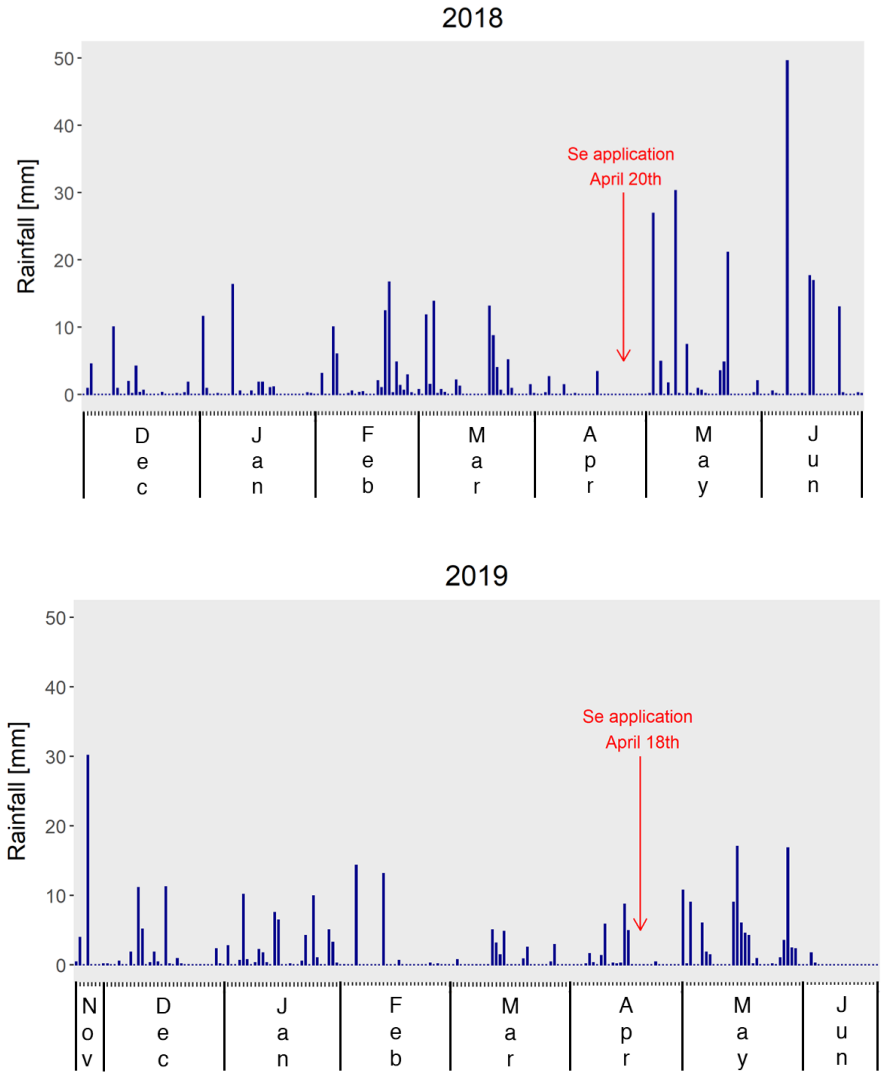

Figure 1. Daily rainfall for the two growing seasons.

2.2. Selenium Analyses

At harvesting, grain samples for the Se analysis were taken from the three field replicates each year. Analyses were performed on three replicates for each field grain sample and considered in the final expression of the results. The whole grain flour used in the analysis was obtained by ground grain sample using a Cyclotec Sample Mill 1093 (Foss Tecator, Hillerød, Denmark).

2.2.1. Determination of the Total Se Content by ICP-MS

The Se content in grain was determined using ICP-MS after acid digestion in a microwave oven (MSP 1000, CEM, Matheus, NC, USA) procedure described by Moreno-Martin et al. [40], with minor modifications. About 0.5 g of whole grain flour were weighed in an Xpress tube (volume 10 mL), 5 mL of concentrated nitric acid (Merck, Madrid, Spain), and 1.0 mL of 30% (*v*/*v*) hydrogen peroxide (Panreac, Barcelona, Spain) were added. The mixture was held for 30 min at room temperature and then heated at the maximum power of 800 W, first at 130 °C for 5 min, after a temperature ramp of 15 °C·min^{-1}, and finally at 160 °C for 15 min, increasing the temperature to 10 °C·min^{-1}. After digestion, the mixture was diluted with Milli-Q water to 25 mL, filtered through a 0.22 µm syringe Nylon filter (Scharlab, Barcelona, Spain), and analyzed. Se content in the digests was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., Santa Clara, CA, USA) by monitoring ^{77}Se, ^{78}Se, and ^{80}Se isotopes under continuous acquisition mode. The experimental conditions were as follows: RF power of 1550 W, sample depth of 8 mm, plasma gas flow rate of 15.0 L·min^{-1}, an auxiliary gas flow rate of 0.90 L·min^{-1}, and conical nebulizer with a flow rate of 1.01 L·min^{-1}. Se quantification was performed by external calibration with the isotope ^{78}Se.

