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Proceeding Paper

Calcium Biofortification in *Solanum tuberosum* L.: Assessing the Influence of Calcium Nitrate and Calcium Chloride on Yield †

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Abstract: Potato (*Solanum tuberosum* L.) is a widely consumed and essential food crop globally, making it an ideal food matrix for biofortification. Agronomic biofortification is one of the strategies used to enhance Ca content in edible parts of crops, considering the adverse health issues associated with Ca deficiency. This study aims to investigate the impact of Ca agronomic biofortification through four foliar applications after the beginning of tuberization on the yield of tubers of *Solanum tuberosum* L. (Picasso variety) produced in Lourinhã (Portugal) in 2018, focusing on the application of calcium chloride or, alternatively, calcium nitrate at different concentrations (calcium chloride—1, 3, 6 and 12 kg/ha or calcium nitrate—0.5, 1, 2 and 4 kg/ha). Control plants and plants subjected to the different Ca treatments were placed in plots of 20 × 24 m; the treatments were carried out in quadruplicate (compass 60–80 cm). As such, the Ca content in tubers was quantified by atomic absorption spectrophotometry in the different treatments. The Ca biofortification index with calcium chloride or calcium nitrate ranged between 5 and 40%, with the treatment with 6 kg/ha $CaCl_2$ being the one which presented the highest Ca content in tubers at harvest and 1 kg/ha $CaCl_2$ being the treatment with the lowest Ca biofortification index. However, 6 kg/ha $CaCl_2$, despite presenting the highest Ca content, was not the treatment that presented the highest yield. Indeed, all the calcium nitrate treatments demonstrated a substantial increase in tubers yield, which varied between 2.3 (4 kg/ha $Ca(NO_3)_2$) and 24.3% (2 kg/ha $Ca(NO_3)_2$). Statistical analysis was carried out in all the analyses using one-way ANOVA to assess differences among treatments in *Solanum tuberosum* L. (Picasso variety), followed by Tukey's analysis for mean comparison, with a 95% confidence level. Furthermore, these findings emphasize the potential of Ca biofortification, especially calcium nitrate treatments, in enhancing the yield of *Solanum tuberosum* L. tubers.

Keywords: calcium biofortification; calcium nitrate; calcium chloride; *Solanum tuberosum* L.; yield

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1. Introduction

Calcium is a highly abundant mineral element present in the human body [1] and has a crucial role in various bodily functions, including in the development of bones and teeth, in muscle contraction, and in the circulatory system [1–5]. The required amount of Ca intake varies depending on the age and physical conditions of the individual, with higher intake recommended for pregnant women and toddlers [5]. Moreover, Ca is obtained though dietary sources, necessitating a daily intake through foods, especially the naturally rich ones such as milk or leafy vegetables [1,5]. Considering that Ca deficiency can lead to various health issues, including osteoporosis and rickets [2], it is important to have strategies for combating it. As such, when it comes to combating Ca deficiency, agronomic biofortification is one of those strategies, considering that it has been employed to enhance mineral content [6], enriching the edible portions of food crops. Indeed, agronomic biofortification has emerged as a fast, reliable, and cost-effective approach for the goal of increasing mineral content in edible parts of plants [6], particularly through foliar applications [7]. However, despite the importance of obtaining an increase in the mineral content of crops, it is also essential not to reduce their yield.

In this context, considering that the potato (*Solanum tuberosum* L.) is the third most consumed staple food crop worldwide, following rice and wheat [8], and ranks as the fourth most cultivated crop, after rice, wheat, and maize [9,10], it stands out as the perfect food matrix for biofortification. As such, the objective of this study is to investigate the effects of Ca biofortification on *Solanum tuberosum* L. tubers of Picasso variety through foliar applications and their subsequent yield, assessing the impact of four different concentrations of calcium chloride (1, 3, 6 and 12 kg/ha) and calcium nitrate (0.5, 1, 2 and 4 kg/ha).

