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Proceeding Paper

Monitoring a Zinc Biofortification Workflow in an Experimental Field of *Triticum aestivum* L. Applying Smart Farming Technology †

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Abstract: The strong increase of the human population worldwide is demanding a food production meeting quality standards. In this context, the agronomic biofortification with Zn is widely used in staple food crops as a strategy to surpass micronutrient deficiencies. Conversely, as bread wheat is one of the most produced and consumed cereal, this staple food biofortification can be an opportunity to create an added value product. In this context, a workflow for Zn biofortification of *Triticum aestivum* L. (cvs Paiva and Roxo) crops was implemented in an experimental field located in Beja, Portugal, and smart farming techniques were introduced. Images were collected with cameras coupled to an Unmanned Aerial Vehicle before Zn foliar applications. Grain yield, test weight, and thousand kernel weight were analyzed (post-harvest) after two foliar applications of $ZnSO_4$. Three levels of the factor were used (control–0, 8.1 and 18.2 kg.ha^{-1}) at booting and heading stages. In general, when applying higher concentrations of foliar Zn, grain yield, test weight, and thousand kernel weight decreased slightly and Paiva presented higher values compared to Roxo. Nevertheless, the Normalized Difference Vegetation Index (NDVI) did not reveal a direct correlation between its higher values or the increase of grain yield. However, it was concluded that using drones coupled with specific cameras is of utmost importance to decide whether an experimental field is qualified to implement a biofortification workflow.

Keywords: agronomic biofortification; bread wheat; grain yield; NDVI; test weight; thousand kernel weight

1. Introduction

It is estimated that the human population will reach the milestone of approximately 9.7 billion inhabitants in 2050 and about 10.9 billion in 2100 [1]. To feed the growing population, it is crucial to find new strategies to increase food production in a sustainable way, as well as to reduce nutritional deficiencies. Biofortification is a strategy that can diminish nutritional deficiencies in micronutrients, aiming to increase the content and bioavailability of a nutrient in the edible parts of plants [2–4]. There are already several studies [5–7] for biofortification with different nutrients (namely Zn, Fe, I, Mg) and several staple crops, such as rice, grapes, carrot, onion, and kale. Zinc is an essential micronutrient and its deficiency can lead to losses of brain function, changes in growth, complications in newborns, and weakening of the immune system. This micronutrient interacts with a high number of enzymes, playing a fundamental role in several levels (structural, regulatory, and functional) [8,9]. *Triticum aestivum* L. is considered one of the staple crops, which is consumed on a large scale worldwide as it is estimated that world wheat will reach, in 2020/2021, a production of 761.7 million tons, being, for this reason, biofortified in micronutrients [10]. One way to increase crop productivity, predict disease, and monitor the plant development cycle is through the implementation of precision agriculture. Smart precision agriculture is transforming the most traditional agricultural practices, using new technologies, such as the use of Unmanned Aerial Vehicles (UAVs) and the internet of things (IoT) [11]. Precision agriculture is defined as a form of agriculture that aims to optimize agriculture, improving its efficiency and protecting the environment through the management of practices carried out in time, place, and in the right way [12]. The use of UAVs makes possible the measurement of some vegetation indices, such as NDVI, GNDVI, and RENDVI, as well as other indices, such as NDRER, GRVI, RGRI, and MCARI, which allow the monitoring of the status of crops and making decisions in real time to restore balance [13].

2. Materials and Methods

2.1. Experimental Field

Triticum aestivum L. (cv. Roxo and Paiva) was cultivated in Beja (Portugal) at 37°58′56.10″ N; 7°44′18.38″ W. The experimental field was sown on 13 January 2020 (with a rate of 350 seeds/m^2) in a randomized block design with four repetitions, where the experimental field presented 24 plots, with an area of 12 m^2 (10 m × 1.2 m) each, comprising 0.4 m between plots and 3 m between repetitions. Before sowing, the field was fertilized with 50 kg Zn.ha^{-1} and with NPK fertilization (25 UN; 45 UP; 30 UK). The harvest took place on 19 June 2020, with a plot harvester combine (Hege). During April, the agronomic biofortification comprised $ZnSO_4$ foliar pulverization at booting and heading stages, with three different concentrations applied (0–control (T0), 8.1 (T1), and 18.2 (T2) kg.ha^{-1}) and with the application of 45 UN at the tillering stage. From sowing to harvest, the average maximum and minimum temperatures were 20 °C and 10 °C, respectively. The total rainfall accumulation was about 280 mm (with a daily maximum of 43 mm), and the maximum and minimum averages values of air humidity were 97% and 54%, respectively.

2.2. Grain Yield, Test Weight, and Thousand Kernel Weight (TKW) of Triticum aestivum L. Grains

After harvesting, the grain yield (expressed as kg.ha^{-1}) [14,15] was determined, as well as test weight (as kg.hL^{-1}) [15,16] and thousand kernel weight (TKW), expressed in grams [15,17] in *Triticum aestivum* L. grains.

