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Proceeding

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 yield of tubers of *Solanum tuberosum* L. (Picasso variety) produced in Lourinhã (Portugal) in 2018, focusing on the use of calcium chloride or alternatively, calcium nitrate at different concentrations applied (calcium chloride - 1, 3, 6 and 12 kg/ha or calcium nitrate - 0.5, 1, 2 and 4 kg/ha). Control plants and plants submitted to the different Ca treatments were implemented in plots of 20 x 24 m, having been carried out in quadruplicate (compass 60-80 cm). As such, 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 – 40 %, being the treatment with 6 kg/ha $CaCl_2$ the one which presented the highest Ca content in tubers at harvest and 1 kg/ha CaCl2 the treatment with the lowest Ca biofortification index. However, 6 kg/ha $CaCl_2$ despite presenting the highest Ca content wasn't 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 $Ca(NO_3)_2$)–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 the development of bones and teeth, muscle contraction and in the circulatory system [1-5]. The required amount of Ca intake varies depending on 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's important to have strategies for combating it. As such, to combat Ca deficiency, agronomic biofortification is one of those strategies, considering that 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 that goal of increasing mineral content in edible parts of plants [6], particularly through foliar applications [7]. However, despite being important to obtain an increase in mineral content of crops, it's also essential not to reduce their yield.

In this context, considering that potato (*Solanum tuberosum* L.) holds the third most consumed staple food crop worldwide position, following rice and wheat [8] and ranks as the fourth most cultivated crop, after rice, wheat, and maize [9,10], 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 the Western of Portugal was used to cultivate *Solanum tuberosum* L. (Picasso variety). Throughout the agricultural period from 15th May (planting date) to 25th September 2018 (harvest date) the average daily temperatures oscillated between 15 ^{o}C and 23^{o}C. Four foliar applications after the beginning of tuberization were carried out with 8 to 12 days interval with $CaCl_2$ (1,3,6 and 12 kg/ha) or $Ca(NO_3)_2$ (0.5,1,2 and 4 kg/ha). Moreover, Control plants (which remained untreated with calcium chloride or calcium nitrate) and plants submitted to the different Ca treatments were implemented in plots of 20 x 24 m, having been carried out in quadruplicate (compass 60-80 cm).

2.2. Calcium content in tubers

Tubers after being harvest, were washed, dried at 60^{o}C until constant weight and grounded 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 a AA WinLab software.

2.3. Total yield

In tubers of Picasso variety after harvest, 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

Calcium content in tubers submitted to 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 $Ca(NO_3)_2$, 2kg/ha $Ca(NO_3)_2$ and 4 kg/ha $Ca(NO_3)_2$ presented significantly higher values relative to control. Moreover, with Ca biofortification treatments, the biofortification index varied between 5 – 40 %, being 6 kg/ha $CaCl_2$ the treatment which presented the highest Ca content compared to the remain treatments (**Figure 1**).

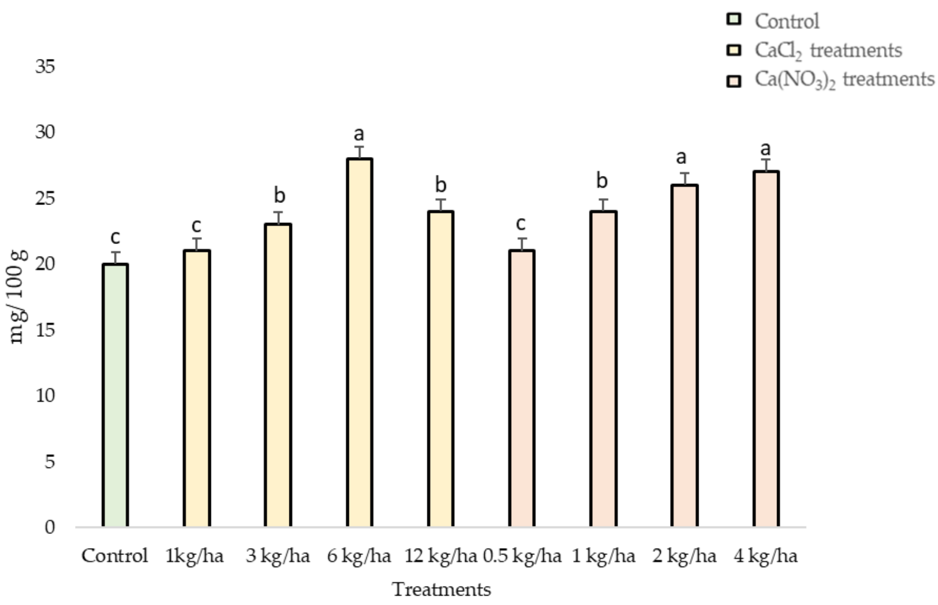

Figure 1. Calcium content (considering dry weight) in tubers of *Solanum tuberosum* L. (Picasso variety) at harvest. Mean values (n=4) ± SE (standard error). Different letters (a,b,c) indicate significant different between treatments.

3.2. *Yield*

Considering the total yield of *Solanum tuberosum* L. tubers from Picasso variety (**Table 1**), only treatments with calcium chloride with the concentrations of 1 and 12 kg/ha presented lowest yield value relatively to control. Moreover, 2 kg/ha of $Ca(NO_3)_2$ treatment showed the highest yield in Picasso variety and $CaCl_2$ treatments only presented an increase in yield between 0.1 – 3.4 % and $Ca(NO_3)_2$ treatments showed an increase which varied between 2.3 – 24.3 %. Additionally, calcium nitrate treatments (0.5, 1, 2 and 4 kg/ha) always showed a yield increase, relatively to 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's obtained through dietary sources [1,5] and being important not only to increase mineral content in crops as well to improve their yield, this study aimed to assess the impact of Ca content (**Figure 1**) and yield (**Table 1**) in *Solanum tuberosum* L. tubers from Picasso variety. As such, the study reveals 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. from Picasso variety. However, contrary to the expected, this treatment did not exhibit the highest yield compared to control (**Table 1**). In fact, 6 kg/ha $CaCl_2$ treatment showed similar values of yield relatively to control, while 3 kg/ha $CaCl_2$ treatment only showed an increase of yield of 3.4 %. Indeed, our data is not in accordance with a previous study carried out with two foliar sprays with 2 and 4 kg/ha of $CaCl_2$ showed an enhanced in tuber yield [13] or even according with another study carried out by [14], which showed improves in tuber yield by foliar sprays with calcium chloride. Furthermore, the third treatment with the highest Ca content (2kg/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 relatively to control (**Table 1**), independently of treatment and the Ca content obtained in tubers (**Figure 1**). In fact, the increase in tuber yield with application of calcium nitrate was already reported by different studies [15,16]. In this context, Ca biofortification, specifically with calcium nitrate treatments can increase tubers yield of *Solanum tuberosum* L. (Picasso variety), despite the Ca biofortification index in 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 of 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 a 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 of future optimization of a biofortification workflow with calcium nitrate treatments not only in Picasso but also in other varieties of *Solanum tuberosum* L..

Supplementary Materials: Not applicable.

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