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Agronomic biofortification of *Eruca sativa* L. with iron in nutrient film technique hydroponic cultivation

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Abstract

The global need for greater production of biofortified foods has increased especially in the case of hidden hunger. The agronomic biofortification of arugula with iron has become a strategy to solve the low intake of this nutrient in population. Therefore, the objective of this study was to evaluate the agronomic biofortification of two arugula cultivars in a hydroponic-NFT system, in two seasons (winter and summer), under the effect of different concentrations of iron in the nutrient solution. The experimental design was carried out in randomized blocks, in a factorial scheme with 4 concentrations of chelated iron (EDDHA) (1.8, 3.6, 5.4 and 7.2 g 1000 L^{-1}) in the nutrient solution with fertilizers and 2 arugula cultivars (Astro and Roka), and four repetitions. The harvest was To carried out 25 days after transplanting (DAT) in winter, and in summer at 20 DAT. The agronomic analyzes were: plant diameter, number of leaves per plant, length and width of leaves, package height, fresh root mass and fresh shoot mass productivity in m², and iron content in shoots and roots. We found that the cultivation period influenced the plant's ability to accumulate iron. In summer, the faster metabolism of plants together with greater transpiration caused a faster daily saturation of Fe content by the roots. The plants showed an intense green color, with an increase in the iron concentration in the nutrient solution of both cultivars. We concluded that agronomic biofortification of arugula is possible in both cultivars. We observed that a concentration of 3.6 mg 1000L^{-1} of iron (iron content 9.70% above control) in winter is suitable for Astro. For cv.Roka, we indicated a concentration of 7.2 mg 1000 L^{-1} of iron (iron content 47.9% higher than the control), in summer. Both are recommended for consuming iron from plants for human nutrition.

Keywords: *Eruca sativa* Miller L., Plant nutrition, EDDHA, NFT, Hydroponics, Sustainability.

Introduction

Hidden hunger or non-explicit nutritional deficiency, in one or more micronutrients, is identified as the most prevalent nutritional problem in the world (Rush et al., 2019). It is greatly affected by wage inequality, in addition to the fast pace of urban life. Cases of food insecurity and hidden hunger have increased around the world, a scenario that is amplified by the population increase, in which by 2050, could reach 10 billion people (Vilela et al., 2019), thus increasing the demand for food on the same portion of cultivable land.
Regarding iron, estimates indicate that approximately two billion people around the world have some type of anemia (Fisberg et al., 2018), which can cause delays in motor and mental functions in children, impair memory in adolescents and cause fatigue in adults, interfering with the ability to perform physical activities on a daily basis (Clark, 2008). Another example is pregnant women, who need Fe supplementation during pregnancy, as during pregnancy there is a risk of premature birth of babies, which is prone to health problems and can lead to death (Clark, 2008). Furthermore, vegetarian diets have been associated with iron deficiency (Kabata-Pendias et al., 2007). This is because vegetable iron is in the non-heme form, which is less absorbed than the heme iron present in foods of animal origin. For this non-heme iron to be absorbed, it is necessary to ingest a citrus food, such as orange juice.
Rocket (*Eruca sativa*) has approximately 1.4 mg of iron in every 100 g of fresh mass (Genuncio et al., 2011), whereas meat has 15 mg in a portion of 150 g (Pedreira, 2006). Therefore, to absorb the same amount of iron, it would be necessary to consume 1 kg of fresh mass of rocket. Since this does not happen, there is a need for biofortification of plants with Fe in the culture or planting system. In Brazil, there is an increasing market for vegan and vegetarian consumers. In 2017, it reached 8 %, and in 2018 reached 14 % of population (Aydar et al., 2019; Silva et al., 2020).
However, this deficiency is easily resolved by diet diversification, supplements and medicines, these treatments may not be available for the population in general because of geographic and financial limitations. Rocket becomes a possible alternative source of iron in human nutrition, as it is a crop that is easy to grow in home gardens and hydroponics.
Among leafy vegetables, lettuce is the most planted and most consumed by the Brazilian population. However, since the end of the 90s, rocket consumption has been increasing (Purgueiro et al., 2007), currently becoming the second most

