

RESEARCH ARTICLE

# Characterization of growth and visual symptoms of nitrogen, potassium and magnesium deficiencies in arugula

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## ABSTRACT

Arugula is a short-cycle species with a high demand for nutrients. In the plant, each nutrient performs one or more specific functions, so its deficiency will negatively impact the crop growth and development, being important to recognize the nutritional symptoms to help in decision making. In this context, the objective of this study was to describe the evolution of N, K, and Mg deficiencies symptoms and their effect on the growth of arugula plants. One at a time, the nutrients were omitted from the nutrient solution. At 13, 16, 18, and 20 days after transplanting (DAT) the seedlings to the - Nutrient Film Technique (NFT) system, plants were collected, symptoms characterized, and leaves were scanned in JPEG (1.200 pixels). At 10 DAT of arugula, plants grew in a nutrient solution N-depleted shown the initial visual symptoms of N deficiency and at 16 DAT was recorded the first symptoms of K and Mg depletion in nutrient solution. At 20 DAT, the plants showed severe N deficiency symptoms, the old leaves presented intense and generalized chlorosis, followed by necrosis. Deficient plants in K exhibited chlorosis at the edge of old leaves advancing to the center of the leaves and small white dots. For Mg, the older leaves developed interveinal chlorosis from the leaf tip towards the central vein. The omission of nitrogen resulted in plants with lower height, leaf area, and number of leaves when compared to arugula plants grown in a complete nutrient solution.

**Keywords:** *Eruca sativa* Miller, nutritional deficiency, physiological disorder

## INTRODUCTION

The arugula (*Eruca sativa* Miller) is a leafy green with a high yield potential. Its cultivation has been rapidly increasing due to its nutritional characteristics, which is rich in iron, sulfur, and vitamins A and C (Nunes et al., 2013), being widely consumed by people with healthy food (Bjorkman et al., 2011; Traka and Mithen, 2011; Oliveira et al., 2015). Arugula is a short-cycle species with a high demand for nutrients, especially potassium (K), nitrogen (N), and magnesium (Mg) the most accumulated nutrients in this crop (Grangeiro et al., 2011).

In plant metabolism, each nutrient performs one or more specific functions (ENGELS et al., 2012; Taiz et al., 2017). Nutrient in the plant tissue below the necessary rate for optimal growth and development take the plant to nutritional deficiency. (Epstein and Bloom, 2006).

Symptoms of nutrient deficiency appear in plants grown in a medium (soil, substrate or nutrient solution) with low-nutrient availability, that can be caused, for example, for the presence of a nutrient in a chemical form that the plant cannot absorb, or a decline in the roots capacity to absorb the element (Hawkesford et al., 2012). In a severe nutrient deficiency, plants will exhibit characteristic symptoms for each nutrient (Römheld, 2012; Li et al., 2020). With this, is possible to identify the deficient nutrient in the plant, helping technicians, researchers, and producers to diagnose the nutrient status of plants to proceed with possible corrections (Epstein and Bloom, 2006). This way of diagnosing the plant nutritional status is called visual diagnosis (Taiz et al., 2017; LI et al., 2020).

In general, some researches already described the main symptoms of nutrient deficiencies for some leafy vegetables, such as lettuce (Goodall et al., 1997; Malavolta et al., 1997; Gazula et al., 2007) and cabbage (Pizetta et al., 2005), even so,

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there are still variations in the appearance of interspecies and intra-species nutrient disorders (Hawkesford et al., 2012). The description of the nutrient deficiencies symptoms for the arugula crop was not found in the literature.

Based on the above, the objective of this study was to describe the evolution of N, K, and Mg deficiencies symptoms and their effect on the growth of arugula plants.

## MATERIAL AND METHODS

### Experimental site

The study was carried out from August 28 to October 12, 2018, using a *nutrient film technique* (NFT) hydroponic system at UNESP, campus Jaboticabal, São Paulo - Brazil (21°15'22" S, 48°15'58" W; 615 m a.s.l.).

During the experimental period the mean maximum, mean minimum, and average temperatures were 39.9 °C, 26.4 °C, and 32.5 °C. The mean maximum, mean minimum, and average relative humidity were 67.6%, 17.0%, and 43.8%. The average solar radiation was 848,2 MJ m<sup>-2</sup>.

