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Precision Agriculture as input for the Rice Grain (*Oryza sativa* L.) Biofortification with Selenium[†]

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Abstract: With population growth worldwide, the production of quality and quantity food is increasingly pressing**.** As such, it becomes essential to develop new agricultural technologies to increase productivity. Under this assumption, an agronomic workflow for Se biofortification of two genotypes resulting from genetic breeding (OP1505 and OP1509) were selected for evaluation through foliar fertilization with sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) with different concentrations (300 and 500 g $Se.ha^{-1}$). Aiming to characterize, through precision agriculture, the experimental fields production and monitor the state of the culture (slope, surface drainage, water lines and normalized differences vegetation index - NDVI), an Unmanned Aerial Vehicles (UAVs) synchronized by global positioning system (GPS) was used. It was found that after sown, the water drainage pattern became profoundly altered, following the artificial pattern, created by the grooves between plots. NDVI values, compared to the control, did not show significant differences. These data were correlated with physiological monitoring during biofortification. In fact, as shown by the eco-physiological data obtained through leaf gas exchanges, the application of 300 g $Se.ha^{-1}$ did not show any toxicity effects in the biofortified plants. In a context of innovation, it was concluded that the use of precision agriculture techniques in conjunction with leaf gas exchanges measurements allowed an efficient monitoring of the field conditions and culture to implement a rice biofortification itinerary.

Keywords: leaf gas exchanges; photosynthesis; precision agriculture; rice genotypes; selenium biofortification

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1. Introduction

Agriculture is incorporating several last generation technologies, such as precision agriculture, to enhance the productivity: meeting the growing food needs with the limited resources of the planet [1]. Precision agriculture involves the acquisition and processing of data related, not only to the field (water surface drainage, elevation and slope), but also to the plant health at multiple growth levels (the presence of pests and weeds, the content of chlorophyll in plants and some climatic conditions) [1,2].

The presence of Se in plants is scarce but it is an essential element in the human diet [3]. Staple foods, namely rice, with a low content of micronutrients, like Se, is to some extend adding deficiencies in more than half of the world population [4], yet through agronomic biofortification it is possible to increase the its intake by humans [5]. The application of Se (in the form of sodium selenate and sodium selenite) is a well-known method to increase the Se concentration in food crops such as rice [6]. Considering the increasing importance of remote sensing and *Oryza sativa* L., this work aimed to use new state-of-the-art technologies to complement an workflow of agronomic biofortification for rice grains (by foliar application of sodium selenate and sodium selenite), while evaluating the morphological conditions of the field, plant vigor, photosynthetic metabolism and the accumulation of Se in whole flour.

2. Materials and Methods

2.1. Experimental Fields

The trial was conducted, from 04 June to 23 October of 2019. Field trials were carried out at the Rice Technological Center (COTArroz) located in the middle of the Lezíria Ribatejana – Portugal. Two new advanced rice lines (OP1505 and OP1509) of the breeding program carried out by the Instituto Nacional de Investigação Agrária e Veterinária (INIAV, Elvas, Portugal) were used. During that period, air temperatures reached an average daily of 26 ◦C and 17 ◦C (with maximum and minimum values of 35 ◦C and 22 ◦C, respectively). The average rainfall was 0.49 mm, with a daily maximum of 24 mm and an accumulation of 0.5 mm. Biofortification was carried out by foliar application of sodium selenate and sodium selenite with three replicates per genotype. The experimental design was performed in randomized blocks and a factorial arrangement (2 concentrations, 2 forms selenium, 2 genotypes, 4 replicates in a total of 32 plots). The plot size for each replication was (8 m × 1.2 m = 9.6 m^2). The agronomic management of trials, namely the application of control of weeds, nitrogen fertilizers, diseases, insect pests and the water irrigation were typically and recommended used for rice crops. First selenium application (occurred at the end of booting) with a concentration of 500 g $Se \cdot ha^{-1}$. In the second application (occurred at anthesis) and the third (at the milky grain stage) the concentration was reduced to 300 g $Se \cdot ha^{-1}$ because the plants showed visual symptoms of phytotoxicity. Grain harvest occurred at 23 October of 2019 for both genotypes.

2.2. Precision Agriculture – Characterize the experimental fields production and monitor the state of the culture

The experimental field was flow over twice with Unmanned Aerial Vehicle (equipped with altimetric measurement sensors) synchronized by GPS, described by Coelho et al. [7]. The first flight was performed before applying the biofortification itinerary for morphological characterization (slopes, surface drainage and water lines) at 18 July 2019, while the second meant to characterize the vegetation index (NDVI), at 25 June 2019, for monitoring the differences in vigor between plants.

