

## NANOMAGNETIC IRON OXIDE SOLUTION FOR FERTILIZATION ON WHEAT PLANTS

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### ABSTRACT

Wheat is the most cultivated plant and an important source of carbohydrates in the world. The Fe deficiency reduces quality of grain wheat leading to Fe deficiency in human. The purpose of this study was to investigate the effects of foliar and ground application of iron oxide nanoparticles (made in Romania) on growth components, yield and morphological and anatomical modifications of wheat plants.

The ground application of iron oxide decreased height of plant, length of root and increased root volume and chlorophyll content more than foliar application.

For the wheat plants fertilized with iron oxide nanoparticles, the decrease of root length was compensated by an increase of radicular density, which led to the development of new adventitious roots that could help the plants have a better uptake of water and nutrients. This meant that the production was not negatively influenced by the treatments performed, regardless of the application method.

Our studies revealed that the fertilized wheat plants (foliar and root zone) presented anatomical changes in relation to control plants.

The studies presented in this paper can contribute to achieve the necessary framework for the innovative development strategy regarding the efficiency of magnetic nanoparticles in foliar and ground fertilization of different crops.

**Keywords:** anatomy, fertilization, iron oxide, plant growth, wheat.

### INTRODUCTION

In the last years, the World Health Organization reported that in developing countries there is an increase of iron, zinc and vitamin A deficiencies in human population (Zia-ur-Rehman et al., 2018). Wheat crop provides 60% of the daily calorie intake and is the most important cereal crop in the world and in Romania, too with regard to total production, cultivated area and human consumption (Cakmak et al., 2004; Șerban et al., 2019).

The deficiency of iron in the soil causes

reduction in wheat grain and quality leading to nutrition disorder (Fe deficiency) in human. The enhancement of wheat grain quality by increasing the micronutrients quantity of grains can be achieved by breeding or foliar and soil fertilization methods.

In the last years, the use of nanotechnology in various fields such as agriculture and life sciences have been steadily increasing (Zia-ur-Rehman et al., 2018; Negrilă et al., 2018; Predoi et al., 2018, 2018; Zhu et al., 2008). Depending on the types of nanoparticles and the plant species under study, both positive and negative

effects have been reported (Bondarenko et al., 2013; Nair et al., 2012). Foliar fertilization with solutions containing iron oxide nanoparticles can help plants to a better growth and development and fight against diseases and pests. But, most of the current published studies regarding nanoparticles and plants are centered around the effects of this on seed germination and vegetative plant growth. So, Zhu et al. (2008) demonstrated that the use of iron oxide nanoparticles as a spraying solution for wheat leaves increased their germination rate by 41%. Furthermore, iron is one of the essential nutrients for plant growth and it is the cofactor of various enzymes that accelerate plant growth and development processes. Therefore, root and plant growth as well as the formation of seeds had been accelerated by using appropriate concentrations of iron oxide nanoparticles (10-40 mg/l) for rice, cucumber and wheat (Zia-ur-Rehman et al., 2018). It was observed that the effect of iron oxide was more obvious in the case of seedlings and offshoots due to easier iron oxide nanoparticles absorption and transfer via root cells. Thus, the studies conducted until present have found that iron oxide nanoparticles to play an important role in the plant growth process due to the stimulus effect of iron when applied in a certain range of concentrations (Zia-ur-Rehman et al., 2018). Iron oxide nanoparticles with a large surface area have been reported to be able to bind with transport proteins or organic substances and to be absorbed by plant tissues (Xu et al., 2011). A hydroponic study showed that iron oxide nanoparticles had the potential to be transported through plant tissues after being absorbed from nutritive solution (Hong et al., 2005). It was believed that iron oxide nanoparticles accumulated near root and leaf tissues of plants (Zhu et al., 2008). However, a microscopic study of the same experiment revealed the presence of iron oxide nanoparticles in the xylem vessel, a water transport channel - vital for plants. In addition, it was found that both absorption of nanoparticles and their accumulation and transfer were reduced in the lime bean case than in the pumpkin case. It was reported that

the absorption, transfer and accumulation of iron oxide nanoparticles varied, depending on crop types and species (Zhu et al., 2008). Accumulation of iron oxide particles occurs in various plant tissues, depending on plant species, particles' size and the exposure time. In previous studies on effects of superparamagnetic iron oxide nanoparticles on photosynthesis and growth of the aquatic plant *Lemna gibba*, Barhoumi et al. (2015), reported an increase of the iron oxide nanoparticles concentration of 10 times in plants exposed to 400 µg/ml iron oxide nanoparticles for 7 days as compared to the first day of exposure. Following a detailed scientific and technical study (Corredor et al., 2010), it was shown that iron oxide nanoparticles were present in the stem epidermis, near the trichomes, on the outside of the cell wall and in the cells' cytoplasm. Corredor et al. (2010) in their studies on carbon iron magnetic nanoparticles for agronomic use in plants promising and still a long way to go showed that after 48 hours, a transfer of iron oxide nanoparticles within the interior of stem cells such as parenchymal cells occurred.

