



# Natural Enrichment of *Solanum tuberosum* L. with Calcium— Monitorization of Mineral Interactions in Plant Tissues †

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**Abstract:** Calcium is an essential nutrient for plants and is required for maintenance of plant structures (such as the membranes and the cell wall). Although most of the Ca is obtained via the xylem (taken up by roots from the soil), in potatoes the accumulation of minerals also depends on phloem transport. So, it is crucial to deepen on the interactions of calcium with other minerals in tuber tissues. In this context, this study aimed to monitor the mineral interactions in tubers and leaves of *Solanum tuberosum* L. (Agria variety) after two foliar sprays with solutions of calcium chloride (1, 3, 6 and 12 kg.ha<sup>-1</sup>) and calcium nitrate (0.5, 1, 2 and 4 kg.ha<sup>-1</sup>), in order to improve naturally the Ca content. Calcium content was assessed and presented different increases regarding the two fertilizers. Considering the leaves, Ca content was higher with calcium nitrate 2 kg.ha<sup>-1</sup> treatment and in tubers with calcium chloride 12 kg.ha<sup>-1</sup> treatment. Moreover, Ca accumulation showed (in some treatments) a synergetic interaction with Mg in leaves, and with P, K, and S in tubers. In conclusion, in tubers and leaves, there was a heterogeneous interaction between minerals in the middle of a natural enrichment with Ca in *Solanum tuberosum* L. plants.

**Keywords:** calcium biofortification; mineral interactions; natural enrichment with calcium; *Solanum tuberosum* L.

## 1. Introduction

Calcium's one of the most abundant mineral elements in the human body [1] and plays an important role in bone and teeth development, skeletal mineralization, muscle contraction, fluid balance within cells and it's crucial for the normal functioning of the circulatory system [1–5]. This element is only obtained through dietary sources [1] and must be ingested daily by eating a healthy and balanced diet in which the consumption of food naturally rich in Ca prevails (namely, milk, leafy vegetables, nuts) [5]. Calcium requirement is dependent on age and physical condition (i.e., toddlers and pregnant women needs a higher Ca intake) [5]. Additionally, Ca intake it's also dependent on the state of the individual's Ca metabolism (mainly regulated by intestinal absorption, renal reabsorption, and bone turnover) [1]. Nevertheless, Ca deficiency can lead to different pathologies (namely, osteoporosis and rickets) [2]. As such, to surpass Ca deficiency, agronomic biofortification is a way to increase mineral content [6] allowing its enrichment

in the edible part of food crops. Different studies have been carried out with different minerals (namely calcium) in potato (*Solanum tuberosum* L.), considering that it is one of the most consumed food crops worldwide [7–10].

In plants, Ca is also an essential nutrient [11], is required as  $\text{Ca}^{2+}$  and has a central task in stress responses [12], plays an indispensable role in structural (such as in the membranes and in the cell wall) [4,11] and signaling [4]. In plants the accumulation of minerals (including Ca) is mainly obtained via the xylem (taken up by roots from the soil solution) [13], however, in low-transpiring organs such as potato tubers [14], they receive minerals and other nutrients mainly through redistribution from above-ground tissues via phloem [13,15]. However, the most mobile minerals in phloem tissue are Mg, S, P, and K, having Zn and Cu intermediate mobility and Ca, Fe, and Mn low mobility [13]. In this context, the aim of this study is to monitor the mineral interactions in potato tubers and leaves of *Solanum tuberosum* L. (Agria variety) after two foliar sprays with calcium solutions (calcium chloride and calcium nitrate) with different concentrations, to improve naturally the Ca content.

## 2. Materials and Methods

### 2.1. Biofortification Itinerary

The experimental potato-growing field, located in the Western of Portugal (GPS coordinates: 39°16'38,816" N; 9°15'9128" O), was used to growth Agria variety (*Solanum tuberosum* L.). Planting date was on 4 May and harvest was on 4 September 2018 date (after four foliar sprays with 8–10 days interval). The first foliar spray occurred after the beginning of tuberization, on 6 July and the second after 10 days. The biofortification was performed with foliar sprays with  $\text{CaCl}_2$  (1, 3, 6 and 12  $\text{kg}\cdot\text{ha}^{-1}$ ) or  $\text{Ca}(\text{NO}_3)_2$  (0.5, 1, 2 and 4  $\text{kg}\cdot\text{ha}^{-1}$ ). Control plants were not sprayed at any times with  $\text{CaCl}_2$  or  $\text{Ca}(\text{NO}_3)_2$  (being several meters apart from the biofortified plants). Each treatment was performed in quadruplicate (compass, 60–80 cm), in a plot 20 × 20 m. During the agriculture period air temperatures oscillated between an average of 15–23 °C.

