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Proceeding Paper

Precision Agriculture in Rice (*Oryza sativa* L.) Biofortified with Selenium †

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Abstract: Remote sensing data is a powerful tool that contributes to sustainability and efficiency in crop management. Rice (*Oryza sativa* L.) is widely recognized as one of the most important crops in terms of economic and social impact. The aim of this study was to evaluate the efficiency of the use of an Unmanned Aerial Vehicles (UAVs) in two rice varieties (Ariete and Ceres) submitted to a biofortification workflow with two types of selenium (sodium selenate and sodium selenite) in providing valuable information regarding plant health and status. In this context, through the use of an UAVs synchronized, the state of the culture was further assessed, digital elevation model, water lines, slope classes / infiltration suitability and Normalized Difference Vegetation Index (NDVI) were considered. Additionally, leaf gas exchange measurements were conducted during the biofortification process and Se content in rice was quantified. The NDVI index ranged from 0.76 to 0.80 without significant differences regarding control. It was observed that the water drainage pattern following the artificial pattern created by grooves between plots. Furthermore, selenite application up to 100 g Se.ha^{-1} did not exhibit toxicity effects on the biofortified plants and presented a grain enriched of 16.09 $\mu g\ g^{-1}$ (Ariete) and 15.46 $\mu g\ g^{-1}$ (Ceres). In conclusion, through precision agriculture techniques and utilizing data from leaf gas exchanges allows an efficient monitoring of the experimental field conditions and is a highly useful tool in decision making.

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1. Introduction

Several state-of-the-art technologies linked to remote sensing have been incorporated into agriculture [1], including the use of Unmanned Aerial Vehicles (UAVs) images. Using this technology, it is possible to obtain orthophotomaps, digital elevation models, water surface drainage, and slope useful for delimiting cultivation areas. In addition, assessing the condition of plants, detecting pests, and locating weeds is also possible using UAVs [2]. Normalized difference vegetation index (NDVI) is used to monitor different crops such as rice (*Oryza sativa* L.), maize, barley, and oats. In addition, strategies have been developed such as agronomic biofortification, which with the application of sodium selenite and sodium selenate increases the Selenium (Se) content in staple foods such as rice [3, 4].

Considering the importance of remote sensing data, this work aimed to use precision agriculture to evaluate the conditions of paddy rice field, and monitor the vigor status of the plants submitted to Se biofortification.

2. Materials and Methods

2.1. Experimental Fields and Selenium Biofortification

The trials were conducted in the middle of Ribatejo (Portugal) at the Rice Competence Center (COTArroz) located in Salvaterra de Magos. The varieties Ariete and Ceres were used as a system test. During the crop growing season (30 th May to 2 nd November 2018), the agronomic biofortification using sodium selenate and sodium selenite were applied at 25, 50, 75, and 100 g $Se.ha^{-1}$ through foliar pulverization. Selenium applications occurred at the end of booting, anthesis, and at the milky grain stages. The experimental design was performed in a factorial arrangement (5 concentrations x 2 forms selenium x 2 varieties x 4 replicates in a total of 80 plots). The plot size for each replication was 8 m length × 1.2 m width = 9.6 m^2.

In the Ariete variety foliar fertilizations with Se occurred on 23 rd August, 31st August, and 14 th September, whereas in the Ceres variety, the applications were made on 28 th August, 6 th and 20 th September.

2.2. Precision Agriculture - Experimental fields and monitor the state of the rice culture

The experimental field was flow with Unmanned Aerial Vehicles (UAVs) synchronized by GPS as described by [5]. For morphological characterization (digital elevation model, water lines, and slope classes / infiltration suitability) the flight was performed before the implementation of the culture in the field on 18 th May. To monitor the vigor of the different plants submitted to the biofortification, UAVs were used to characterize the vegetation index (NDVI), on 12 th November.

2.3. Leaf Gas Exchange Measurements

According to the methods described by [6], the leaf gas exchange parameters were determined in the trial rice field, using 4 – 6 randomized leaves per treatment, on 12 th September (after 2nd Se application).

Leaf rates of net photosynthesis - Pn, stomatal conductance to water vapor – gs, and transpiration - E were obtained under photosynthetic steady-state conditions (after *ca.* 2 h of illumination). A portable open-system infrared gas analyzer (Li-Cor 6400, LiCor, Lincoln, NE, USA) was used under environmental conditions, with photosynthetic photon flux density (PPFD) of *ca.* 1000 $\mu mol\ m^{-2}.s^{-1}$ and external CO_2.