2.2.2. Extraction Se-Species and HPLC-ICP-MS and HPLC-ESI-MS/MS Analysis

After the evaluation of total Se in grain, its speciation was performed on Cappelli and Nadif genotypes with Se60 application throughout HPLC-ICP-MS analysis after the extraction of Se species with enzymatic hydrolysis, according to the method reported in Moreno-Martin et al. [40], with minor modifications. Briefly, 0.1 g of whole grain flour was treated with 20 mg of non-specific protease Streptomyces griseus (Protease XIV) (Sigma-Aldrich, Madrid, Spain) and 5 mL of 30 mmol·L^{-1} tris-(hydroxymethyl)-aminomethane (Sigma-Aldrich, Madrid, Spain) at pH 7.5 (adjusted with 0.05 M HCl) in a polypropylene tube for 24 h at 37 °C and without shaking. The reaction mixture was then centrifuged at 11,000× *g* rpm for 15 min (Eppendorf 5804 F34-6-38 centrifuge, Hamburg, German) and filtered through a 0.22 µm syringe Nylon filter. The different enzymatic extracts were analyzed by ICP-MS to determine the total extracted Se content and HPLC-ICP-MS to detect and identify the Se species. An evaluation of Se species in Protease XIV was also performed to check for impurities. With respect to the HPLC-ICP-MS analysis, the HPLC unit consisted of a PU-2089 HPLC pump (JASCO, Tokyo, Japan) fitted with a six-port injection valve (model 7725i, Rheodyne Rohner Park, CA, USA) and with an injection loop whose volume varied depending on the chromatographic column employed. In order to obtain an unambiguous identification of the Se species, the extracts were run through two different columns. The first column was a Hamilton PRP-X100 (250 × 4.1 mm, 10 µm) with an anion exchange mechanism and a 100 µL injection loop, with a mobile phase consisting of 10 mM citric acid at pH 5.0 adjusted with ammonia and 2% methanol (Scharlab, Barcelona, Spain). The elution mode was isocratic with a flow rate of 1 mL·min^{-1}. The second column was a Phenomenex Kintex EVO C18 (150 × 3.0 mm, 5 µm) with a reversed-phase mechanism, and a 20 µL injection loop was employed. The mobile phase consisted of 0.1% formic acid at pH 3.2 and 0.5% methanol. The elution mode was also isocratic with a flow rate of 0.5 mL·min^{-1}. The outlet of both columns was connected directly to the conical nebulizer of ICP-MS with PEEK tubing. Analyses were performed by ICP-MS in time-resolved analysis mode by monitoring ^{77}Se, ^{78}Se, and ^{80}Se isotopes and employing the operating conditions summarized in the section determination of the total Se content

by ICP-MS. Se species were identified after separation in both chromatographic columns by comparing their retention time with those of standard solutions: Seleno-L-methionine (SeMet; Sigma-Aldrich, Madrid, Spain) and Selenomethylseleno-L-cysteine (SeMetSeCys; Sigma-Aldrich, Madrid, Spain). Se quantification was performed by external calibration with the isotope ^{78}Se. Finally, the confirmation of the identity of the Se species was carried out by HPLC-ESI-MS/MS. The analysis was performed using a Shimadzu LC-MS-8030 triple quadrupole system (Shimadzu Scientific Instrument, Columbia, MD, USA) equipped with a Nexera LC-30AD solvent delivery unit, a Nexera SIL 30AC autosampler with a temperature-controlled tray, and a CTO-20AC column oven. Separation was performed with a Phenomenex Kinetex EVO C18 column. The analyses were carried out at room temperature, using a mobile phase consisting of a mixture of 0.1% formic acid aqueous solution at pH 3.2 and methanol. Isocratic elution was performed using 0.5% methanol, the flow rate was 0.30 mL·min^{-1}, and the injection volume was set at 10 µL. The instrument operated in positive electrospray ionization (ESI) mode. Nitrogen was used as both nebulizing (1.5 L·min^{-1}) and drying (15.0 L·min^{-1}) gas. Collision-induced dissociation was performed using argon as the collision gas at a pressure of 230 kPa in the collision cell, and the collision energy voltage applied was in the range of 10–33 eV. The ionization voltage for ESI was set at 4.5 kV, the interface current was fixed at 4.4 µA, and the detector voltage at 2.10 kV. The transitions selected for the confirmation of selenium species were 196.0–167.9, 196.0–121.9 and 196.0–94.9 for oxide SeMet; 198.0–153.0, 198.0–135.0 and 198.0–109.0 for SeMet, and 184.1–166.9, 184.1–149.0, 184.1–139.0, and 184.1–73.0 for SeMetSeCys.

2.3. Statistical Analysis

The dataset was analyzed using a linear mixed model (LMM). 'Growing season', 'Variety', 'Organic fertilization', and 'Se application' as well as their interactions were included as fixed effects. The blocks within years, the main plots within blocks, and the plot within main plots were included as random effects to account for the clustering of observations and ensure the independence of model residuals. A graphical inspection of normality and homoscedasticity of residuals never suggested any apparent violation of the basic assumptions for mixed models, and therefore analyses were performed on untransformed data. The significance of fixed effects was tested via ANOVA. The estimated marginal means and significant differences between means with Tukey's honest significant difference (HSD) were estimated using the emmeans R package [41]. Plots were drawn using ggplot2 R Package [42].

3. Results and Discussion

Before seeding, the total Se in soil was 0.056 ± 0.0085 mg kg^{-1} (mean ± SD) in 2018 and 0.087 ± 0.004 mg kg^{-1} in 2019. Thus, according to the classification by Tan [43], the soils can be considered deficient in total Se (<0.125 mg kg^{-1}). As expected, the effect of Se application on total Se content in grain was highly significant (Table 1).

Indeed, after biofortification with foliar Se (Se60), the Se content in grain increased about three times more with respect to the control plants (Se0) in both years (Figure 2). Moreover, it is essential to note that Se foliar application has always increased Se content in grain to levels that meet the Se requirements of humans and animals (food and fodder should contain >50–100 µg Se kg^{-1}) [44], indicating that the foliar application of selenate was effective in raising durum wheat Se content in grain. In our study, the Se contents in grain obtained from the control plants (Se0) were higher than those observed by De Vita et al. [15], who in the same cultivation area (Apulia, Southern Italy) reported a Se content in grain of 0.16 mg kg^{-1} under a conventional fertilization system without Se application. On the other hand, our result agrees with AL-Ghumaiz et al. [45], who found a higher Se content in spring wheat cultivated under an organic fertilization system with respect to a conventional one. This may be due to higher soil pH values (8.2 and 8.4 in 2018 and 2019, respectively) often associated with organic fertilizers [46]; indeed, Se is more available for plants in alkaline soils, where it is mostly present in the form of selenate [47].

Table 1. F-value growing season (GS), variety (V), organic fertilization (OF), selenium application (Se), and their interactions on the Se content in grain resulting from analysis of variance (ANOVA).

