biology and life sciences forum

MDPI

Proceeding Paper

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

Ana Rita F. Coelho [1,2,*], Ana Coelho Marques [1,2], Cláudia Campos Pessoa [1,2], Diana Daccak [1,2], Inês Carmo Luís [1,2], Maria Manuela Silva [2,3], Manuela Simões [1,2], Fernando H. Reboredo [1,2], Maria F. Pessoa [1,2], Paulo Legoinha [1,2], José C. Ramalho [2,4], Paula Scotti Campos [2,5], Isabel P. Pais [2,5], José N. Semedo [2,5] and Fernando C. Lidon [1,2]

1 Earth Sciences Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; amc.marques@campus.fct.unl.pt (A.C.M.); c.pessoa@campus.fct.unl.pt (C.C.P.); d.daccak@campus.fct.unl.pt (D.D.); idc.rodrigues@campus.fct.unl.pt (I.C.L.); mmsr@fct.unl.pt (M.S.); fhr@fct.unl.pt (F.H.R.); mfgp@fct.unl.pt (M.F.P.); pal@fct.unl.pt (P.L.); fjl@fct.unl.pt (F.C.L.)
2 GeoBioTec Research Center, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; abreusilva.manuela@gmail.com (M.M.S.); cochichor@mail.telepac.pt (J.C.R.); paula.scotti@iniav.pt (P.S.C.); isabel.pais@iniav.pt (I.P.P.); jose.semedo@iniav.pt (J.N.S.)
3 Escola Superior de Educação Almeida Garrett (ESAG-COFAC), Avenida do Campo Grande 376, 1749-024 Lisboa, Portugal
4 Plant Stress & Biodiversity Lab, Centro de Estudos Florestais (CEF), Instituto Superior Agronomia (ISA), Universidade de Lisboa (ULisboa), Quinta do Marquês, Avenida da República, 2784-505 Oeiras and Tapada da Ajuda, 1349-017 Lisboa, Portugal
5 Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Avenida da República, Quinta do Marquês, 2780-157 Oeiras, Portugal
* Correspondence: arf.coelho@campus.fct.unl.pt; Tel.: +351-212-948-573
† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

Abstract: Calcium is an essential nutrient for plants and is required for the 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. Thus, it is crucial to deepen our knowledge on the interactions of calcium with other minerals in tuber tissues. In this context, this study aimed to monitor the mineral interactions in the 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^{-1}) and calcium nitrate (0.5, 1, 2 and 4 kg.ha^{-1}), in order to improve the Ca content naturally. 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^{-1} treatment and in tubers with calcium chloride 12 kg.ha^{-1} 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.

Citation: Coelho, A.R.F.; Marques, A.C.; Pessoa, C.C.; Daccak, D.; Luís, I.C.; Silva, M.M.; Simões, M.; Reboredo, F.H.; Pessoa, M.F.; Legoinha, P.; et al. Natural Enrichment of *Solanum tuberosum* L. with Calcium—Monitorization of Mineral Interactions in Plant Tissues. *Biol. Life Sci. Forum* **2022**, *11*, 28. https://doi.org/10.3390/IECPS2021-11972

Academic Editor: Dimitris L. Bouranis

Published: 30 November 2021

1. Introduction

Calcium is 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 is 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 Ca^{2+} and has a central task in stress responses [12] and plays an indispensable role in structural maintenance (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, Zn and Cu having 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 the Ca content naturally.

2. Materials and Methods

2.1. Biofortification Itinerary

The experimental potato-growing field, located in the west of Portugal (GPS coordinates: 39°16′38,816″ N; 9°15′9128″ W, was used to growth the Agria variety (*Solanum tuberosum* L.). The planting date was on 4 May and the harvest date was on 4 September 2018 (after four foliar sprays with 8–10-day intervals). 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 $CaCl_2$ (1, 3, 6 and 12 kg.ha^{-1}) or $Ca(NO_3)_2$ (0.5, 1, 2 and 4 kg.ha^{-1}). Control plants were not sprayed at any times with $CaCl_2$ or $Ca(NO_3)_2$ (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], the 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 is important to perform a soil analysis. In this context, the chemical composition (macro and microelements) of the potato-growing field soil was determined (Figure 1A,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.