2.4. Analysis of Selenium content

The quantification of Se content in the samples of paddy rice (controls and after foliar spraying with Na_2SeO_4 / Na_2SeO_3) was measured by Energy Dispersive X-Ray Fluorescence (μ-EDXRF system, M4 Tornado™) following Cardoso et al. [7]. To improve the quantification of Se, a set of filters of three foils of Al / Ti / Cu was used between the X-ray tube and the sample.

2.4. Statistical Analysis

A One-way ANOVA ($p \leq 0.05$) was performed with the IBM SPSS Statistics 20 program. and the Tukey's test for mean comparison was used considering 95 % confidence level.

3. Results

The elevation model (Figure 1) shows the average and minimum elevation zones associated with the location of the paddy rice field. The direction of water lines suggests that if surface drainage is present, it is likely to follow the trajectory of the estimated water lines. The experimental field has a slope of about 5 %, which results in reduced surface drainage.

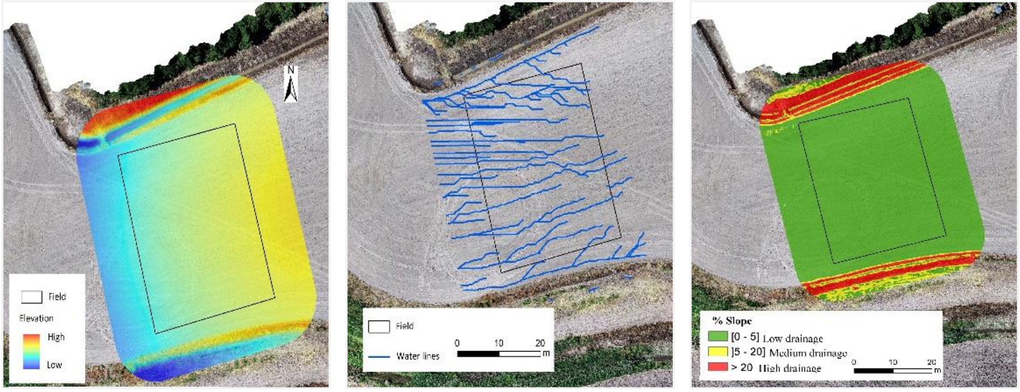

Figure 1. Orthophotomaps of digital elevation model (1), water lines (2) and slope classes / infiltration suitability (3) at 18th May.

Regarding NDVI values, no significant changes were observed in the selenium (Se) treatments when compared to the control in the different varieties (Figure 2). The values ranged from 0.76 to 0.80. The maximum value was obtained in the control plants and with selenite application, in both varieties.

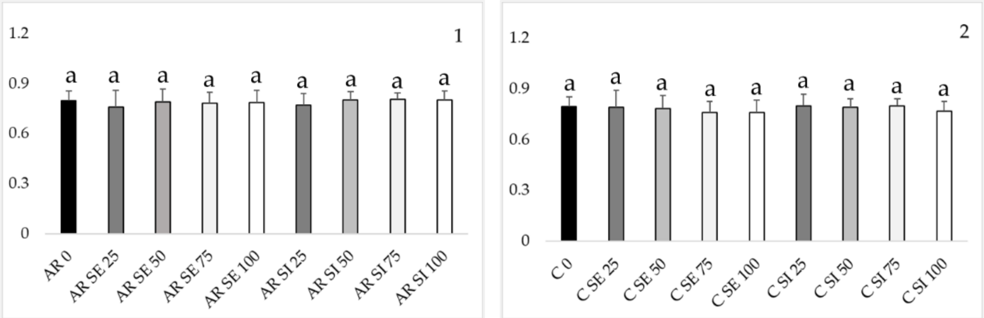

Figure 2. Mean values of normalized vegetation index (NDVI) ± standard deviation. Information collected at 12th September. Obtained from images of UAVs (n = 12), from *Oryza sativa* L. (Ariete and Ceres variety) submitted to foliar fertilization with sodium selenate and sodium selenite. Letter *a* revealed the absence of significant differences among treatments of each variety (single factor ANOVA test - $p \leq 0.05$).

