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Can Precision Agriculture be Used in the Management of a Fe and Zn Biofortification Workflow in Organic Tomatoes (*Lycopersicum esculentum* L.)?

Ana Rita F. Coelho[1,2]*, Ana Coelho Marques[1,2], Cláudia Campos Pessoa[1,2], Diana Daccak[1,2], Inês Carmo Luís[1,2], João Caleiro[1], Maria Brito[1,2], José Kullberg[1,2], Maria Manuela Silva[2,3], Manuela Simões[1,2], Fernando H. Reboredo[1,2], Maria F. Pessoa[1,2], Paulo Legoinha[1,2], Maria J. Silva[2,4], Ana P. Rodrigues[4], José C. Ramalho[2,4], Paula Scotti-Campos[2,5], José N. Semedo[2,5], Isabel P. Pais[2,5] and Fernando C. Lidon[1,2]

1. Earth Sciences Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal; arf.coelho@campus.fct.unl.pt (A.R.F.C.); amc.marques@campus.fct.unl.pt (A.C.M.); c.pessoa@campus.fct.unl.pt (C.C.P.); d.daccak@campus.fct.unl.pt (D.D.); idc.rodrigues@campus.fct.unl.pt (I.C.L.); mgb@fct.unl.pt (M.B.); jck@fct.unl.pt (J.K.); mmsr@fct.unl.pt (M.S.); fhr@fct.unl.pt (F.H.R.); mfgp@fct.unl.pt (M.F.P.); pal@fct.unl.pt (P.L.); fjl@fct.unl.pt (F.C.L.);
2. GeoBioTec Research Center, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal; abreusilva.manuela@gmail.com (M.M.S.); paula.scotti@iniav.pt (P.S.C.); jose.semedo@iniav.pt (J.N.S.); isabel.pais@iniav.pt (I.P.P.);
3. Escola Superior de Educação Almeida Garrett, Lisboa, Portugal;
4. PlantStress & Biodiversity Lab, Centro de Estudos Florestais, Instituto Superior Agronomia, Universidade de Lisboa, Oeiras, Portugal; mjsilva@isa.ulisboa.pt (M.J.S.); anadr@isa.ulisboa.pt (A.P.R.); cochichor@mail.telepac.pt; cochichor@isa.ulisboa.pt (J.C.R.)
5. INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal

* Correspondence: arf.coelho@campus.fct.unl.pt

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Abstract: Is expected that the population worldwide might exceed the 9 billion by 2050, being therefore imperative to increase food production. As such, the development of smart farming technology is an important key food production issue. In fact, through the use of UAVs (Unmanned Aerial Vehicles), it's possible to create normalized difference vegetation index (NDVI) maps, that can indicate, namely, health and vegetation vigor. In this context, this study aimed to assess the state of three tomato varieties (beef heart, "chucha" and apple) in the framework of a biofortification workflow with Fe and Zn, following an organic production mode. In a tomato experimental production field (GPS coordinates - 39° 41′ 48,517′′ N; 8°35′ 45,524′′W), six foliar spraying were carried out during the production cycle, with a mix of Zitrilon (15%) (0.40 and 1.20 kg.ha^{-1}) and Maxiblend (1 and 4 kg.ha^{-1}). NDVI was determined 7 days before the first foliar spraying and showed a maximum of 0.86 (on a scale from -1 to 1). After the 3rd foliar spraying, no changes were detected in the color of freshly harvest tomatoes (assessed through spectrophotometric colorimeter), but an increase of Fe and Zn content was found in the leaves and of Zn in tomatoes (except in "chucha" variety). The use of precision agriculture techniques in correlation with the other analysis is discussed.

Keywords: Biofortification; Iron; *Lycopersicum esculentum* L.; NVDI; Organic tomato production; Zinc.

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1. Introduction

Worldwide population is expected to exceed the 9 billion by 2050 [1] and, as such, food productions must increase by 25 – 70% to be able to feed the future world population [2]. Agriculture has change over the years, and nowadays the digital era is considered the future of this sector. In fact, the development of smart farming technology can monitor continuously the states of plants, soils, and the needs for productions inputs (such as water) [3], being an important tool for food production. UAVs (Unmanned Aerial Vehicles) are being used in monitoring and measuring bio-physical parameters [4]. Nevertheless, through the data obtained by UAVs, it's possible to create NDVI (Normalized Difference Vegetation Index) maps (ranging from -1 to 1) [5], being one of the most used and implemented calculated indices. This type of index can characterize health and vegetation vigor [6].

