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Proceeding Paper

Monitoring of a Calcium Biofortification Workflow for Tubers of *Solanum tuberosum* L. cv. Picasso Using Smart Farming Technology †

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Abstract: Due to the rapid growth of the population worldwide and the need to provide food safety in large crop productions, UAVs (unmanned aerial vehicles) are being used in agriculture to provide valuable data for decision making. Accordingly, through precision agriculture, efficient management of resources, using data obtained by the technologies, is possible. Through remote sensed data collected in a crop region, it is possible to create NDVI (normalized difference vegetation index) maps, which are a powerful tool to detect stresses, namely, in plants. Accordingly, using smart farm technology, this study aimed to assess the impact of Ca biofortification on leaves of *Solanum tuberosum* L. cv. Picasso. As such, using an experimental production field of potato tubers (GPS coordinates: 39°16′38,816″ N; 9°15′9128″ W) as a test system, plants were submitted to a Ca biofortification workflow through foliar spraying with $CaCl_2$ or, alternatively, chelated calcium (Ca-EDTA) at concentrations of 12 and 24 kg·ha^{-1}. A lower average NDVI in Ca-EDTA 12 kg·ha^{-1} treatment after the fourth foliar application was found, which, through the application of the CieLab scale, correlated with lower L (darker color) and hue parameters, regarding control plants. Additionally, a higher Ca content was quantified in the leaves. The obtained data are discussed, and it is concluded that Ca-EDTA 12 kg·ha^{-1} triggers lower vigor in Picasso potatoes leaves.

Keywords: calcium biofortification; NDVI; precision agriculture; *Solanum tuberosum* L.

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1. Introduction

Agriculture has evolved in recent years, due to advances in mainly the areas of chemistry and robotics [1]. This evolution is associated with the rapid increase in the

population, with forecasts of the worldwide population reaching nine billion by 2050 [2]. Additionally, this sector is one of the most susceptible, mainly due to challenges related to climate changes [1,3]. Yet, to feed the worldwide population in 2050, agricultural products must increase by 70% [2]. As such, some RGB and multispectral cameras coupled to UAVs (unmanned aerial vehicles) are being used in agriculture, providing valuable data for decision making, namely, to extract vegetation indices that allow the crop status, growth, and vigor to be monitored [1,4].

The normalized difference vegetation index (NDVI) is one of the most used indices [5], being a powerful tool to detect stresses in plants, characterize growth or vigor [4], and to provide information concerning crop diseases, infestations, or nutrient deficiencies [1], and it can estimate primary productivity [6]. In fact, through remote sensed data, vegetation information obtained by NDVI maps is interpreted by considering the differences between the green color of the plant's leaves [4] and the low values corresponding to stress vegetation [5].

Recently, some studies used remote sensing technology to monitor potato crops [6–9]. Potato (*Solanum tuberosum* L.) is one of the most staple food crops consumed worldwide, after rice and wheat [10], and it is the fourth most cultivated (after rice, wheat, and maize) [11,12]. Considering the major consumption of potato all over the world, different studies of mineral enrichments have been carried out, namely, with selenium [13–15], zinc [16,17], iron [17], and calcium [18]. As such, biofortified food has been marketed has functional food, providing a potentially positive effect on human health [19,20]. Nevertheless, considering the new reality of the COVID-19 pandemic crisis, beyond the fact that food safety has a major role in avoiding the spread of the virus between agri-food chain systems, it is expected that there will be an increase in the demand of functional foods [21]. In fact, by 2027, the global market of functional foods is expected to reach USD 309 billion [20,22].

In this context, and taking into account the fact that calcium is an essential mineral for the human body, and has a vital role in anatomy, physiology, and biochemistry [23], associated with the need to improve the production of food with functional characteristics, the aim of this study is to assess the impact of Ca biofortification on *Solanum tuberosum* L. plants of cv. Picasso, using precision agriculture technology.

2. Materials and Methods

2.1. Biofortification Itinerary

The experimental potato field, located in Western Portugal (GPS coordinates: 39°16′38,816″ N; 9°15′9128″ W), was used to grow cv. Picasso (*Solanum tuberosum* L.). After the beginning of tuberization, seven foliar spraying applications (with 6–8 day interval) were performed between 30 May and 12 July with $CaCl_2$ (12 and 24 kg·ha^{-1}). As Ca-EDTA might become highly toxic to plants, only one foliar application of 24 kg·ha^{-1} with Ca-EDTA was carried out, whereas with 12 kg·ha^{-1} seven spraying applications were performed. Control plants were not sprayed at any time with $CaCl_2$ or Ca-EDTA. All treatments were performed in quadruplicate.

During the agricultural period, from 21 March (planting date) to 9 August 2019 (harvest date), air temperatures varied between 13.8 and 21.9 °C and the average rainfall was 0.51 mm, with a daily maximum of 10.4 mm.