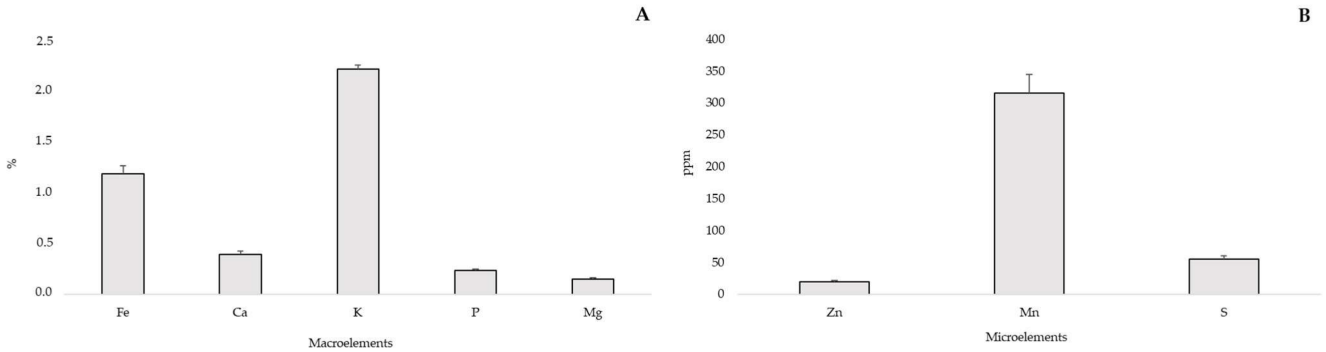

Figure 1. (**A**,**B**) Mean values (n = 9) of macroelements (**A**) and microelements (**B**) of the soil of the experimental potato-growing field selected for Ca biofortification of *Solanum tuberosum* L., Agria variety.

After two foliar sprays, different minerals (Ca, P, K, S, Zn, and Mg) were analyzed in the 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 $Ca(NO_3)_2$ 2 kg.ha^{-1} treatment (obtaining a Ca biofortification index of 73.9%). On the other hand, the lowest Ca content was obtained in $CaCl_2$ 6 kg.ha^{-1} treatment. Additionally, it was possible to identify a synergetic interaction between Ca and Mg in three different treatments ($Ca(NO_3)_2$ 1 and 2 kg.ha^{-1} and $CaCl_2$ 6 kg.ha^{-1}). In the control leaves, K was the only mineral that showed a higher content. Yet, K and Mg were also the only ones that did not obtain a lower content in the $CaCl_2$ 12 kg.ha^{-1} treatment.

Table 1. Mean values ± S.E. (n = 4) of Ca, P, K, S, Zn, and Mg contents in the 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 $Ca(NO_3)_2$ (0.5, 1, 2, and 4 kg.ha^{-1}) and $CaCl_2$ (1, 3, 6, and 12 kg.ha^{-1}). Control was not sprayed.