### 2.2. Mineral Content in Soils, Potato Tubers and Leaves

Mineral contents were determined in soil samples (16 samples, 100 g picked up at 30 cm depth in the experimental field), following [7], before the implementation of the culture. Following [16], quantification of mineral elements in potato tubers and leaves after two foliar sprays was carried out by X-ray fluorescence, using a XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere.

### 2.3. Colorimetric Parameters

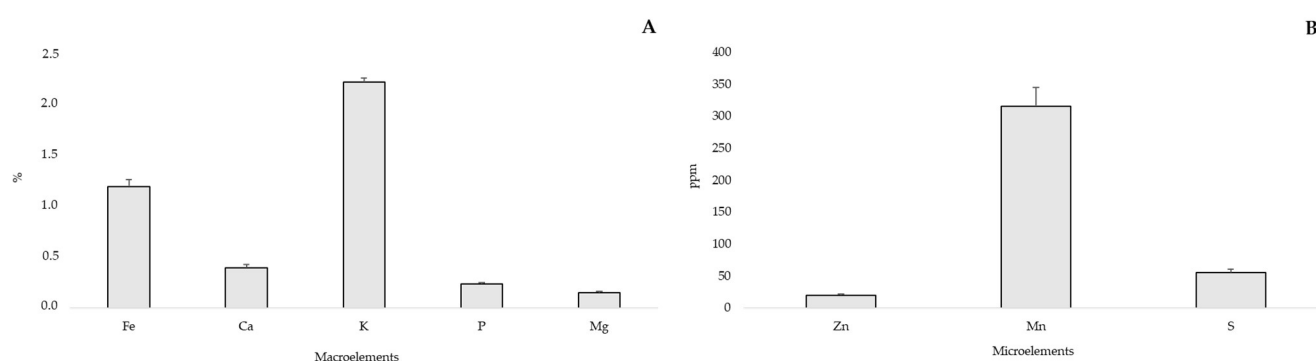
Colorimetric parameters were determined in fresh tubers of *Solanum tuberosum* L., Agria variety after two foliar sprays, using a Minolta CR 400 colorimeter (Minolta Corp., Ramsey, NJ, USA) coupled to a sample vessel (CR-A504), according to [8]. Measurements were carried out in quadruplicate.

### 2.4. Statistical Analysis

Data were statistically analyzed using a One-Way ANOVA to assess differences among treatments in Agria variety, followed by a Tukey's for mean comparison. A 95% confidence level was adopted for all tests.

## 3. Results

To understand the mineral interactions in potato plants, it's important to perform a soil analysis. In this context, chemical composition (macro and microelements) of the potato-growing field soil was determined (Figure 1 A,B). It was found that K had the highest content in soil followed by Fe and Ca. Among microelements, Mn presented the highest content, followed by S and Zn.



**Figure 1.** (A,B) Mean values (n = 9) of macroelements (A) and microelements (B) of the soil of the experimental potato-growing field selected for Ca biofortification of *Solanum tuberosum* L., Agria variety.

After two foliar sprays, different minerals (Ca, P, K, S, Zn and Mg) were analyzed in dry leaves of Agria variety (Table 1). Moreover, not considering P, all the minerals analyzed showed significant differences between treatments. Calcium content presented different increases regarding the two fertilizers, having a significantly highest content in  $\text{Ca}(\text{NO}_3)_2$  2  $\text{kg}\cdot\text{ha}^{-1}$  treatment (obtaining a Ca biofortification index of 73.9%). In the other hand, the lowest Ca content was obtained in  $\text{CaCl}_2$  6  $\text{kg}\cdot\text{ha}^{-1}$  treatment. Additionally, was possible to identified a synergetic interaction between Ca and Mg in three different treatments ( $\text{Ca}(\text{NO}_3)_2$  1 and 2  $\text{kg}\cdot\text{ha}^{-1}$  and  $\text{CaCl}_2$  6  $\text{kg}\cdot\text{ha}^{-1}$ ). In control leaves, K was the only mineral that showed a higher content. Yet, also K and Mg were the only ones that did not obtain a lower content in the  $\text{CaCl}_2$  12  $\text{kg}\cdot\text{ha}^{-1}$  treatment.