	DF	Se Content in Grain
Growing seasons	1	12.0832 *
Variety	3	42.9841 ***
Organic fertilization	3	39.4836 ***
Selenium application	1	3599.0316 ***
GS × V	3	22.5878 ***
Gs × OF	3	10.0963 ***
V × OF	9	6.8474 ***
GS × Se	1	19.5236 ***
V × Se	3	18.7205 ***
OF × Se	3	18.8835 ***
GS × V × OF	9	4.5424 ***
GS × V × Se	3	10.2129 ***
GS × OF × Se	3	1.5649 n.s.
V × OF × Se	9	0.7306 n.s.
GS × V × OF × Se	9	1.0679 n.s.

*** $p < 0.001$; * $p < 0.05$; n.s. not significant.

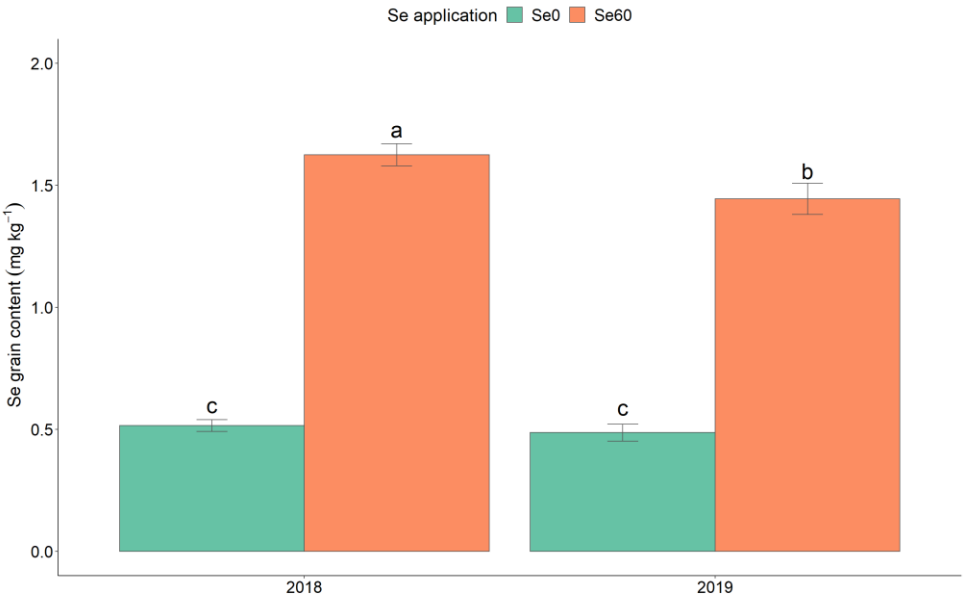

Figure 2. Effect of interaction growing season × selenium application on Se content in grain. Se0, no selenium application; Se60, selenium application. Tukey HSD significant differences ($p < 0.05$) are indicated by different letters. Vertical bars indicate standard errors (n = 48).

Moreover, Se content in grain was significantly influenced by the interaction growing season × selenium application (Table 1). Under Se foliar application (Se60), the highest value was observed in the first wetter growing season, while when the Se was absorbed only from the indigenous soil source (Se0), no significant differences were observed between the two growing seasons (Figure 2). The higher grain yield observed in 2018 with respect to 2019 [39] excluded a possible dilution effect; thus, it seems clear that the rainfall was a crucial factor influencing the absorption of foliar Se and its accumulation into the grain. Rodrigo et al. [48] found an inverse correlation between rainfall during the days before Se foliar spray fertilization and the Se accumulation in grain. Indeed, the highly negative osmotic potential may occur in the plant under dry conditions, promoting an efficient absorption through the stomata of the Se foliar fertilizer applied. In our study, the lower rainfall amount in 2018 in the ten days before the Se foliar application (3.5 mm in 2018 vs. 21.2 mm in 2019; Figure 1) probably also caused a higher Se accumulation in the grain. Therefore, due to the irregular precipitation distribution typically in the Mediterranean

environment [49], it is necessary to pay special attention to the Se fertilizer management to obtain a Se biofortification as effective as possible, mainly when Se was provided as foliar application.

The interaction organic fertilization × selenium application significantly affected the Se content in grain (Table 1). The similar values observed under CTR and CTR + N in both Se0 and Se60 plants (Figure 3) showed the lack of effect of organic N on Se content in grain, probably due to the low N concentration in the organic fertilizer [39] even if similar results were also found in previous studies under the conventional mineral system [50,51]. Adding organic foliar S alone (CTR + S) or in combination with organic N (CTR + NS), a decrease of 24% and 25% Se content in grain with respect to CTR was observed in Se60 plants, respectively (Figure 3). Considering that in our study the fertilizers were applied via foliar, the results allow us to suppose that the competitive relationship between S and Se in plants [17–19] is not confined only to root uptake by the soil, but physiological mechanisms might also be involved at leaf level. Moreover, since the Se content in grain was similar in Se0 plants without foliar Se application, under all the different foliar organic fertilization strategies adopted, we can argue that foliar application of organic S fertilizer did not interact with the uptake of Se from the soil. Thus, the competitive behavior between S and Se is evident only when both elements are given via foliar or soil. Further investigations are necessary to study this aspect deeply.

