*Article*

# Biofortification of Common Wheat Grains with Combined Ca, Mg, and K through Foliar Fertilisation

Francesca Cobalchin [1], Mirko Volpato [2], Alberto Modena [3], Luisa Finotti [4], Francesco Manni [4], Anna Panozzo [1,*] and Teofilo Vamerali [1]

1   Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padua, Legnaro, 35020 Padova, Italy; francesca.cobalchin@unipd.it (F.C.); teofilo.vamerali@unipd.it (T.V.)
2   Grandi Molini Italiani SpA, Viale dell'Elettricità, 13, Marghera, 30175 Venezia, Italy; MVolpato@grandimolini.it
3   Fomet SpA, Via Vialarga, 25, S. Pietro in Morubio, 37050 Verona, Italy; a.modena@fomet.it
4   Promolog SRL, Via Aldo Moro, 6, 45100 Rovigo, Italy; lfinotti@grandimolini.it (L.F.); fmanni@promolog.it (F.M.)
*   Correspondence: anna.panozzo@unipd.it

**Abstract:** Common wheat grains are characterised by low concentrations of Ca, K, and Mg, which can be partially removed with the bran during milling processes. This preliminary study investigated the effects of foliar fertilisation at the earing stage with nitrates of Ca, Mg, and K contemporarily, together with a small amount of urea and protein hydrolysate as potential carriers, in two contrasting common wheat varieties, i.e., Solehio (medium proteins content) and Vivendo (high proteins content). Based on the preliminary grain-to-straw concentration ratio of these minerals, two biofortification targets were applied in order to increase their grain contents by +20% and +40%, in comparison with untreated controls. Here, we demonstrate that the highest fertilisation dose was effective in increasing grain K by 13% and Mg by 16% in Vivendo, and Ca by 7% in Solehio, with no boosting effects of the co-formulants urea and protein hydrolysate. In addition to some qualitative benefits due to nitrates supply, negligible phytotoxicity symptoms were observed, as revealed by the NDVI vegetational index dynamics. Although the biofortification target was not fully achieved, this study firstly reports the possibility to increase at the same time Mg and K, and to a lower extent Ca in wheat grains. It is concluded that efficient multiple biofortification should consider a variety-depend response, while further studies are necessary to investigate the effects of different fertilisation timings and doses for improving the poor mineral translocation to the grains.

**Keywords:** agronomic biofortification; cation nitrates; foliar fertilisation; grain quality; vegetational indexes

## 1. Introduction

Biofortification is a process by which the content of some desirable nutrients can be increased in edible plants through sustainable and cost-effective methods, such as agronomic fertilisation or plant breeding [1]. The aim of plant biofortification is to produce staple foods containing higher amounts of bioavailable minerals and some nutritional compounds, such as folate [2,3], vitamin B1 [4], vitamin B6 [5], and vitamin E [6] in edible parts of plants. As an alternative, artificial fortification consists of the addition of desired minerals to food products, such as iodine in food salt or iron and zinc in flours [7,8]. The major drawback of this technique is that these compounds have limited stability in the food [9]. For instance, iron-fortified foods are susceptible to oxidation and can also cause taste alteration [8]; folate-fortified rice partially decreases its folate content during boiling due to its increased solubility [10]. Furthermore, the absorption of oral supplementation also depends on the type of food ingested [11].

Agronomic biofortification by foliar application is receiving increasing attention as cultivation techniques to deliver essential nutrients to plants in order to improve their

quality in terms of nutrient contents in food products, avoiding the direct artificial food fortification. While improving plant nutritional status, foliar application of adequate concentrations of target nutrients can also have the potential to increase the yield and quality of various crops as an alternative to traditional soil-applied fertilisers [12,13]. The nutrients applied through foliar fertilisation, in particular macro-nutrients such as nitrogen (N), phosphorus (P), and potassium (K) can penetrate directly into the leaf or through cellular layers such as the cuticle or stomata. However, some micro-nutrients, such as iron (Fe) or molybdenum (Mo), are less mobile across plant tissues. Key factors for effective absorption of the minerals are leaf age and pH of spraying solutions [14]. As regards leaf anatomy, the absence of plasmadesmatic connections between guard cells and epidermal cells has to be considered for possible interaction among nutrients [15]. For instance, calcium (Ca) plays an important role in the absorption of essential ions such as K and boron (B), as well as of toxic elements, such as aluminium (Al), cadmium (Cd), and lead (Pb), and the maintenance of the integrity of selective ion transport proteins [16]. Calcium is capable of reducing the toxic effect of some cations and modulating the absorption and translocation of certain essential elements such as N, P and K, to achieve concentrations that do not prevent the absorption of other nutrients such as manganese (Mn) and zinc (Zn) [17–19]. With regard to the age of the leaf, mineral nutrients applied at the stage of leaf development rapidly permeate through the cuticle. Polar characteristics of the cuticle and pectin layers, which are part of an outer cell wall, are determined mainly by their negative charge due to the presence of –OH and –COOH groups, which enable cation absorption [20]. Additionally, the negative charge of these layers contributes to a more efficient translocation of apolar molecules (such as urea) and cations rather than anions. For this reason, a lower efficiency is observed with foliar spraying of mineral nutrients delivered in anion form than as cations [12].

In grain cereals, the kernel endosperm is the most important source of calories for human nutrition, providing about 23%, 17%, and 10% of total global calories, respectively, by wheat, rice, and maize [11,21]. Mineral intake with cereal grains is highly dependent on the milling process, while mineral bioavailability depends on the abundance of anti-nutritional factors. In wheat, a significant fraction of minerals is lost during milling since they are more concentrated in external kernel layers [22]. Most of P, K, and Mg content is indeed retained in the aleurone and scutellum, while lower concentrations characterise the endosperm. Calcium is predominantly present in the bran, likely due to its function in structural maintenance [23,24]. Potassium is also mostly concentrated within the pericarp, together with Ca and Mg [25].

The bioavailability of minerals is also generally poor in wheat products, as a consequence of high contents of phytic acid, a strong inhibitor of mineral absorption [26], which is formed during grain maturation. Phytates are mostly found in the embryo and aleurone layers, which are richer in mineral elements. Chelation of cations, such as Mg and Ca, by the phytic acid could decrease the absorption of these mineral elements during food digestion [27], due to the absence of phytase enzymes in the gastrointestinal tract [28]. Literature highlights that wheat straw contains approximately three and eight times higher amounts of K and Mg, respectively, compared to the grains, and Ca is equally concentrated in straw and grains [29]. Potassium is generally present at relatively high concentrations in green tissue and reproductive organs [30]. After root uptake, both K and Mg are rapidly translocated through the plant, while Ca tends to be present at low concentrations in the phloem sap with significant amounts retained by mature and senescent organs [31]. The translocation of Ca through the phloem from leaves to storage organs, such as fruits, seeds, and tubers, is generally small, and this makes its biofortification challenging [31–34].

Among the most interesting strategies to enhance wheat quality, late-season (between earing and flowering) foliar nitrogen spraying has shown promising results in terms of increased grain protein content [35]. The rate and timing of N application are crucial factors to achieve yield improvements, as well as enhance protein content, gluten quality, and rheological parameters [36–38]. There is currently attention on biofortification practices

targeting nutrients malnutrition, i.e., by enriching plants with desirable elements against the so-called *hidden hunger* [39–41]. In this regard, there is a lack of scientific knowledge on foliar application of minerals such as Ca, Mg, and K in cereals crops.

Given this background, the present study aimed at investigating the effectiveness of combined Ca + K + Mg biofortification through foliar fertilisation to enhance the nutritional value of wheat grains while maintaining high grain quality standards and avoiding phytotoxicity. Based on preliminary investigations on the straw-to-grain mineral concentration ratio of the largely cultivated wheat var. Bologna (SIS, Bologna, Italy), we investigated two biofortification targets, i.e., +20% and +40% of Ca, Mg, and K, applied through foliar spraying at the earing stage in two varieties with different grain protein contents. The study aimed at (i) assessing the impact of biofortification on grain yield and quality, (ii) highlighting any phytotoxic effect on the canopy, and (iii) quantifying the mineral concentrations of Ca, Mg, and K in grains and straw for developing biofortified bakery products. Moreover, the study aimed at verifying whether the additional application of a small amount of nitrogen as organic fertiliser can improve cations translocation to the grains.

## 2. Materials and Methods

### 2.1. Experimental Design

A field trial was carried out at the "Lucio Toniolo" experimental farm of the University of Padua (Legnaro, Padua, NE, Italy) during the 2019–2020 growing season. Here, the soil was silty loam, with 19% clay, 65% silt, 16% sand, 1.65% organic matter, 0.1% total N content, CEC of 11.4 cmol (+) kg$^{-1}$, and pH 7.75. Two common wheat varieties were sown at the end of October 2019, i.e., var. Solehio (ISTA, Potenza, Italy) with medium grain protein content and classified as ordinary bread-making wheat, and var. Vivendo (RAGT, Ferrara, Italy), characterised by a high grain protein content and classified as superior bread-making wheat. The sowing rate was 220 kg ha$^{-1}$ for Vivendo and 245 kg ha$^{-1}$ for Solehio, with 0.12 m apart rows. In both varieties, seeds were treated with the Celest Trio fungicide (Syngenta, Basel, Switzerland) containing fludioxonil, difenoconazole, and tebuconazole as active ingredients (a.i.). Pre-sowing fertilisation consisted of 32 kg ha$^{-1}$ of N, 96 kg ha$^{-1}$ of $P_2O_5$, and 96 kg ha$^{-1}$ of $K_2O$ (as ternary fertiliser) incorporated into the soil through harrowing. During the crop cycle, N was supplied twice as ammonium nitrate, for a total amount of 146 kg N ha$^{-1}$. The crop was protected against fungal pathogens by spraying a.i. azoxystrobin and cyproconazole at the beginning of May 2020, recommended as local agronomic practice. Var. Solehio was harvested on 25 June 2020 and var. Vivendo on 26 June 2020. The experimental design was completely randomised with 6 treatments per variety and 3 replications per treatment. Each replication consisted of an 11 m long and 5 m width (55 m$^2$ area) plot.