**Table 1.** Mean values  $\pm$  S.E. (n = 4) of Ca, P, K, S, Zn and Mg contents in dry leaves of *Solanum tuberosum* L., Agria variety after the 2nd foliar application. Different letters indicate significant differences, of each parameter, between treatments ( $p \leq 0.05$ ). Foliar spray was carried out with four concentrations of  $\text{Ca}(\text{NO}_3)_2$  (0.5, 1, 2 and 4  $\text{kg}\cdot\text{ha}^{-1}$ ) and  $\text{CaCl}_2$  (1, 3, 6 and 12  $\text{kg}\cdot\text{ha}^{-1}$ ). Control was not sprayed.

Treatments	Ca (%)	P (%)	K (%)	S (%)	Zn (ppm)	Mg (ppm)
Control	5.13 $\pm$ 0.89 bc	0.58 $\pm$ 0.11 a	11.0 $\pm$ 1.49 a	1.18 $\pm$ 0.14 ab	33.7 $\pm$ 2.20 ab	4.25 $\pm$ 0.28 abc
$\text{Ca}(\text{NO}_3)_2$	0.5 $\text{kg}\cdot\text{ha}^{-1}$	5.56 $\pm$ 1.14 abc	0.58 $\pm$ 0.06 a	9.26 $\pm$ 0.71 ab	1.32 $\pm$ 0.17 ab	51.6 $\pm$ 3.15 a
	1 $\text{kg}\cdot\text{ha}^{-1}$	8.41 $\pm$ 1.01 ab	0.53 $\pm$ 0.10 a	9.88 $\pm$ 0.77 ab	1.53 $\pm$ 0.13 a	37.0 $\pm$ 1.67 ab
	2 $\text{kg}\cdot\text{ha}^{-1}$	8.92 $\pm$ 0.46 a	0.70 $\pm$ 0.03 a	10.1 $\pm$ 0.48 ab	1.46 $\pm$ 0.04 a	37.0 $\pm$ 4.05 ab
	4 $\text{kg}\cdot\text{ha}^{-1}$	5.81 $\pm$ 0.13 abc	0.73 $\pm$ 0.07 a	9.76 $\pm$ 0.12 ab	1.60 $\pm$ 0.10 a	37.1 $\pm$ 8.73 ab
$\text{CaCl}_2$	1 $\text{kg}\cdot\text{ha}^{-1}$	6.80 $\pm$ 0.52 abc	0.63 $\pm$ 0.07 a	9.08 $\pm$ 0.56 ab	1.17 $\pm$ 0.07 ab	20.1 $\pm$ 2.43 b
	3 $\text{kg}\cdot\text{ha}^{-1}$	6.54 $\pm$ 0.50 abc	0.45 $\pm$ 0.03 a	8.75 $\pm$ 0.81 ab	1.13 $\pm$ 0.02 ab	29.8 $\pm$ 2.22 b
	6 $\text{kg}\cdot\text{ha}^{-1}$	4.82 $\pm$ 0.12 c	0.42 $\pm$ 0.04 a	7.51 $\pm$ 0.65 ab	0.90 $\pm$ 0.02 b	23.3 $\pm$ 1.13 b
	12 $\text{kg}\cdot\text{ha}^{-1}$	7.49 $\pm$ 0.89 abc	0.44 $\pm$ 0.05 a	6.87 $\pm$ 0.74 b	1.13 $\pm$ 0.09 ab	28.4 $\pm$ 1.96 b