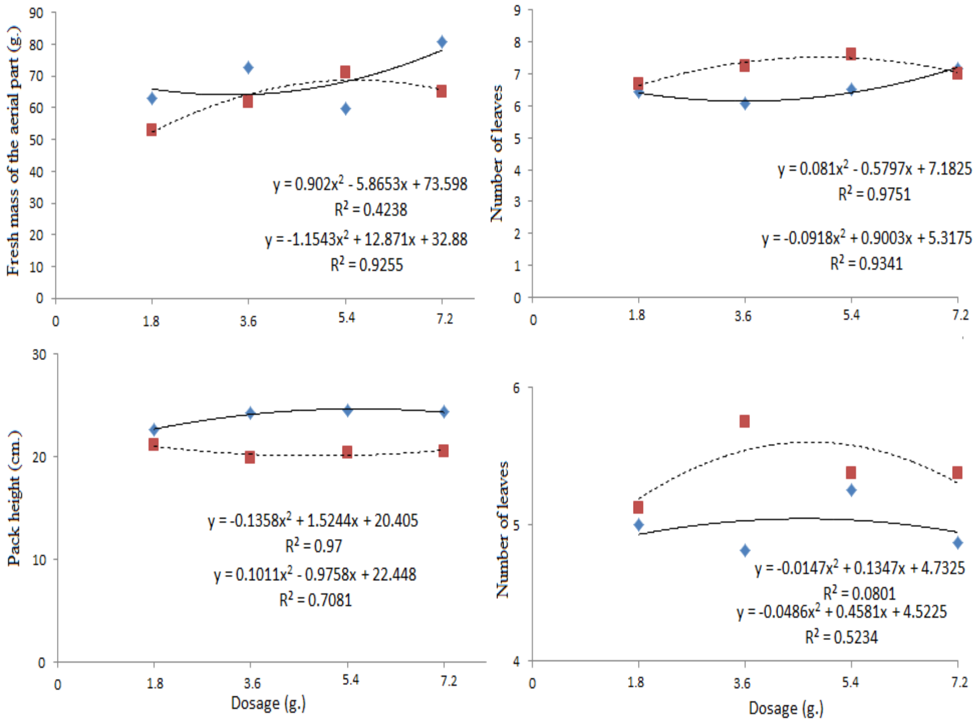

Figure 1. Fresh mass of the aerial part, number of leaves in the Winter and pack height and number of leaves in the Summer, of the cultivars Astro (—◆—) and Roka (- ■ -) as a function of iron concentration in the nutrient solution. UFSCar, Araras (SP) - 2021.

planted leafy vegetable in NFT hydroponic systems in Brazil, due to its short cycle.

Agronomic and genetic biofortification can increase the concentrations and/or bioavailability of mineral elements in the edible part of plants (Cakmak et al., 2004; Graham, 2007; Pfeiffer; Mcclafferty, 2007; Cakmak, 2008; White; Broadley, 2009; Kumar et al., 2017, Moraes et al., 2022)

Agronomic biofortification is an earlier technique and with faster results, which serves to increase the nutritional quality of the foods. This has already been studied in lettuce (*Lactuca sativa*) with zinc (Moraes et al., 2022), and beet (*Beta vulgaris*) (Carmona, 2020) and kidney bean (*Phaseolus vulgaris*) (Petry et al. 2015; Cambraia, 2019) with iron (Laurett, 2017; Gioia, 2019; Giordano, 2019).

The agronomic biofortification in leafy vegetables can be performed with fertilizer applications in the soil, in a nutrient solution (hydroponics) or in the leaves. Nevertheless, in hydroponics it is a very feasible technique, given the practicality of the application, and for being a production system without soil in a closed environment, allowing the generation of products with equal or superior quality to the field, especially in tropical environments.

In brassica microgreens, Gioia et al. (2019) increased 64 % in the iron concentration in rocket. With kidney beans (*Phaseolus vulgaris*), Petry et al. (2015) provided 30 % to 50 % of the daily iron requirement in 100 g of bean, and in lettuce (*Lactuca sativa*), Moraes (2022) provided zinc in 50 g of lettuce for the cultivars Vanda (20 %) and Saladela (43 %).