The lack of essential nutrients, as Fe and Zn, in human diets are a current global problem [7], which can lead to the development of several pathologies namely, anemia (for Fe deficiency) [8] or problems related to immune system, gastrointestinal, central nervous and reproductive system (for Zn deficiency) [9].

Zinc supports normal growth and development during different stages of life (mainly during pregnancy, childhood, adolescence). In fact, the daily adequate intake for pregnant and lactation woman ranges between 11-13 mg, since birth to 8 years varied between 2-5 mg and for individuals from 9 years the daily dose varied between 8-11 mg (depending on whether it is a man or woman) [10]. In the other hand, Fe is an essential component of hemoglobin, supports muscle metabolism and plays an essential role, namely, in physical growth and cellular functioning. Iron daily adequate intake varied between whether it is a man, woman, pregnant or if are lactating, being needed in large quantities during pregnancy (27 mg) [11]. As such, being edible agriculture products the main source of minerals [12], biofortification has been carried out over the years, aiming to attain biofortified food crops [13], where contents of target minerals increase in the edible part of plants.

Tomato is considered one of the most popular and consumed vegetables worldwide [14] and organic food consumption worldwide continues to grow and organic produce counts on a growing market [15]. Furthermore, with the restriction of applied products for pest and disease control [16] implies a more accurate monitorization of crops to avoid total losses. In this context, this study aimed to assess thought precision agriculture, the state of three tomato varieties (beef heart, "chucha" and apple) biofortified with Fe and Zn, following an organic production mode.

2. Materials and Methods

2.1. Biofortification Itinerary

The experimental tomato-growing field, located in the Western of Portugal (39° 41′ 48,517′′ N; 8°35′ 45,524′′O), was used to growth three tomato varieties ("Beef Heart", "Chucha" and "Apple") (*Lycopersicum esculentum* L.) following an organic production mode. During the agricultural period, from 22nd May (planting date) to 3rd September of 2020 (harvest date), air temperatures reached an average daily of 30.6 and 13.3 ^{o}C (with minimum and maximum values varying between 5.3 and 40.6 ^{o}C, respectively). Foliar spraying with Fe and Zn was carried out with two treatments during the production cycle, with six foliar sprays (with 10 – 11 days interval). Treatments were carried out with a mix of two products (Zitrilon- 15% and Maxiblend), in which treatment 1 (T1) corresponds to a mix of 0.40 kg.ha^{-1} Zitrilon (15%) and 1 kg.ha^{-1} Maxiblend and treatment 2 (T2) corresponds to a mix of 1.20 kg.ha^{-1} Ziltrilon (15%) and 4 kg.ha^{-1} Maxiblend. Control plants were not sprayed at any time with Fe and Zn. Each treatment was performed in quadruplicate.

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 on 26th June (seven days before the 1st foliar spraying) to characterize vegetation indexes, to monitor differences in vigor between control and sprayed plants. The images were processed in ArcGIS Pro and the NDVI maps were obtained.

2.3. Iron and Zinc contents in leaves and Zinc Content in Tomatoes

Iron and Zinc contents in leaves and Zn content in tomatoes were determined after the 3rd foliar spraying (23rd July) after 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 [17].

2.4. Colorimetric Parameters

Colorimetric parameters were determined in fresh tomatoes per treatment with a scanning spectrophotometric colorimeter, according to [18]. Measurements were carried out in quadrupled.

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

Concerning the management of Fe and Zn biofortification workflow in organic tomatoes, the NDVI map was obtained 7 days after the 1st foliar spraying (Figure 1), with the aim of verifying if the culture was in good health conditions to be biofortified. In fact, the NDVI map ranged between 0.19 and 0.86, corresponding the lower NDVI to the soil, since the tomatoes plants were in an early vegetative state.

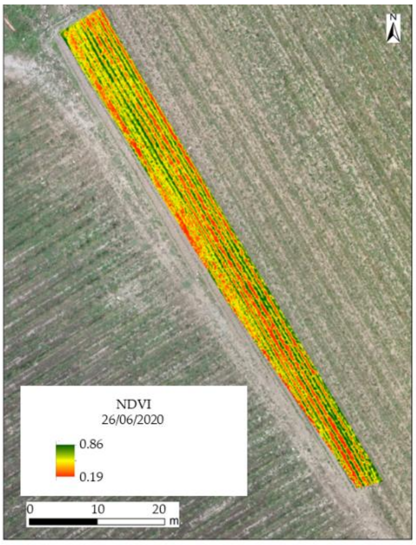

Figure 1. NDVI (Normalized Difference Vegetation Index) map in plants of *Lycopersicum esculentum* L. (considering the three varieties), 7 days before the 1st foliar spraying with Fe and Zn.