Mineral content in potato tubers after two foliar applications was also assessed (Table 2). Regarding the minerals analyzed (Ca, P, K, and S) all had a higher content in the highest treatment applied with calcium chloride. Considering Ca content, the biofortification index varied between varied between 6.4% ( $\text{Ca}(\text{NO}_3)_2$  2  $\text{kg}\cdot\text{ha}^{-1}$ ) and 35.3% ( $\text{CaCl}_2$  12  $\text{kg}\cdot\text{ha}^{-1}$ ) at this stage of biofortification process. In  $\text{CaCl}_2$  6  $\text{kg}\cdot\text{ha}^{-1}$  treatment, Ca and P showed a lower content and Ca accumulation showed a synergetic interaction with P, K, and S in some treatments (namely, in  $\text{CaCl}_2$  12  $\text{kg}\cdot\text{ha}^{-1}$  and  $\text{Ca}(\text{NO}_3)_2$  0.5 treatment). Potassium and S showed a lower content in  $\text{CaCl}_2$  1  $\text{kg}\cdot\text{ha}^{-1}$  and 3, respectively.

**Table 2.** Mean values  $\pm$  S.E. (n = 4) of Ca, P, K and S contents in dry tubers of *Solanum tuberosum* L., Agria variety after the 2nd foliar application. Different letters indicate significant differences, of each parameter, between treatments ( $p \leq 0.05$ ). Foliar spray was carried out with four concentrations of  $\text{Ca}(\text{NO}_3)_2$  (0.5, 1, 2 and 4  $\text{kg}\cdot\text{ha}^{-1}$ ) and  $\text{CaCl}_2$  (1, 3, 6 and 12  $\text{kg}\cdot\text{ha}^{-1}$ ). Control was not sprayed.

Treatments	Ca (%)	P (%)	K (%)	S (%)
Control	0.122 $\pm$ 0.006 cd	0.204 $\pm$ 0.006 abc	3.36 $\pm$ 0.099 ab	0.173 $\pm$ 0.005 ab
$\text{Ca}(\text{NO}_3)_2$	0.5 $\text{kg}\cdot\text{ha}^{-1}$	0.143 $\pm$ 0.012 abc	0.218 $\pm$ 0.005 ab	3.45 $\pm$ 0.086 a
	1 $\text{kg}\cdot\text{ha}^{-1}$	0.159 $\pm$ 0.005 ab	0.181 $\pm$ 0.004 c	3.35 $\pm$ 0.025 ab
	2 $\text{kg}\cdot\text{ha}^{-1}$	0.130 $\pm$ 0.007 bcd	0.205 $\pm$ 0.009 abc	3.22 $\pm$ 0.060 ab
	4 $\text{kg}\cdot\text{ha}^{-1}$	0.136 $\pm$ 0.007 abcd	0.185 $\pm$ 0.006 bc	3.31 $\pm$ 0.238 ab
$\text{CaCl}_2$	1 $\text{kg}\cdot\text{ha}^{-1}$	0.120 $\pm$ 0.007 cd	0.186 $\pm$ 0.011 bc	2.83 $\pm$ 0.086 b
	3 $\text{kg}\cdot\text{ha}^{-1}$	0.113 $\pm$ 0.000 cd	0.176 $\pm$ 0.006 c	3.27 $\pm$ 0.067 ab
	6 $\text{kg}\cdot\text{ha}^{-1}$	0.107 $\pm$ 0.006 d	0.170 $\pm$ 0.002 c	3.16 $\pm$ 0.035 ab
	12 $\text{kg}\cdot\text{ha}^{-1}$	0.165 $\pm$ 0.001 a	0.237 $\pm$ 0.011 a	3.58 $\pm$ 0.157 a

In fresh tubers of Agria variety, colorimetric parameters were determined after two foliar sprays of Ca (Table 3). The parameters analyzed (L,  $a^*$ , and  $b^*$ ) did vary significantly. Regarding  $\text{Ca}(\text{NO}_3)_2$  2  $\text{kg}\cdot\text{ha}^{-1}$  treatment,  $b^*$ (yellow/blue) and L (brightness/luminosity) parameters showed a lower value, however,  $a^*$  (red/green) parameter showed a highest value. In L and  $b^*$  parameter,  $\text{Ca}(\text{NO}_3)_2$  4  $\text{kg}\cdot\text{ha}^{-1}$  and  $\text{CaCl}_2$  12  $\text{kg}\cdot\text{ha}^{-1}$  treatments showed a higher value, respectively. Regarding  $a^*$  parameter, the lowest value were obtained in  $\text{Ca}(\text{NO}_3)_2$  1  $\text{kg}\cdot\text{ha}^{-1}$  treatment.