2.2. NDVI (Normalized Difference Vegetation Index) in the Experimental Field

The experimental field was flown over once with UAV (unmanned aerial vehicle), equipped with altimetric measurement sensors, and synchronized by GPS. The flight was performed on 25 June (four days after the 4th foliar application) to characterize vegetation indexes, to monitor differences in vigor between control and sprayed plants. The images were processed in ArcGIS Pro.

2.3. Calcium Content in Leaves

Calcium contents were determined in leaves after the 4th foliar application (21 June), being cut, dried (at 60 °C, until constant weight) and grounded, using a XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, according to [24].

2.4. Colorimetric Parameters

Colorimetric parameters were determined in leaves (dried at 60 °C, until constant weight), after the 4th foliar application, using a Minolta CR 400 colorimeter (Minolta Corp., Ramsey, NJ, USA) coupled to a sample vessel (CR-A504), according to [25]. Measurements were carried out in quadruplicate.

2.5. Statistical Analysis

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

3. Results

To monitor the calcium biofortification workflow in the experimental field, the NDVI map was obtained four days after the fourth foliar application (Figure 1), with a lower NDVI being identified in area 1, corresponding to the treatment with 12 kg·ha^{-1} Ca-EDTA.

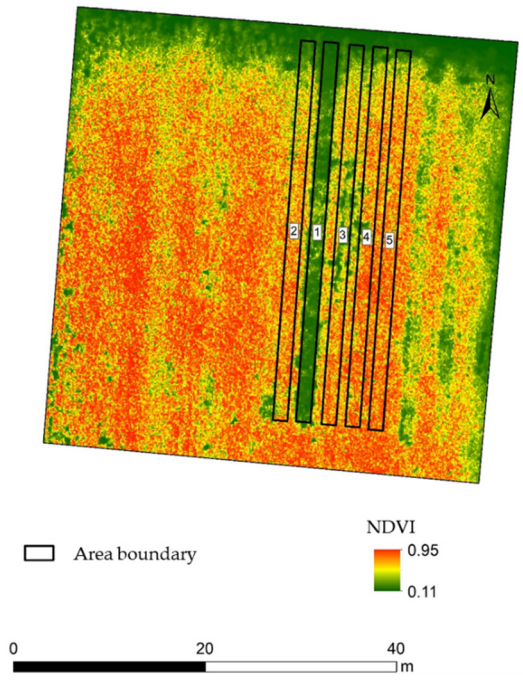


Figure 1. NDVI (normalized difference vegetation index) map in plants of *Solanum tuberosum* L., cv. Picasso (obtained on 25 June 2019), after the 4th foliar application (1—plants sprayed with Ca-EDTA 12 kg·ha^{-1}; 2—plants sprayed with Ca-EDTA 24 kg·ha^{-1}; 3—control plants (not sprayed); 4—plants sprayed with $CaCl_2$ 24 kg·ha^{-1}; 5—plants sprayed with $CaCl_2$ 12 kg·ha^{-1}).

Additionally, from the NDVI map, the average NDVI for each treatment was calculated (Figure 2). The treatment with 12 kg·ha^{-1} Ca-EDTA (Figure 1) showed a lower average NDVI, relative to the remaining treatments, and was the only treatment that showed a lower average than the control plants. A higher average NDVI was obtained with 12 kg·ha^{-1} $CaCl_2$.

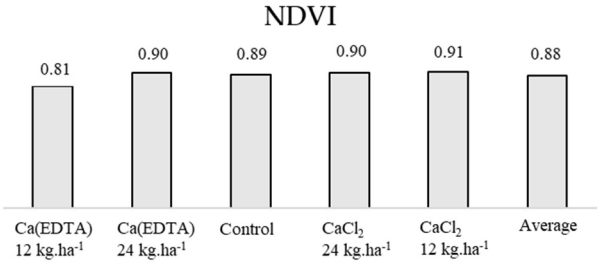


Figure 2. Values of the average NDVI of each treatment and the average of the different treatments and control in plants of *Solanum tuberosum* L., cv. Picasso (obtained on 25 June 2019), after the 4th foliar application.

Calcium content was assessed in leaves of *Solanum tuberosum* L., cv. Picasso after the fourth foliar application (Table 1). Relative to the control, the contents of Ca in the leaves was significantly higher in all the treatments, with an increase ranging between 29.8 and 89.7%. The maximum Ca content in the leaves was obtained with 12 kg·ha^{-1} Ca-EDTA, despite this showing a lower NDVI.

Table 1. Mean values ± S.E. (n = 4) of Ca in leaves of *Solanum tuberosum* L., cv. Picasso, after the 4th foliar application.