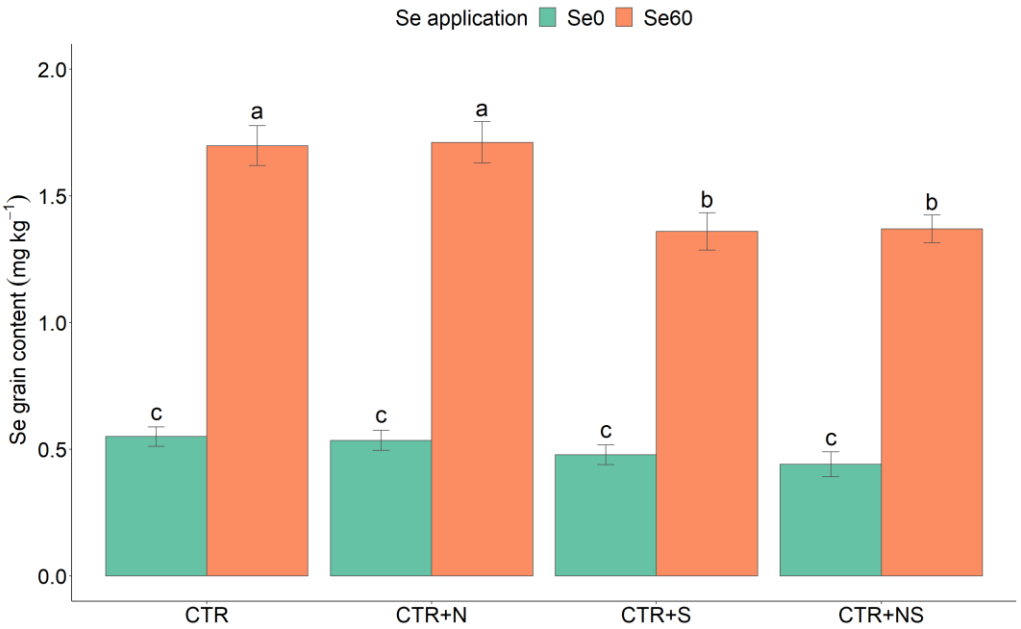

Figure 3. Effect of interaction organic fertilization × selenium application on Se content in grain. Se0, no selenium application; Se60, selenium application; CTR, control fertilized with dry blood meal at seeding; CTR + N, control plus N foliar application at the beginning of heading stage (BBCH stage 51); CTR + S, control plus S foliar application at flag leaf sheath opening stage (BBCH stage 47); CTR + NS, control plus the combination of S and N foliar applications at flag leaf sheath opening stage and at the beginning of heading (BBCH stage 47 and 51), respectively. Tukey HSD significant differences ($p < 0.05$) are indicated by different letters. Vertical bars indicate standard errors (n = 24).

The four durum wheat varieties under study differently accumulated Se in the grain with the Se application (Table 1 and Figure 4). No differences were observed among the varieties without Se application, except for the landrace old Saragolla, which showed the lowest value. Under Se application, Nadif showed the highest value between the two modern varieties, and Cappelli the highest value between the two old varieties. Our results confirmed the variety-dependent response for Se accumulation under Se fertilization, in agreement with De Vita et al. [15], who observed genetic variation in Se concentration and uptake among different winter wheat and spring wheat cultivars. On the other hand, other

studies suggested slight genetic variation in Se concentrations in grain across bread wheat genotypes [52,53]. Moreover, Zhao et al. [53] and De Vita et al. [54] highlighted that the old varieties had significantly higher mineral concentrations in grain than the modern varieties. Our results partially agree with these authors since even if the old variety Cappelli showed higher values than the modern Marco Aurelio, the highest value was observed for the modern Nadif.

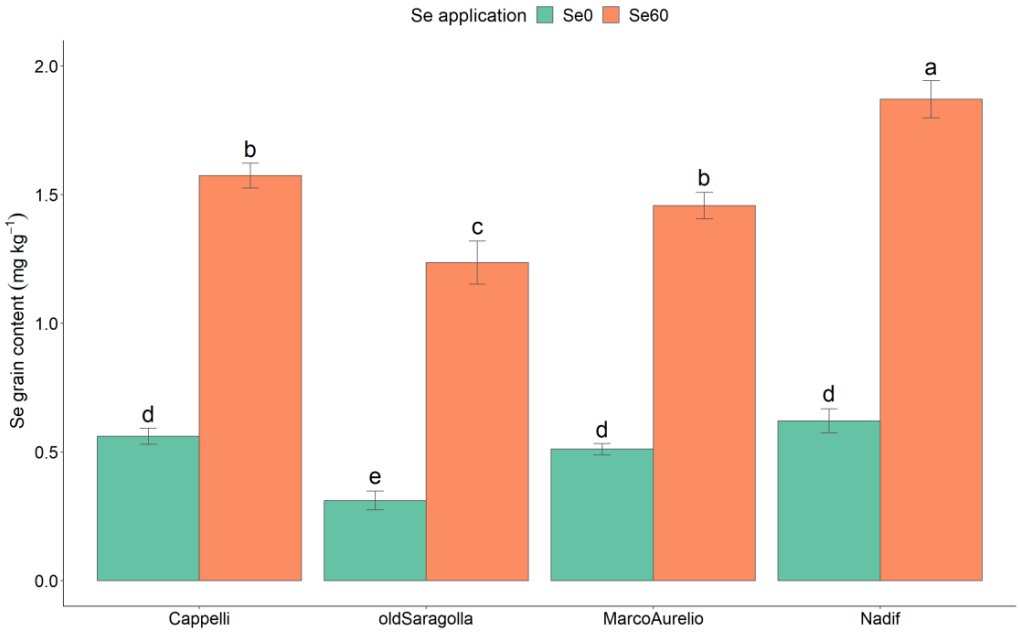

Figure 4. Effect of interaction variety × selenium application on Se content in grain. Se0, no selenium application; Se60, selenium application. Tukey HSD significant differences ($p < 0.05$) are indicated by different letters. Vertical bars indicate standard errors (n = 24).

After crop uptake, Se is transformed into different species by the plant. Therefore, the bioavailability of Se for humans and animals largely depends on the species of Se consumed rather than the total Se content [55]; for this reason, we have also investigated the Se speciation in the old variety Cappelli and in the modern Nadif, which showed the highest Se content in grain values under Se60 application (Figure 4).