Physiological data were acquired after the 2nd foliar fertilization with Se in rice (Table 1). In the Ariete variety, the net photosynthesis (P_n) values did not show significant differences between treatments. However, in the Ceres variety, the values were higher than the control in all treatments, with the maximum values obtained in the treatment with 100 g Se.ha^{-1} of selenite (17.82 µmol CO_2 m^{-2}.s^{-1}). This positive effect on Pn was found along with higher stomatal conductance to water vapor (gs) and lower instantaneous water use efficiency (iWUE). The maximum gs value in the Ariete variety was 368.6 mmol H_2O m^{-2}.s^{-1} in plants spayed with 100 g Se.ha^{-1} of selenite while transpiration (E) showed 6.66 mmol H_2O m^{-2}.s^{-1} . In the Ceres variety, it was in the same treatment that the highest value of E was obtained (6.81 mmol H_2O m^{-2}.s^{-1}). Regarding transpiration (E) was observed an increase in both varieties regarding control. A significant and gradual decrease in lower instantaneous water use efficiency (iWUE) was observed in all plants.

Table 1. Leaf gas exchange parameters: net photosynthesis (Pn), stomatal conductance to water vapor (gs), transpiration (E) rates, and instantaneous water use efficiency (iWUE = Pn/E). Analyses performed on leaves of the Ariete and Ceres varieties at 12th September, after 2nd Se application, of sodium selenate (selenate) and sodium selenite (selenite) at 50 and 100 g Se.ha^{-1}.

Fertilization	**Ariete**	**Ceres**
Pn (µmol CO_2 m^{-2}.s^{-1})		
Control	15.80 ± 0.24a[1]	16.66 ± 0.32c
Selenate 50	16.89 ± 0.70a	17.20 ± 0.33ab
Selenate 100	16.43 ± 0.36a	17.60 ± 0.31a
Selenite 50	16.70 ± 0.21a	16.84 ± 0.68bc
Selenite 100	16.18 ± 0.24a	17.82 ± 0.10a
gs (mmol H_2O m^{-2}.s^{-1})		
Control	182.4 ± 5.9c	242.9 ± 8.7b
Selenate 50	307.4 ± 11.0ab	266.0 ± 3.3ab
Selenate 100	320.7 ± 18.7b	275.3 ± 4.4ab
Selenite 50	280.6 ± 1.4b	263.4 ± 24.8ab
Selenite 100	368.6 ± 23.0a	313.7 ± 15.3a
E (mmol H_2O m^{-2}.s^{-1})		
Control	3.81 ± 0.06d	5.86 ± 0.13b
Selenate 50	5.56 ± 0.10c	5.99 ± 0.01b
Selenate 100	5.90 ± 0.20b	6.28 ± 0.09ab
Selenite 50	5.13 ± 0.02bc	6.10 ± 0.29ab
Selenite 100	6.66 ± 0.20a	6.81 ± 0.15a
iWUE (mmol CO_2 m^{-2}.s^{-1} H_2O)		
Control	4.15 ± 0,01a	2.84 ± 0.01ab
Selenate 50	3.03 ± 0,06c	2.86 ± 0.05a
Selenate 100	2.81 ± 0,08d	2.80 ± 0.03b
Selenite 50	3.25 ± 0,03b	2.77 ± 0.03b
Selenite 100	2.44 ± 0,05e	2.63 ± 0.05b

[1] Letters *a*, *b*, *c*, *d* and *e* indicate significant differences between treatments for each variety (single factor ANOVA test - $p \leq 0.05$).

The application of increasing concentrations of Se, in both forms, allowed the gradual increase of this element in the paddy rice grain (Figure 3). In both varieties, selenate application showed significant differences compared to the control, however, the increment of Se in the grain was lower when compared to the selenite form. In the Ariete variety, by applying selenite at 100 g Se.ha^{-1}, 16.09 µg g^{-1} was obtained in the grain. Ceres variety showed a higher value in selenite treatment (15.49 µg g^{-1}), while in selenate treatment the maximum value was 6.25 µg g^{-1}.