Treatments		Ca (%)	P (%)	K (%)	S (%)	Zn (ppm)	Mg (ppm)
Control		5.13 ± 0.89 bc	0.58 ± 0.11 a	11.0 ± 1.49 a	1.18 ± 0.14 ab	33.7 ± 2.20 ab	4.25 ± 0.28 abc
$Ca(NO_3)_2$	0.5 kg.ha^{-1}	5.56 ± 1.14 abc	0.58 ± 0.06 a	9.26 ± 0.71 ab	1.32 ± 0.17 ab	51.6 ± 3.15 a	5.25 ± 0.97 abc
	1 kg.ha^{-1}	8.41 ± 1.01 ab	0.53 ± 0.10 a	9.88 ± 0.77 ab	1.53 ± 0.13 a	37.0 ± 1.67 ab	5.40 ± 0.52 ab
	2 kg.ha^{-1}	8.92 ± 0.46 a	0.70 ± 0.03 a	10.1 ± 0.48 ab	1.46 ± 0.04 a	37.0 ± 4.05 ab	7.44 ± 0.59 a
	4 kg.ha^{-1}	5.81 ± 0.13 abc	0.73 ± 0.07 a	9.76 ± 0.12 ab	1.60 ± 0.10 a	37.1 ± 8.73 ab	4.12 ± 1.05 abc
$CaCl_2$	1 kg.ha^{-1}	6.80 ± 0.52 abc	0.63 ± 0.07 a	9.08 ± 0.56 ab	1.17 ± 0.07 ab	20.1 ± 2.43 b	5.13 ± 0.81 abc
	3 kg.ha^{-1}	6.54 ± 0.50 abc	0.45 ± 0.03 a	8.75 ± 0.81 ab	1.13 ± 0.02 ab	29.8 ± 2.22 b	3.43 ± 1.12 bc
	6 kg.ha^{-1}	4.82 ± 0.12 c	0.42 ± 0.04 a	7.51 ± 0.65 ab	0.90 ± 0.02 b	23.3 ± 1.13 b	1.59 ± 0.35 c
	12 kg.ha^{-1}	7.49 ± 0.89 abc	0.44 ± 0.05 a	6.87 ± 0.74 b	1.13 ± 0.09 ab	28.4 ± 1.96 b	3.51 ± 0.61 bc

The mineral content in the potato tubers after two foliar applications was also assessed (Table 2). Regarding the minerals analyzed (Ca, P, K, and S), they all had a higher content in the highest treatment applied with calcium chloride. Considering Ca content, the biofortification index varied between 6.4% ($Ca(NO_3)_2$ 2 kg.ha^{-1}) and 35.3% ($CaCl_2$ 12 kg.ha^{-1}) at this stage of the biofortification process. In $CaCl_2$ 6 kg.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 $CaCl_2$ 12 $kg.ha^{-1}$ and $Ca(NO_3)_2$ 0.5 treatment). Potassium and S showed a lower content in $CaCl_2$ 1 $kg.ha^{-1}$ and 3, respectively.

Table 2. Mean values ± S.E. ($n = 4$) of Ca, P, K, and S contents in the 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 $Ca(NO_3)_2$ (0.5, 1, 2, and 4 $kg.ha^{-1}$) and $CaCl_2$ (1, 3, 6, and 12 $kg.ha^{-1}$). Control was not sprayed.

Treatments		Ca (%)	P (%)	K (%)	S (%)
Control		0.122 ± 0.006 cd	0.204 ± 0.006 abc	3.36 ± 0.099 ab	0.173 ± 0.005 ab
$Ca(NO_3)_2$	0.5 $kg.ha^{-1}$	0.143 ± 0.012 abc	0.218 ± 0.005 ab	3.45 ± 0.086 a	0.186 ± 0.005 a
	1 $kg.ha^{-1}$	0.159 ± 0.005 ab	0.181 ± 0.004 c	3.35 ± 0.025 ab	0.182 ± 0.008 a
	2 $kg.ha^{-1}$	0.130 ± 0.007 bcd	0.205 ± 0.009 abc	3.22 ± 0.060 ab	0.180 ± 0.002 ab
	4 $kg.ha^{-1}$	0.136 ± 0.007 abcd	0.185 ± 0.006 bc	3.31 ± 0.238 ab	0.179 ± 0.006 ab
$CaCl_2$	1 $kg.ha^{-1}$	0.120 ± 0.007 cd	0.186 ± 0.011 bc	2.83 ± 0.086 b	0.157 ± 0.006 ab
	3 $kg.ha^{-1}$	0.113 ± 0.000 cd	0.176 ± 0.006 c	3.27 ± 0.067 ab	0.148 ± 0.004 b
	6 $kg.ha^{-1}$	0.107 ± 0.006 d	0.170 ± 0.002 c	3.16 ± 0.035 ab	0.163 ± 0.004 ab
	12 $kg.ha^{-1}$	0.165 ± 0.001 a	0.237 ± 0.011 a	3.58 ± 0.157 a	0.188 ± 0.013 a