To reduce the negative (toxic) impact of iron oxide nanoparticles and to increase the positive effect on plant development (Wang et al., 2011), both the methods of applying the magnetic solutions and the iron concentration in these solutions should be optimized.

The aim of this research was to compare the effect of foliar and ground applications of new nanomagnetic iron oxide solution performed in Romania on plant growth, chlorophyll content and yield of winter wheat as well as on anatomical modifications in wheat plants.

## MATERIAL AND METHODS

### Materials

The nanoparticles of iron oxide in the form of a solution were obtained through an adapted coprecipitation method (Prodan et al., 2013; Iconaru et al., 2013; Predoi and Valsangiacom, 2007; Predoi, 2010; Predoi et al., 2017). The magnetic solution obtained was made in order to be used as a fertilizer for wheat plants. The precursors used to

obtain the magnetic solution were ferric chloride and ferrous chloride. A solution of ferrous chloride tetrahydrate was mixed with a solution of ferric chloride hexahydrate at 90°C. The ratio of the iron ions of the final solution was  $Fe^{2+}/Fe^{3+} = 1/2$ . The attainment of the iron oxide nanoparticles based on the co-precipitation method was performed starting from Massart's method (Massart, 1981).

In order to study the effect of iron oxide fertilization on some physiological traits and yield of wheat, seeds of wheat were sown in vegetation house in pots filled with a soil-sand mixture (3:1). The experimental variants were *control*: pots which were watered with tap water, *foliar treatment*: pots which were foliar sprayed with iron oxide at 4 leaves stage and *ground treatment*: pots which were watered with iron oxide at 4 leaves stage.

In order to study the effect of iron oxide fertilization on anatomical of wheat plants, during growth period, wheat plants were fertilized with iron oxide solutions at both root zone and foliar levels.

### Analyses

Biometric measurements regarding the height of plant (mm), leaf area (cm<sup>2</sup>), length of main root (mm), dry matter (g/plant), volume of roots (cm<sup>3</sup>), yield (g/plant) were achieved at two weeks after treatments. Chlorophyll content was measured with Chlorophyll meter Minolta device and results are expressed in SPAD units.

Compared to classical mechanisms of plant analyses, in recent years new approaches for the analysis of vegetal matter based on non-destructive methods such as Fourier-transform infrared spectroscopy (FTIR), optical microscopy and scanning electron microscopy (SEM) were developed. The need of using these methods in the

analyses of vegetal matter was imperative with the emergence of new chemical fertilization methods that led to a quality improvement of plants intended for human and animal consumption. The analysis of dried wheat leaves and the resulting ash (following calcination) were performed using a FTIR Perkin Elmer SP100 spectrometer. Morphological studies were performed by electronic scanning microscopy (SEM) using a HITACHI S4500 microscope equipped with an EDX system.

Observations regarding the anatomy of the youngest leaves of wheat plants were made with a Leica DM 1000 LED optic microscope equipped with a video camera Leica DFC 295 at 40 X and 100 X objective lenses. Leaf sections were clarified with chloral hydrate for 24 hours. All leaves samples had both upper and lower epidermis as well as the mesophyll measured.

Dried wheat leaves consist of the following compounds: cellulose, hemicellulose and lignin. FTIR spectroscopy followed the evolution of the molecular bands (specific to these compounds) in the spectra obtained in the case of fertilized (root zone and leaf levels) and unfertilized plants. Furthermore, the ashes resulted following calcination were also analyzed.

Data were subjected to analysis of variance respectively linear regressions.

## RESULTS AND DISCUSSION

The effect of iron oxide fertilization on some physiological traits and yield of wheat.

The results of the analysis of variance showed that the height of plants, length of main root, root volume and chlorophyll content were affected by the iron oxide solution at the 5 and 1% level of significance (Table 1).