One of the most critical aspects when performing Se speciation is that the selenium species extraction should be quantitative. In this regard, the extraction efficiency of the enzymatic process employed was (108 ± 8%) in both genotypes. Once Se species were extracted quantitatively, the resulting extracts were analyzed by HPLC-ICP-MS to determine and quantify the Se species. For this purpose, two chromatographic separation mechanisms were used, anion exchange (PRP-X100) and reversed- phase (EVO-C18). The species identified using PRP-X100 (Figure 5a,b) and EVO-C18 (Figure 5c,d) mechanisms was SeMet, whose content was between 0.62 and 0.75 mg Se kg^{-1} in all samples, with lower values under organic S fertilization and higher values under organic N fertilization. This result agrees with the data obtained for the Se content in grain (Figure 3), suggesting that the application of organic S also reduces the concentration of SeMet. Moreover, Duncan et al. [16] also found lower SeMet values in wheat grain when soil S availability increased. In addition, the analysis of Protease XIV by both columns did not detect the presence of Se impurities in the enzyme. Although HPLC-ICP-MS is a sensitive technique for Se detection, the confirmation of species identity is not entirely reliable. For this purpose, enzyme extracts were analyzed by HPLC-ESI-MS/MS using the reversed-phase column (EVO-C18). These analyses confirmed the transformation of SeO_4^{2-} to SeMet through the detection of oxidized SeMet (Figure 5e). The concentration of this Se species coincided with that determined by HPLC-ICP-MS. In addition, the presence of SeMetSeCys (Figure 5f), a non-proteinogenic selenoamino acid that was not detected by HPLC-ICP-MS, was also

identified by this technique with similar values in both variety under all organic fertilizer treatment. The concentration of this species was between 0.77 and 0.84 mg Se kg^{-1}. Based on these results, selenium recovery after analysis of its species was (85% ± 10%). Although generally, the SeMet is the dominant organic form of Se in cereals grown under the conventional system [14,56–59], SeMetSeCys can also be found when the plant is exposed to high concentrations of selenium, which can be toxic. In this sense, plants activate a detoxification mechanism to transform selenium into SeMetSeCys, a species that does not form part of proteins and allows the plant to continue growing without developing symptoms of toxicity [60–62].

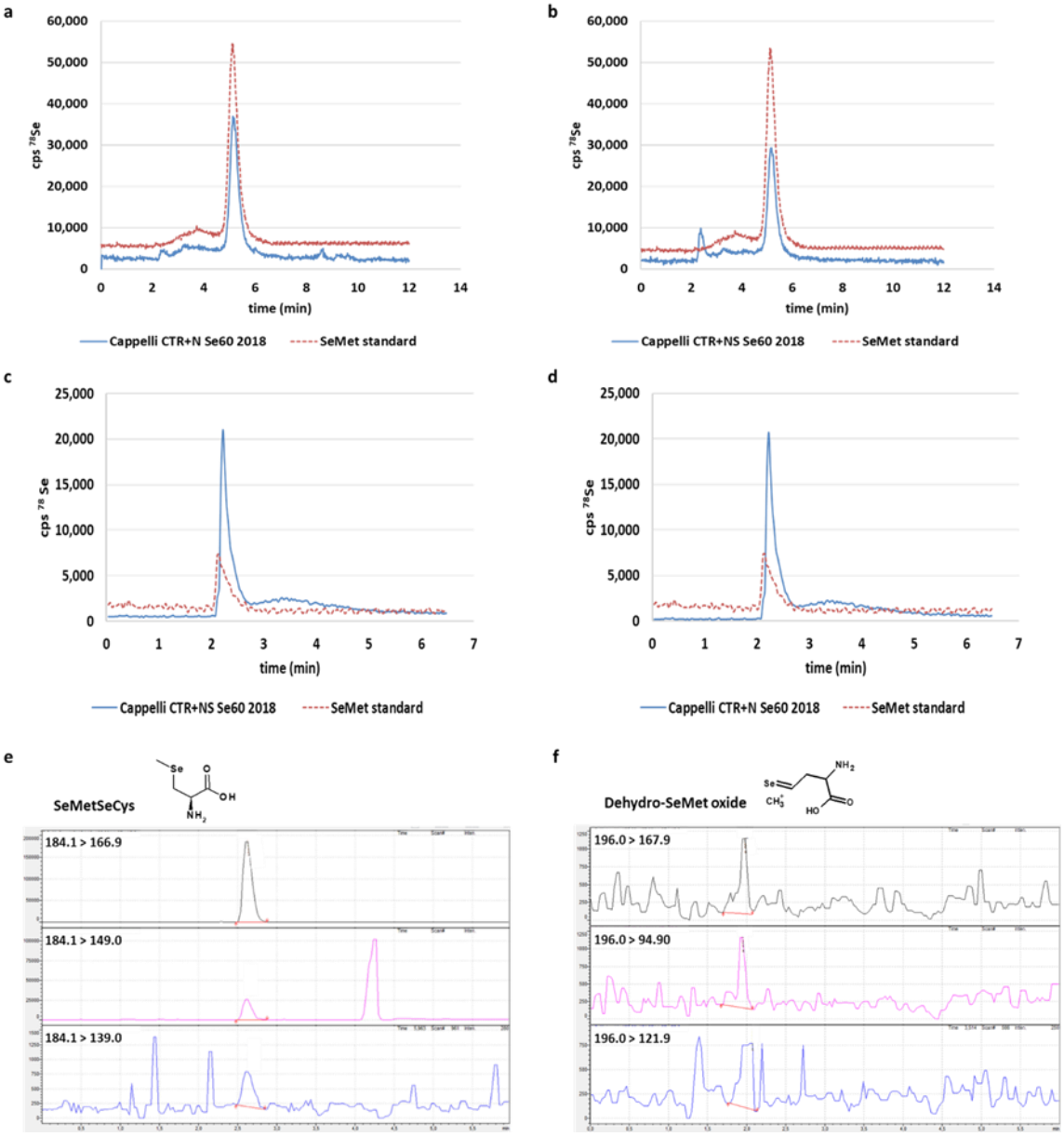

Figure 5. Chromatographic profile obtained by anion-exchange HPLC-ICP-MS from (**a**) Cappelli CTR + N Se60 2018 and (**b**) Cappelli CTR + NS Se60 2018. Chromatographic profile obtained by reverse phase HPLC-ICP-MS from (**c**) Cappelli CTR + N Se60 2018 and (**d**) Cappelli CTR + NS Se60 2018. In the chromatograms, the discontinuous lines correspond to a SeMet standard containing 50 µg L^{-1}. HPLC-ESI-MS/MS analysis to confirm the presence of (**e**) SeMetSeCys and (**f**) dehydro-SeMet oxide in the Cappelli variety.