Treatments	Ca (%)
Control	4.29 d ± 0.17
$CaCl_2$ (12 kg ha^{-1})	6.05 b ± 0.00
$CaCl_2$ (24 kg ha^{-1})	7.94 a ± 0.01
Ca-EDTA (12 kg ha^{-1})	8.14 a ± 0.02
Ca-EDTA (24 kg ha^{-1})	5.57 c ± 0.01

Different letters indicate significant differences of each parameter between treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$). Foliar spray application was carried out with two concentrations (12 and 24 kg·ha^{-1}) of $CaCl_2$ and Ca-EDTA. Control was not sprayed.

Independently of the Ca content in the leaves, colorimetric parameters were assessed in dry leaves (Table 2). The control showed significantly higher values in L, chroma and hue parameters. Regarding L and chroma, the 24 kg·ha^{-1} $CaCl_2$ treatment showed significantly lower values, followed by 12 kg·ha^{-1} $CaCl_2$ and 12 kg·ha^{-1} Ca-EDTA. Regarding the hue parameter, the 12 kg·ha^{-1} Ca-EDTA treatment showed a significantly lower value.

Table 2. Mean values ± S.E. (n = 4) of colorimetric parameters (L, chroma and hue) in dry leaves of *Solanum tuberosum* L., cv. Picasso, after the 4th foliar application.

Treatments	L	Chroma	Hue
Control	42.61 a ± 0.03	28.77 a ± 0.01	112.2 a ± 0.02
$CaCl_2$ (12 kg ha^{-1})	33.25 d ± 0.00	16.66 d ± 0.00	95.66 c ± 0.05
$CaCl_2$ (24 kg ha^{-1})	32.62 e ± 0.00	14.85 e ± 0.00	85.05 d ± 0.01
Ca-EDTA (12 kg ha^{-1})	33.50 c ± 0.01	17.68 c ± 0.01	83.19 e ± 0.04
Ca-EDTA (24 kg ha^{-1})	38.22 b ± 0.01	22.80 b ± 0.00	106.3 b ± 0.00

Different letters indicate significant differences of each parameter between treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$). Foliar spray application was carried out with two concentrations (12 and 24 kg·ha^{-1}) of $CaCl_2$ and Ca-EDTA. Control was not sprayed.

4. Discussion

Calcium is an essential macronutrient for plants and for humans [18,23]. This nutrient plays an important role in several physiological processes, namely, growth and development in plants. In fact, Ca^{2+} is implicated in the regulation of the photosynthetic pathway [26–28]. In potato tubers, Ca accumulation depends on xylem and phloem delivery (despite the fact that they are almost immobile). Foliar spraying with Ca in potato

plants can complement the xylem mass flow of Ca through phloem redistribution [18]. In this context, when a Ca biofortification workflow is implemented, it is important to monitor the growth and development of plants. As such, four days after the fourth foliar application of Ca, using the UAV, the NDVI maps were obtained, as well as the quantification of NDVI in each treatment (Figures 1 and 2). The area corresponding to the treatment with 12 kg·ha^{-1} Ca-EDTA (Figure 1) shows a lower NDVI, relative to the remaining treatments. Additionally, by calculating the average NDVI in each treatment (Figure 2), it is possible to verify an NDVI lower than the control plants. In fact, in biofortification workflows, foliar spraying with Ca is more often applied as a salt, mainly as $CaCl_2$ [29]. Although, recently, some studies have been carried out with chelated Ca sources, mainly Ca-EDTA [29–31]; this compound can cause toxicity (promoting chlorosis and necrosis on the edges of leaves) [29]. As such, the NDVI map (Figure 1) shows the beginning of toxicity symptoms in potato plants with 12 kg·ha^{-1} Ca-EDTA treatment, after four foliar applications. Yet, globally, considering the average of all the treatments, potato plants showed a positive response after the fourth foliar application with $CaCl_2$ and Ca-EDTA, showing a medium/high NDVI (>0.8).

Regarding the Ca content in the leaves of potato plants (Table 1), all the treatments sprayed with Ca showed a higher content than the control. Yet, considering the plants sprayed with 12 kg·ha^{-1} Ca-EDTA, despite the symptoms of toxicity (Figure 1), they showed a higher content of Ca. This tendency of Ca accumulation with 12 kg·ha^{-1} Ca-EDTA was observed in potatoes at harvest [25]. Considering colorimetric parameters (Table 2), higher values of the L, chroma and hue parameters were obtained in the control leaves, corresponding to the lower Ca content (Table 1). Yet, despite that, the 12 kg·ha^{-1} Ca-EDTA treatment showed the highest Ca content, but revealed the lowest values of the hue parameter, and an intermediate value in the L and chroma parameters.

5. Conclusions

The biofortification workflow showed an increase in Ca content in the leaves of *Solanum tuberosum* cv. Picasso, relative to the control plants. In this context, it was possible to conclude that the NDVI values allowed more accurate monitoring than the analysis of the colorimetric parameters of the leaves, regarding the beginning of toxicity symptoms (i.e., lower vigor in potatoes leaves). As such, smart farming technology is an important tool for decision making, regarding crop monitoring.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECAG2021-09660/s1.

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