2.3. Experimental Characterization–Unamanned Aerial Vehicle (UAV)

The experimental field was flown on 28 February 2020 with an Unmanned Aerial Vehicle (UAV) synchronized with GPS before $ZnSO_4$ foliar applications. The data collected by the UAV was used to produce orthophotomaps and, consequently, to determine the Normalized Difference Vegetation Index (NDVI). In this way, it was possible to analyze the field and decide whether it would be ready to proceed with the foliar applications. The UAV was equipped with a multispectral Parrot Sequoia camera (with five electromagnetic spectral bands–NIR, REG, Green, Red, and RGB). The images were processed and the NDVI was determined using ArcGIS PRO from the data obtained from the camara [18,19].

2.4. Statistical Analyses

Data was statistically analyzed using software R (version 3.6.3) to obtain the correlation matrix of the coefficients Pearson and Spearman of the NDVI, grain yield, test weight. and TKW.

3. Results

The plots with the highest NDVI value showed greater plant vigor and, in addition, plots with the lowest NDVI standard deviation (STD) showed greater homogeneity in the vigor. In general, it appeared that there were some plots scattered around the experimental field with low NDVI values. Plots R0S1, P0S1, and P1S4 had average NDVI values below 0.44, while in plots R0S4, R2S2, P0S4, P1S3, and P2S4, the average values were greater than 0.55 (Table 1). Plots R1S4, R2S1, P0S1, and P2S1 had grain yield values below 500 kg.ha^{-1}, while plots R0S2, P0S3, and P1S2 had values above 1000 kg.ha^{-1}. Plots R0S4, R1S2, R2S1, P0S2, P0S3, and P2S3 had test weight values less than 70 kg.hL^{-1}, while plots R0S1, R0S2, R0S3, R1S1, R2S3, R2S4, and P0S1 had values greater than 75 kg.hL^{-1}. Plots R0S1, R0S2, R0S3, R2S1, and R1S2 had TKW values below 33 g, while plots P1S1 and P2S2, and all plots of Paiva control variety, had values above 38 g. Furthermore, R2S1 and R1S2 presented lower values in grain yield (except R1S2), test weight, and TKW.

Table 1. Grain yield, test weight, and thousand kernel weight (TKW) of *Triticum aestivum* L. (cv Paiva and Roxo) grains for the experimental field. With the foliar application of $ZnSO_4$: T0 = control; T1 correspond to 8.1 and T2 to 18.2 kg.ha^{-1}. Normalized Difference Vegetation Index (NDVI) acquired by ArcGIS PRO software from UAVs images of experimental field (28 February 2020, before $ZnSO_4$ foliar applications and after sowing) (P = Paiva; R = Roxo; = 0, 1, 2 = Treatments; S = $ZnSO_4$; 1–4 = Replicates).

Variety	Treatment	Replicated	Grain Yield (kg.ha^{-1})	Test Weight (kg.hL^{-1})	TKW (g)	NDVI ± STD
Paiva (P)	T0	1	452	75.9	42.3	0.431 ± 0.162
		2	802	40.5	38.7	0.489 ± 0.153
		3	1005	69.2	39.9	0.532 ± 0.151
		4	950	72.2	39.7	0.598 ± 0.135
	T1	1	621	74.1	38.3	0.458 ± 0.140
		2	1092	70.1	37.6	0.472 ± 0.149
		3	890	73.2	36.2	0.564 ± 0.138
		4	586	73.6	36.5	0.343 ± 0.185
	T2	1	447	74.2	37.1	0.517 ± 0.138
		2	647	71.7	38.5	0.525 ± 0.144
		3	916	67.4	35.8	0.495 ± 0.175
		4	579	73.4	36.8	0.557 ± 0.152
Roxo (R)	T0	1	582	76.3	33.4	0.388 ± 0.164
		2	1284	76.8	35.8	0.508 ± 0.157
		3	932	77.2	35.7	0.521 ± 0.154
		4	905	64.4	34.9	0.551 ± 0.154
	T1	1	766	76.0	32.8	0.474 ± 0.163
		2	971	68.1	32.0	0.500 ± 0.168
		3	679	73.9	31.8	0.462 ± 0.155
		4	472	74.4	33.2	0.538 ± 0.163
	T2	1	304	65.7	32.1	0.482 ± 0.154
		2	657	73.6	31.0	0.573 ± 0.135
		3	514	75.5	32.4	0.488 ± 0.176
		4	566	75.8	32.9	0.519 ± 0.158

There was a strong and positive correlation between NDVI and grain yield, for the Pearson (CP) and Spearman (CS) coefficients, Paiva T0, and Roxo T2, as well as for Paiva T1 (between NDVI and grain yield for CS) and for Roxo T0 and Roxo T1 (between NDVI and TKW for CP and CS, respectively). For Paiva T1 and Roxo T1 samples, there was a null correlation between NDVI and TKW (only in CP) and between NDVI and test weight (only in CS), respectively. In addition, there were weak positive correlations for Paiva T0 (between NDVI and test weight for CP), Paiva T2 (between NDVI and TKW for CP), and Roxo T0 (between NDVI and grain yield and between NDVI and TKW, both for CS). Furthermore, there were weak negative correlations for Paiva T1 (between NDVI and test weight for CP), Roxo T1 (between NDVI and grain yield-CS and between NDVI and TKW-CP), Paiva T0 (between NDVI and test weight for the CS), Roxo T0 (between NDVI and test weight for the CS), and for the Roxo T2 sample (between NDVI and TKW for the CS). All the other samples had an intermediate correlation with NDVI, whether positive or negative (Table 2).