4. Conclusions

The foliar selenium biofortification applied to durum wheat (*Triticum turgidum* spp. *durum*) grown under organic fertilization management increased the Se values in grain to those requested by humans and animals. Our results confirmed that the impact of the rainfall occurring in the days before the Se foliar application reduced the effectiveness of Se biofortification. This aspect is particularly relevant under the variable climatic condition of the Mediterranean area. Concerning fertilization management, the negative effect of organic foliar S on Se accumulation in grain was observed, probably due to competitive behavior between S and Se also under foliar fertilization. Moreover, the Se foliar application led to a variety-dependent response for Se accumulation in grain. The modern variety Nadif showed the highest value, followed by the old Cappelli; thus, in our study, it is demonstrated that modern varieties can also have significantly high mineral concentrations in grain. Finally, the analysis by HPLC-ICP-MS and HPLC-ESI-MS/MS showed the transformation of SeO_4^{2-} into two Se species, SeMet and SeMetSeCys, by all the samples. The application of organic N or S seems to have differently altered the concentration of SeMet. Further research is necessary to go deep inside some aspects, including the investigation of the physiological mechanism that inhibits the Se enrichment in combination with organic S fertilization, when both of them were applied via foliar, and the possible interaction relative to Se speciation and the aggregation level of the gluten protein, which is essential for durum wheat quality.

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Data Availability Statement: The data used and/or analyzed during the current study will be available from the authors on reasonable request.

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

References

1. FAO. FAO Launches 2020 as the UN's International Year of Plant Health. 2020. Available online: http://www.fao.org/news/story/pt/item/1253551/icode/#:~{}:text=Plants%20make%20up%2080%20percent%20of%20the%20food%20we%20eat,to%20plant%20pests%20and%20diseases (accessed on 5 September 2021).
2. Martin, C.; Li, J. Medicine is not health care, food is health care: Plant metabolic engineering, diet and human health. *New Phytol.* **2017**, *216*, 699–719. [CrossRef]
3. Lowe, N.M. The global challenge of hidden hunger: Perspectives from the field. *Proc. Nutr. Soc.* **2021**, *80*, 283–289. [CrossRef] [PubMed]
4. Schiavon, M.; Nardi, S.; Dalla Vecchia, F.; Ertani, A. Selenium biofortification in the 21st century: Status and challenges for healthy human nutrition. *Plant Soil* **2020**, *453*, 245–270. [CrossRef]
5. Marek, K.; Stanisław, B. Current Knowledge on the Importance of Selenium in Food for Living Organisms: A Review. *Molecules* **2016**, *21*, 609. [CrossRef]
6. Wang, X.; Li, H.; Yang, L.; Kong, C.; Wang, J.; Li, Y. Selenium nutritional status of rural residents and its correlation with dietary intake patterns in a typical low-selenium area in China. *Nutrients* **2020**, *12*, 3816. [CrossRef]
7. Petrović, M. Selenium: Widespread yet scarce, essential yet toxic. *ChemTexts* **2021**, *7*, 1–17. [CrossRef]
8. Jones, G.D.; Droz, B.; Greve, P.; Gottschalk, P.; Poffet, D.; McGrath, S.P.; Winkel, L.H. Selenium deficiency risk predicted to increase under future climate change. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 2848–2853. [CrossRef]
9. Izydorczyk, G.; Ligas, B.; Mikula, K.; Witek-Krowiak, A.; Moustakas, K.; Chojnacka, K. Biofortification of edible plants with selenium and iodine–A systematic literature review. *Sci. Total Environ.* **2021**, *754*, 141983. [CrossRef] [PubMed]