Table 1. Analysis of variances for physiological traits studied

Source of variance	DF	Height of plant		Length of main root		Root volume		Chlorophyll content	
		Mean square	F value and significance	Mean square	F value and significance	Mean square	F value and significance	Mean square	F value and significance
Factors (treatments)	2	1849	12.38**	1022.11	187.73***	0.0661	14.60**	46.77	60.14***
Error	4	149.33		5.44		0.0045		0.77	

Iron oxide application decreased size of plant and length of main root. Plant sizes registered a reduction of 13 mm in foliar treatments and 48 mm in ground treatments.

In the case of control plants, the main root length was 175 mm, while for the fertilized plants with foliar application of iron oxide was 167 mm. In the case of plants fertilized with iron oxide solution applied at soil, the main root length recorded the smallest growth (140 mm) (Table 2). Several studies showed that nano-iron oxide increased plant height and yield in sunflower or soybean

(Sheykhbaglou et al., 2010; Ghodsi et al., 2012).

A positive effect on the root volume and chlorophyll content both for foliar and ground application of iron oxide was registered.

The highest volume of root per plant was obtained in the case of soil fertilized foliar with iron oxide solution (0.54 cm<sup>3</sup>/plant). Plants fertilized foliar with iron oxide solution had a root volume of 0.41 cm<sup>3</sup>/plant while that the untreated plants had a root volume of 0.25 cm<sup>3</sup>/plant.

Table 2. Effect of iron oxide treatments on physiological traits studied

Variants	Height of plant		Length of main root		Root volume		Chlorophyll content	
	mm	Differences from control	mm	Differences from control	cm <sup>3</sup>	Differences from control	SPAD units	Differences from control
Control	273	0	175	0	0.25	0	30	0
Foliar treatment	225	-48	140	-35	0.54	+29	37	+7
Ground treatment	260	-13	167	-8	0.41	+16	34	+4

Foliar iron oxide application produced the highest chlorophyll contents followed by ground applications (Table 3). In concordance with our results, Liu et al. (2005) reported that nano- Fe<sub>2</sub>O<sub>3</sub> application increased chlorophyll content of peanut and Amanullah et al. (2012), showed that foliar spray increased chlorophyll content in maize. Increased in chlorophyll content of wheat in

our experiment could be due to promotion of the absorption and utilization of nutrients such as nitrogen by nano-Fe compound as concluded by Liu et al. (2005).

Concerning the effect of iron oxide on the yield our results shown that application of iron oxide increased the yield in wheat (Table 3).

Table 3. Analysis of variances for yield

Source of variance	DF	Height of plant	
		Mean square	F value and significance
Factors (treatments)	2	0.292	12.38**
Error	4	0.014	19.58***

Between the length of the main root and the volume of the root is a negative correlation, the correlation coefficient being  $r = -0.93$  \*\*\* (Table 4), which means that in the case of plants fertilized with iron oxide

solutions, the reduction of root length was compensated by the increase of the root system density. Between root volume and yield was a positive correlation ( $r = 0.91$ \*\*\*, Table 4).

Table 4. Relationships between the yield and physiological traits studied

Physiological trait	Yield	Length of root
Root volume	0.91***	-0.93***
Chlorophyll content	0.92***	
Length of root		

Following these observations we can say that fertilization with iron oxide solutions (regardless of how it were applied) led to the development of new adventives roots, which helped the plants have a better absorption of water and nutrients. This also explains the positive effect on production, to which is added that of chlorophyll content (positive correlation coefficient,  $r = 0.92^{***}$ ).