In the 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 $Ca(NO_3)_2$ 2 $kg.ha^{-1}$ treatment, b* (yellow/blue) and L (brightness/luminosity) parameters showed a lower value; however, the a* (red/green) parameter showed the highest value. In the L and b* parameters, $Ca(NO_3)_2$ 4 $kg.ha^{-1}$ and $CaCl_2$ 12 $kg.ha^{-1}$ treatments showed a higher value, respectively. Regarding the a* parameter, the lowest value was obtained in $Ca(NO_3)_2$ 1 $kg.ha^{-1}$ treatment.

Table 3. Mean values ± S.E. ($n = 4$) of colorimetric parameters (L, a* and b*) in the fresh tubers of *Solanum tuberosum* L., Agria variety after the 2nd foliar application. Letters a and b indicate significant differences, of each parameter, between treatments ($p \leq 0.05$). Foliar spray was carried out with four concentrations of $Ca(NO_3)_2$ (0.5, 1, 2, and 4 $kg.ha^{-1}$) and $CaCl_2$ (1, 3, 6, and 12 $kg.ha^{-1}$). Control was not sprayed.

Treatments		Colorimetric Parameters		
		L	a*	b*
Control		64.5 ± 0.53 a	1.42 ± 0.02 b	12.7 ± 0.09 ab
$Ca(NO_3)_2$	0.5 $kg.ha^{-1}$	63.6 ± 1.56 ab	1.77 ± 0.07 ab	12.4 ± 0.33 ab
	1 $kg.ha^{-1}$	62.4 ± 0.98 ab	1.40 ± 0.08 b	12.3 ± 0.20 ab
	2 $kg.ha^{-1}$	58.1 ± 0.68 b	2.11 ± 0.10 a	11.7 ± 0.12 b
	4 $kg.ha^{-1}$	66.3 ± 0.14 a	1.73 ± 0.13 ab	13.3 ± 0.28 a
$CaCl_2$	1 $kg.ha^{-1}$	64.5 ± 1.87 a	1.89 ± 0.02 a	12.5 ± 0.51 ab
	3 $kg.ha^{-1}$	63.9 ± 0.63 ab	1.79 ± 0.06 ab	13.1 ± 0.10 a
	6 $kg.ha^{-1}$	66.5 ± 0.93 a	1.44 ± 0.12 b	12.9 ± 0.19 ab
	12 $kg.ha^{-1}$	64.4 ± 2.10 a	1.77 ± 0.02 ab	13.5 ± 0.38 a

4. Discussion

In potato plants, mineral element uptake mainly occurs from the soil solution [13] and is important to correlate nutrient accumulation with the soil composition. In this context, nutrients in the soil were assessed (Figure 1). Potassium was found in greater quantity in the soil (Figure 1A), being one of the most soluble elements in the soil–plant system, its absorption through soils is highly efficient and its transport through plants very fast [17]. In plants, K is required as K^+ (from soil by roots) and is considered the most abundant inorganic cation in plants [18] (as seen in Table 2). It is required for the activation of various enzymes and plays a vital role in cell metabolism and tissue growth [18]. Regarding Fe, it is also an important soil element, showing an 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 plant growth, being required (as Fe^{2+} and/or Fe^{3+}) for several cellular processes (namely, respiration, photosynthesis, and it is a cofactor for various enzymes) [20]. Calcium was the third element in greatest quantity (Figure 1A), due to the fact that it is the third most available nutrient in soil [21]. In plants, Ca is considered an essential nutrient (required as Ca^{2+}) for plant growth and development, and 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 on xylem workflow [22].