Nevertheless, from the NDVI map, the minimum, maximum and average of NDVI was calculated (Table 1). As such, the average of NDVI was 0.44, 7 days before the 1st foliar spraying with Fe and Zn.

Table 1. NDVI ((Normalized Difference Vegetation Index) of the three varieties of *Lycopersicum esculentum* L., (obtained on 26th June 2020), 7 days before the 1st foliar spraying with Fe and Zn.

Minimum NDVI	Maximum NDVI	Average NDVI	SD
0.19	0.86	**0.44**	0.15

The colorimetric analysis of tomatoes after the 3rd foliar spraying with Fe and Zn showed the highest value at 650 nm, which corresponds to the red color (Figure 2). Only T2 treatment of apple variety showed higher transmittance compared to control and T1 treatment.

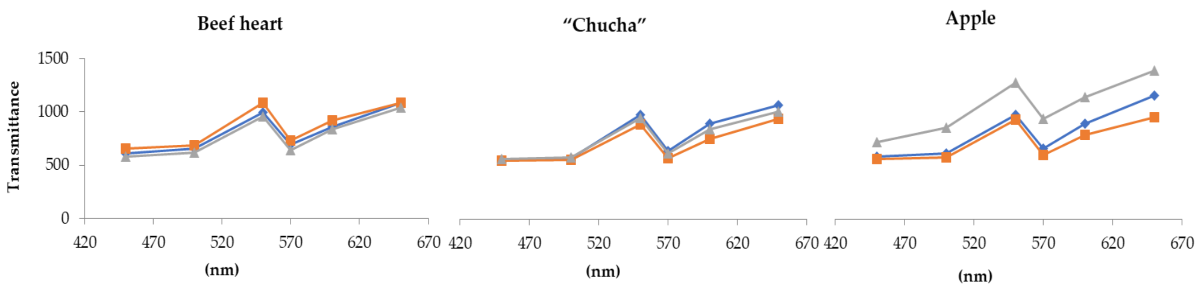

Figure 2. Visible spectra showing the average of transmittance (n = 4) in tomatoes of *Lycopersicum esculentum* L. of the three varieties, after the 3rd foliar spraying (● Control, ■ T1 and ▲ T2).

Mineral content of leaves and tomatoes was assessed after the 3rd foliar spraying (Table 2). Relatively to control leaves, T2 showed the highest content of Fe and Zn, followed by T1 in "Chucha" and "Apple" varieties. However, in "Beef Heart" variety, Zn content in leaves only showed a higher content that control in T2 treatment. In "Apple" variety, Zn content in tomatoes were significantly higher in T2 treatment. Yet, in "Beef Heart" variety all the treatments with Fe and Zn showed higher content than control, although T1 showed a significantly higher content of Zn compared to T2. At this stage of the biofortification process, "Chucha" variety did not showed a higher content of Zn compared to control tomatoes.

Table 2. Mean values ± S.E. (n = 4) of Fe and Zn in leaves and Zn in tomatoes of *Lycopersicum esculentum* L. ("Beef heart", "Chucha" and "Apple"), after the 3rd foliar spraying.

		Leaves		Fruits
Variety	**Treatments**	**Fe (ppm)**	**Zn (ppm)**	**Zn (ppm)**
Beef heart	Control	<50	74.92b ± 1.79	38.59c ± 0.43
	T1	<50	62.29c ± 3.97	77.16a ± 0.18
	T2	<50	200.8a ± 1.96	63.12b ± 1.57
"Chucha"	Control	<50	61.67c ± 0.99	30.30a ± 1.98
	T1	140.2b ± 7.94	140.0b ± 3.14	28.76a ± 1.32
	T2	255.0a ± 10.5	167.0a± 2.26	13.71b ± 0.86
Apple	Control	73.90c ± 10.4	54.23c ± 0.70	30.35b ± 2.71
	T1	113.7b ± 3.84	121.7b ± 1.64	33.27b ± 1.43
	T2	273.9a ± 8.55	285.3a ± 2.57	64.32a ± 1.64

Different letters indicate significant differences, of each variety, between treatments (statistical analysis using the single factor ANOVA test, P ≤0.05). Foliar spray was carried out with two concentrations (T1 and T2). Control was not sprayed.