The aim of this study was to evaluate the effect of iron concentrations in the nutrient solution, aiming at biofortifying rocket cultivated in NFT hydroponics.

Results and Discussion

Agronomic characteristics of structure analyses

At the concentration of 3.6 g of iron, the highest fresh mass of the aerial part (FMAP) was observed with 72.6 g of fresh aerial mass and the concentration of 7.2 g of iron, with 80.9 g of fresh mass aerial part of the Astro variety and in the concentration of 5.4 g of iron with 71.1 g of fresh mass of the aerial part of the Roka variety. The results of the work of Gioia et al. (2019), with rocket microgreens at increasing iron concentrations, showed that applying more than 20 g of iron for 1000 L^{-1}, a lowest fresh mass (60 g) was obtained. According to Giordano et al. (2019), studying iron concentrations in green and red Salanova® lettuce, a lowest fresh mass showed, with an increased in iron concentration from 0.015 to 2.0 mM, in the cultivars in NFT hydroponics. Although the concentration of 3.6 g of iron for Astro presented FMAP, leaf number (LN) was smaller, because the pH maintained around 5.5 (ideal for a greater iron absorption) may have led to a smaller availability of phosphorus and boron, fundamental nutrients in the processes of cell division and cell increase (Hammond; White, 2008; Santos et al 2015; Taiz et al., 2017). On the other hand, in the summer, the concentration of 5.4 g of iron for Astro resulted in 52.8 g of FMAP (10.67 % more than the control) and in Roka the highest FMAP was at 7.2 g of iron, with 49.8 g (9.36 % more than the control). Nonetheless, Roka was inferior to Astro in this feature, which is a genetic characteristic of this cultivar (Figure 2). It is highlighted that Roka is greatly used in the market of minimally processed in Brazil, given the genetic characteristics of thicker and crispy leaves, with a lower susceptibility to the mechanical damages of minimal processing, in the steps of centrifugation and drying, besides a longer postharvest by the slow water loss by transpiration or lower oxidation.

In the winter, LN was superior to seven at the concentration of 7.2 g of iron in Astro and at the concentrations of 3.6 g; 5.4 g and 7.2 g of iron of Roka, being superior to 7 leaves, generating an increase of 10.4% at the concentration of 7.2 g of iron in Astro in comparison with the control, and of 7.8 % (3.6 g), 12.3 % (5.4 g) and 4.5 % (7.2 g) greater in Roka (Figure 1). Different results were obtained by Giordano et al.

Table 1. Fe content in a portion of 50 g of fresh mass of the aerial part (FMAP) of the rocket cultivars Astro and Roka in the period of winter and summer, and contribution of this portion in percentage for the recommended daily intake of Fe, as a function of iron concentration in the nutrient solution. UFSCar, Araras (SP) - 2021.

Fe Concentration (g 1000L^{-1})	Fe in 50g of FMAP (mg)		Daily recommendation of Fe** (%)	
		Astro		
	Winter	Summer	Winter	Summer
1.8	14.6	10.2	71.7	19.9
3.6	16.0	8.3	88.3	1.7
5.4	10.1	10.0	19.6	18.0
7.2	12.0	10.5	41.9	23.3
Fe Concentrations (g 1.000L^{-1})	Fe in 50g of FMAP (mg)		Daily recommendation of Fe** (%)	
		Roka		
	Winter	Summer	Winter	Summer
1.8	14.6	10.3	71.4	21
3.6	11.7	9.4	37.3	10.4
5.4	12.7	12.5	49.4	41.5
7.2	12.8	15.1	50.3	77.3

*Recommendation of ingestion from 5.5 to 11.1 mg of Fe mg day^{-1} on an omnivorous diet, for the content of 1 to 2 mg of iron to occur in the organism. In traditional rocket, the amount of iron present is of 0.9 mg every 100g of fresh mass (TACO, 2011). **Mean of 8.5 mg of Fe day^{-1}, equivalent to 46.9 mg to 62.7mg of Fe.