2. Materials and Methods

2.1. Calcium Biofortification Workflow

The experimental field located in western Portugal was used to cultivate *Solanum tuberosum* L. (Picasso variety). Throughout the agricultural period from 15 May (planting date) to 25 September 2018 (harvest date), the average daily temperatures oscillated between 15 °C and 23 °C. Four foliar applications after the beginning of tuberization were carried out at 8- to 12-day intervals with $CaCl_2$ (1, 3, 6 and 12 kg/ha) or $Ca(NO_3)_2$ (0.5, 1, 2 and 4 kg/ha). Moreover, the control plants (which remained untreated with calcium chloride or calcium nitrate) and plants subjected to the different Ca treatments were placed in plots of 20 × 24 m the treatments were carried out in quadruplicate (compass 60–80 cm).

2.2. Calcium Content in Tubers

After being harvested, the tubers were washed and dried at 60 °C until they reached a constant weight and were ground using an agate mortar. Subsequently, an acid digestion procedure was carried out using a mixture of HNO_3–$HClO_4$ (4:1), as described in [11,12]. Following the filtration of samples, Ca content was quantified using atomic absorption spectrophotometry (Perkin Elmer AAnalyst 200 model). The absorbance was determined in mg/L using AA WinLab software (version 32).

2.3. Total Yield

In tubers of the Picasso variety, after harvest, the total yield was carried out considering 57 plants for each treatment (control and Ca biofortification treatments).

2.4. Statistical Analysis

Statistical analysis was carried out using one-way ANOVA to assess differences among treatments in *Solanum tuberosum* L. (Picasso variety), followed by Tukey's analysis for mean comparison. A 95% confidence level was adopted for all tests.

3. Results

3.1. Ca Content in Tubers at Harvest

The calcium content in tubers that underwent Ca biofortification treatments presented higher values compared to control (Figure 1). In fact, 3 kg/ha $CaCl_2$, 6 kg/ha $CaCl_2$, 12 kg/ha $CaCl_2$, 1 kg/ha $Ca(NO_3)_2$, 2 kg/ha $Ca(NO_3)_2$, and 4 kg/ha $Ca(NO_3)_2$ presented significantly higher values relative to control. Moreover, as a result of the Ca biofortification treatments, the biofortification index varied between 5 and 40%, with 6 kg/ha $CaCl_2$ being the treatment which presented the highest Ca content compared to the remaining treatments (Figure 1).

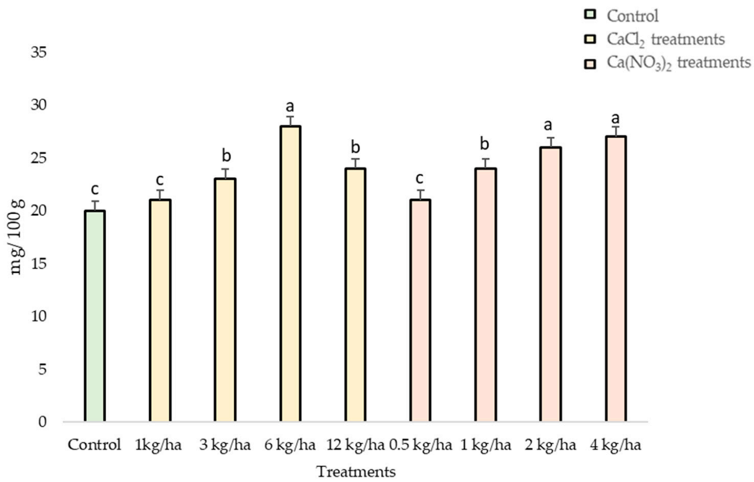

Figure 1. Calcium content (considering dry weight) in tubers of *Solanum tuberosum* L. (Picasso variety) at harvest. Mean values (n = 4) ± SE (standard error). Different letters (a–c) indicate significant differences between treatments.