### Experimental design and treatments

The experiment was carried out in randomized block design, with three replicates. The treatments consisted of providing four distinct nutrient solutions for arugula growth: a complete nutrient solution (SC) and equal solutions but without nitrogen (-N), magnesium (-Mg), and potassium (-K). The sources and amounts of nutrients used for each nutrient solution are described in Table 1. For micronutrients, the following amounts of fertilizers were used in all treatments: 1.76 (boric acid); 0.15 (copper sulfate); 1.5 (manganese sulfate); 0.30 (zinc sulfate); 0.10 (ammonium molybdate) g L<sup>-1</sup> and 0,06 mL L<sup>-1</sup> (iron) (Furlani, 1998). The experimental unit consisted of a 1.3 m long with four-channel bench. Container with 100 L capacity was nutritive solution reservoir. Recirculation of nutrient solution was performed by a submerged pump at flow rate of 1000 L h<sup>-1</sup>.

### Installation and conduction of the experiment

The arugula 'Folha Larga' was sown on August 28 in a 2 x 2 x 2 cm block of phenolic foam, previously washed in running water for approximately ten minutes, in order to eliminate some contaminant that could impairing planting. During the seed germination, the phenolic foams remained in a greenhouse with a sprinkling system being irrigated with water (Phase I).

On September 5<sup>th</sup>, eight days after sowing (DAS), when the seedlings displayed expanded cotyledons, the phenolic foam were individualized, and the seedlings were transplanted to a 5 cm diameter polypropylene channels in the NFT system (Phase II). The channels of this structure called "nursery"

had 5% declivity. The supply of the nutrient solution was intermittent, alternating 15 minutes with circulation and 15 minutes without circulation. During this phase, plants received a complete nutrient solution recommended by Furlani (1998).

Later, when the plants stated 3 leaves (Phase II) were transplanted on September 22 (25 DAS) to the final growth channel (10 cm in diameter), with spacing between channels of 0.25 m and 0.05 m between plants within a channel, totaling 240 plants per plot (Phase III). At this phase, the treatments corresponding to the complete nutrient solution and solutions without N, K, and Mg were applied throughout the crop cycle (Table 1).

In Phase III, the activation of pumps utilized to recirculate the nutrient solution was regulated by a timer, beginning at 07:00 h and ending at 18:00 h, without interruption.

The pH was maintained between 5.5 and 6.5 using sodium hydroxide and hydrochloric acid to raise and lower pH, respectively. The nutrient solution was renewed when the electrical conductivity (EC) of the treatments reached 70% of the initial EC (dS m<sup>-1</sup>). Three renovations were made during the experiment.

### Evaluated Characteristics

The biometric characteristics evaluated were: plant height, measured from the base of the plant to the highest end of the foil; number of leaves; and leaf area, using a LI-COR 3100 electronic meter.

### Nitrogen content (-N)

The plant was divided into three parts (20 DAT): old leaf (OL), intermediate leaf (IL), and new leaf (NL). After washing and cleaning the leaves, they were packed in paper bags and identified, then placed to dry in an oven with forced air circulation at 65 ± 5°C until a constant weight were reached. After drying, the material was ground and weighed (0.1 g). The sulfuric digestion was performed, and the N content was determined according to Miyazawa et al. (2009).

### Images acquiring

Plants were collected and scanning at 13, 16, 18, and 20 DAT to perform the identification of nutrient deficiencies symptoms.

Ten plants were randomly collected per bench and washed in deionized water and then dried on paper towels. The leaves were scanned using a conventional desktop scanner, acquiring images with a horizontal and vertical resolution of 1.200 DPI. The images were stored with dimensions of width and height equal to 10.200 x 14.040 pixels and saved in JPEG compression format (*Joint Photographic Experts Group*).