(2019), where no differences were observed in LN among the iron concentrations for the cultivation of lettuce varieties. Laurett (2017), observed a reduction in LN with the rise in iron concentration in the nutrient solution ofrocket , in other words, in the present work, a better response was obtained than in the study, since the concentrations tested by the author were higher, in a different cultivar (cv. Rococó), season of the year (fall) and with a greater harvest time (27 DAT). In the summer, the concentration of 5.4 g of iron in Astro and 3.6 g of iron in Roka presented the highest LN (Figure 1), producing 4.7 % and 10.9 % more, respectively. This result was little different from Laurett (2017), who obtained a reduction in LN for lettuce and rocket, with the increase in Fe concentration in the solution, 70.5 % and 38.7 % less for lettuce and rocket, respectively. Nevertheless, the values observed by Queiroz et al. (2014) in hydroponic lettuce in the summer had a mean of 17.1 leaves per plant, being the highest LN.

For plant diameter (DI) in the cultivation in the summer, all treatments presented values above 20 cm, except for the concentration of 5.4 g of iron, in both cultivars, which presented values inferior to the others, with a difference of 9.4 % less in Astro and 7.4 % less in Roka in comparison to the concentration of 1.8 g (control).

For Height (HGT) in the cultivation in the winter, the mean of the concentration 7.2 g of iron presented the greatest height compared to others, representing 5.5 % more than the control. In the summer, a higher HGT was observed deriving from the rise in the iron concentration (Figure 1), with Astro presenting the greatest height in comparison with Roka, presenting the values of 24.5 cm and 21.1 cm, a difference of 3.3 cm (13.7 % more between the cultivars), respectively. The opposite was verified by Laurett (2017) for rocket, where plant height presented a reduction of 34.9 % at the highest Fe concentration compared to the control, using three seeds per cell, probably causing the most pronounced phytotoxicity and consequently, lower plant height.

As iron acts in reactions during photosynthesis (Kerbauy, 2008) and in the formation of chlorophyll, these factors may be responsible for greater plant growth, since their photosynthetic efficiency occurs and, consequently, the growth and adaptability to different environments. in response to chlorophyll content. The chlorophyll content is used to estimate the photosynthetic potential of plants, given its direct link with the absorption and transfer of light energy, as a plant with a high concentration of chlorophyll can achieve even higher photosynthetic rates (Rêgo, 2004), thus generating greater growth. However, it must be considered that the morphological characteristics of the genetic materials were different, but in both cases it was possible to increase HGT in relation to the control. Therefore, as hydroponic rockets are sold in conical polyethylene packaging, they can give the visual appearance of a larger plant on the supermarket counter.

As in the study of Laurett (2017), the highest iron content provided the greatest accumulation in the roots (Figure 3). The values of iron absorbed between both cultivars and translocated to the aerial part were between 203.8 and 321.0 mg kg^{-1}. The iron content in the root (FeR) was greater than in the aerial part (FeAP), around 10-20 times, given the fact that iron has small mobility in the plant, getting accumulated in the root system, and thus not being translocated to the aerial part (Laurett, 2017). In the summer, it was observed that there was a drop in iron content in the roots, from 3782.7 mg kg^{-1} to 3112.7 mg kg^{-1} (21.5% less), as the iron concentration rose in the nutrient solution, in the cultivar Roka. This happened because, in the summer, with the higher temperature and solar radiation, the ability of a plant of removing water from the soil and moving it to its edible parts, as well as of losing it by transpiration, depends on water potential, in other words, the water status of the plant and the water availability in the soil (Taiz; Zeiger, 2017); therefore, the iron concentration in the roots must have decreased, given the greater movement of the nutrient solution from the root to the shoot.

Figure 2. Comparison of Astro (A) (left) and Roka (R) (right) as a function of iron concentration in the nutrient solution, in the summer.

