IECAG 2021

Proceedings

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

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† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021.

Citation: Luís, I.C.; Coelho, A.R.F.; Pessoa, C.C.; Daccak, D.; Marques, A.C.; Caleiro, J.; Patanita, M.; Dôres, J.; Simões, M.; Almeida, A.S.; et al. Monitoring a Zinc Biofortification Workflow in an Experimental Field of *Triticum aestivum* L. Applying Smart Farming Technology. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

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 being 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 $ZnSO_4$, in three concentrations (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, in which Paiva presented higher values compared to Roxo. Nevertheless, the Normalized Difference Vegetation Index (NDVI) did not reveal a direct correlation between its higher values and the increase of grain yield. Yet, 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), 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, weakening of the immune system. This micronutrient interacts with a high number of enzymes, playing a fundamental role at 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 monitoring the status of crops and make 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 of 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. The harvest took place on 19 June of 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 46% urea. 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 of 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 Normalized Difference Vegetation Index (NDVI). In this way it was possible to analyze the field and to 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 show greater plant vigor and, in addition, plots with the lowest NDVI standard deviation (STD), show greater homogeneity in the vigor. In general, it appears that there are some plots scattered around the experimental field with low NDVI values. Plots R0S1, P0S1 and P1S4 have average NDVI values below 0.44, while in plots R0S4, R2S2, P0S4, P1S3 and P2S4 the average values are greater than 0.55 (Table 1). Plots R1S4, R2S1, P0S1 and P2S1 have grain yield values below 500 kg·ha^{-1}, while plots R0S2, P0S3 and P1S2 have values above 1000 kg·ha^{-1}. Plots R0S4, R1S2, R2S1, P0S2, P0S3 and P2S3 have test weight values less than 70 kg·hL^{-1}, while plots R0S1, R0S2, R0S3, R1S1, R2S3, R2S4 and P0S1 have values greater than 75 kg·hL^{-1}. Plots R0S1, R0S2, R0S3, R2S1 and R1S2 have TKW values below 33 g, while plots P1S1, P2S2 and all plots of Paiva control variety have 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 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 of February of 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 is a strong and positive correlation between NDVI and grain yield, for the Pearson (CP) and Spearman (CS) coefficients, for Paiva T0 and Roxo T2. Furthermore, 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 is a null correlation between NDVI and TKW (only in CP) and between NDVI and test weight (only in CS), respectively. In addition, there are 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 are 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 have 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 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)

Paiva T0	Grain Yield	Test Weight	TKW	NDVI
Grain Yield	1	-0.4	-0.2	0.8
Test Weight	-0.156	1	0.8	-0.2
TKW	-0.761	0.74	1	-0.4
NDVI	0.858	0.111	-0.569	1

d)

Roxo T0	Grain Yield	Test Weight	TKW	NDVI
Grain Yield	1	0.6	1	0.2
Test Weight	0.081	1	0.6	-0.2
TKW	0.895	0.084	1	0.2
NDVI	0.656	-0.5	0.809	1

b)

Paiva T1	Grain Yield	Test Weight	TKW	NDVI
Grain Yield	1	-0.8	0	0.8
Test Weight	-0.895	1	0.4	-0.6
TKW	-0.053	-0.099	1	-0.4
NDVI	0.59	-0.18	-0.093	1

e)

Roxo T1	Grain Yield	Test Weight	TKW	NDVI
Grain Yield	1	-0.4	-0.4	-0.2
Test Weight	-0.689	1	0.6	0
TKW	-0.623	0.527	1	0.8
NDVI	-0.426	-0.165	0.679	1

c)

Paiva T2	Grain Yield	Test Weight	TKW	NDVI
Grain Yield	1	-1	-0.4	-0.4
Test Weight	-0.986	1	0.4	0.4
TKW	-0.503	0.504	1	0.4
NDVI	-0.564	0.695	0.331	1

f)

Roxo T2	Grain Yield	Test Weight	TKW	NDVI
Grain Yield	1	0.4	-0.2	1
Test Weight	0.826	1	0.8	0.4
TKW	-0.332	0.192	1.0	-0.2
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 to 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 is in line with the values presented in the Table 1, as when the grain yield rises/fall so does 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 show 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 occur 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 are less than 0.44, it may be due to the fact that sowing did not took place in the usual way, with flaws appearing in these plots.

5. Conclusions

In overall, grain yield, test weight and TKW decreased slightly, when applying higher concentrations of foliar Zn (with Paiva presenting higher values relatively 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 just 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.

Author Contributions: Conceptualization, I.C.L., M.P., M.M.S. and F.C.L.; methodology, M.P., J.C.K., M.G.B. and F.C.L.; software, I.C.L. and M.G.B.; formal analysis, I.C.L., A.R.F.C.; C.C.P.; D.D.; A.C.M.; J.C.; M.S.; A.S.A.; M.F.P.; F.H.R.; P.L.; I.P.P.; P.S.C.; J.C.R.; J.C.K. and M.G.B.; resources, M.P., J.D., J.C.K. and M.G.B.; writing—original draft preparation, I.C.L.; writing—review and editing, I.C.L., M.P., M.G.B. and F.C.L.; supervision, M.P., M.M.S and F.C.L.; project administration, F.C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by PDR2020, grant number 101-030835.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: The authors thanks to Francisco Palma, Instituto Politécnico de Beja and Associação de Agricultores do Baixo Alentejo for facilities in bread wheat field. We also thank to the research center (GeoBioTec) UIDB/04035/2020 for lab facilities.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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