Regardless of soil composition, different interactions and contents of mineral elements were observed in leaves and potato tubers (Tables 1 and 2), probably due to the different mobility of minerals through phloem and xylem pathways. For instance, K is considered to be highly mobile within plants [18] and probably because of that showed higher contents in leaves and potato tubers (Tables 1 and 2). Phosphorus is essential for plant growth and its concentration in plants tissue varies 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 obtained in the 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 the potato tubers. Sulfur is considered as a key nutrient (together with N and P) needed for the growth and development of crops, making its requirements similar to P [25] (as seen in our data—Table 2).

For instance, the treatment with the highest Ca content in leaves ($Ca(NO_3)_2$ 2 $kg.ha^{-1}$ treatment) (Table 1) also presented the highest Mg content. This synergetic relationship (observed in some treatments—there is not a clear trend) is not usually verified, due to the subsistence of a cationic antagonism between Ca and Mg or K (when one increases, it can lead to a decrease in the other) [7,26]. Additionally, there is not a clear trend regarding Ca and K and no trend of antagonism or synergetic relationship in the leaves and potato tubers. Nevertheless, regarding vegetable crops, 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 presented higher values compared to other studies carried out at harvest [8]. Moreover, Ca accumulation showed (in some treatments, namely in $CaCl_2$ 12kg.ha^{-1} and $Ca(NO_3)_2$ 0.5 $kg.ha^{-1}$) a synergetic interaction with P, K, and S. In fact, when Ca content increased, S content also increased, and this behavior was 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 increase in Ca content.

Regardless, it is important to understand that many factors affect the mineral composition of potatoes, namely, the stage of development, soil, irrigation, fertilization, and genotype [28]. In this case, the potato tubers were not fully developed/not ready to be harvested for human consumption, and was a monitoring process to understand if there was Ca accumulation in the early stages 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 ($CaCl_2$ 6 $kg.ha^{-1}$) showed the higher value of L (more brightness) and $CaCl_2$ 12 $kg.ha^{-1}$ treatment (higher Ca content) presented a higher value of b* (more yellow). Nevertheless, compared to the same variety, the L parameter in our study showed higher data compared to other studies carried out at harvest [9,30]. However, our data of the L parameter showed lower values compared to another study [31]. Additionally, 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 the tubers' pulp at this stage of the 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, it 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 the potato tubers) any cationic antagonism between Ca and Mg or K did not occur. In fact, in some treatments, it was possible to identify a synergetic relationship between Ca and Mg in potato leaves at this stage. Additionally, in some treatments, a synergetic relationship also occurred with P, K, and S in tuber tissues. Nevertheless, the concentrations of mineral elements in potato tubers are influenced by Ca supply and Ca accumulation occurring 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 the tubers' pulp.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECPS2021-11972/s1.

Author Contributions: Conceptualization, F.C.L.; methodology, F.C.L.; software, A.R.F.C.; formal analysis, A.R.F.C., A.C.M., C.C.P., I.C.L. and D.D.; resources, M.M.S., M.S., F.H.R., M.F.P., P.L., J.C.R., P.S.C., I.P.P. and J.N.S.; writing—original draft preparation, A.R.F.C. and F.C.L.; writing—review and editing, A.R.F.C. and F.C.L.; supervision, F.C.L.; project administration, F.C.L.; funding acquisition, F.C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by PDR2020, grant number 101-030719. Funding from Fundação para a Ciência e Tecnologia (FCT) UI/BD/150806/2020 is also greatly acknowledged.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Eng. Nuno Cajão (Cooperativa de Apoio e Serviços do Concelho da Lourinhã—LOURICOOP) for technical assistance in the agricultural parcel as well as to project PDR2020-101-030719for the financial support. We also thank the research centers (GeoBioTec) UIDB/04035/2020 and (CEF) UIDB/00239/2020.