Table 2. Correlation matrix of Pearson (the bottom of the diagonal) and Spearman (the top of the diagonal) coefficients of the NDVI, grain yield, test weight, and TKW of *Triticum aestivum* L. (cv Paiva and Roxo) grains for the experimental field. With the foliar application of $ZnSO_4$: T0 = control ((a) and (d)); T1 correspond to 8.1 ((b) and (e)) and T2 to 18.2 kg.ha^{-1} ((c) and (f)).

(a)					(d)				
Paiva T0	**Grain Yield**	**Test Weight**	**TKW**	**NDVI**	**Roxo T0**	**Grain Yield**	**Test Weight**	**TKW**	**NDVI**
Grain Yield	1	−0.4	−0.2	0.8	**Grain Yield**	1	0.6	1	0.2
Test Weight	−0.156	1	0.8	−0.2	**Test Weight**	0.081	1	0.6	−0.2
TKW	−0.761	0.74	1	−0.4	**TKW**	0.895	0.084	1	0.2
NDVI	0.858	0.111	−0.569	1	**NDVI**	0.656	−0.5	0.809	1

(b)					(e)				
Paiva T1	**Grain Yield**	**Test Weight**	**TKW**	**NDVI**	**Roxo T1**	**Grain Yield**	**Test Weight**	**TKW**	**NDVI**
Grain Yield	1	−0.8	0	0.8	**Grain Yield**	1	−0.4	−0.4	−0.2
Test Weight	−0.895	1	0.4	−0.6	**Test Weight**	−0.689	1	0.6	0
TKW	−0.053	−0.099	1	−0.4	**TKW**	−0.623	0.527	1	0.8
NDVI	0.59	−0.18	−0.093	1	**NDVI**	−0.426	−0.165	0.679	1

(c)					(f)				
Paiva T2	**Grain Yield**	**Test Weight**	**TKW**	**NDVI**	**Roxo T2**	**Grain Yield**	**Test Weight**	**TKW**	**NDVI**
Grain Yield	1	−1	−0.4	−0.4	**Grain Yield**	1	0.4	-0.2	1
Test Weight	−0.986	1	0.4	0.4	**Test Weight**	0.826	1	0.8	0.4
TKW	−0.503	0.504	1	0.4	**TKW**	−0.332	0.192	1	−0.2
NDVI	−0.564	0.695	0.331	1	**NDVI**	0.83	0.376	−0.683	1

4. Discussion

Bearing in mind that NDVI values refer to a date prior to the two applications of $ZnSO_4$ (which occurred during the month of April), analysis can only be drawn regarding the comparison between the two varieties Paiva and Roxo and the differences presented by all plots (considering all of them as "control", as $ZnSO_4$ foliar applications did not occur at the time of the flight). For samples Paiva T0, Paiva T1, and Roxo T2, the correlation between NDVI and grain yield was in line with the values presented in the Table 1, as when the grain yield rose/fell, so did the values of NDVI (Tables 1 and 2). This is supported by several authors [20–22], as NDVI is directly correlated to grain yield in wheat. Nevertheless, the samples Roxo T0 and T1 showed a weak correlation between NDVI and grain yield, since when the NDVI was lower, the grain yield was higher, comparing the four plots of the sample. This might have occurred because some plants possibly had more grain stored than others, resulting in higher grain yield values and lower values of NDVI, as the plots presented less plants (i.e., lower values of NDVI). The opposite can happen by having higher plant density in the plots but a smaller number of grains stored in each plant, resulting in lower values of grain yield and higher values of NDVI, when comparing the four plots of the same sample. In plots where the NDVI values were less than 0.44, it may have been due to the fact that sowing did not take place in the usual way, with flaws appearing in these plots.

5. Conclusions

Overall, grain yield, test weight, and TKW decreased slightly when applying higher concentrations of foliar Zn (with Paiva presenting higher values relative to Roxo). The NDVI did not reveal a direct correlation between its higher values and test weight and TKW. Nevertheless, grain yield showed a strong and positive correlation with NDVI for both coefficients (Pearson and Spearman) in some samples, but only when averaging the four plots of samples and not in separated plots. To sum up, using UAVs was of utmost importance to decide whether this experimental field was qualified to implement the biofortification workflow of *Triticum aestivum* L.

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECAG2021-09724/s1.

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