### The effect of iron oxide fertilization on anatomical of wheat plants

In Figure 1, it was shown the transmission spectrum of dried wheat leaves. The bands from  $670\text{ cm}^{-1}$  and  $895\text{ cm}^{-1}$  (Shang et al., 2012; Cui et al., 2012) were characteristics vibrations of the cellulose structure. The molecular bands from the  $1060\text{--}1200\text{ cm}^{-1}$  spectral area were attributed to vibrations caused by expanse and distortion of C-O bonds from cellulose, lignin and residual cellulose. The molecular bands at  $1040\text{ cm}^{-1}$  and  $1160\text{ cm}^{-1}$  were attributed to C-O-C binding vibrations between cellulose and hemicellulose glycosidic interconnections (Shang et al., 2012; Cui et al., 2012). The  $1245\text{ cm}^{-1}$  band belongs to the hemicellulose vibrations and the  $1365\text{ cm}^{-1}$  band belongs to the asymmetrical distorted vibrations of the C-H bonds. This molecular band can decrease during calcination process. The molecular vibrations of the basic structure of the aromatic functional groups of lignin occurred at  $1627$  and  $1425\text{ cm}^{-1}$ . The molecular band at  $1735\text{ cm}^{-1}$  was attributed to acetyl or uronic ester groups of hemicellulose or ester interconnections of the carboxyl groups of ferulic and p-coumaric acids present in lignin and hemicellulose. This molecular band may disappear with the increase of temperature, showing that hemicellulose was removed (Shang et al., 2012; Cui et al., 2012). The molecular bands at  $2864$  and  $2916\text{ cm}^{-1}$  were assigned to aliphatic C-H bonds. These

bands did not change significantly with the temperature changes. The IR band at  $3300\text{ cm}^{-1}$  corresponded to the vibrations of the O-H groups (Ciobanu et al., 2016; Predoi et al., 2018, 2019; Cornell and Schwertmann, 2003). The width of this band indicated the hydrophilic tendency of wheat leaves. In the spectra from Figures 2a-c were noticed some differences related to the presence of Fe-O bonds between root zone and foliar fertilized wheat plants. The molecular band at  $698\text{ cm}^{-1}$  of the spectrum of leaves belonging to foliar and root zone fertilized wheat plants, indicated the absorption of the iron oxide (Popa et al., 2016; Iconaru et al., 2012; Prodan et al., 2013). The molecular band at  $560\text{ cm}^{-1}$ , also attributed to Fe-O bonds, was more pronounced in the IR spectrum of leaves belonging to foliar fertilized wheat plants. The intensity of the molecular band at  $698\text{ cm}^{-1}$  was more pronounced in the case of FTIR spectra of leaves belonging to root zone fertilized wheat plants. Upon calcination at  $650^\circ\text{C}$  (Figures 2d-e), FTIR spectra of dried wheat leaves changed. The main advantage of calcination is that the anorganic elements and their molecular bonds are better observed by FTIR spectroscopy. In the Figures 2d-e, spectral bands from the  $1200\text{--}3500\text{ cm}^{-1}$  region were strongly reduced in intensity, and the subsequent changes were invisible in the spectral range of  $400\text{--}2000\text{ cm}^{-1}$ . In the spectral area  $1200\text{--}1800\text{ cm}^{-1}$ , the band intensities, specific to the vibrations of the cellulose and hemicellulose bonds, were significantly reduced. The molecular bands at  $670$  and  $1160\text{ cm}^{-1}$  completely disappeared, and the molecular bands at  $895$  and  $1040\text{ cm}^{-1}$  were strongly diminished. In the spectra from Figures 2d-e, the molecular bands at  $590\text{ cm}^{-1}$  and  $470\text{ cm}^{-1}$ , attributed to the Fe-O bonds, were identified. The molecular band at  $560\text{ cm}^{-1}$ , observed in the IR spectrum of calcined wheat leaves, was more emphasized.