**Table 3.** Mean values  $\pm$  S.E. (n = 4) of colorimetric parameters (L,  $a^*$  and  $b^*$ ) in fresh tubers of *Solanum tuberosum* L., Agria variety after the 2nd foliar application. Letters a and b indicates significant differences, of each parameter, between treatments ( $p \leq 0.05$ ). Foliar spray was carried out with four concentrations of  $\text{Ca}(\text{NO}_3)_2$  (0.5, 1, 2 and 4  $\text{kg}\cdot\text{ha}^{-1}$ ) and  $\text{CaCl}_2$  (1, 3, 6 and 12  $\text{kg}\cdot\text{ha}^{-1}$ ). Control was not sprayed.

Treatments	Colorimetric parameters		
	L	$a^*$	$b^*$
Control	64.5 $\pm$ 0.53 a	1.42 $\pm$ 0.02 b	12.7 $\pm$ 0.09 ab
$\text{Ca}(\text{NO}_3)_2$	0.5 $\text{kg}\cdot\text{ha}^{-1}$	63.6 $\pm$ 1.56 ab	1.77 $\pm$ 0.07 ab
	1 $\text{kg}\cdot\text{ha}^{-1}$	62.4 $\pm$ 0.98 ab	1.40 $\pm$ 0.08 b
	2 $\text{kg}\cdot\text{ha}^{-1}$	58.1 $\pm$ 0.68 b	2.11 $\pm$ 0.10 a
	4 $\text{kg}\cdot\text{ha}^{-1}$	66.3 $\pm$ 0.14 a	1.73 $\pm$ 0.13 ab
$\text{CaCl}_2$	1 $\text{kg}\cdot\text{ha}^{-1}$	64.5 $\pm$ 1.87 a	1.89 $\pm$ 0.02 a
	3 $\text{kg}\cdot\text{ha}^{-1}$	63.9 $\pm$ 0.63 ab	1.79 $\pm$ 0.06 ab
	6 $\text{kg}\cdot\text{ha}^{-1}$	66.5 $\pm$ 0.93 a	1.44 $\pm$ 0.12 b
	12 $\text{kg}\cdot\text{ha}^{-1}$	64.4 $\pm$ 2.10 a	1.77 $\pm$ 0.02 ab

#### 4. Discussion

In potato plants, mineral elements uptake occurs mainly from the soil solution [13], being important to correlate nutrients accumulation with the soil composition. In this context, nutrients in soil were assessed (Figure 1). Potassium was found in greater quantity in the soil (Figure 1A), being one of the most soluble elements in soil-plant system, its absorption through soils is highly efficient and its transport through plants very fast [17]. In plants, K is required as  $\text{K}^+$  (from soil by roots) and is considered the most abundant inorganic cation in plants [18] (as seen in Table 2). It's required for the activation of various enzymes, plays a vital role in cell metabolism and tissue growth [18]. Regarding Fe, it's also an important soil element, being its average of 3.5% in soils [19] and regarding our data (Figure 1A), Fe has a lower content than the average. Iron is considered an essential

element for plants growth, being required (as  $\text{Fe}^{2+}$  and/or  $\text{Fe}^{3+}$ ) for several cellular processes (namely, respiration, photosynthesis, and it's a cofactor for various enzymes) [20]. Calcium was the third element in greatest quantity (Figure 1A), being accordingly with the fact that it is the third most available nutrient in soil [21]. In plants, Ca is considered an essential nutrient (required as  $\text{Ca}^{2+}$ ) for plant growth and development, plays a central role in different plant mechanisms, namely in plant signaling, water relations [12], stress responses [11,12] and in maintenance of plant structures [4,11]. Its delivery is dependent of xylem workflow [22].