3.2. Yield

Considering the total yield of *Solanum tuberosum* L. tubers of the Picasso variety (Table 1), treatments with calcium chloride with concentrations of 1 and 12 kg/ha presented the lowest yield value relative to the control. Moreover, 2 kg/ha of $Ca(NO_3)_2$ treatment showed the highest yield for the Picasso variety and $CaCl_2$ treatments only presented an increase in yield between 0.1 and 3.4%, while $Ca(NO_3)_2$ treatments showed an increase which varied between 2.3 and 24.3%. Additionally, calcium nitrate treatments (0.5, 1, 2 and 4 kg/ha) always showed a yield increase relative to the control. On the other hand, considering the calcium chloride treatments, only 3 and 6 kg/ha treatment showed an increase in yield.

Table 1. Total yield at harvest of *Solanum tuberosum* L. tubers (Picasso variety).

Treatments	Yield (kg)
Control	106.5
1 kg/ha $CaCl_2$	96.1
3 kg/ha $CaCl_2$	110.1
6 kg/ha $CaCl_2$	106.6
12 kg/ha $CaCl_2$	104.2
0.5 kg/ha $Ca(NO_3)_2$	116.6
1 kg/ha $Ca(NO_3)_2$	131.2
2 kg/ha $Ca(NO_3)_2$	132.4
4 kg/ha $Ca(NO_3)_2$	109

4. Discussion

Considering the importance of Ca in the human body [1–5], the fact that it is obtained through dietary sources [1,5], and its importance not only for increasing the mineral content of crops, but also for improving their yield, this study aimed to assess the impact of Ca content (Figure 1) and yield (Table 1) in *Solanum tuberosum* L. tubers of the Picasso variety. As such, this study revealed that among the different treatments and products evaluated (Figure 1), 6 kg/ha $CaCl_2$ treatment resulted in the highest Ca content in tubers of *Solanum tuberosum* L. of the Picasso variety. However, contrary to expectations, this treatment did not exhibit the highest yield compared to the control (Table 1). In fact, the 6 kg/ha $CaCl_2$ treatment showed similar values of yield relative to the control, while 3 kg/ha $CaCl_2$ treatment only showed a 3.4% increase in the yield. Indeed, our data are not in accordance with a previous study that used two foliar sprays containing 2 and 4 kg/ha of $CaCl_2$, which enhanced the tuber yield [13], or even according to another study carried out by [14], which showed improvements in tuber yield resulting from foliar sprays containing calcium chloride. Furthermore, the third treatment with the highest Ca content (2 kg/ha $Ca(NO_3)_2$ treatment) (Figure 1) was the one with the highest yield (Table 1). Also, all the calcium nitrate treatments presented a higher yield relative to the control (Table 1), independently of treatment and the Ca content obtained from tubers (Figure 1). In fact, the increase in tuber yield with the application of calcium nitrate was previously reported by different studies [15,16]. In this context, Ca biofortification, specifically with calcium nitrate treatments, can increase the tubers yield of *Solanum tuberosum* L. (Picasso variety), despite the Ca biofortification index of the tubers.

5. Conclusions

Considering the data obtained in this study, potato (*Solanum tuberosum* L.) is a highly suitable candidate for biofortification, namely in Ca, despite showing different variations in the Ca biofortification index considering the product used (calcium chloride or calcium nitrate). Moreover, the concentration of 6 kg/ha of $CaCl_2$ showed the highest Ca content in Picasso tubers. Additionally, all the treatments with calcium nitrate presented increases in tuber yield, suggesting that calcium nitrate biofortification holds potential for increasing yield in *Solanum tuberosum* L. tubers of Picasso variety. Furthermore, this study highlights the effectiveness of Ca biofortification in enhancing mineral content in *Solanum tuberosum* L. tubers and the need for future optimization of a biofortification workflow with calcium nitrate treatments not only in the Picasso variety, but also in other varieties of *Solanum tuberosum* L.

Supplementary Materials: The presentation materials can be downloaded at: https://www.mdpi.com/article/10.3390/IOCAG2023-15920/s1.

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