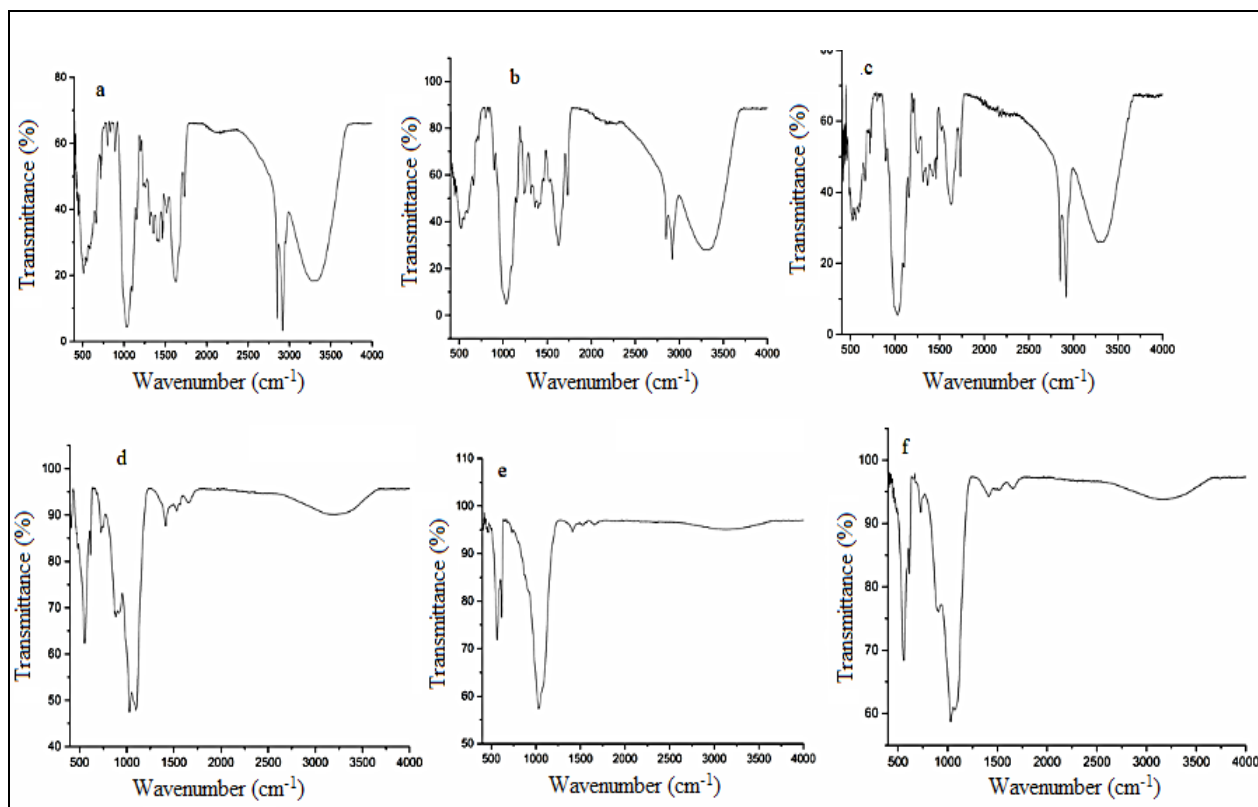
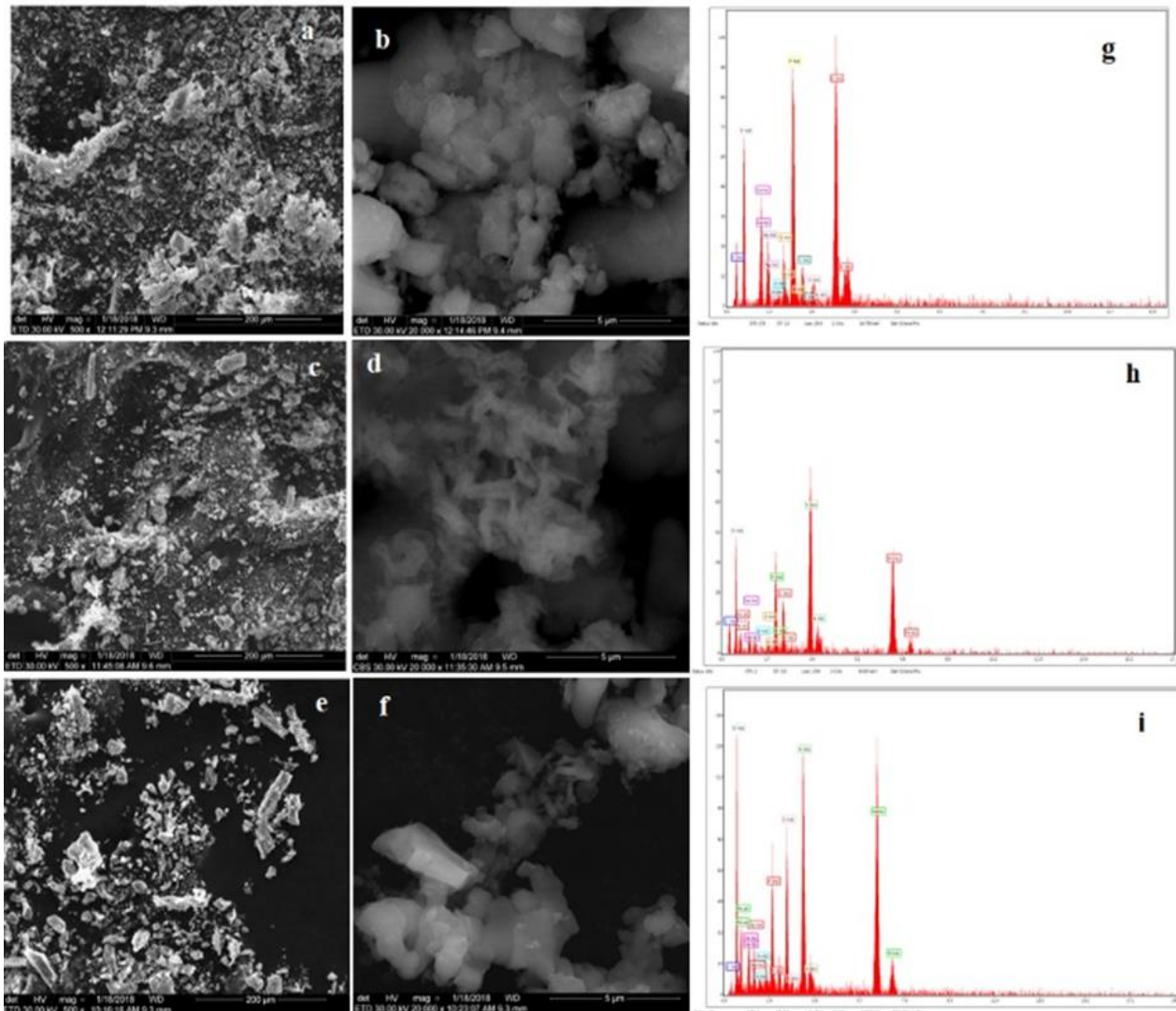