2.3. Leaf Gas Exchange Measurements and Analysis of Selenium contents

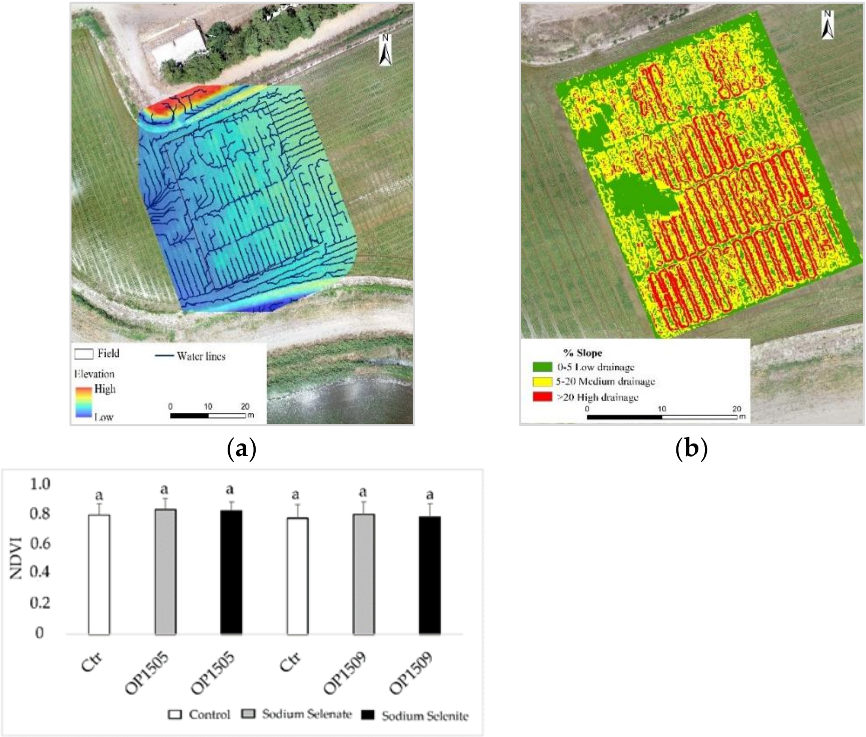
Leaf gas exchange parameters, determined in the trial field, using 6 randomized leaves per treatment, on 17 September, 12 and 15 October, followed the methods described [8]. Selenium content in the whole flour was determined using an XRF analyzer (model XL3t 950 He GOLDD +) under helium atmosphere [9]. For each sample, measurements were carried out in triplicate with emission of radiation for 180 s. For data analysis, the software NITON Data Transfer (XL 3t-36653) was used.

2.4. Statistical Analysis

Statistical analysis of the data was performed with the IBM SPSS Statistics 20 program, through a one-way analysis of variance and the Tukey's test for mean comparison. A value of $P \leq 0.05$ was considered to be significant.

3. Results

The water lines observed in the experimental field are associated with intermediate elevation zones (Figure 1a). The direction of the estimated water lines suggests that if surface drainage exists, it should follow the trend of the estimated water lines for WSE (Figure 1a). After seeding, the drainage pattern is profoundly altered following the artificial pattern created by the existing furrows between plots. After slopes calculation of each plot of land surface drainage zones were differentiated into classes in map (Figure 1b). As expected, given the location and soft morphology of the land, as well as the crop typology, the COTArroz field presents slopes of less than 5%, for the entire limit of the cultivation area, and is therefore considered suitable for rice cultivation, given the estimated potential for infiltration of surface water. The field (Figure 1b) has moderate drainage capabilities therefore promoting runoff (5 - 20 % slop). The field also has areas with 0 - 5 % and more than 20% slope. Regarding NDVI values there were no significant changes (Figure 1c) regarding control. In OP1505 the highest value was in the plants where selenate would be applied showed greater vigor (0.820) while in OP1509 where the selenite treatment was to be applied (0.788), compared to the control.



(**a**) (**b**)

(**c**)

Figure 1. Orthophotomaps of water lines (a), slope / surface drainage (b) - Information collected at 18 June 2019 and mean values of normalized vegetation index (NDVI) ± standard deviation (c) - Information collected at 25 June 2019. Obtained from images of UAVs (n=12) from *O. sativa* OP1505 and OP1509 in the plants where testing with selenate and selenite would be implemented. Letter a revealed the absence of significant differences among treatments of each genotype.