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

References

1. Peacock, M. Calcium Metabolism in Health and Disease. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*, S23–S30. [CrossRef] [PubMed]
2. IOM-Institute of Medicine. *Dietary Reference Intakes for Calcium and Vitamin D*; The National Academies Press: Washington, DC, USA, 2011; ISBN 978-0-309-16395-8.
3. Buchowski, M.S. Calcium in the context of dietary sources and metabolism. In *Calcium: Chemistry, Analysis, Function and Effects*; Preedy, V.R., Ed.; Food and Nutritional Components in Focus–Book Series; Royal Society of Chemistry: Cambridge, UK, 2015; Chapter 1; pp. 3–20, ISBN 978-1-78262-213-0.
4. Sharma, D.; Jamra, G.; Singh, U.M.; Sood, S.; Kumar, A. Calcium Biofortification: Three Pronged Molecular Approaches for Dissecting Complex Trait of Calcium Nutrition in Finger Millet (*Eleusine coracana*) for Devising Strategies of Enrichment of Food Crops. *Front. Plant Sci.* **2017**, *7*, 2028. [CrossRef] [PubMed]
5. Pravina, P.; Sayaji, D.; Avinash, M. Calcium and its role in human body. *IJRPBS* **2013**, *4*, 659–668.
6. Bouis, H.E.; Saltzman, A. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Glob. Food Secur.* **2017**, *12*, 49–58. [CrossRef]
7. Coelho, A.R.F.; Lidon, F.C.; Pessoa, C.C.; Marques, A.C.; Luís, I.C.; Caleiro, J.; Simões, M.; Kullberg, J.; Legoinha, P.; Brito, M.; et al. Can Foliar Pulverization with $CaCl_2$ and $Ca(NO_3)_2$ Trigger Ca Enrichment in *Solanum tuberosum* L. Tubers? *Plants* **2021**, *10*, 245. [CrossRef]