4. Discussion

Organic production uses lower levels of pesticides [16], being important to monitor the culture. As such, before implementation of the biofortification workflow, it was necessary to ensure the best conditions of the culture. The average of NDVI was 0.44 and the maximum was 0.86 (Figure 1; Table 1). Regarding the average of NDVI, moderate values (between 0.2 to 0.5) were found [5], corresponding to the early vegetative state of tomato plants and the spacing between plants. Additionally, the maximum NDVI of the culture was 0.86, corresponding to high values (i.e., varying between 0.6 to 0.9) [5]. As such, the use of this parameter the culture revealed that the culture had a healthy phenological development allowing the implementation of the biofortification workflow.

In the middle of the biofortification workflow (after the 3rd foliar spraying with Fe and Zn), the color of tomatoes (control and sprayed with Fe and Zn – T1 and T2) was assessed (Fig. 2). All the treatments of the three varieties showed a highest value at 650 nm, corresponding to the red color [18] and being an indicator of high lycopene content [19]. Thus, the use of this technology allowed an objective definition of the maturation state of the fruits, since red is an indication of 10 times more lycopene content than yellow [20].

Nevertheless, the mineral content of the leaves, after the 3rd foliar spraying (Table 2), pointed that after foliar spraying "Chucha" and "Apple" varieties, they became biofortified with Fe and Zn (relatively to control, revealing a higher content in T2, followed by T1). However, in the "Beef Heart" variety, Zn content only revealed higher content than the control T2, which may be due to the heterogeneity during foliar spraying or because the accumulation of minerals varies depending on the genotype [20].

Despite the heterogeneity of tomato genotypes, it is crucial to increase the contents of healthy compounds in the fruits [20]. In this context, it was found that in the middle of the implementation of the biofortification workflow Fe content was under the limits of detection, whereas Zn content revealed different accumulation patterns (Table 2). "Apple" variety, relatively to the control, only showed a significantly higher content of Zn in T2, whereas "Beef Heart", revealed significantly higher contents of Zn in T1 and T2 (Table 2). Moreover, "Chucha" variety at this stage of the biofortification process did not revealed a biofortification pattern, nevertheless it should be noted that Zn contents in tomatoes can be dependent of the maturation of the fruit and the variety [21].

5. Conclusions

Through the use of cameras coupled to UAV, it was possible to obtain NDVI values and assess the vegetative stage of the different varieties of the tomato culture before the implementation of the biofortification workflow. In fact, the use of Smart Farm techniques before biofortification, can help the management of the culture and decision-making in real time, namely by assessing this organic culture health before foliar spraying.

Considering this technical workflow of foliar spraying with Fe and Zn in an organic production mode, it was observed that the content of these minerals in leaves and fruits varied among tomato varieties. "Apple" variety showed better relatively to "Beef Heart" and "Chucha" varieties. Nevertheless, foliar spraying with Fe and Zn can increase these chemical elements in tomato leaves and fruits (without major changes in the color of fruits).

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References

1. Alexandratos, N.; Bruinsma, J. World agriculture towards 2030/2050: the 2012 revision. Agricultural Development Economics Division **2012**, 12-03. (accessed on http://www.fao.org/fileadmin/templates/esa/Global_persepctives/world_ag_2030_50_2012_rev.pdf)
2. Hunter, M.C.; Smith, R.G.; Schipanski, M.E.; Atwood, L.W.; Mortensen, D.A. Agriculture in 2050: Recalibrating targets for sustainable intensification. *BioScience* **2017**, *67*, 386–391. doi:10.1093/biosci/bix010
3. Walter, A.; Finger, R; Huber, R.; Buchmann, N. Opinion: Smart farming is key to developing sustainable agriculture. *Proceedings of the National Academy of Sciences* **2017**, *114*, 6148-6150. doi: 10.1073/pnas.1707462114
4. Senthilnath, J.; Dokania, A.; Kandukuri, M.; Ramesh, K.N.; Anand, G.; Omkar, S. N. Detection of tomatoes using spectral-spatial methods in remotely sensed RGB images captured by UAV. *Biosystems Engineering* **2016**, *146*, 16–32. doi:10.1016/j.biosystemseng.2015.12.003

