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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 of providing 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, an 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, namely stresses in plants. Accordingly, using some Smart Farm technology, this study aimed to access the impact of Ca biofortification process in leaves of *Solanum tuberosum* L. cv. Picasso. As such, using as a test system, an experimental production field of potato tubers (GPS coordinates - 39º 16′ 38,816′′ N; 9º 15′ 9,128′′W), 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}. It was found a lower average of NDVI in Ca(EDTA) 12 kg.ha^{-1} treatment after the 4th foliar application, which through the application of the CieLab scale correlated with a lower L (darker color) and hue parameters, regarding control plants. Additionally, a higher Ca content was quantified in the leaves. The obtained data is discussed, being concluded that Ca(EDTA) 12 kg.ha^{-1} triggers a 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 mainly in chemistry and robotics areas [1]. This evolution is associated with the rapid increase of the population, with forecasts of a worldwide population reaching 9 billion by 2050 [2]. Additionally, this sector it's 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 allows monitoring the crop status, growth, and vigor [1,4].

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 can estimate primary productivity [6]. In fact, through remote sensed data, vegetation information obtained by NDVI maps it's interpreted considering the differences between the green color of the plants leaves [4], with 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's the 4th 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 a COVID-19 pandemic crisis, beyond the fact that food safety has a major role in avoid the spreading of the virus between agri-food chain system, it's expected an increase in the demand of functional foods [21]. In fact, by 2027 the global market of functional food is expected to reach $309 billion [20,22].

In this context, being calcium an essential mineral for human body and having a vital role in the anatomy, physiology, and biochemistry [23], associated with the need of improving the food production with functional characteristics, the aim of this study is to access the impact of Ca biofortification process in *Solanum tuberosum* L. plants of cv. Picasso, using some precision agriculture technology.

2. Materials and Methods

2.1. Biofortification itinerary

The experimental potato field, located in the Western of Portugal (GPS coordinates - 39º 16′ 38,816′′ N; 9º 15′ 9,128′′W), was used to growth cv. Picasso (*Solanum tuberosum* L.). After the beginning of tuberization, seven foliar spraying (with 6-8 days interval) were performed between 30th May and 12th 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 21st March (planting date) to 9th August of 2019 (harvest date), air temperatures varied between 13.8 – 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 flow over once with UAV (Unmanned Aerial Vehicle), equipped with altimetric measurement sensors and synchronized by GPS. The flight was performed in 25th 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 (21th 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 60ºC, 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 a One-Way ANOVA to assess differences among treatments in cv. Picasso, followed by a Tukey's for mean comparison. A 95% confidence level was adopted for all tests.

3. Results

To monitore the calcium biofortification workflow in the experimental field, the NDVI map was obtained four days after the 4th foliar application (Figure 1), being possible to identify a lower NDVI in area 1, corresponding to the treatment with 12 kg.ha-1 Ca-EDTA.

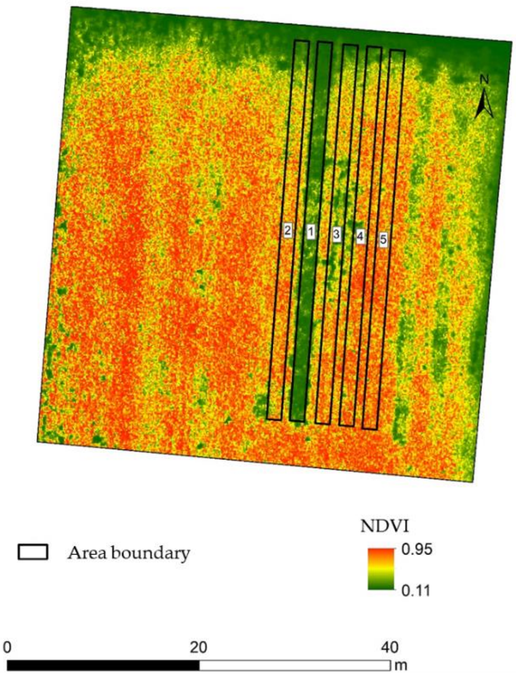

Figure 1. NDVI (Normalized Difference Vegetation Index) map in plants of *Solanum tuberosum* L., cv. Picasso (obtained on 25th June 2019), after the 4th foliar application (1- plants sprayed with Ca(EDTA) 12 $kg.ha^{-1}$; 2- plants sprayed with Ca(EDTA) 24 $kg.ha^{-1}$; 3- Control plants (not sprayed); 4- plants sprayed with $CaCl_2$ 24 $kg.ha^{-1}$ and 5- plants sprayed with $CaCl_2$ 12 $kg.ha^{-1}$).