Figure 1. FTIR spectra of dried wheat leaves (a) control plants; (b) soil (root zone) fertilized plants with iron oxide solution during the growth process; (c) foliar fertilized plants with iron oxide solutions during the growth process; FTIR spectra of the calcined wheat plants samples at 650°C; (d) control plants; (e) soil (root) fertilized plants with iron oxide solution during the growth process; (f) foliar fertilized plants with iron oxide solutions during the growth process

The ashes obtained following dried leaves calcination were analyzed by SEM and energy-dispersive X-ray spectroscopy (EDX). In Figure 2a, it was observed the particles of calcined dried leaves from control wheat plants. Moreover, it was noted that these particles are unevenly agglomerated. In Figure 2b, the uneven particle sizes and the non-homogeneous particle structures are better evidenced. Figure 2c shows the morphological analysis results from the ashes of the dried leaves of plants fertilized with iron oxide solutions at root zone and leaf levels. Unlike unfertilized ones, they displayed a different dispersion of particles. Figure 2d, obtained at a high resolution of the microscope,

showed a different organization of vegetal particle layers. Figures 2e-f showed the microscopic analysis of particles of calcined dried leaves from root zone fertilized plants. They showed a similar behavior to foliar fertilized plants. The EDX analyses of the wheat samples were also presented. In Figure 2g, it was observed the absence of iron from the leaves of control wheat plants. However, in the fertilized wheat plants, the presence of iron was evidenced (Figures 2h-i). The spectral lines specific to iron  $K\alpha_1$ ,  $K\beta_1$ ,  $L\alpha_1$ ,  $L\beta_1$  indicated its absorption both in the case of root zone and foliar fertilization. If the case of root zone fertilized plants, the iron mineral spectral lines were more evident.



*Figure 2.* SEM images and EDX Spectrum

SEM images of control plants samples, calcined at 650°C (a-b); SEM images of iron oxide foliar fertilized plants, calcined at 650°C (c-d); SEM images of iron oxide root zone fertilized plants, calcined at 650°C (e-f); EDX spectrum of control plant dried leaves (g); EDX spectrum of calcined leaves of iron oxide foliar fertilized plants (h); EDX spectrum of calcined leaves of iron oxide root zone fertilized plants (i)

The leaf anatomy was examined in all three types of wheat plants (root zone and foliar fertilized as well as control plants) and presented in Figures 3a-c. In all analyzed samples it was observed that the structure of the lamina was represented by upper epidermis, mesophyll and lower epidermis, in accordance with previous studies (Zanoschi and Toma, 1985). The microscopic examination revealed that the both epidermis were single layered, having the cells arranged

in parallel rows. The parallel rows contain elongated cells, alternating with shorter, quadratic cells, with silicified walls. The upper epidermis (adaxial) was curled, forming vallecules and costas, while the lower epidermis (abaxial) was nearly flat. Both epidermides presented stomata and trichomes. The presence of stomata in both epidermides was also observed by Desheva et al. (2018) in their previous studies on wheat plants.