Physiological data were acquired after the 2nd an 3rd leaf application of Se fertilization in rice (Table 1). In the 1st (after 2nd application) analysis at OP1505 the net photosynthesis (P_n) values increased regardless of the form applied. A significant (and gradual) decrease of Pn and stomatal conductance to water vapor (gs) and transpiration (E) was observed in all plants as the end of their life cycle approached. In OP1509 (1st analysis) Pn values increased in selenite treated plants. The positive effect on Pn of applying 500 g Se ha^{-1} (1st application) and 300 g Se ha^{-1} (2nd and 3rd applications) of selenite was extended in the next two evaluations along with higher gs and lower instantaneous water use efficiency (iWUE). The application of selenate had no impact (positive or negative) regarding to the control.

Table 1. Leaf gas exchange parameters - net photosynthesis (Pn), stomatal conductance to water vapor (gs), transpiration (E) rates, and as well as variation in the instantaneous water use efficiency (iWUE=Pn/E) in leaves of *O. sativa* (OP1505 and OP1509) at 1st analysis - after 2nd leaf application (17 September 2019); 2nd and 3rd analysis - after 3rd leaf application (2 and 15 October 2019).

Fertilization	**OP1505**			**OP1509**		
	1st Analysis	**2nd Analysis**	**3rd Analysis**	**1st Analysis**	**2nd Analysis**	**3rd Analysis**
Pn (µmol CO_2 $m^{-2}s^{-1}$)						
Control	15.53 ± 0.75aA[1]	12.43 ± 0.64bA	5.57 ± 0.46cA	13.57 ± 0.33aB	9.61 ± 0.44bB	3.65 ± 0.17cB
Selenate	18.36 ± 0.18aA	11.04 ± 0.76bA	6.17 ± 0.44cA	13.71 ± 0.69aB	7.81 ± 1.06bB	3.11 ± 0.30cB
Selenite	19.20 ± 1.60aA	11.84 ± 0.68bA	6.48 ± 0.28cA	18.19 ± 2.08aA	12.80 ± 0.97bA	8.03 ± 0.81cA
gs (mmol H_2O $m^{-2}s^{-1}$)						
Control	190.0 ± 22.5aB	306.4 ± 41.3aA	150.5 ± 22.3aA	173.1 ± 24.5aB	170.4 ± 18.5aB	87.9 ± 12.9aA
Selenate	332.8 ± 60.8aAB	226.1 ± 33.8abA	120.7 ± 15.5bA	339.0 ± 32.9aA	104.8 ± 17.0bB	84.7 ± 4.6bA
Selenite	418.8 ± 27.2aA	278.7 ± 78.6abA	168.0 ± 48.2bA	399.1 ± 22.9aA	276.0 ± 44.4bA	136.4 ± 15.2cA
E (mmol H_2O $m^{-2}s^{-1}$)						
Control	2.31± 0.23aB	3.62 ± 0.44aB	1.65 ± 0.19aA	2.19 ± 0.30aC	2.49 ± 0.25aB	1.15 ± 0.55aA
Selenate	3.02 ± 0.30aB	2.85 ± 0.31aB	2.07 ± 0.38bA	3.22 ± 0.20aB	1.69 ± 0.22bB	1.70 ± 0.22bA
Selenite	5.12 ± 0.82aA	4.03 ± 0.52aB	1.81 ± 0.17aA	5.80 ± 0.57aA	4.06 ± 0.42aA	1.97 ± 0.16bA
iWUE (mmol CO_2 $m^{-2}s^{-1}$ H_2O)						
Control	7.71 ± 0.75aA	4.12 ± 0.46bA	4.25 ± 0.63bA	8.89 ± 1.39aA	4.60 ± 0.54bA	3.87 ± 0.45bA
Selenate	6.54 ± 0.68aB	4.06 ± 0.29abA	3.50 ± 0.17bA	4.34 ± 0.18aB	4.75 ± 0.58aA	1.91 ± 0.14aA
Selenite	4.18 ± 0.42aC	3.16 ± 0.26aA	3.91 ± 0.67aA	3.14 ± 0.14aB	3.23 ± 0.14aA	4.16 ± 0.32aA

[1] Different letters indicate significant differences between treatments (a, b, c) ant between different moments of analyses (A, B, C), for each genotype (single factor ANOVA test, $P \leq 0.05$).

It was found that, at harvest, the average yields (in kg ha^{-1}) were for OP1505, 7296 and 6785 and for OP1509, 7409 and 6168 (for both genotypes, after application of selenate and selenite, respectively). Foliar fertilization with both forms promoted the accumulation of Se in the whole flour compared to the control (Figure 2). Genotype OP1505 present higher value in selenate treatment (11.9 mg.Kg^{-1}) while OP1509 is statistically differences in both treatments, in particular selenite treatment (8.60 mg.Kg^{-1}), compared to the control.