Furthermore, the lowest concentration generated the best result, since it did not saturate the root system in an exacerbated way, thus being more diluted and more susceptible to translocation in the plant (Malavolta et al.,1989). The same happened for Astro, where the greatest iron contents in the roots were in the control (4579.1 mg Kg^{-1}). This greater FeR accumulation derives from the probable low mobility of iron and its precipitation in the older leaves as insoluble oxides. This iron precipitation decreases the mobility into the phloem, with a consequent decrease in the long-distance transport (Taiz; Zeiger, 2017). According to Malavolta et al. (1989), the capacity of the root to absorb ions is limited, since, when the internal concentration of an ion increases, the absorption rate declines and vice versa (Faquin, 2005). Stein et al. (2009) reported that the reduced iron translocation from the roots to the aerial part has been indicated as a mechanism of defense against the stress caused by the toxicity of iron.

Considering the results of the winter, it was possible to biofortify rocket with iron, since the concentration of 3.6 g of iron in Astro presented a far greater iron content than the other treatments, being 9.7 % more than the control. Nevertheless, for Roka, it was not possible to verify an increase in the content of absorbed iron. Conversely, in the summer, although not presenting statistical difference, numerically, the concentration of 7.2 g of iron in Astro presented the greatest accumulation in the aerial part (210.3 mg kg^{-1} or 9.7 % more than the control), whereas only the concentration of 7.2 g of iron in Roka had the highest accumulation of FeAP (302.3 mg kg^{-1} or 47.9 % more than the control), differing from the others by the statistical analysis performed. However, this concentration of iron in the leaves could have been higher, if the pH of the solution were maintained at 5.5, thus indicating that biofortification may be related to the genetic material, iron concentration in the nutrient solution and period of cultivation. Nonetheless, studies must be performed with different types of iron chelates and in different forms of application, aiming at enhancing the content of this ion by the plant.

Considering the estimate of contribution of the rocket biofortified with Fe on the recommended daily intake from 5.5 to 11.1 mg of Fe, it was observed that the Fe amounts in the portion of 50 g of mass of the aerial part obtained in the winter were higher in Astro, at the concentration of 3.6 mg 1000 L^{-1} of iron in the nutrient solution. Comparing the treatment of 3.6 g of iron with the Fe naturally present in rocket (1.4 mg 100 g^{-1}) (Taco, 2011), an increase of 88.3 % in daily intake was verified (Table 1). Conversely, in the summer, with the estimate of contribution of the rocket biofortified with Fe on the recommended daily intake from 5.5 to 11.1 mg of Fe, it was observed that the Fe concentration in the portion of 50 g obtained in the summer were the highest at the concentration of 7.2 g of iron in Roka, at the concentration of 7.4 g 1000 L^{-1}. Comparing the treatment of 7.2g of iron with the Fe naturally present in rocket (1,4 mg 100 g^{-1}), an increase of 77.3 % was verified for daily intake. Nevertheless, the accumulation of iron in the leaves must be better studied, since it is necessary to verify whether the efficiency of the bioavailability of this nutrient in the human organism is adequate.

Instrumental color analyses

According to the results of leaf area (AF) in the winter period (Table 2), there was no difference between treatments of the same variety, except for the concentration of 1.8 g of iron in Astro. However, as the concentration of iron in the nutrient solution increased, except for the concentration of 5.4 g of iron in Astro and 7.2 g of iron in Roka, there was a difference in leaf area. However, in the study of Laurett et al. (2017), as the iron concentration in the nutrient solution increased, LA decreased, and it was also described that the rockets with higher LA presented higher FMAP. This was not observed in this work, possibly because the plants developed in a shaded environment are bigger and contain more chlorophyll in each reaction center; nevertheless, they present a lower thickness than those under full sun (Taiz; Zeiger, 2017), which can result in a lower fresh mass, since, according to Dias-Filho (1997) and Reich et al. (1998), shading leads to alterations that maximize the capture of light, increasing leaf area, decreasing the ratio between the root and the aerial part and reducing leaf mass. However, in comparison with Novo et al. (2010) for commercial leaves,

Table 2. Means of leaf area (L.A.), total chlorophyll index (T.C.I.), instrumental color (*a**), (b*) and L of the rockets produced in an NFT hydroponic system with different iron concentrations, in the winter and summer. UFSCar, Araras (SP) - 2021.