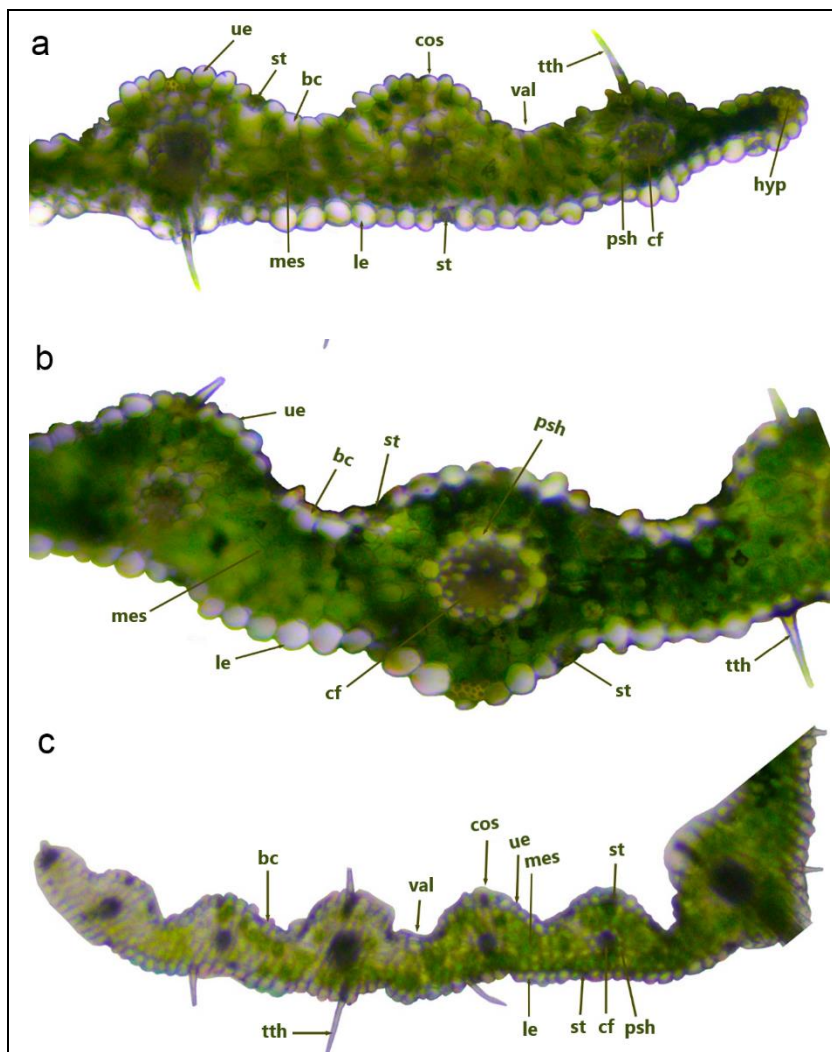


Figure 3. Leaf section

control plants (a), foliar fertilized plants (b) and root zone fertilized plants (c) with iron oxide solution [ue - upper epidermis; le - lower epidermis; st - stoma; mes - mesophyll; hyp - hypodermis; bc - bulliform cells; tth - tector trichomes; cf - conductive fascicle (bundle); psh - parenchyma sheath; val - vallecula; cos - costa]

The observed stomata were dumbbell-shaped type, with the upper epidermis being several stomata that delineate bullous cell strips. Each band consisted of 3-5 bulliform, thin-walled cells. At root zone fertilized wheat plants it was observed a larger number of trichomes on the lower epidermis (Figure 3c). For all the studied wheat plants (Figures 3a-c), the leaf mesophyll found between the two epidermides was homogeneous, consisting of 4-5 rows of elongated cells, with few intercellular spaces. In the leaf mesophyll were found collaterally closed vascular bundles of different sizes. All conductive bundles (fascicles) were surrounded by a colorless parenchyma sheath. The smaller vascular bundles contained more phloem.

The mechanical tissue was missing, however the edge of the lamina presented hypodermic cells with lignified cell walls.