Biofortification treatments consisted of multiple mineral cations (Ca + Mg + K) applications on wheat plants as nitrate salts. Two biofortification doses were tested, referred to a target increase in the three cations by +20% (D1) and +40% (D2) through foliar fertilisation. The nitric form was chosen for its high solubility in water, as compared to sulphates, while chlorides were excluded for the possible phytotoxicity by chlorine. The doses of minerals cations to be distributed were calculated from the content of Ca, K and Mg in grains and straw of a reference var. "Bologna", as revealed in previous trials in the same experimental site. D2 was defined as the maximum applicable dose of nitrate salts in order to guarantee their complete solubility in the spraying water volume of 600 L ha$^{-1}$. The doses of each mineral cation are reported in Table 1. The cations were applied in combination with a nitrogen fertiliser, in order to provide a total amount of 25 kg ha$^{-1}$ of N in all the treatments. For each variety and cation doses (D1 and D2), two different nitrogen fertilisers were applied together with cations, i.e., urea (named Urea) or urea + organic fertiliser (named Urea + Org). Urea has 46% of N, while organic nitrogen consisted of a protein hydrolysed-based form (8% of N on FW). Foliar spraying of the 4 treatments (D1_Urea; D1_Urea + Org; D2_Urea; D2_Urea + Org) occurred on 14 May 2020, in the morning, at the earing stage (BBCH 58) in both the varieties Solehio and Vivendo.

**Table 1.** Quantitative mineral cations per hectare (kg ha$^{-1}$) and dosages of Ca, Mg, K, urea (g L$^{-1}$), and organic nitrogen (mL L$^{-1}$) applied in D1 or D2 treatments, considering 600 L ha$^{-1}$ as irrigation volume. Percentage in brackets indicates salts purity.

| Biofortification Treatments | Ca (kg ha$^{-1}$) | K (kg ha$^{-1}$) | Mg (kg ha$^{-1}$) | Ca Nitrate (78%, g L$^{-1}$) | Mg Nitrate (98.8%, g L$^{-1}$) | K Nitrate (57%, g L$^{-1}$) | Urea (g L$^{-1}$) | Organic N Fertiliser (mL L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| D1 | 3.7 | 15.6 | 2.1 | 32.3 | 38.0 | 68.1 | 52.1 | 38.3 |
| D2 | 7.3 | 31.2 | 4.3 | 64.7 | 76.0 | 136.2 | 13.5 | 38.3 |

The 4 biofortification treatments were compared with two controls (Table 2) without cations, one receiving 25 kg of N as urea (C_Urea) and the other one 23.1 kg of N as urea and 1.9 kg as an organic liquid fertiliser (C_Urea + Org). The control plots were fertilised by foliar spraying on the same date of biofortification treatments.

**Table 2.** List of treatments with the amount of N (Kg ha$^{-1}$) and chemical form applied, together with cations by foliar spraying, and reference controls.

| List of Treatments | N as Nitrates (kg ha$^{-1}$) | N as Urea (kg ha$^{-1}$) | N as Organic (kg ha$^{-1}$) | Total N (kg ha$^{-1}$) |
|---|---|---|---|---|
| C_Urea | - | 25 | - | 25 |
| C_Urea + Org | - | 23.15 | 1.85 | 25 |
| D1_Urea | 10.6 | 14.6 | - | 25 |
| D1_Urea + Org | 10.6 | 12.75 | 1.85 | 25 |
| D2_Urea | 21.3 | 3.7 | - | 25 |
| D2_Urea + Org | 21.3 | 1.85 | 1.85 | 25 |

*2.2. Climatic Conditions during the Trial*

Foliar fertilisation occurred on a sunny cloudless day (14 May 2020), with 23.8 MJ solar global radiation. The daily minimum and maximum air temperatures were 11.3 °C and 23.6 °C, respectively, with a mean of 18.7 °C, as recorded by the local meteorological station (ARPAV, Teolo, Padua, Italy).

During the field trial, the average monthly temperature was quite similar to the reference 10-year mean (2010–2020) (Figure S1A), while large differences were found for precipitation (Figure S1B). Compared with the historical mean, rainfall was higher in November (150 mm vs. 104.7 mm) and December (90 mm vs. 39.3 mm) after wheat germination and emergence, and lower from January to May, particularly in February (4.8 mm vs. 67.4 mm). In June, the precipitation was much higher than the historical mean (142.2 mm vs. 68 mm) and caused some lodging.

*2.3. Plant Analysis and Mineral Concentrations*

2.3.1. Leaf Vegetational Index NDVI

Starting from biofortification time to maturity, the normalised difference vegetation index (NDVI) was measured twice a week on the wheat canopy of each plot by means of an active handheld Greenseeker spectrometer (Ntech Industries, Ukiah, CA, USA). The sensor measures the canopy reflectance at wavelengths 590 nm (refRED) and 880 nm (refNIR) and provides a ratio value as follows:

$$NDVI = \frac{refNIR - refRED}{refNIR + refRED} \tag{1}$$

This vegetational index provides an accurate indication of the presence of chlorophyll in the crop canopy, which correlates with plant health/phytotoxicity and soil coverage by green vegetation. The index may vary from 0 to +1.

### 2.3.2. Grain Yield and Quality

Wheat grain yield was measured at maturity in each plot ($n$ = 3) by collecting the grains with a plot combine harvester. The harvest index (HI, grain-to-total shoot weight ratio) was determined in a sampling area of 1 m$^2$ of each plot, where grains and straw were separated and weighed after oven-drying at 105 °C for 36 h. The testing weight of wheat grains was determined with the equipment GAC 500XT (Dickey-John, Auburn, IL, USA). For each variety/treatment/replicate, three samples of 1000 grains were weighed for calculating the thousand kernel weight (TKW).

N concentration in the grains and straw was determined according to the Kjeldahl method, while Ca, Mg, and K concentrations by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (SPECTRO CirOS Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) on 0.4 g microwave acid-digested (7 mL HNO$_3$ 65% *v/v* and 1 mL H$_2$O$_2$ 30% *v/v*) samples (Milestone ETHOS 900, Bergamo, Italy) according to the EPA method 3052 [42]. Measurement accuracy was ensured with certified reference materials (ERM-CD281 and BRC-402; JRC-IRMM, Geel, Belgium).

### 2.4. Statistical Analysis

Biological data are the means of measurements performed on three replicates per treatment. The analysis of variance (ANOVA) was performed by CoStat software v. 6.204 (Manugistics, Rockville, MD, USA) with the Student–Newman–Keuls test in order to determine significant differences among means at $p \leq 0.05$.

Principal component analysis (PCA) and factorial discriminant analysis (Multigroup Discriminant Analysis (MDA) with Wilks' lambda and Pillai's trace tests [43] were carried out with MS Excel XLSTAT (Addinsoft, Paris, France) to describe the wheat response to biofortification as a function of cations' dose and variety choice. Before analysis, multivariate data normality was verified by the Shapiro test using R 3.0.1 software [44], and data were standardised by subtracting the mean and dividing by the standard deviation for each variable.

## 3. Results

### 3.1. Normalised Difference Vegetation Index (NDVI)

In both wheat varieties, the normalised difference vegetation index (NDVI) slightly decreased after foliar fertilisation, as compared to controls (C_Urea and C_Urea + Org), significantly during May and at half June ($p \leq 0.05$) (Figure 1). This effect was somewhat more evident in the var. Vivendo, with anticipate leaf senescence, and with the highest cations dose (D2), regardless of the additional application of urea or urea + organic hydrolysate. The average seasonal NDVI of control C_Urea was 0.59 and 0.60 in var. Solehio and Vivendo, respectively, while under D1 and D2, it was 0.58 and 0.57 in both varieties.

### 3.2. Grain Yield and Quality Parameters

Grain yield was not significantly affected by the biofortification treatment in both varieties, except for the unexpected decrease observed in the var. Solehio under D1_Urea + Org treatment (−14%, $p \leq 0.05$). A general improvement (not significant) of grain yield was revealed in the var. Vivendo following biofortification, as compared to the control C_Urea, particularly under D1_Urea + Org (+14%) and D2_Urea (+7%) (Table 3).