	Astro				Roka				
	Winter								
	1.8	**3.6**	**5.4**	**7.2**	**1.8**	**3.6**	**5.4**	**7.2**	**CV(%)**
L.A.	595.2b	697.4ª	799.6a	750.5a	498.8b	520.1b	639.7b	549.9b	17.3
T.C.I.*	38.8b	41.4b	37.4b	40.1b	42.4b	41.3b	41.5b	52.1a	12.1
Color a*	-12.5a	-14.3b	-12.5a	-11.1a	-11.6a	-11.7a	-11.6a	-10.6a	8.9
Color b*	22.6a	26.3a	22.8b	18.5b	19.0b	18.3b	19.9b	17.1b	12.1
Color L	45.0a	47.0a	43.8b	42.2b	43.1b	42.5b	43.7b	42.2b	3.15
	Astro				**Roka**				
	Summer								
	1.8	**3.6**	**5.4**	**7.2**	**1.8**	**3.6**	**5.4**	**7.2**	**CV(%)**
L.A.	569.3b	647.7a	719.4a	551.8b	558.7b	515.2b	587.0b	606.2b	12.0
T.C.I.*	33.9b	31.5b	29.2b	29.3b	39.7a	40.2a	41.2a	37.9a	12.0
Color a*	-13.2a	-12.4a	-12.9a	-12.7a	-12.0a	-10.6b	-10.8b	-11.7b	7.6
Color b*	25.4a	23.8a	24.3a	23.8a	21.2b	18.1b	18.1b	20.5b	11.22
Color L	48.3a	47.9a	46.5a	47.1a	46.2a	46.2a	44.4a	45.8a	3.43

Means followed by distinct letters on the lines differ from each other ($p \leq 0.05$) by the Scott-Knott test. L = Luminosity (0= dark and 100= white); *a** = red/green coordinate (+a indicates red and –a indicates green); *b** = yellow/blue coordinate (+b indicates yellow and –b indicates blue). * SPAD units.

the values of this study are close to the varieties with the biggest leaves (cultivar Orelha de elefante and cultivar Vale das Garças), between 500 and 650 cm^2. In the summer, the results of leaf area did not present difference among the treatments of Roka, confirming what was obtained in the winter harvest.
At a concentration of 7.2 g of iron, during the winter, it showed a higher mean total chlorophyll index (TCI) in leaves of the Roka cultivar (Table 2). The intense green color provided by chlorophyll can make it more attractive for consumers. The variation from light green to intense green color among the treatments (1.8, 3.6, 5.4 and 7.2 g of Fe. 1000 L^{-1} of water) and varieties (Astro and Roka) was from 37.4 to 52.1 mg g^{-1}, as the SPAD index increases one unit. This was not observed by Laurett et al. (2017), analyzing TCI, who did not perceive a difference with the increase in iron concentration. This happened because the concentrations assayed in the research were inferior to the limit concentration recommended by Laurett et al. (2017), so that phytotoxicity does not occur due to excess iron. Therefore, it was possible to equilibrate the ionic balance of the nutrient solution and, thus, increase chlorophyll content in the leaves. In the summer, the chlorophyll index in the rocket leaves was higher in Roka than in Astro and without significant difference among the treatments of the same cultivar, being similar to the study of Laurett (2017) described previously.
For coordinate a^*(Table 2), red/green (+a indicates red and –a indicates green) in the winter, the concentration of 3.6 g of iron in Astro presented a value superior to the others (-14.4), and for Roka there was no significant difference. Coordinate *a* in the summer indicated 1.8 g of Roka as superior to the other treatments applied in the cultivar (-12.0), and among the treatments in Astro there was no significant difference. The values obtained were numerically similar in both seasons.
In the present research, as well as in the research of Laurett et al. (2017), it was possible to observe that there were no symptoms of phytotoxicity, demonstrating it is possible to biofortify rocket with iron at low concentrations in the nutrient solution.