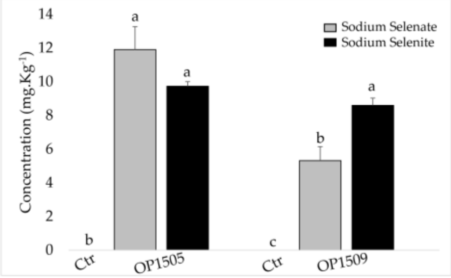


Figure 2. Mean values of Se contents ± S.D. (n = 4) of whole flour of O. *sativa* control (Ctr), genotypes OP1505 and OP1509. Different letters (a, b) indicate significant differences between treatments for each genotype (single factor ANOVA test, $P \leq 0.05$).

4. Discussion

Geomorphology of rice fields strongly affects water surface drainage. After seeding, the drainage pattern is profoundly altered following the artificial pattern created by the existing furrows between plots (Figure 1a). These slope maps were obtained shortly after crop implementation, they allowed the assessment of the initial land state of the field (direct observation of the organoleptic properties of the land state and acquisition an altimetry model). Slope maps were classified in slope classes to differentiate the surface drainage zones from the planar zones (that accumulates surface water). The field also had planar zones with 0 - 5 % slope where low drainage and good conditions for accumulation surface water and, consequently, promote its infiltration (Figure 1b). Additionally, it had areas with a slope greater than 20% where there were opposite conditions to the previous ones. The vegetation index, NDVI, of spectra images captured by drones is used to find the vigor of plants, thus monitoring the health of the crop [10, 11]. The NDVI values are near to 1, ranged between 0.784 – 0.820 in OP1505 and 0.764 – 0.788 in OP1509 (Figure 1c). Based on this, it can be pointed that the plants have greater photosynthetic capacity and consequently higher vigor, which indicates that the drainage network in the experimental field did not negatively affect the vigor of the plants. The data obtained allow us to assume that, at the time of image collection, the plants of both genotypes in each plot were ready for the implementation of the biofortification itinerary. The leaf gas exchange analyses were performed in situ after the 2nd and 3rd foliar Se applications (Table 1). In the genotype OP1505 (after the 2nd application), regardless of the form, the P_n values has increased in the treated plants. The gs rise was paralleled with significant E increases, whereas iWUE was reduced, thus indicating that Se stimulate net photosynthesis. This study also showed that P_n, gs and E values decrease at the end of the life cycle of all plants. In the genotype OP1509 the application of selenate did not show any impact compared to the control during the trial. Additionally, the result of the application of 500 and 300 g Se ha^{-1} of selenite was shown in the consistent positive effect on P_n, higher gs and lower iWUE. These results suggest that the application of selenite preserves the photosynthetic machinery until the end of the life cycle of these plants. Therefore, selenite application in this genotype promoted a positive systematic effect that was reflected in a greater increase of Se in the grain (8.60 mg.Kg^{-1}) and consequently positive impact on grain quality. This study showed that for both genotypes, both forms of the Se applied promoted biofortification (Figure 2). Other studies reported that, in rice, foliar application of sodium selenite is more effective than sodium selenate [12]. The maximum content of Se in the whole flour of OP1509 was with the application of selenite, which is in agreement with the previous study. In the same context, it was observed that the selenite is very mobile and easily absorbed by the plants [13]. Other studies have reported that selenate biofortification is more efficient that selenite [14]. In fact, for the OP1505, the highest biofortification was achieved in selenate treatment (11.9 mg.Kg^{-1}) and consequently higher productivity (7296 Kg.ha^{-1}). This result agrees with the performance of the photosynthetic machinery that suggested the application of this form for this genotype. In general, the dose 300 g Se ha^{-1} can be applied in both genotypes to maximize Se absorption without compromising the photosynthetic machinery.

5. Conclusions

The use of drones with multispectral cameras attached allowed characterizing the field morphology and vigor of rice plants OP1505 and OP1509 for the implementation of biofortification itinerary with Se forms (sodium selenate and sodium selenite). Thereafter, promoting Se biofortification with 500 g Se.ha^{-1} revealed visual symptoms of toxicity but with 300 g Se.ha^{-1} inhibitions at the photosynthetic machinery could not be found. It was concluded that it was possible to obtain higher Se content in rice grain of OP1505 and OP1509 with foliar application of selenate and selenite, respectively.

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