As regards the thousand kernel weight (TKW), var. Solehio showed higher values than var. Vivendo, this parameter being 44.9 g and 39.7 g in the control C_Urea plots of the two varieties, respectively. The biofortification treatments did not have a significant impact on the TKW; however, a decreasing trend was observed following cations application, particularly at the highest cation dose D2, and in the var. Vivendo (−22% under D2_Urea).
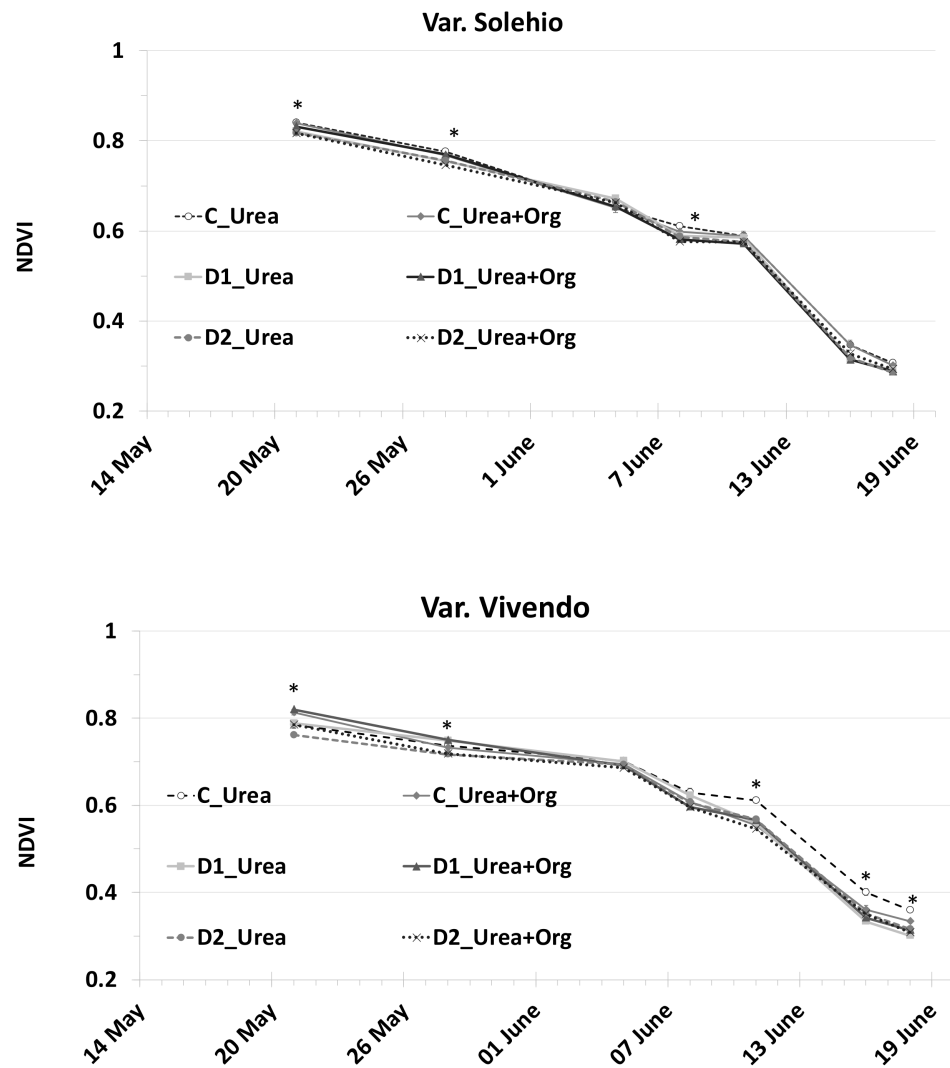
**Figure 1.** Dynamics of normalised difference vegetation index (NDVI) from earing to maturity (*n* = 3) in wheat varieties Solehio and Vivendo following the biofortification treatment (on 14 May) at D1 and D2 doses of Ca + Mg + K and in the controls C_Urea and C_Urea + Org. Asterisks indicate significant differences between treatments within each date (Student–Newman–Keuls test; $p \leq 0.05$).

The testing weight also varied slightly in the var. Solehio (76.3 kg hL$^{-1}$ as the average of the two controls), with little increase after the D2 biofortification treatment, significantly when the cations were applied together with urea + organic nitrogen fertiliser (Urea + Org: +5%, $p \leq 0.05$). Instead, no differences were observed in the var. Vivendo for this parameter, except for the 7% decrease ($p \leq 0.05$) under D2_Urea treatment.

As regards the harvest index (HI), var. Solehio showed higher values than Vivendo in the control plots (C_Urea: 0.47 vs. 0.43, respectively), and no significant differences were observed after the biofortification treatments.

As expected, var. Vivendo had higher grain protein content than var. Solehio in the reference C_Urea plots, i.e., 13.8% vs. 12.5%; the biofortification treatments did not cause any variation to this qualitative parameter of the grain according to the same foliar N dose across all the treatments (Table 3). In this regard, the application of a small amount of nitrogen as protein hydrolyzates (Org) did not have any significant effect on yield and quality parameters.

**Table 3.** Grain quantitative and qualitative parameters (means ± S.E.; *n* = 3) in the wheat varieties Solehio (A) and Vivendo (B) following the biofortification treatment with D1 and D2 doses of Ca + Mg + K, applied with urea (Urea) and urea + organic (Urea + Org) nitrogen fertiliser, and in the two controls C_Urea and C_Urea + Org. TKW: thousand kernel weight; DW: dry weight.

| Treatment | Yield (t ha⁻¹) | | | TKW (g) | | | Testing Weight (kg hL⁻¹) | | | Harvest Index | | | Grain Proteins (% DW) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Var. SOLEHIO | | | | | | | | |
| C_Urea | 8.69 ± 0.18 | a | (Ref.) | 44.9 ± 2.8 | ab | (Ref.) | 75.7 ± 1.01 | bc | (Ref.) | 0.47 ± 0.01 | a | (Ref.) | 12.5 ± 0.08 | ab | (Ref.) |
| C_Urea + Org | 8.32 ± 0.32 | ab | (−4%) | 38.0 ± 2.18 | b | (−15%) | 76.9 ± 1.20 | abc | (+2%) | 0.46 ± 0.02 | a | (−2%) | 12.8 ± 0.39 | a | (=) |
| D1_Urea | 8.81 ± 0.34 | a | (+1%) | 45.3 ± 3.18 | ab | (+1%) | 74.0 ± 0.30 | c | (−2%) | 0.47 ± 0.006 | a | (−1%) | 12.1 ± 0.18 | abc | (=) |
| D1_Urea + Org | **7.51 ± 0.38** | b | (−14%) | 47.0 ± 3.2 | a | (+4%) | 76.3 ± 0.26 | abc | (+1%) | 0.46 ± 0.01 | a | (−2%) | 12.3 ± 0.08 | ab | (=) |
| D2_Urea | 8.58 ± 0.21 | a | (−1%) | 40.8 ± 1.9 | ab | (−9%) | 79.3 ± 1.36 | a | (+5%) | 0.48 ± 0.01 | a | (+2%) | 12.3 ± 0.32 | ab | (=) |
| D2_Urea + Org | 8.34 ± 0.48 | ab | (−4%) | 41.5 ± 0.3 | ab | (−8%) | 78.0 ± 1.47 | ab | (+3%) | 0.46 ± 0.005 | a | (−1%) | 12.2 ± 0.20 | bc | (−1%) |
| Mean | 8.37 | A | | 42.9 | A | | 76.7 | A | | 0.46 | A | | 12.3 | A | |
| | | | | | | | Var. VIVENDO | | | | | | | | |
| C_Urea | 7.09 ± 1.17 | a | (Ref.) | 39.7 ± 3.06 | ab | (Ref.) | 76.7 ± 2.09 | a | (Ref.) | 0.43 ± 0.02 | a | (Ref.) | 13.8 ± 0.29 | a | (Ref.) |
| C_Urea + Org | 7.38 ± 0.81 | a | (+4%) | 40.0 ± 2.3 | a | (+1%) | 76.5 ± 1.03 | a | (=) | 0.41 ± 0.07 | a | (−5%) | 13.6 ± 0.77 | a | (=) |
| D1_Urea | 7.37 ± 1.04 | a | (+4%) | 40.6 ± 3.7 | a | (+2%) | 76.7 ± 1.73 | a | (=) | 0.44 ± 0.02 | a | (+1%) | 13.3 ± 0.52 | a | (=) |
| D1_Urea + Org | 8.08 ± 0.31 | a | (+14%) | 32.5 ± 0.16 | ab | (−18%) | 76.6 ± 1.15 | a | (=) | 0.46 ± 0.01 | a | (+5%) | 12.9 ± 0.66 | a | (−1%) |
| D2_Urea | 7.58 ± 1.47 | a | (+7%) | 31.0 ± 0.7 | b | (−22%) | 71.7 ± 1.95 | b | (−7%) | 0.40 ± 0.03 | a | (−6%) | 13.6 ± 0.61 | a | (=) |
| D2_Urea + Org | 6.93 ± 0.69 | a | (−2%) | 35.3 ± 4.1 | ab | (−11%) | 77.4 ± 0.13 | a | (+1%) | 0.44 ± 0.04 | a | (+2%) | 12.3 ± 0.62 | a | (−1%) |
| Mean | 7.41 | B | | 36.5 | B | | 75.9 | A | | 0.43 | A | | 13.2 | A | |

Lower case letters: significant differences among treatments within the same parameter (Student–Newman–Keuls test; $p \leq 0.05$). Capital letters: comparisons among varieties (main effect "Variety") within the same parameter. In brackets: % variation of treatments vs. urea control (C_Urea). In bold: values statistically different from the controls C_Urea.

### 3.3. Mineral Concentration in Grains and Straw

#### 3.3.1. Calcium

In the var. Solehio, grain Ca concentration was not affected by the biofortification treatments, although a not significant ($p > 0.05$) +7% (547 mg kg⁻¹) improvement was observed under D2_Urea + Org, compared to C_Urea (Figure 2A). In the straw of this wheat variety, there was a general decrease in Ca concentration in all the biofortification treatments as a response to the cation supply, with a significant reduction in D1_Urea vs. C_Urea + Org (Figure 2B), although it was not translated into grain improvements.

In the var. Vivendo, Ca concentration was slightly increased in the grains (up to +3%) and straw (up to +12%), but the differences were not statistically significant (Figure 2C,D). Similarly, in the two varieties, a small increase in Ca concentration in the straw was observed after the application of urea + organic nitrogen fertiliser without cations (C_Urea + Org: +7% and +10%, respectively, in Solehio and Vivendo; $p > 0.05$).

#### 3.3.2. Potassium

In the var. Solehio, grain K concentration did not show any significant variation after the biofortification treatments, as compared to C_Urea, with the highest increase being observed under D2_Urea + Org (+4%, $p > 0.05$) (Figure 3A). In the straw of this wheat variety, K concentration was slightly decreased, compared to the reference control (down to −11% in D1_Urea ($p \leq 0.05$), while a little increment (+2%; $p > 0.05$) was observed in D1_Urea + Org (Figure 3B).