The data presented in Tables 5-7 showed higher dimensions of the mesophyll of foliar fertilized plants (114.82  $\mu\text{m}$  - costas and 67.05  $\mu\text{m}$  - vallecules) and for root zone fertilized plants (116.31  $\mu\text{m}$  - costas and 50.37  $\mu\text{m}$  - vallecules) compared to control plants (87.75  $\mu\text{m}$  - costas and 45.19  $\mu\text{m}$  - vallecules). Also, the size of the lower epidermis was increased for the foliar fertilized plants (19.47  $\mu\text{m}$ ) and root zone fertilized plants (22.11  $\mu\text{m}$ ) compared to control plants (15.24  $\mu\text{m}$ ). Thus, a positive influence of the iron oxide on the lower epidermis and the mesophyll was evidenced.



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*Table 5. Foliar fertilized plants ( $\mu\text{m}$ )*

Upper Epidermis	Tector Trichomes/ Upper Epidermis	Mesophyll		Lower Epidermis	Tector Trichomes/ Lower Epidermis
		Costa	Vallecula		
13.88	122.21	97.32	60.74	16.84	45.54
23.82	66.18	126.64	63.75	22.98	39.81
19.50	31.01	112.33	67.71	24.16	63.20
15.83	59.78	108.82	72.86	16.16	48.70
19.52	55.04	128.98	70.21	17.22	50.20
18.51	66.84	114.82	67.05	19.47	49.49

*Table 6. Root zone fertilized plants ( $\mu\text{m}$ )*

Upper Epidermis	Tector Trichomes/ Upper Epidermis	Mesophyll		Lower Epidermis	Tector Trichomes/ Lower Epidermis
		Costa	Vallecula		
12.46	39.66	102.92	40.36	23.60	79.35
17.80	60.01	112.14	47.70	17.88	52.86
13.88	59.73	133.34	61.09	24.36	60.87
14.63	45.18	115.48	57.13	22.34	48.91
13.60	52.82	118.45	45.61	22.39	58.61
14.47	51.48	116.31	50.37	22.11	58.72

*Table 7. Control plants ( $\mu\text{m}$ )*

Upper Epidermis	Tector Trichomes/ Upper Epidermis	Mesophyll		Lower Epidermis	Tector Trichomes/ Lower Epidermis
		Costa	Vallecula		
16.16	57.07	122.93	55.61	10.92	56.47
17.80	62.51	98.22	55.92	20.54	35.56
14.07	62.12	68.55	35.15	17.62	62.12
14.82	62.12	73.15	40.35	16.16	40.01
12.50	59.93	75.91	48.94	10.98	57.47
15.07	60.75	87.75	45.19	15.24	50.33

Our research revealed a variation in the frequency of stomata, depending on how the nutrient solutions were applied. Equiza et al. (2001), revealed a variation in leaf stomata. Previous studies (Equiza et al., 2001), showed that stomatal frequency varied, depending on the plant variety and on the atmospheric temperature. Menghiu et al. (2012) showed that the density of stomata might vary between individuals of the same species. Moreover, previous studies showed that density of stomata might vary within the leaves belonging to the same plant and even within the same leaf of a plant. In addition, the variation in the number of stomata can be influenced by other factors such as air humidity, light intensity, water availability and atmospheric CO<sub>2</sub> concentration (Ianovici, 2012).

Our results showed that the solutions of iron oxide, used as foliar and root zone

fertilizers, increased the concentration of iron intake in wheat plants. Therefore, increasing the iron concentration in wheat plants could contribute to the reduction of serious problems such as anemia cases (encountered especially in children), iron being an indispensable element of the living organism, supporting the organism growth process and reducing the risk of illness.

## CONCLUSIONS

The main objective of this study was to create the necessary framework for the substantiation of the innovative development strategy on the efficiency of magnetic nanoparticles in foliar and root zone fertilization. Iron oxide nanoparticles stabilized in solution were obtained by an adapted co-precipitation method. Furthermore, in this research, the dried leaves, from both

iron oxide fertilized (foliar and root zone levels) and control plants were analyzed by FTIR spectroscopy. Following these studies, it was observed that iron was adsorbed after foliar and root zone fertilization of wheat plants. FTIR spectra obtained in the case of calcined samples better outlined the presence of adsorbed iron. The data obtained in this research showed that the fertilization with iron oxide solutions can have a positive influence on the young wheat plants by amassing the amount of dry matter, and by increasing the size of the plant mesophyll, which can lead to better plant growth and development processes. Following the fertilization with foliar and ground application, it was revealed an increase of chlorophyll content and the root system density by developing new adventitious roots that could contribute to a better absorption of water and nutrients. The results of this study could help to prevent iron deficiency and increase the body resistance to diseases with the help of a balanced diet.

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