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

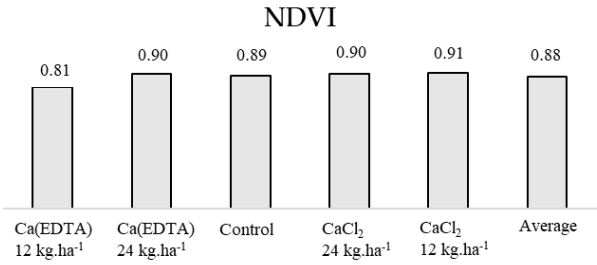

Figure 2. Values of the average of NDVI of each treatment and the average of the different treatments and control, in plants of *Solanum tuberosum* L., cv. Picasso (obtained in 25th June 2019), after the 4th foliar application.

Calcium content was assessed in leaves of *Solanum tuberosum* L., cv. Picasso, after the 4th foliar application (Table 1). Relatively to the control, the contents of Ca in the leaves was significantly higher in all treatments, with an increase ranging between 29.8 - 89.7%. Besides, the maximum Ca content in the leaves was obtained with 12 kg.ha^{-1} Ca-EDTA, in spite of 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	4.29d ± 0.17
$CaCl_2$ (12 kg ha^{-1})	6.05b ± 0.00
$CaCl_2$ (24 kg ha^{-1})	7.94a ± 0.01
Ca(EDTA) (12 kg ha^{-1})	8.14a ± 0.02
Ca(EDTA) (24 kg ha^{-1})	5.57c ± 0.01

Different Letters indicates significant differences, of each parameter, between treatments (statistical analysis using the single factor ANOVA test, P ≤0.05). Foliar spray 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). Control showed significant higher values in L, Chroma and Hue parameter. Regarding L and Chroma, 24 kg.ha^{-1} $CaCl_2$ treatment showed significant lower values, followed by 12 kg.ha^{-1} $CaCl_2$ and 12 kg.ha^{-1} Ca-EDTA. Considering Hue parameter, the 12 kg.ha^{-1} Ca-EDTA treatment showed a significant 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	42.61a ± 0.03	28.77a ± 0.01	112.2a ± 0.02
$CaCl_2$ (12 kg ha^{-1})	33.25d ± 0.00	16.66d ± 0.00	95.66c ± 0.05
$CaCl_2$ (24 kg ha^{-1})	32.62e ± 0.00	14.85e ± 0.00	85.05d ± 0.01
Ca(EDTA) (12 kg ha^{-1})	33.50c ± 0.01	17.68c ± 0.01	83.19e ± 0.04
Ca(EDTA) (24 kg ha^{-1})	38.22b ± 0.01	22.80b ± 0.00	106.3b ± 0.00

Different letters indicates significant differences, of each parameter, between treatments (statistical analysis using the single factor ANOVA test, P ≤0.05). Foliar spray 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 as it is 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 delivery and phloem (despite it is almost immobile). Yet, foliar spraying with Ca in potato plants can complement the xylem mass flow of Ca though phloem redistribution [18]. In this context, when a Ca biofortification workflow is implemented, it's important to monitor the growth and development of plants. As such, four days after the 4th foliar application of Ca, thought UAV, the NDVI maps were obtained, as well as the quantification of NDVI in each treatment (Figure 1 and 2). The area corresponding to the treatment with 12 $kg.ha^{-1}$ Ca-EDTA (Figure 1) shows a lower NDVI relatively to the remaining treatments. Additionally, thought the average of NDVI in each treatment (Figure 2) it is possible to verify a NDVI lower than control plants. In fact, in biofortification workflows, foliar spraying with Ca is applied more often as salts, 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 treatments, potato plants showed a positive response after the 4th 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 higher content than control. Yet, considering the plants sprayed with 12 $kg.ha^{-1}$ Ca-EDTA, despite the symptoms of toxicity (Figure 1), showed the higher content of Ca. This tendency of Ca accumulation with 12 $kg.ha^{-1}$ Ca-EDTA was observed in potatoes at harvest [25]. Considering the colorimetric parameters (Table 2) the higher values in L, Chroma and Hue parameters were obtained in 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 Hue parameter and an intermediate value in L and Chroma parameters.

5. Conclusion

Biofortification workflow showed an increased in Ca content in leaves for *Solanum tuberosum* cv. Picasso, relatively to the control plants. In this context it was possible to concluded that NDVI values allowed a 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). In this context, the use of smart farming technology is an important tool for decision making, regarding crop monitoring.

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