Regardless of soil composition, different interactions and contents of mineral elements were observed in leaves and potato tubers (Table 1 and 2), probably due to the different mobility of minerals through phloem and xylem pathways. For instance, K is considered a highly mobile within plants [18] and probably because of that, showed higher contents in leaves and potato tubers (Table 1 and 2). Phosphorus is essential for plant growth and its concentration in plants tissue vary between 0.4 and 1–5% of the dry matter [23], being in accordance with the data obtain in the leaves (Table 1) and not with the data obtain in potato tubers (Table 2) (lower content in our data). Additionally, P is relatively immobile in the soil [22] and poorly mobile in plants [24] and probably because of that our data showed lower values in potato tubers. Sulfur is considered as a key-nutrient (together with N and P) needed for the growth and development of crops, being its requirements similar to P [25] (as seen in our data—Table 2).

For instance, in the treatment with the highest Ca content in leaves ( $\text{Ca}(\text{NO}_3)_2$  2 kg  $\text{ha}^{-1}$  treatment) (Table 1), presented also the highest Mg content. This synergetic relationship (observed in some treatments—there isn't a clear trend) is not usually verified, due to the subsistence of a cationic antagonism between Ca and Mg or K (when one increases, can lead to a decrease in the other) [7,26]. Additionally, there isn't also a clear trend regarding Ca and K and no trend of antagonism or synergetic relationship in the leaves and potato tubers. Nevertheless, regarding vegetable crop, potato plants require more K than any other [27]. In potato tubers (Table 2) K is one of the main minerals [28], showing similar values to another study with Ca enrichment carried out with the same variety (at harvest) [8]. After K, P is the main mineral in tubers [28] and present higher values compared to other study carried out at harvest [8]. Moreover, Ca accumulation showed (in some treatments, namely in  $\text{CaCl}_2$  12kg. $\text{ha}^{-1}$  and  $\text{Ca}(\text{NO}_3)_2$  0.5 kg  $\text{ha}^{-1}$ ) a synergetic interaction with P, K, and S. In fact, when Ca content increased, S content also increased, being this behavior previously reported by [29]. As seen in our study (Table 2) and reported by another study [8], K and P also increased in potato tubers with the increased of Ca content.

Regardless, it's important to understand that many factors affect the mineral composition of potatoes, namely, stage of development, soil, irrigation, fertilization, and genotype [28]. In this case, the potato tubers were not fully developed/not ready to be harvest for human consumption, which was a monitor process to understand if there were Ca accumulation in the early stage of the biofortification process.

The color index is an important indicator of quality, affecting the acceptability of consumers [30]. As such, colorimetric parameters were analyzed (Table 3), showing that apparently, the treatment with the lower Ca content ( $\text{CaCl}_2$  6 kg. $\text{ha}^{-1}$ ) showed the higher value of L (more brightness) and  $\text{CaCl}_2$  12 kg. $\text{ha}^{-1}$  treatment (higher Ca content) presented a higher value of  $b^*$  (more yellow). Nevertheless, compared to the same variety, L parameter in our study showed higher data compared to other studies carried out at harvest [9,30]. However, our data of L parameter showed lower values compared to another study [31]. Also, regarding  $a^*$  and  $b^*$  parameters, our data presented higher and lower values, respectively, compared to a study carried out with the same variety [31]. However, there are no relevant changes in colorimetric parameters of tubers pulp at this stage of tubers' development, as seen in another study carried out with Ca foliar applications with calcium chloride or calcium nitrate [8].

## 5. Conclusions

Through the monitorization of mineral interactions in leaves and potato tubers, was possible to identify that natural enrichment of Ca (after two foliar sprays with calcium chloride or calcium nitrate) showed different relationships between minerals. In our study (at this stage of development of potato tubers) didn't occurred any cationic antagonism between Ca and Mg or K. In fact, in some treatments, was possible to identify a synergetic relationship between Ca and Mg in potato leaves at this stage. Additionally, in some treatments, synergetic relationship also occurred with P, K and S in tubers tissues. Nevertheless, the concentrations of mineral elements in potato tubers are influenced by Ca supply and Ca accumulation occurred through xylem mass flow and phloem redistribution, as seen in more phloem mobile elements (Mg, S, P, and K). Furthermore, mineral interactions and Ca natural enrichment did not show relevant changes in colorimetric paraments in tubers' pulp.

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