10. De Santis, M.A.; Giuliani, M.M.; Giuzio, L.; De Vita, P.; Flagella, Z. Assessment of grain protein composition in old and modern Italian durum wheat genotypes. *Ital. J. Agron.* **2018**, *13*, 40–43. [CrossRef]
11. Di Mola, I.; Ottaiano, L.; Sacco, A.; Senatore, M.; Mori, M. Organic versus mineral fertilization: Assessing of yield and quality of durum wheat in marginal lands. *Ital. J. Agron.* **2021**, *16*, 117–126. [CrossRef]
12. Giacco, R.; Vitale, M.; Riccardi, G. Pasta: Role in diet. *Encycl. Food Health* **2016**, 242–245. [CrossRef]
13. Galinha, C.; Freitas, M.D.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]
14. 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] [PubMed]
15. De Vita, P.; Platani, C.; Fragasso, M.; Ficco, D.B.M.; Colecchia, S.A.; Del Nobile, M.A.; Petrozza, A. Selenium-enriched durum wheat improves the nutritional profile of pasta without altering its organoleptic properties. *Food Chem.* **2017**, *214*, 374–382. [CrossRef] [PubMed]
16. Duncan, E.G.; Maher, W.A.; Jagtap, R.; Krikowa, F.; Roper, M.M.; O'Sullivan, C.A. Selenium speciation in wheat grain varies in the presence of nitrogen and sulphur fertilisers. *Environ. Geochem. Health* **2017**, *39*, 955–966. [CrossRef]
17. Deng, X.; Zhao, Z.; Lv, C.; Zhang, Z.; Yuan, L.; Liu, X. Effects of sulfur application on selenium uptake and seed selenium speciation in soybean (*Glycine max* L.) grown in different soil types. *Ecotoxicol. Environ. Saf.* **2020**, *209*, 111790. [CrossRef]
18. Liu, X.; Yang, Y.; Deng, X.; Li, M.; Zhang, W.; Zhao, Z. Effects of sulfur and sulfate on selenium uptake and quality of seeds in rapeseed (*Brassica napus* L.) treated with selenite and selenate. *Environ. Exp. Bot.* **2017**, *135*, 13–20. [CrossRef]
19. Silva, V.M.; Wilson, L.; Young, S.D.; Broadley, M.R.; White, P.J.; Reis, A.R.D. Interaction between sulfur and selenium in agronomic biofortification of cowpea plants under field conditions. *Plant Soil* **2022**, 1–17. [CrossRef]
20. Pompa, M.; Giuliani, M.M.; Giuzio, L.; Gagliardi, A.; Di Fonzo, N.; Flagella, Z. Effect of sulphur fertilization on grain quality and protein composition of durum wheat (Triticum durum Desf.). *Ital. J. Agron.* **2009**, *4*, 159–170. [CrossRef]
21. Ercoli, L.; Lulli, L.; Arduini, I.; Mariotti, M.; Masoni, A. Durum wheat grain yield and quality as affected by S rate under Mediterranean conditions. *Eur. J. Agron.* **2011**, *35*, 63–70. [CrossRef]
22. Gupta, U.C.; Gupta, S.C. Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. *Commun. Soil Sci. Plant Anal.* **2000**, *31*, 1791–1807. [CrossRef]
23. Läuchli, A. Selenium in plants: Uptake, functions, and environmental toxicity. *Bot. Acta* **1993**, *106*, 455–468. [CrossRef]
24. Buchner, P.; Stuiver, C.E.E.; Westerman, S.; Wirtz, M.; Hell, R.; Hawkesford, M.J.; De Kok, L.J. Regulation of sulfate uptake and expression of sulfate transporter genes in Brassica oleracea as affected by atmospheric H2S and pedospheric sulfate nutrition. *Plant Physiol.* **2004**, *136*, 3396–3408. [CrossRef]
25. Smith, F.W.; Ealing, P.M.; Hawkesford, M.J.; Clarkson, D.T. Plant members of a family of sulfate transporters reveal functional subtypes. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 9373–9377. [CrossRef] [PubMed]
26. Li, H.F.; McGrath, S.P.; Zhao, F.J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **2008**, *178*, 92–102. [CrossRef] [PubMed]
27. Sors, T.G.; Ellis, D.R.; Salt, D.E. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* **2005**, *86*, 373–389. [CrossRef]
28. Terry, N.; Zayed, A.M.; De Souza, M.P.; Tarun, A.S. Selenium in higher plants. *Annu. Rev. Plant Biol.* **2000**, *51*, 401–432. [CrossRef]
29. Ellis, D.R.; Salt, D.E. Plants, selenium and human health. *Curr. Opin. Plant Biol.* **2003**, *6*, 273–279. [CrossRef]
30. Chan, Q.; Afton, S.E.; Caruso, J.A. Selenium speciation profiles in selenite-enriched soybean (Glycine Max) by HPLC-ICPMS and ESI-ITMS. *Metallomics* **2010**, *2*, 147–153. [CrossRef]
31. Gagliardi, A.; Carucci, F.; Masci, S.; Flagella, Z.; Gatta, G.; Giuliani, M.M. Effects of genotype, growing season and nitrogen level on gluten protein assembly of durum wheat grown under mediterranean conditions. *Agronomy* **2020**, *10*, 755. [CrossRef]
32. Ben Mariem, S.; González-Torralba, J.; Collar, C.; Aranjuelo, I.; Morales, F. Durum wheat grain yield and quality under low and high nitrogen conditions: Insights into natural variation in low-and high-yielding genotypes. *Plants* **2020**, *9*, 1636. [CrossRef] [PubMed]
33. Rehim, A.; Khan, M.; Imran, M.; Bashir, M.A.; Ul-Allah, S.; Khan, M.N.; Hussain, M. Integrated use of farm manure and synthetic nitrogen fertilizer improves nitrogen use efficiency, yield and grain quality in wheat. *Ital. J. Agron.* **2020**, *15*, 29–34. [CrossRef]
34. Chen, Y.; Liang, D.; Song, W.; Lei, L.; Yu, D.; Miao, S. Effects of nitrogen application on selenium accumulation, translocation and distribution of winter wheat at different growth periods. *J. Plant Nutr.* **2016**, *22*, 395–402. [CrossRef]
35. Klikocka, H.; Szostak, B.; Barczak, B.; Kobiałka, A. Effect of sulphur and nitrogen fertilization on the selenium content and uptake by grain of spring wheat. *J. Elem.* **2017**, *22*, 985–994. [CrossRef]
36. Zhou, X.; Yang, J.; Kronzucker, H.J.; Shi, W. Selenium biofortification and interaction with other elements in plants: A review. *Front. Plant Sci.* **2020**, *11*, 586421. [CrossRef] [PubMed]
37. Benton, T.G.; Froggatt, A.; Wellesley, L.; Schröder, P. *The Ukraine War and Threats to Food and Energy Security*; Royal Institute of International Affairs: London, UK, 2022.
38. Ullah, H.; Liu, G.; Yousaf, B.; Ali, M.U.; Irshad, S.; Abbas, Q.; Ahmad, R. A comprehensive review on environmental transformation of selenium: Recent advances and research perspectives. *Environ. Geochem. Health.* **2019**, *41*, 1003–1035. [CrossRef]
39. Carucci, F.; Gatta, G.; Gagliardi, A.; De Vita, P.; Bregaglio, S.; Giuliani, M.M. Agronomic strategies to improve N efficiency indices in organic durum wheat grown in Mediterranean area. *Plants* **2021**, *10*, 2444. [CrossRef]