A greater positive effect of foliar K supply was recorded in the grains of var. Vivendo, which showed general grain K improvements, as compared to C_Urea, i.e., from +8% (D1_Urea) up to +13% (D2_Urea, 5119 mg kg⁻¹), the latter being significantly higher ($p \leq 0.05$) (Figure 3C). In the straw (Figure 3D) of var. Vivendo, the variations of tissue K concentration were generally small and not statistically significant, while a significant improvement was observed in D2_Urea as in the grains (+13%; $p > 0.05$) (Figure 3D).
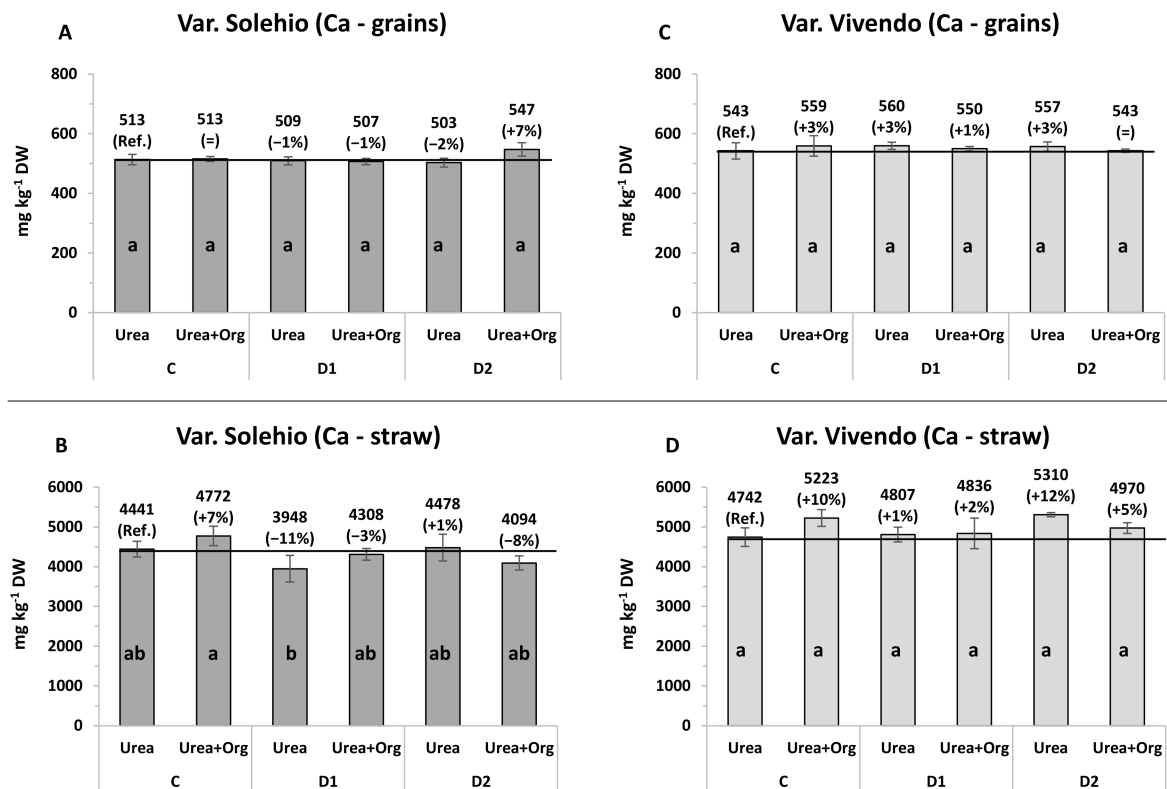
**Figure 2.** Calcium concentration (mean $\pm$ S.E.; $n = 3$) in grains and straw at harvest in the wheat varieties Solehio (**A**,**B**) and Vivendo (**C**,**D**) following the biofortification treatment with D1 and D2 doses of Ca + Mg + K, applied together with urea (Urea) and urea + organic (Urea + Org) nitrogen fertiliser, and in the two controls C_Urea and C_Urea + Org without cations. Letters above histograms indicate significant differences between treatments (Student–Newman–Keuls test; $p \leq 0.05$). In brackets: % variation of treatments vs. urea control (C_Urea).

### 3.3.3. Magnesium

In the var. Solehio, similar to K, grain Mg concentration did not show any significant variation, with the cation dose D2 allowing to reach the highest Mg accumulation (+3% and +7% in D2_Urea and D2_Urea + Org, respectively, $p > 0.05$) (Figure 4A). The same figure was drawn for the straw of this variety, with stable or slightly reduced values; the lowest concentration was measured in D1_Urea (−11% vs. C_Urea) and the highest one in the urea + organic controls (C_Urea + Org: +12%) (Figure 4B).

In the var. Vivendo, similarly to K, also grain Mg concentration was increased by foliar biofortification, with D2 leading the highest values ($p > 0.05$), as compared to D1 and controls. Again, the treatment D2_Urea was the most efficient one, allowing to reach the highest grain Mg concentration (1526 mg kg$^{-1}$, +16% vs. C_Urea, $p \leq 0.05$) (Figure 4C). In the crop residues of this variety, Mg concentration was also generally increased under all the biofortification treatments, as compared to C_Urea, particularly under D2 and the urea + organic control (C_Urea + Org). However, such variations in the straw were not significant according to the Student–Newman–Keuls test (Figure 4D).
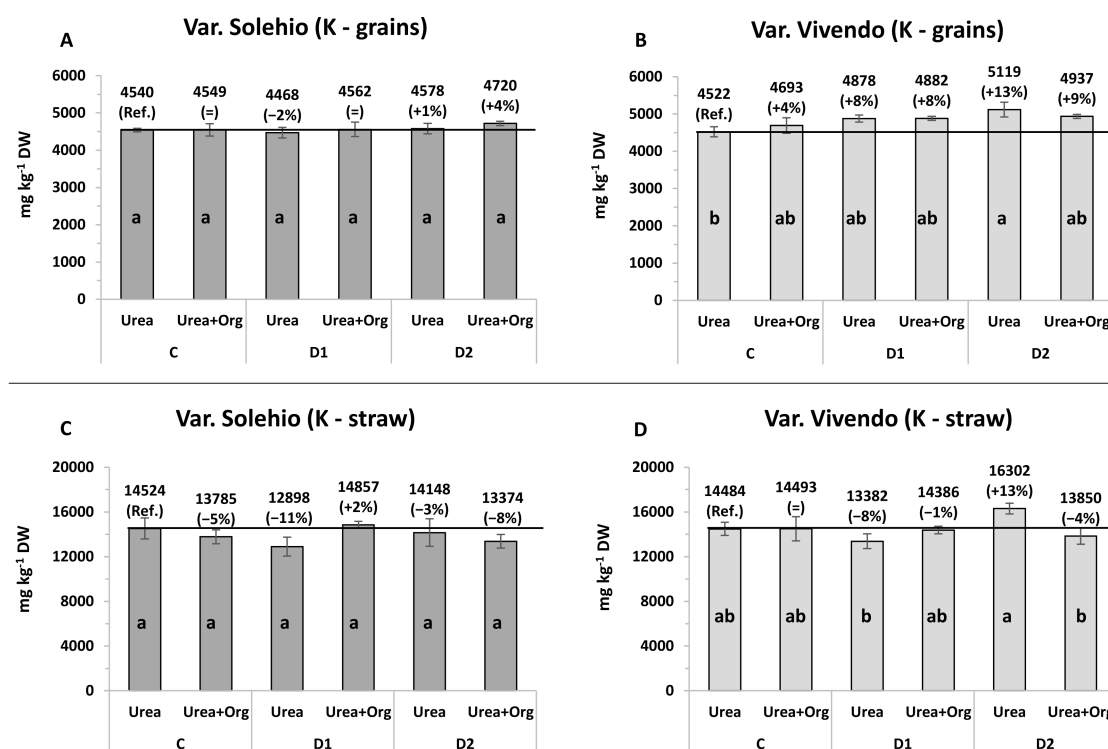
**Figure 3.** Potassium concentration (mean ± S.E.; *n* = 3) in grains and straw at harvest in the wheat varieties Solehio (**A**,**B**) and Vivendo (**C**,**D**) following the biofortification treatment with D1 and D2 doses of Ca + Mg + K, applied, together with urea (Urea) and urea + organic (Urea + Org) nitrogen fertiliser, and in the two controls C_Urea and C_Urea + Org without cations. Letters above histograms indicate significant differences between treatments (Student–Newman–Keuls test; *p* ≤ 0.05). In brackets: % variation of treatments vs. urea control (C_Urea).

### 3.4. Straw-to-Grain Element Concentration Ratio

As regards the straw-to-grain element concentration ratio, the highest values were observed for Ca, it having a mean value of 8.42 in var. Solehio and 9.04 in var. Vivendo. As compared to the reference control (C_Urea), a general decrease in this ratio was observed in the var. Solehio after foliar supply of cations, down to −13% (*p* > 0.05, n.s.) in D2_Urea + Org. In the var. Vivendo, the straw-to-grain [Ca] ratio was also not statistically affected, although there was a general opposite increasing trend, with the greatest value under D2_Urea (+9%, *p* > 0.05, n.s.) (Table 4).

An intermediate straw-to-grain element concentration ratio, i.e., with better translocation to grains, was revealed for K, which average value approximated 3 in both the varieties. A general decrease in this ratio was favourably noticed in all the treatments and both varieties, with statistically significant reductions under D1_Urea and D2_Urea + Org in the var. Vivendo (−15% and −12% vs. C_Urea, respectively).