Materials and methods

Plant materials
The rocket cultivars used were Astro, characterized by having large leaves with less cultivated areas, and more early cycle, with vigorous plants and high productivity, good visual quality also moderate resistance to early bolting. The other rocket was called Roka, which has a shorter plant, dark green and tender leaves, perfect for minimal processing also has a good root system adapting very well to hydroponic cultivation.

Experimental site
The experiments were conducted in a protected environment in hydroponic system in the experimental area of the Department of Biotechnology and Plant and Animal Production, Horticulture sector, at the Center for Agrarian Sciences of the Federal University of São Carlos, in Araras, SP (22° 21'S, 47°23'W; 640 m of altitude).

Experimental design
The experimental design was of randomized blocks, in factorial scheme with four concentrations of chelated iron (EDDHA) in the nutrient solution (1.8; 3.6; 5.4 and 7.2 g of iron 1000 L^{-1} in Furlani (1998) solution) and two rocket cultivars (Astro and Roka), with four repetitions.

Experimental procedure
Two experiments were performed, the first in the period of winter (June/July, 2021) and the second in the summer (November/December, 2021).
The rocket seedlings were produced in polypropylene trays with 128 cells, with 12 seeds per cell. Subsequently, they were transplanted to the hydroponic profiles on Jun/18/2021, in the winter, and Nov/11/21, in the summer.
The protected environment had a structure of greenhouse, where the right foot was with 3.5 meters, 15 meters in length and 7 meters in width, covered in the superior part by Aluminet® silver 20% retractable (providing better conditions for the cultivation of rocket, which is little tolerant to high radiation), sides with 20% red ChromatiNet® Leno mesh, and

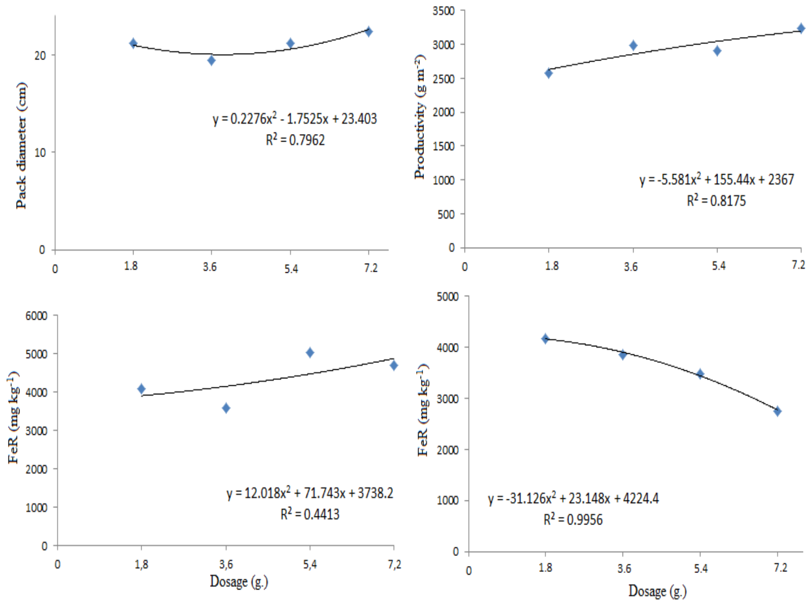

Figure 3. Means of the features of pack diameter, productivity (Prod. (g m^{-2}) in the Winter and summer, and iron content in the root (FeR) (mg kg^{-1}), in the Winter and iron content in the root (FeR) in the Summer, of the cultivars Astro and Roka as a function of iron concentration in the nutrient solution. UFSCar, Araras (SP) - 2021.

the nutrient solution was pumped every 15 minutes in the period from 6-10h and only 3 times at night.