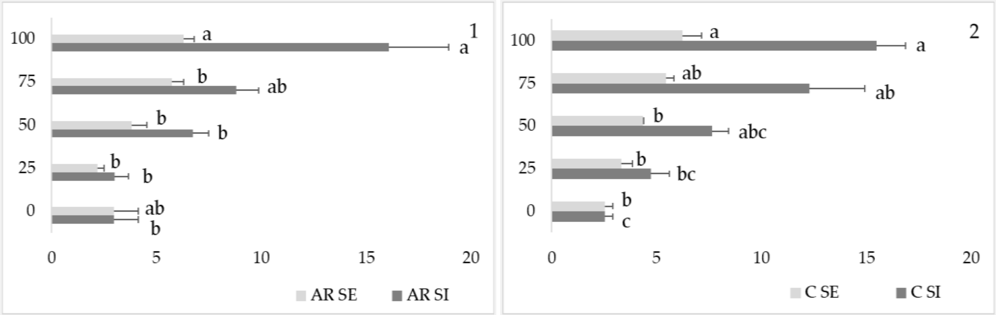

Figure 3. Mean values of Se contents ± S.D. (n = 4) in paddy rice of O. *sativa* control, Ariete (1) and Ceres (2) varieties. Letters *a* and *b* indicate significant differences between treatments for each variety (single factor ANOVA test - $p \leq 0.05$).

4. Discussion

Studies showed that the morphology of the terrain, namely the slope and orientation of the terrain, directly influences the water runoff pattern [8]. In this study, the results indicate that the paddy rice field has an elevation ranging from minimal to medium (Figure 1). In addition, the runoff pattern created by the water lines is visible and follows the elevation of the field (Figure 1). The field is suitable for growing this cereal, considering its location, soft morphology, slope variation, and the estimated potential for surface water infiltration. Considering 5% of water infiltration capacity, the field presents reduced surface drainage. Thus, water accumulation is promoted, a fundamental aspect of the practices used in rice cultivation [9]. The use of NDVI data in agriculture provides useful information about crop monitoring and aids decision-making. Studies have linked NDVI values with yields of maize, wheat, and rice [10]. Other studies, have used NDVI to monitor vegetation density and relate declines in rice yield to increases in nocturnal temperature [11]. In our study, the NDVI values ranged from 0.76 – 0.80 without significant differences regarding control (Figure 2). The highest NDVI values were obtained in control plants and after application of the selenium (Se) biofortification which indicates healthy rice plants. The plants did not show a negative impact on net photosynthesis (P_n) after Se pulverization, regardless of the dose, however, show a marginal increase in both varieties. The Ceres plants showed a positive impact on P_n, and a slight increase, regarding control (Table 1). In addition, the increase in stomatal conductance to water vapor (g_s) and transpiration (E) values, followed the increase in applied concentrations. Leaf instantaneous water-use efficiency (iWUE) represents the units of assimilated CO_2 per unit of water lost through transpiration and was calculated as the P_n/E ratio. The decrease in this parameter is associated with the increase in the applied concentration of these forms. Comparing NDVI data with gas exchange parameters it is possible to infer that Se stimulates net photosynthesis. The literature reports that damage to the photosynthetic apparatus can be reduced by the addition of suitable levels of Se in cereals [12], namely rice [13]. Additionally, plant growth is also promoted to increase crop quality [14]. Both varieties showed a significant increase in Se compared to the applied form (Figure 3). The highest contents were obtained by applying 100 g Se.ha^{-1} of Na_2SeO_3 in the Ariete (16.09 µg g^{-1}) and Ceres (15.49 µg g^{-1}) varieties. These results are in agreement with other studies on rice that demonstrated the higher efficiency of Na_2SeO_3 than Na_2SeO_4 [15]. Thus, the vigor of the plant was not affected by the biofortification route, allowing the increase of Se in the grain without interfering negatively with the photosynthetic mechanism.

5. Conclusions

Using the Unmanned Aerial Vehicle (UAVs), it was possible to map the site where the rice (*Oryza sativa* L.) biofortification itinerary was implemented. Normalized Difference Vegetation Index (NDVI) data, photosynthesis analysis, and selenium (Se) concentration in the grain were integrated. Furthermore, Se application up to 100 g Se.ha^{-1} did not exhibit toxicity effects on the biofortified plants. With the application of selenite, grain enriched in 16.09 $\mu g\ g^{-1}$ (Ariete) and 15.46 $\mu g\ g^{-1}$ (Ceres) was obtained. In conclusion, precision agriculture techniques and utilizing data from leaf gas exchanges allow an efficient monitoring of the experimental field conditions and is a highly useful tool in decision making.

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