The lowest straw-to-grain ratio was observed for Mg concentration, which was, on average, 1.0 in Solehio and 1.14 in Vivendo. In var. Solehio, all the biofortification treatments led to a decrease in this ratio, particularly under D1_Urea and D2_Urea + Org treatments (−12% and −9%, respectively). In var. Vivendo, the variations of the straw-to-grain Mg concentration ratio were generally modest and not significant, although the use of the organic fertiliser without cations in controls (C_Urea + Org) reduced Mg translocation to the grains, as indicated by the 16% increase in this ratio (*p* > 0.05), compared to C_Urea (Table 4).
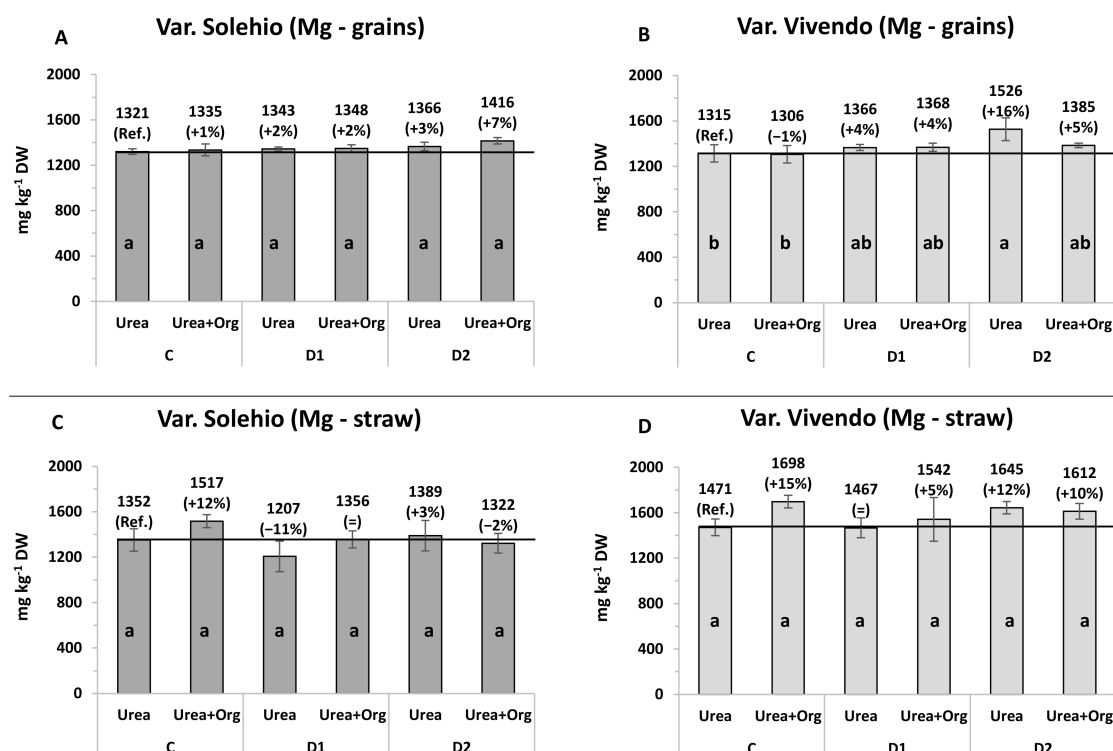
**Figure 4.** Magnesium concentration (mean ± S.E.; *n* = 3) in grains and straw at harvest in the varieties Solehio (**A**,**B**) and Vivendo (**C**,**D**) following the biofortification treatment with D1 and D2 doses of Ca + Mg + K, applied with urea (Urea) and urea + organic (Urea + Org) nitrogen fertiliser, and in the two controls C_Urea and C_Urea + Org without cations. Letters above histograms indicate significant differences between treatments (Student–Newman–Keuls test; *p* ≤ 0.05). In brackets: % variation of treatments vs. urea control (C_Urea).

**Table 4.** Straw-to-grain element concentration ratio (mean ± S.E.; *n* = 3) at harvest in the wheat varieties Solehio and Vivendo following the biofortification treatment with D1 and D2 doses of Ca + Mg + K, applied with urea (Urea) and urea + organic (Urea + Org) nitrogen fertiliser, and in the two controls C_Urea and C_Urea + Org without cations. Small letters indicate significant differences between treatments within the same wheat variety (Student–Newman–Keuls test; *p* ≤ 0.05). Capital letters: comparisons among varieties within the same element (main effect "Variety").

| | Var. Solehio | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatments | Ca | | | K | | | Mg | | |
| C_Urea | 8.65 ± 0.10 | abc | Ref. | 3.20 ± 0.21 | a | Ref. | 1.02 ± 0.06 | ab | Ref. |
| C_Urea + Org | 9.26 ± 0.58 | a | (+7%) | 3.03 ± 0.16 | a | (−5%) | 1.14 ± 0.07 | a | (+12%) |
| D1_Urea | 7.73 ± 0.47 | bc | (−11%) | 2.88 ± 0.14 | a | (−10%) | 0.89 ± 0.09 | b | (−12%) |
| D1_Urea + Org | 8.50 ± 0.36 | abc | (−2%) | 3.26 ± 0.10 | a | (+2%) | 1.00 ± 0.07 | ab | (−1%) |
| D2_Urea | 8.90 ± 0.61 | ab | (+3%) | 3.10 ± 0.32 | a | (−3%) | 1.01 ± 0.07 | ab | (−1%) |
| D2_Urea + Org | 7.48 ± 0.29 | c | (−13%) | 2.83 ± 0.11 | a | (−12%) | 0.93 ± 0.04 | ab | (−9%) |
| Mean | 8.42 | B | | 3.05 | A | | 1.00 | B | |
| | Var. Vivendo | | | | | | | | |
| Treatments | Ca | | | K | | | Mg | | |
| C_Urea | 8.78 ± 0.65 | a | Ref. | 3.20 ± 0.14 | a | Ref. | 1.12 ± 0.07 | a | Ref. |
| C_Urea + Org | 9.38 ± 0.42 | a | (+7%) | 3.08 ± 0.13 | ab | (−4%) | 1.30 ± 0.04 | a | (+16%) |
| D1_Urea | 8.61 ± 0.52 | a | (−2%) | 2.74 ± 0.08 | b | (−15%) | 1.07 ± 0.08 | a | (−4%) |
| D1_Urea + Org | 8.78 ± 0.60 | a | (=) | 2.94 ± 0.09 | ab | (−8%) | 1.12 ± 0.13 | a | (=) |
| D2_Urea | 9.55 ± 0.32 | a | (+9%) | 3.18 ± 0.05 | a | (−1%) | 1.08 ± 0.03 | a | (−4%) |
| D2_Urea + Org | 9.15 ± 0.16 | a | (+4%) | 2.80 ± 0.17 | b | (−12%) | 1.16 ± 0.03 | a | (+3%) |
| Mean | 9.04 | A | | 2.99 | A | | 1.14 | B | |

Letters: significant differences among treatments within the same parameter (Student–Newman–Keuls test; *p* ≤ 0.05). In brackets: % variation of treatments vs. urea control (C_Urea).

### 3.5. PCA and MDA

Principal Component Analysis (PCA) allowed the identification of two synthetic variables, F1 and F2, summarizing ~40% and 36% of the overall variability (Figure 5). Significant variables (loadings > |0.4|) were NDVI, grain protein content, and grain cation (Ca, K, Mg) concentrations. Multigroup discriminate analysis (MDA) and PCA revealed a different behaviour in the two investigated wheat varieties. According to the centroids position and cluster separation in MDA, the effects of the biofortification treatments on Solehio, i.e., the wheat variety with medium proteins content, were mostly associated with variations in yield and its components, particularly the harvest index (HI). On the contrary, the variations observed among treatments in the var. Vivendo, i.e., characterised by high proteins content, were mostly linked to the mineral content in the grains, particularly Mg and K. A dosing effect of biofortification was also highlighted, with the highest cation dose D2 associated with the highest content of Mg and K in the grains of var. Vivendo. The application of a small amount of nitrogen as organic fertiliser (Urea + Org) did not exert evident positive effects, either on quantitative or qualitative parameters, in comparison with the application of ureic nitrogen only (Urea).
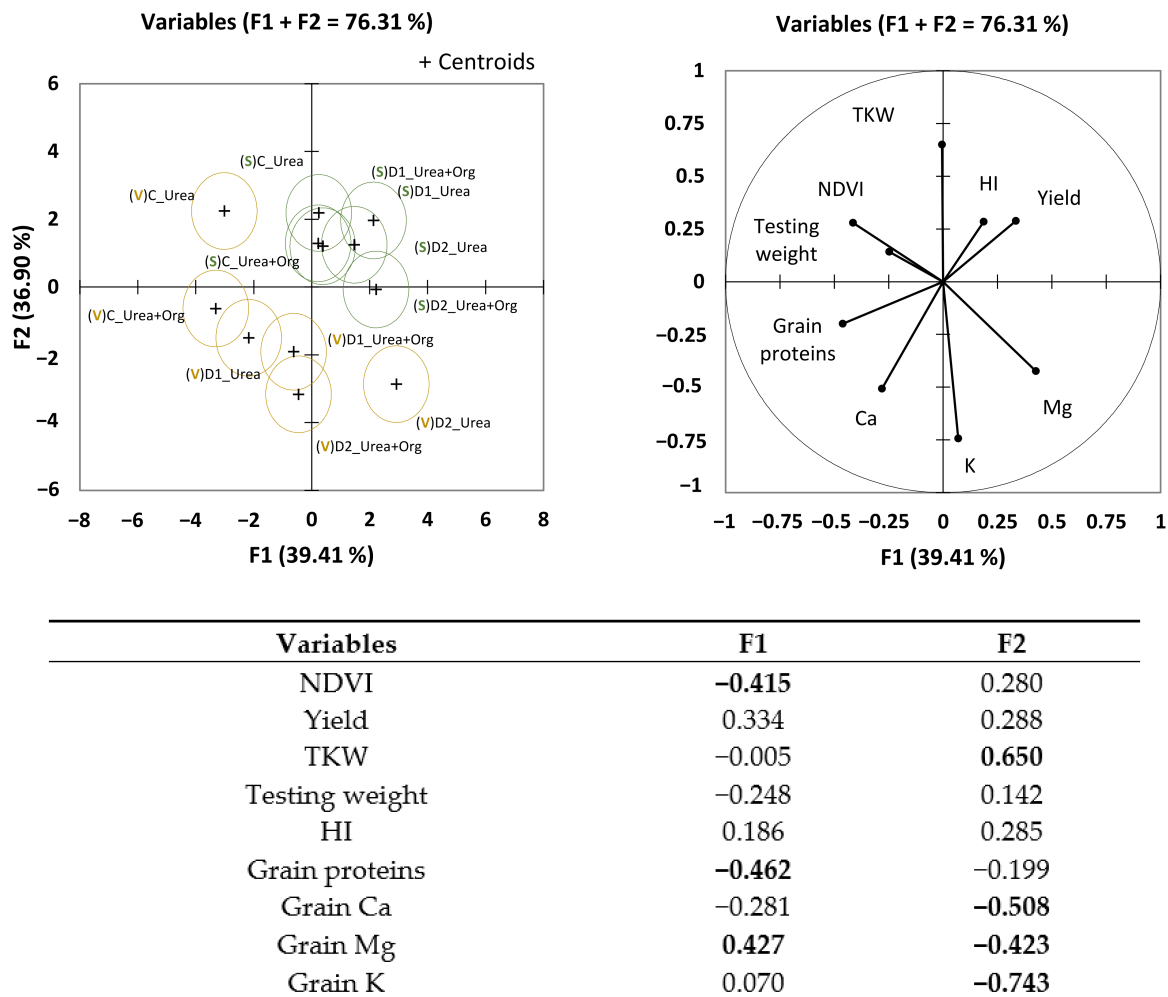