8. Coelho, A.; Pessoa, C.; Marques, A.; Luís, I.; Daccak, D.; Silva, M.M.; Simões, M.; Rebo-redo, F.; Pessoa, M.; Legoinha, P.; et al. Na-tural mineral enrichment in *Solanum tuberosum* L. cv. Agria: Accumulation of Ca and interaction with other nutrients by XRF analysis. In Proceedings of the 1st International Electronic Conference on Plant Science, Online, 1–15 December 2020; Volume 1, p. 15. [CrossRef]
9. Coelho, A.R.F.; Marques, A.C.; Pessoa, C.C.; Luís, I.C.; Daccak, D.; Simões, M.; Reboredo, F.H.; Pessoa, M.; Silva, M.M.; Legoinha, P.; et al. Calcium Biofortification in *Solanum tuberosum* L. cv. Agria: A Technical Workflow. In Proceedings of the 1st International Conference on Water Energy Food and Sustainability (ICoWEFS 2021), Leiria, Portugal, 10–12 May 2021; Springer: Cham, Switzerland, 2021. [CrossRef]
10. Coelho, A.; Luís, I.C.; Marques, A.C.; Pessoa, C.; Daccak, D.; Caleiro, J.; Brito, M.; Kullberg, J.; Silva, M.; Simões, M.; et al. Monitoring of a calcium biofortification workflow for tubers of *Solanum tuberosum* L. cv. Picasso using smart farming technology. In Proceedings of the 1st International Electronic Conference on Agronomy, Online, 3–17 May 2021; MDPI: Basel, Switzerland, 2021. [CrossRef]
11. White, P.J.; Broadley, M.R. Calcium in Plants. *Ann. Bot.* **2003**, *92*, 487–511. [CrossRef]
12. Hocking, B.; Tyerman, S.D.; Burton, R.A.; Gilliham, M. Fruit Calcium: Transport and Physiology. *Front. Plant Sci.* **2016**, *7*, 569. [CrossRef]
13. Subramanian, N.K.; White, P.; Broadley, M.; Ramsay, G. The three-dimensional distribution of minerals in potato tubers. *Ann. Bot.* **2011**, *107*, 681–691. [CrossRef]
14. Busse, J.S.; Palta, J.P. Investigating the in vivo calcium transport path to developing potato tuber using 45Ca: A new concept in potato tuber calcium nutrition. *Physiol. Plant.* **2006**, *128*, 313–323. [CrossRef]
15. Baker, D.A.; Moorby, J. The Transport of Sugar, Water, and Ions into Developing Potato Tubers. *Ann. Bot.* **1969**, *33*, 729–741. [CrossRef]
16. Pelica, J.; Barbosa, S.; Reboredo, F.; Lidon, F.; Pessoa, F.; Calvão, T. The paradigm of high concentration of metals of natural or anthropogenic origin in soils—The case of Neves-Corvo mine area (Southern Portugal). *J. Geochem. Explor.* **2018**, *186*, 12–23. [CrossRef]
17. Reitemeier, R. Soil Potassium. *Adv. Agron.* **1951**, *3*, 113–164.
18. White, P.J.; Karley, A.J. Potassium. In *Plant Cell Monographs*; Springer: Singapore, 2010; Volume 17, pp. 199–224.
19. Kabata-Pendias, A.; Pendias, H. *Trace Elements in Soils and Plants*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2001.
20. Kobayashi, T.; Nozoye, T.; Nishizawa, N.K. Iron transport and its regulation in plants. *Free Radic. Biol. Med.* **2019**, *133*, 11–20. [CrossRef] [PubMed]
21. D'Imperio, M.; Renna, M.; Cardinali, A.; Buttaro, D.; Serio, F.; Santamaria, P. Calcium biofortification and bioaccessibility in soilless "baby leaf" vegetable production. *Food Chem.* **2016**, *213*, 149–156. [CrossRef]
22. Weinl, S.; Held, K.; Schlücking, K.; Steinhorst, L.; Kuhlgert, S.; Hippler, M.; Kudla, J. A plastid protein crucial for Ca 2+ -regulated stomatal responses. *New Phytol.* **2008**, *179*, 675–686. [CrossRef]
23. White, P.J.; Hammond, J.P. Phosphorus nutrition of terrestrial plants. In *The Ecophysiology of Plant-Phosphorus Interactions*; Springer: Dordrecht, The Netherlands, 2008; pp. 51–81.
24. Hinsinger, P.; Brauman, A.; Devau, N.; Gérard, F.; Jourdan, C.; Laclau, J.-P.; Le Cadre-Barthélémy, E.; Jaillard, B.; Plassard, C. Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? *Plant Soil* **2011**, *348*, 29–61. [CrossRef]
25. Stipp, S.R.; Casarin, V. A importância do enxofre na agricultura brasileira. *Inf. Agron.* **2010**, *129*, 14–20.
26. Rhodes, R.; Miles, N.; Hughes, J.C. Interactions between potassium, calcium and magnesium in sugarcane grown on two contrasting soils in South Africa. *Field Crops Res.* **2018**, *223*, 1–11. [CrossRef]
27. Khan, M.Z.; Akhtar, M.E.; Safdar, M.N.; Mahmood, M.M.; Ahmad, S.; Ahmed, N. Effect of source and level of potash on yield and quality of potato tubers. *Pak. J. Bot.* **2010**, *42*, 3137–3145.
28. Navarre, D.A.; Goyer, A.; Shakya, R. Nutritional Value of Potatoes. *Adv. Potato Chem. Technol.* **2009**, *4*, 395–424. [CrossRef]
29. Aulakh, M.S.; Dev, G. Interaction effect of calcium and sulphur on the growth and nutrient composition of alfalfa (*Medicago sativa* L. pers.), using35S. *Plant Soil* **1978**, *50*, 125–134. [CrossRef]
30. Xiao, Q.; Bai, X.; He, Y. Rapid Screen of the Color and Water Content of Fresh-Cut Potato Tuber Slices Using Hyperspectral Imaging Coupled with Multivariate Analysis. *Foods* **2020**, *9*, 94. [CrossRef] [PubMed]
31. Cabezas-Serrano, A.B.; Amodio, M.L.; Cornacchia, R.; Rinaldi, R.; Colelli, G. Suitability of five different potato cultivars (*Solanum tuberosum* L.) to be processed as fresh-cut products. *Postharvest Biol. Technol.* **2009**, *53*, 138–144. [CrossRef]