40. Moreno-Martín, G.; Sanz-Landaluze, J.; León-González, M.E.; Madrid, Y. Insights into the accumulation and transformation of Ch-SeNPs by Raphanus sativus and Brassica juncea: Effect on essential elements uptake. *Sci. Total Environ.* **2020**, *725*, 138453. [CrossRef]
41. R Core Team. R: A Language and Environment for Statistical Computing. 2018. Available online: https://www.R.-project.org (accessed on 9 May 2021).
42. Wickham, H. *Elegant Graphics for Data Analysis-ggplot2*; Springer: New York, NY, USA, 2009. Available online: https://cran.r-project.org/web/packages/ggplot2/citation.html (accessed on 9 May 2021).
43. Tan, J.A. *The Atlas of Endemic Diseases and Their Environments in the People's Republic of China*; Science Press: Beijing, China, 1989.
44. Gissel-Nielsen, G.; Gupta, U.C.; Lamand, M.; Westermarck, T. Selenium in soils and plants and its importance in livestock and human nutrition. *Adv. Agron.* **1984**, *37*, 397–460. [CrossRef]
45. AL-Ghumaiz, N.S.; Motawei, M.I.; Abd-Elmoniem, E.M.; Al-Otayk, S.M. Selenium and zinc concentrations in spring wheat (Triticum aestivum) genotypes under organic and inorganic fertilization. *J. Plant Nutr.* **2020**, *43*, 1980–1987. [CrossRef]
46. Laurent, C.; Bravin, M.N.; Crouzet, O.; Pelosi, C.; Tillard, E.; Lecomte, P.; Lamy, I. Increased soil pH and dissolved organic matter after a decade of organic fertilizer application mitigates copper and zinc availability despite contamination. *Sci. Total Environ.* **2020**, *709*, 135927. [CrossRef]
47. Manojlović, M.S.; Lončarić, Z.; Cabilovski, R.R.; Popović, B.; Karalić, K.; Ivezić, V.; Singh, B.R. Biofortification of wheat cultivars with selenium. *Acta Agric. Scand. B Soil Plant Sci.* **2019**, *69*, 715–724. [CrossRef]
48. Rodrigo, S.; Santamaria, O.; Chen, Y.; McGrath, S.P.; Poblaciones, M.J. Selenium speciation in malt, wort, and beer made from selenium-biofortified two-rowed barley grain. *J. Agric. Food Chem.* **2014**, *62*, 5948–5953. [CrossRef] [PubMed]
49. Yang, C.; Fraga, H.; van Ieperen, W.; Santos, J.A. Assessing the impacts of recent-past climatic constraints on potential wheat yield and adaptation options under Mediterranean climate in southern Portugal. *Agric. Syst.* **2020**, *182*, 102844. [CrossRef]
50. Soltanpour, P.N.; Olsen, S.R.; Goos, R.J. Effect of nitrogen fertilization of dryland wheat on grain selenium concentration. *Soil Sci. Soc. Am. J.* **1982**, *46*, 430–433. [CrossRef]
51. Lyons, G.H.; Lewis, J.; Lorimer, M.F.; Holloway, R.E.; Brace, D.M.; Stangoulis, J.C.; Graham, R.D. High-selenium wheat: Agronomic biofortification strategies to improve human nutrition. *Food Agric. Environ.* **2004**, *2*, 171–178.
52. Lyons, G.H.; Genc, Y.; Stangoulis, J.C.; Palmer, L.T.; Graham, R.D. Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. *Biol. Trace Elem. Res.* **2005**, *103*, 155–168. [CrossRef]
53. Zhao, F.J.; Su, Y.H.; Dunham, S.J.; Rakszegi, M.; Bedo, Z.; McGrath, S.P.; Shewry, P.R. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal Sci.* **2009**, *49*, 290–295. [CrossRef]
54. De Vita, P.; Nicosia, O.L.D.; Nigro, F.; Platani, C.; Riefolo, C.; Di Fonzo, N.; Cattivelli, L. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. *Eur. J. Agron.* **2007**, *26*, 39–53. [CrossRef]
55. D'Amato, R.; Regni, L.; Falcinelli, B.; Mattioli, S.; Benincasa, P.; Dal Bosco, A.; Businelli, D. Current knowledge on selenium biofortification to improve the nutraceutical profile of food: A comprehensive review. *J. Agric. Food Chem.* **2020**, *68*, 4075–4097. [CrossRef]
56. Stadlober, M.; Sager, M.; Irgolic, K.J. Effects of selenate supplemented fertilisation on the selenium level of cereals—Identification and quantification of selenium compounds by HPLC–ICP–MS. *Food Chem.* **2001**, *73*, 357–366. [CrossRef]
57. Cubadda, F.; Aureli, F.; Ciardullo, S.; D'Amato, M.; Raggi, A.; Acharya, R.; Prakash, N.T. Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *J. Agric. Food Chem.* **2010**, *58*, 2295–2301. [CrossRef]
58. Ogra, Y.; Kitaguchi, T.; Ishiwata, K.; Suzuki, N.; Toida, T.; Suzuki, K.T. Speciation of selenomethioninemetabolites in wheat germ extract. *Metallomics* **2009**, *1*, 78–86. [CrossRef]
59. Olson, O.E.; Novacek, E.J.; Whitehead, E.I.; Palmer, I.S. Investigations on selenium in wheat. *Phytochemistry* **1970**, *9*, 1181–1188. [CrossRef]
60. Hart, D.J.; Fairweather-Tait, S.J.; Broadley, M.R.; Dickinson, S.J.; Foot, I.; Knott, P.; Hurst, R. Selenium concentration and speciation in biofortified flour and bread: Retention of selenium during grain biofortification, processing and production of Se-enriched food. *Food Chem.* **2011**, *126*, 1771–1778. [CrossRef] [PubMed]
61. Shao, S.; Mi, X.; Ouerdane, L.; Lobinski, R.; García-Reyes, J.F.; Molina-Díaz, A.; Dernovics, M. Quantification of Se-methylselenocysteine and its γ-glutamyl derivative from naturally Se-enriched green bean (Phaseolus vulgaris vulgaris) after HPLC-ESI-TOF-MS and orbitrap MS n-based identification. *Food Anal. Methods* **2014**, *7*, 1147–1157. [CrossRef]
62. White, P.J. Selenium metabolism in plants. *Biochim Biophys Acta* **2018**, *1862*, 2333–2342. [CrossRef]