| Variables | F1 | F2 |
|---|---|---|
| NDVI | **−0.415** | 0.280 |
| Yield | 0.334 | 0.288 |
| TKW | −0.005 | **0.650** |
| Testing weight | −0.248 | 0.142 |
| HI | 0.186 | 0.285 |
| Grain proteins | **−0.462** | −0.199 |
| Grain Ca | −0.281 | **−0.508** |
| Grain Mg | **0.427** | **−0.423** |
| Grain K | 0.070 | **−0.743** |

**Figure 5.** Multigroup discriminant analysis (MDA; **left**) and principal component analysis (PCA; **right**) for the yield and quality parameters, and the concentration of Ca, Mg, and K in the grains of the varieties Solehio (S, in green) and Vivendo (V, in yellow) after the biofortification treatment with D1 and D2 doses of Ca + Mg + K cations and in the two controls C_Urea and C_Urea + Org without cations. The isodensity confidence circles contain 75% variability. In the bottom table, the highly informative variables (loadings > |0.4|) are highlighted in bold, within synthetic variables F1 and F2. NDVI: normalised difference vegetation index; TKW: thousand kernel weight; HI: harvest index.

## 4. Discussion

Biofortification techniques implemented in agronomic practices are receiving increasing interest to enhance the nutritional value of food, without relying on artificial fortification. Foliar fertilisation is considered a more sustainable and efficient practice to enhance the concentration of nutrients and their bioavailability in edible plants, compared with soil fertilisation. Some essential mineral elements, such as Ca, Mg, and K, are currently still poorly considered in biofortification aims, despite their low abundance and bioavailability in staple crops such as cereals. Indeed, as occurred for micronutrients such as Fe, Zn, I, etc., marked declines of meso- and macro-nutrients concentration in cereal grains have been reported over the past decades, likely owing to yield dilution following the Green Revolution [45].

Given this background, this study aimed at developing an innovative biofortification protocol based on the application of multiple mineral cations (Ca + Mg + K) through foliar fertilisation, to enhance the concentration of Ca, Mg and K contemporarily in common wheat grains. The multiple biofortification approach through foliar supply is a novel aim in this sector, as poorly investigated in the literature, and this study provides useful information of practical use.

The efficiency of foliar fertilisation in maximising nutrients absorption is widely recognised, known to be 8 to 20 times higher than soil fertilisation, with high potential to overcome nutritional deficiencies, improve the nutritional status of plants, and increase grain yield and quality [12]. For instance, several authors have already demonstrated that foliar application of urea, even at low concentrations, allows increasing cuticle hydration, with positive effects on nutrients absorption in many plants [20,46,47].

One essential key aspect to maximise biofortification efficiency is the choice of the chemical form of fertilisers to be applied, and from our results, the use of nitrates seems appropriate to achieve favourable results. The rate of nutrients penetration across the plant tissues is known to be greatly affected by the hydration of cuticles and salt hygroscopicity [48]. In previous studies, the application of K fertilisers did not lead to any precipitation, except for $K_2SO_4$ when the irrigation water contained a high amount of calcium [49]. According to the existing literature, Ca nitrate [$Ca(NO_3)_2$] can be successfully mixed with Mg nitrate [$Mg(NO_3)_2$] and K nitrate ($KNO_3$), similar to our case study. However, it is suggested to avoid mixing Ca with sulphates in order to avoid the formation of insoluble precipitates [50]. With regard to K, $KNO_3$ (solubility: 130–320 g/L) is less soluble than KCl (280–340 g/L) but more soluble than $K_2SO_4$ (70–110 g/L) [51]. As regards the chlorides, they have possible phytotoxic effects and were not considered in this study. For instance, after the application of $CaCl_2$ in apple, an excess of the anion ($Cl^-$) over cation $Ca^{2+}$ uptake led to leaf cell acidification, while a pH decrease in stomatal guard cell led to stomata closure [52].

This preliminary information from the literature suggested pursuing multiple applications of Ca + Mg + K in the form of nitrate salts as a compromise between achieving high salt solubility and minimising phytotoxicity issues. Indeed, in our study wheat canopy phytotoxicity was minimal and mainly regarded the apex of the flag leaf that was clearly visible after 3–4 days from foliar fertilisation. In our trial, the extent of phytotoxicity was acceptable and followed a fertiliser dose-dependent response, as highlighted by the reduction of the vegetational index NDVI during May.

Biofortification of mineral cations through foliar fertilisation also did not have a negative impact on agronomic parameters, compared with the *farmer practice* of applying urea or UAN (ammonium nitrate) at the earing stage for improving wheat quality. Indeed, in our study, with the same amount of late-season foliar nitrogen supply in all the treatments (25 kg N ha$^{-1}$), the yield response was similar, while quality parameters seldom improved with cations supply, such as the testing weight of grains at D2 in both varieties. In this regard, it is recognised that both K and Mg stimulate photosynthesis and sugar/starch accumulation, and this may have improved the testing weight of grains. However, a particular aspect that deserves attention in this research is the possible interaction between

cations accumulation and protein synthesis/accumulation. Our results from var. Vivendo at the most efficient cations dose D2, highlight a stable grain protein content response across treatments, suggesting that the concentration of K and Mg is sufficient to express their positive role in nitrate assimilation and protein synthesis [53], while nitrogen may become a limiting factor.

Similar to our results, previous studies regarding foliar applications of urea and $K_2SO_4$ combined with Zn during stem elongation and earing in wheat did not show impairments in the agronomic parameters [54]. It is reported that foliar application of urea to wheat can enhance plant biomass, the number of kernels per spike, and their length, HI, grain protein content, and can also improve grain yield when applied together with $K_2SO_4$ [55]. Improvements in physiological and agronomic parameters of wheat have also been observed when K was applied during grain filling [56], or Mg during the earing stage as an essential component of chlorophyll [57]. In our study, the application of cation nitrates led to a slightly positive response in grain yield of var. Vivendo despite a decrease in TKW, an effect probably due to improved spike and spikelet fertility with biofortification [58].

Thus far, there are no clear indications on the most suitable phenological stages to maximise biofortification targets according to the choice of the crop and the minerals to be applied, especially when multiple elements are considered. Foliar application of Ca during grain filling of wheat was found to be the most efficient timing to improve the gas exchange properties of the flag leaf, and chlorophyll and carotenoid contents, which contributed to enhanced HI and yield [59]. Foliar fertilisation with Ca also allowed the increase in flag leaf dry weight and leaf area, with positive impacts on photosynthesis [60]. However, Ca is almost immobile in the phloem; as a consequence, fruits, seeds, and tubers rely on its delivery via the xylem and consequently contain low Ca concentration [31,32,61]. This was confirmed in our study, in which biofortification targets of Ca were more difficult to be achieved compared to K and Mg. The highest fertilisation dose D2 we used, with a target increase of +40% in Ca + Mg + K, was partially disregarded. Indeed, this fertilisation dose was effective in increasing grain K by 13% and Mg by 16% in var. Vivendo, an appreciable result probably related to better mobility of these elements within the plant, while Ca increased by only 7% in var. Solehio. Therefore, a variety-depend response was here evinced, with var. Solehio showing better results when the D2 dose was associated with both urea and organic liquid N fertiliser, while var. Vivendo with urea only. Contrarily to our study, Knapowski [62] found a decrease in Mg content in grains following foliar applications of Mg combined with urea at the end of stem elongation and during earing stages. Other authors, instead, showed that the application of Mg at stem elongation and earing could enhance grain Mg concentration by 33% [63,64], while early (30 days from sowing) K foliar application, combined with Fe, improved only yield and quality parameters [56].

In a preliminary investigation, we observed that leaf absorption of cation nitrates at the earing stage is not a limiting factor in biofortification, at least for K and Mg. Our essays demonstrated that within 24 hr from the foliar application, K absorption was 98% and Mg 96%, while Ca was only 60%, (unpublished data). At least for K and Mg, the failure in reaching the biofortification targets should likely be searched in the physiological mechanisms of plant resources remobilisation when wheat approaches senescence and/or other crossing mechanisms and element interactions. According to some authors, K could have an antagonism effect with Ca and Mg absorption at the root level, which depends on the crop and environmental conditions [65–67], although this seems not the case from such preliminary investigations. Results from our trials would also exclude a negative interaction between K and Mg, at least in the wheat var. Vivendo, which reached efficient accumulation of both nutrients in the grains (+13% and +16%, respectively) at the highest fertilisation dose, while variety choice was essential for improving biofortification efficiency. As regards K-Ca antagonism, this may be related to competition between the elements due to the physiological properties of the ions [65,66]. At the root level, calcium enhances

the absorption of P and K under a certain concentration range of ions in the nutrient solution [68].