5. United States Geological Survey (USGS). Available online: https://www.usgs.gov/core-science-systems/eros/phenology/science/ndvi-foundation-remote-sensing-phenology?qt-science_center_objects=0#qt-science_center_objects (accessed on 24 03 2021).
6. Xue, J.; Su, B. Significant remote sensing vegetation indices: A review of developments and applications. *J Sensors* **2017**, 1-17. doi:10.1155/2017/1353691
7. Li, H.; Lian, C.; Zhang, Z.; Shi, X.; Zhang, Y. Agro-biofortification of iron and zinc in edible portion of crops for the global south. *Adv Plant Agric Res* **2017**, *6*, 52-54. doi: 10.15406/apar.2017.06.00210
8. Abbaspour, N.; Hurrell, R.; Kelishadi, R. Review on iron and its importance for human health. *J Res Med Sci* **2014**, *19*, 164. PMID: 24778671; PMCID: PMC3999603
9. Roohani, N.; Hurrell, R.; Kelishadi, R.; Schulin, R. Zinc and its importance for human health: An integrative review. *J Res Med Sci* **2013**, 18, 144-57. PMID: 23914218, PMCID: PMC3724376
10. National Institutes of Health (NIH). Available online: https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/ (accessed on 24 03 2021).
11. National Institutes of Health (NIH). Available online: https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/ (accessed on 24 03 2021).
12. Wang, Z.; Hassan, M.U.; Nadeen, F.; Wu, L.; Zhang, F.; Li, X. Magnesium fertilization improves crop yield in most production systems: A meta-analysis. *Front. Plant Sci.* **2020**, *10*, 17–27. doi:10.3389/fpls.2019.01727
13. Alshaal, T.; El-Ramady, H. Foliar application: From plant nutrition to biofortification. *Environment, Biodiversity and Soil Security* **2017**, 1, 71-83. doi: 10.21608/jenvbs.2017.1089.1006
14. Sainju, U.M.; Dris, R.; Singh, B. Mineral nutrition of tomato. *Food Agric Environ* **2003**, *1*, 176-183.
15. Rizzo, G.; Borrello, M.; Dara Guccione, G.; Schifani, G.; Cembalo, L. Organic food consumption: The relevance of the health attribute. *Sustainability* **2020**, *12*, 595. doi:10.3390/su12020595
16. HelpGuide. Available online: https://www.helpguide.org/articles/healthy-eating/organic-foods.htm (accessed on 24 03 2021).
17. Luís, I.C.; Lidon, F.C.; Pessoa, C.C.; Marques, A.C.; Coelho, A.R.F.; Simões, M.; Patanita, M.; Dôres, J.; Ramalho, J.C.; Silva, M.M.; Almeida, A.S.; Pais, I.P.; Pessoa, M.F.; Reboredo, F.H.; Legoinha, P.; Guerra, M.; Leitão, R.G.; Campos, P.S. Zinc enrichment in two contrasting genotypes of *Triticum aestivum* L grains: Interactions between edaphic conditions and foliar fertilizers. *Plants* **2021**, *10*, 204. doi:10.3390/plants10020204
18. Coelho, A.; Pessoa, C.; Marques, A.; Luís, I.; Daccak, D.; Manuela, M.; Reboredo, F.; Pessoa, M.; Galhano, C.; Legoinha, P.; Ramalho, J.; Scotti-Campos, P.; Pais, I.; Lidon, F. Nutrient interactions in natural fortification of tomato with Mg: An analytical perspective. In *Presented at the 1st International Electronic Conference on Plant Science* **2020**, *1*, 1-15. doi: 10.3390/IECPS2020-08724
19. Jarquín-Enríquez, L.; Mercado-Silva, E.M.; Maldonado, J.L.; Lopez-Baltazar, J. Lycopene content and color index of tomatoes are affected by the greenhouse cover. *Sci. Hortic. Amst.* **2013**, *155*, 43-48. doi:10.1016/j.scienta.2013.03.004
20. Dorais, M.; Ehret, D.L.; Papadopoulos, A.P. Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. *Phytoch Rev* **2008**, *7, 231–250.* doi:10.1007/s11101-007-9085-x
21. Costa, F.; de Lurdes Baeta, M.; Saraiva, D.; Veríssimo, M.T.; Ramos, F. Evolution of mineral contents in tomato fruits during the ripening process after harvest. *Food Anal Method* **2011**, *4*, 410-415. doi:10.1007/s12161-010-9179-8.