The experiments were performed in an NFT system with cultivation profiles of 50 mm in diameter, with benches with 9% of slope composed of 6 profiles/treatment, spacing of 15 (plant) x 20 cm (profile), where each profile was composed of 44 holes, totalizing 264 plants, with 4 repetitions for each cultivar. The nutrient solution used for the cultivation followed the recommendation of Furlani, (1998): 750 g $1000L^{-1}$ of calcium nitrate, 500 g $1000L^{-1}$ of potassium nitrate, 150 g $1000L^{-1}$ of monoammonium phosphate and 400 g $1000L^{-1}$ of magnesium sulfate. The fertilizer ConMicros *Light*® was employed, 20 g $1000L^{-1}$, with the following composition: Cu EDTA (4.1%), Zn EDTA (1.6%), Mn EDTA (4.1%), B (4.1%), Mo (0.9%) and Ni (0.8%). Based on the absence of Fe in the fertilizer ConMicros *Light*®, the Fe chelate commercially named FERRILENE was added, with 6% of iron.

The iron doses were calculated, using as control the dose recommended by Furlani, (1998), with the concentration of 1.8g of iron per $1000L^{-1}$. Four 5L buckets were used as a reservoir of the different iron concentrations at the concentrations of 1.8g (control), 3.6g, 5.4g and 7.2g $1000L^{-1}$. Additionally, two 50L plastic barrels were used, as a solution concentrated in 100 times the dose for 1000L and separated in A with the nitrates and B with the other nutrients and micros, to avoid precipitation by incompatibility.

Daily, electrical conductivity (EC) and pH were verified. When EC was below 2.0 Siemens m^{-1}, a replacement was performed with the concentrated solution until stabilizing at an EC of 2.0 Siemens m^{-1}.

The nutrient solutions at the beginning and at the end of the experiment were collected for each treatment for macro and micronutrient analysis.

Evaluated features

To perform the analyses, the harvest was performed 25 days after transplantation (DAT) in the winter, whereas in the summer, at 20 DAT. Four useful packs of each repetition were analyzed, randomly chosen. After harvest, they were taken to the Laboratory of Horticulture, and evaluated agronomically and biometrically.

The agronomic analyses were: pack diameter (Diam.) (cm); number of leaves per plant (LN) (considering leaves from 1 cm); leaf length (LL) and width (W); pack height (HGT); fresh mass of the root (FMR) and fresh mass of the aerial part (FMAP); productivity in m^{-2} (Product.) calculated by the value of the FMAP by the number of plants in one square meter (Kg.m^{-2}); Iron content in the aerial part (FeAP) and roots (FeR). The dry materials were sent for analysis in a laboratory, being ground in a Willey-type mill for the determination of the Fe content (roots and aerial part) by nitric-perchloric digestion and by reading in inductively coupled plasma – optical emission spectrometry (ICP – OES) and iron accumulation was determined by the product between its content in the plant tissue and the amount of dry mass of the corresponding part (mg $plant^{-1}$).

The instrumental analyses were: leaf area (LA), in the device Li-cor 3000[A], Licor Inc., Lincon, Nebrasca, USA, in four plants of each treatment, using whole leaves. The total chlorophyll index (TCI) was evaluated in the device SPAD-502 Plus, collecting the TCI of two plants at two reading points for each leaf, two leaves per plant, preferably the median, and colorimetric analysis in two leaves per plant per treatment, using the colorimeter Hunterlab, registering the value L (Luminosity), which varies from dark (L = 0) to white (L = 100); value *a**, which varies from the red color (+*a**) to green (-*a**); and value *b**, which varies from yellow (+*b**) to blue (-*b**) (MINOLTA, 2007). For the measurements of leaf area (LA), total chlorophyll index (TCI) and instrumental color analysis (L, *a**, *b**), four useful packs of each repetition/treatment were used.

Statistical analysis

The data obtained were subjected to the analysis of variance (ANOVA) and the regression analysis was performed for the iron concentrations in the nutrient solution of all features, considering the adjustment with the highest coefficient of determination (R^2) and lowest standard error (Epad) as the best one, and for the cultivars, the Tukey's test was performed using the Software Sisvar. For the elaboration of the graphs, the Office package, Excel 2016, was used.

Conclusion

After compiling all the agronomic characteristics, was verified, that was possible to biofortify both rockets cultivars, but at different times and doses. The cultivar Astro can be better biofortified in winter at a dose of 3.6 g, also cultivar Roka was better biofortified in summer at higher doses (7.2 g), being both indicated to consume iron from plants for human nutritional.

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