In our study, the application of mineral cations was combined with N at a standard rate, also verifying the possible contribution of organic N (biostimulant protein hydrolysate) in cation biofortification. According to the literature, both foliar and soil N application can enhance grain Ca concentration [22,69], as well as yield and qualitative parameters, with late-season applications [70]. In our study, the additional application of a small amount of nitrogen as organic fertiliser did not have evident positive effects, on either productivity or qualitative parameters, in comparison with the application of mineral nitrogen only. Although the biofortification targets were not fully achieved, the increase in Ca, Mg, and K concentrations in the grains of both Solehio and Vivendo were much higher, compared to the results observed by other authors with different wheat varieties and agronomic practices [71–74].

## 5. Conclusions

Late-season foliar nitrogen fertilisation is widely recognised as a strategic agronomic practice to efficiently enhance qualitative parameters of wheat grains and seldom yield. This study ascertained that replacing urea, commonly used late in the season as N foliar fertiliser, with cation nitrates allows for multiple biofortification of wheat grains with Mg and K and at a lower extent Ca, without altering yield and quality. To the best of our knowledge, this is the first report on multiple mineral biofortification suggesting a preliminary agronomic protocol for developing a new supply chain of wheat flours for biofortified oven-cooked foods, although it requires further confirmation across other seasons and subsequent adjustments. Compared with a specific biofortification target, an overload of mineral cations is likely necessary to be taken into account for inefficient translocation/remobilisation to the grains, although large differences are expected among varieties. There probably is large scope for improving grain mineral accumulation, suggesting the necessity of further investigations on different fertilisation time settings, cations doses, chemical forms, and various corroborating ingredients.

# References

1. Singh, U.; Praharaj, C.S.; Singh, S.S.; Singh, N.P. *Biofortification of Food Crops*; Springer: New Delhi, India, 2016.
2. Olivares, F.L.; Aguiar, N.O.; Rosa, R.C.C.; Canellas, L.P. Substrate biofortification in combination with foliar sprays of plant growth promoting bacteria and humic substances boosts production of organic tomatoes. *Sci. Hortic.* **2015**, *183*, 100–108. [CrossRef]
3. Strobbe, S.; Van der Straeten, D. Folate biofortification in food crops. *Curr. Opin. Biotech.* **2017**, *44*, 202–211. [CrossRef]
4. Goyer, A. Thiamin biofortification of crops. *Curr. Opin. Biotechnol.* **2017**, *44*, 1–7. [CrossRef]
5. Fudge, J.; Mangel, N.; Gruissem, W.; Vanderschuren, H.; Fitzpatrick, T.B. Rationalising vitamin B6 biofortification in crop plants. *Curr. Opin. Biotechnol.* **2017**, *44*, 130–137. [CrossRef] [PubMed]
6. Mène-Saffrané, L.; Pellaud, S. Current strategies for vitamin E biofortification of crops. *Curr. Opin. Biotechnol.* **2017**, *44*, 189–197. [CrossRef] [PubMed]
7. Rosado, J.L. Zinc and copper: Proposed fortification levels and recommended zinc compounds. *J. Nutr.* **2003**, *133*, 2985S–2989S. [CrossRef] [PubMed]
8. Gómez-Galera, S.; Rojas, E.; Sudhakar, D.; Zhu, C.; Pelacho, A.M.; Capell, T.; Christou, P. Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic Res.* **2010**, *19*, 165–180. [CrossRef]
9. Allen, L.H. Interventions for micronutrient deficiency control in developing countries: Past, present and future. *J. Nutr.* **2003**, *133*, 3877S–3878S. [CrossRef] [PubMed]
10. Brinch-Pederson, H.; Borg, S.; Tauris, B.; Holm, P.B. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J. Cereal Sci.* **2007**, *46*, 308–326. [CrossRef]
11. Rawat, N.; Neelam, K.; Tiwari, V.K.; Dhaliwal, H.S. Biofortification of cereals to overcome hidden hunger. *Plant Breed.* **2013**, *132*, 437–445. [CrossRef]
12. Smoleń, S. Foliar Nutrition: Current State of Knowledge and Opportunities. In *Advances in Citrus Nutrition*; Srivastava, A.K., Ed.; Springer Science & Business Media B.V.: Dordrecht, The Netherlands, 2012; pp. 41–58.
13. Sabbe, W.E.; Hodges, S.C. Interpretation of plant mineral status. In *Physiology of Cotton. National Cotton Council of America, Memphis, Tenn*; Stewart, J.M., Oosterhuis, D.M., Heitholt, J.J., Mauney, J.R., Eds.; Springer: London, UK, 2009; pp. 266–272.
14. Alshaal, T.; El-Ramady, H. Foliar application: From plant nutrition to biofortification. *Environ. Biodivers. Soil Secur.* **2017**, *1*, 71–83. [CrossRef]
15. Kannan, S. Foliar fertilization for sustainable crop production. In *Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming*; Sustainable Agriculture Reviews; Lichtfouse, E., Ed.; Springer Science & Business Media B.V.: Dordrecht, The Netherlands, 2010; Volume 4, pp. 371–402. [CrossRef]
16. Kinraide, T.B. Three mechanisms for the calcium alleviation of mineral toxicities. *Plant Physiol.* **1998**, *118*, 513–520. [CrossRef] [PubMed]
17. Bell, R.W.; Edwards, D.G.; Asher, C.J. Effects of calcium supply on uptake of calcium and selected mineral nutrients by tropical food legumes in 49 solution culture. *Aust. J. Agric. Res.* **1989**, *40*, 1003–1013. [CrossRef]
18. Shah, A.; Ryushi, K.; Fumihito, A.; Shigeru, K.; Shigenao, K. Alleviation of manganese phytotoxicity in barley with calcium source. *J. Plant Nutr.* **2006**, *29*, 59–74.
19. Patel, N.T.; Panchal, N.S.; Pandey, I.B.; Pandey, A.N. Implications of calcium nutrition on the response of *Acacia senegal* (Mimosaceae) to soil 57 salinity. *Anal. Biol.* **2011**, *33*, 23–34.
20. Michałojć, Z.; Szewczuk, C. Theoretical aspects of foliar plant nutrition (Teoretyczne aspekty dolistnego dokarmiania roślin). *Acta Agrophysica* **2003**, *85*, 9–17.
21. Khush, G.S. Productivity improvements in rice. *Nutr. Rev.* **2003**, *61*, S114–S116. [CrossRef] [PubMed]
22. Zhang, P.; Ma, G.; Wang, C.; Zhu, Y.; Guo, T. Mineral elements bioavailability in milling fractions of wheat grain response to zinc and nitrogen application. *Agron. J.* **2019**, *111*, 2504–2511. [CrossRef]
23. Wang, S.; Li, M.; Tian, X.; Li, J.; Li, H.; Ni, Y.; Zhao, J.; Chen, Y.; Guo, C.; Zhao, A. Foliar zinc, nitrogen, and phosphorus application effects on micronutrient concentrations in winter wheat. *Agron. J.* **2015**, *107*, 61–70. [CrossRef]
24. Xue, Y.; Zhang, W.; Liu, D.; Xia, H.; Zou, C. Nutritional Composition of Iron, Zinc, Calcium, and Phosphorus in Wheat Grain Milling Fractions as Affected by Fertilizer Nitrogen Supply. *Cereal Chem. J.* **2016**, *93*, 543–549. [CrossRef]
25. De Brier, N.; Gomand, S.V.; Donner, E.; Paterson, D.; Delcour, J.A.; Lombi, E.; Smolders, E. Distribution of minerals in wheat grains (*Triticum aestivum* L.) and in roller milling fractions affected by pearling. *J. Agric. Food Chem.* **2015**, *63*, 1276–1285. [CrossRef]
26. Gibson, R.S.; Perlas, L.A.; Hotz, C. Improving the bioavailability of nutrients in plant foods at the household level. *Proc. Nutr. Soc.* **2006**, *65*, 160–168. [CrossRef]
27. Vitali, D.; Dragojevic, I.V.; Sebecic, B. Bioaccessibility of Ca, Mg, Mn and Cu from whole grain tea-biscuits: Impact of proteins, phytic acid and polyphenols. *Food Chem.* **2008**, *110*, 62–68. [CrossRef] [PubMed]
28. Iqbal, T.H.; Lewis, K.O.; Cooper, B.T. Phytase activity in the human and rat small intestine. *Gut* **1994**, *35*, 1233–1236. [CrossRef] [PubMed]
29. Jankowski, K.J.; Hulanicki, P.S.; Sokólski, M.; Hulanicki, P.; Dubis, B. Yield and quality of winter wheat (*Triticum aestivum* L.) in response to different systems of foliar fertilization. *J. Elem.* **2016**, *21*, 715–728. [CrossRef]
30. Karley, A.J.; White, P.J. Moving cationic minerals to edible tissues: Potassium, magnesium, calcium. *Curr. Opin. Plant Biol.* **2009**, *12*, 291–298. [CrossRef]
31. White, P.J.; Broadley, M.R. Calcium in plants. *Ann. Bot.* **2003**, *92*, 487–511. [CrossRef] [PubMed]

32. Ho, L.; White, P.J. A cellular hypothesis for the induction of blossom-end rot in tomato fruit. *Ann. Bot.* **2005**, *95*, 571–581. [CrossRef]

33. White, P.J.; Bradshaw, J.E.; Dale, M.F.B.; Ramsay, G.; Hammond, J.P.; Broadley, M.R. Relationships between yield and mineral concentrations in potato tubers. *HortScience* **2009**, *44*, 6–11. [CrossRef]

34. Pfeiffer, W.H.; McClafferty, B. HarvestPlus: Breeding crops for better nutrition. *Crop. Sci.* **2007**, *47*, S88–S105. [CrossRef]

35. Fageria, N.K.; Filho, M.P.B.; Moreira, A.; Guimaraes, C.M. Foliar fertilization of crop plants. *J. Plant Nutr.* **2009**, *32*, 1044–1064. [CrossRef]

36. Borghi, B.; Corbellini, M.; Minoia, C.; Palumbo, M.; Di Fonzo, N.; Perenzin, M. Effects of Mediterranean climate on wheat bread-making quality. *Eur. J. Agron.* **1997**, *6*, 145–154. [CrossRef]

37. Garrido-Lestache, E.; López-Bellido, R.J.; López-Bellido, L. Durum wheat quality under Mediterranean conditions as affected by N rate, timing and splitting, N form and S fertilization. *Eur. J. Agron.* **2005**, *23*, 265–278. [CrossRef]

38. Corbellini, M.; Perenzin, M.; Boggini, G.; Bellocchi, G.; Scudellari, D.; Monotti, M. Balance sheet method assessment for nitrogen fertilization in bread wheat: I. Yield and quality. *Ital. J. Agron.* **2006**, *3*, 331–341. [CrossRef]

39. El-Ramady, H.; Alshaal, T.; Abdalla, N.; Prokisch, J.; Sztrik, A.; Fári, M.; Domokos-Szabolcsy, É. Selenium and nano-selenium biofortified sprouts using micro-farm systems. In Proceedings of the 4th International Conference on Selenium in the Environment and Human Health, Sao Paulo, Brazil, 18–21 October 2015; Bañuelos, G.S., Lin, Z.-Q., Guilherme, L.R.G., dos Reis, A.R., Eds.; CRC, Taylor & Francis Group: London, UK, 2016; pp. 189–190.

40. De Valença, A.W.; Bake, A.; Brouwer, I.D.; Giller, K.E. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Glob. Food Secur.* **2017**, *12*, 8–14. [CrossRef]

41. Díaz-Gómez, J.; Twyman, R.M.; Zhu, C.; Farré, G.; Serrano, J.C.E.; Portero-Otin, M.; Muñoz, P.; Sandmann, G.; Capell, T.; Christou, P. Biofortification crops with nutrients: Factors affecting utilization and storage. *Curr. Opin. Biotechnol.* **2017**, *44*, 115–123. [CrossRef] [PubMed]

42. USEPA; EPA. EPA; EPA. EPA method 3052: Microwave assisted acid digestion of siliceous and organically based matrices. In *Test Methods for Evaluating Solid Waste*, 3rd ed.; USEPA: Washington, DC, USA, 1995.

43. Podani, J. PCA. Ordinamento. In *Analisi ed Esplorazione Multivariata dei dati in Ecologia e Biologia*; Liguori Editore: Napoli, Italy, 2007; pp. 333–334.

44. Ihaka, R.; Gentleman, R. R: A language for data analysis and graphics. *J. Comput. Graph. Stat.* **1996**, *5*, 299–314.

45. Rosanoff, A. Changing crop magnesium concentrations: Impact on human health. *Plant Soil* **2013**, *368*, 139–153. [CrossRef]

46. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic: London, UK, 1995.

47. Smole, S.; Sady, W. Effect of foliar application of urea, molybdenum, benzyladenine, sucrose and salicylic acid on yield, nitrogen metabolism of radish plants and quality of edible roots. *J. Plant Nutr.* **2012**, *35*, 1113–1129. [CrossRef]

48. Schönherr, J. Foliar nutrition using inorganic salts: Laws of cuticular penetration. *Acta Hortic.* **2002**, *594*, 77–84. [CrossRef]

49. Solaimalai, A.; Baskar, M.; Sadasakthi, A.; Subburamu, K. Fertigation in high value crops—A review. *Agric. Rev.* **2005**, *26*, 1–13.

50. Fares, A.; Abbas, F. Irrigation systems and nutrient sources for fertigation. *Soil Crop. Manag.* **2009**, *25*, 1–4.

51. Magen, H. Potassium chloride in fertigation. In Proceedings of the 7th International Conference on Water and Irrigation, ICL Fertilizers, Tel Aviv, Israel, 13–16 May 1996; pp. 13–16.

52. Swietlik, D.; Bunce, J.A.; Miller, S.S. Effect of foliar application of mineral nutrients on stomatal aperture and photosynthesis in apple seedlings. *J. Am. Soc. Hortic. Sci.* **1984**, *109*, 306.

53. Ahmed, A.A.; Motoki, I.; Yoshio, Y. Effects of the supply of K, Ca, and Mg on the absorption and assimilation of ammonium- and nitrate-nitrogen in tomato plants. *Soil Sci. Plant Nutr.* **1991**, *37*, 283–289. [CrossRef]

54. Barut, H. Effects of foliar urea, potassium and zinc sulphate treatments before and after flowering on grain yield, technological quality and nutrient concentrations of wheat. *Appl. Ecol. Environ. Res.* **2019**, *17*, 4325–4342. [CrossRef]

55. Jakhro, A.A.; Jamro, G.H. *Effect of Foliar Fertilization of Urea on the Quantitative and Qualitative Traits of Wheat (cv. Sarsabz)*; Faculty of Crop Production: Tandojam, Pakistan, 2000.

56. Hamouda, H.A.; EI-Dahshouri, M.F.; Manal, F.M.; Thalooth, A.T. Growth, yield and nutrient status of wheat plants as affected by potassium and iron foliar application in sandy soil. *Int. J. Chemtech Res.* **2015**, *8*, 1473–1481.

57. Hlisnikovský, K.; Čermák, P.; Kunzová, E.; Barłóg, P. The effect of application of potassium, magnesium and sulphur on wheat and barley grain yield and protein content. *Agron. Res.* **2019**, *17*, 5.

58. Zoz, T.; Steiner, F.; Seidel, E.P.; Castagnara, D.D.; Souza, G.E. Foliar application of calcium and boron improves the spike fertility and yield of wheat. *Biosci. J.* **2016**, *32*, 873–880. [CrossRef]

59. Zahra, N.; Wahid, A.; Shaukat, K.; Rasheed, T. Role of seed priming and foliar spray of calcium in improving flag leaf growth, grain filling and yield characteristics in wheat (*Triticum aestivum*) a field appraisal. *Int. J. Agric. Biol.* **2020**, *24*, 1591–1600.

60. Hochmal, A.; Schulze, S.; Trompelt, K.; Hippler, M. Calcium-dependent regulation of photosynthesis. *Biochim. Biophys. Acta-Bioenerg.* **2015**, *1847*, 993–1003. [CrossRef]

61. Welch, R.M.; Graham, R.D. A new paradigm for world agriculture: Meeting human needs—Productive, sustainable, nutritious. *Field Crop. Res.* **1999**, *60*, 1–10. [CrossRef]

62. Knapowski, T. Effect of foliar fertilization with nitrogen and magnesium on chemical composition, biological value and baking quality. January. In *Understanding the Requirements for Development of Agricultural Production and of Rural Areas in the Kuyavian-Pomeranian Province as a Result of Scientific Research*; Śliwińska, E., Spychaj-Fabisiak, E., Eds.; University of Technology and Life Sciences Press: Bydgoszcz, Poland, 2009; pp. 359–374.

63. Jarecki, W.; Buczek, J.; Bobrecka-Jamro, D. Response of facultative cultivars of spring wheat to autumn sowing and foliar fertilization. *J. Elem.* **2019**, *24*, 817–828. [CrossRef]

64. Grzebisz, W. Magnesium—Food and human health. *J. Elem.* **2011**, *16*, 299–323. [CrossRef]

65. Johansen, C.; Edwards, D.G.; Loneragan, J.F. Interaction between potassium and calcium in their absorption by intact barley plants. I. Effects of potassium on calcium absorption. *Plant Physiol.* **1968**, *43*, 1717–1721. [CrossRef]

66. Maas, E.V. Calcium uptake by excised maize roots and interactions with alkali cations. *Plant Physiol.* **1969**, *44*, 985–989. [CrossRef] [PubMed]

67. Grunes, D.L.; Huang, J.W.; Smith, F.W.; Joo, P.K.; Hewes, D.A. Potassium effects on minerals and organic acids in three cool season grasses. *J. Plant Nutr.* **1992**, *15*, 1007–1025. [CrossRef]

68. Ishizuka, Y.; Tanaka, A. Studies on the metabolism of nutritional elements in rice plants. *J. Sci. Soil Manure.* **1960**, *31*, 491–494.

69. Wang, Y.X.; Specht, A.; Horst, W.J. Stable isotope labelling and zinc distribution in grains studied by laser ablation ICP-MS in an ear culture system reveals zinc transport barriers during grain filling in wheat. *New Phytol.* **2011**, *189*, 428–437. [CrossRef] [PubMed]

70. Rahman, M.Z.; Islam, M.R.; Karim, M.A.; Islam, M.T. Response of wheat to foliar application of urea fertilizer. *J. Sylhet Agric. Univ.* **2014**, *1*, 39–43.

71. Graham, R.; Senadhira, D.; Beebe, S.; Iglesias, C.; Monasterio, I. Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. *Field Crop. Res.* **1999**, *60*, 57–80. [CrossRef]

72. Ryan, M.; Derrick, J.; Dann, P. Grain mineral concentrations and yield of wheat grown under organic and conventional management. *J. Sci. Food Agric.* **2004**, *84*, 207–216. [CrossRef]

73. Spiegel, H.; Sager, M.; Oberforster, M.; Mechtler, K.; Stüger, H.P.; Baumgarten, A. Nutritionally relevant elements in staple food: Influence of arable site versus choice of variety. *Environ. Geochem. Health* **2009**, *31*, 549–560. [CrossRef] [PubMed]

74. Hussain, A.; Larsson, H.; Kuktaite, R.; Johansson, E. Mineral composition of organically grown wheat genotypes: Contribution to daily minerals intake. *Int. J. Environ. Res. Public Health* **2010**, *7*, 3442–3456. [CrossRef] [PubMed]