**Table 1: Amount of salts and acids used in the preparation of the complete nutrient solution and with omission of nitrogen, potassium and magnesium (FURLANI, 1998).**

Nutritive solution	SM	NA	NK	NC	MAP	MKP	AK	AC	SA	SK	AF	AN
	g 1000L <sup>-1</sup>											
Complete	320	-	400	600	120	-	-	-	-	-	-	-
-N	320	-	-	-	-	135.6	335	495	-	-	-	-
-K	320	80	-	750	-	-	-	-	-	-	68	-
-Mg	-	-	242	600	-	-	-	-	72.4	140	68	100

SM = Magnesium sulphate; NA = Ammonium nitrate; NK = Potassium nitrate; NC = Calcium nitrate; MAP = Monoammonium phosphate; MKP = Monobasic potassium phosphate; AK = Potassium acetate; AC = Calcium acetate; SA = Ammonium sulfate; SK = Potassium sulfate; AF = Phosphoric acid and AN = Nitric acid.

The data were submitted to analysis of variance by the F test ( $p \leq 0.05$ ) and the means compared by the Student t-test ( $p \leq 0.05$ ). The AgroEstat statistics program was used (Barbosa e Maldonado Júnior, 2015).

## RESULTS AND DISCUSSION

The omission of N, affected the plant height, number of leaves, leaf area, and the foliar N content when compared to plants which received the complete nutrient solution (Table 2 and 3).

### Nitrogen (-N)

The first visual symptom of N deficiency was identified at 10 DAT, with greater evidence of visual diagnosis at 13 DAT. In this period, most of the symptoms were observed in the old leaves, through slight chlorosis with greater intensity in the leaf limb, growth reduction, and decrease in emission and leaf expansion (Image 1). The other leaves had a green color considered normal when compared to the plants which received a complete nutrient solution.

N is a structural component of amino acids, proteins, nitrogenous bases, enzymes, and energy transfer materials, such as chlorophyll, ADP, and ATP. Besides that, it plays an important role in the processes of ionic absorption, photosynthesis, respiration, multiplication and cellular differentiation (Hawkesford et al., 2012; Taiz et al., 2017).

As seen, N is a constituent of chlorophyll molecules and in its deficiency takes to decrease in the chlorophyll levels. The main characteristic observed with the N deficiency is the appearance of chlorosis that starts first in older leaves (Image 1), as a result of the high mobility of N (Taiz et al., 2017). This high mobility is due to the fact that proteins, compounds that are in constant synthesis and degradation, release permeable nitrogenous compounds into the phloem, providing N redistribution to new leaves.

At 16 DAT the symptoms were moderate with differences in shades of green for colors that ranged from yellow to

**Table 2: Summary of the variance analysis for plant height (H) and number of leaves (NL) of arugula plants at 20 days (Phase III) as a function of the complete nutrient solution and solution without nitrogen, potassium and magnesium.**

	H (cm)			NL (leaves plant <sup>-1</sup> )		
	-N	-K	-Mg	-N	-K	-Mg
Complete	32.9 a	32.9 a	32.90 a	15.26 a	15.26 a	15.26 a
Omission	21.01 b	30.20 a	31.96 a	12.73 b	14.63 a	14.30 a
Test F	692.90**	15.03 <sup>ns</sup>	11.70 <sup>ns</sup>	23.67*	1.07 <sup>ns</sup>	12.55 <sup>ns</sup>
CV (%)	2.05	2.70	1.03	4.55	5.01	2.26

\*\* 0.01 and \*0.05 level of significance by the F Test; ns: not significant. Averages with different letters in the column differed according to Student's t Test ( $p \leq 0.05$ ).

**Table 3: Summary of the variance analysis for the variables leaf area (LA) and foliar nitrogen content (N) in the new leaf (NL), intermediate leaf (IL) and old leaf (OL), in arugula plants at 20 days after transplanting (Phase III) as a function of the complete nutrient solution and solutions without nitrogen, potassium and magnesium.**

	LA (cm <sup>2</sup> per plant <sup>-1</sup> )			N (g kg <sup>-1</sup> )		
	-N	-K	-Mg	NL	IL	OL
Complete	718.68 a	718.69 a	718.69 a	37.69 a	31.60 a	16.55 a
Omission	253.00 b	543.19 a	678.20 a	18.87 b	10.71 b	6.97 b
Test F	129,94**	13.44 <sup>ns</sup>	0.29 <sup>ns</sup>	19930.38**	296.59**	41.42*
CV (%)	10,29	9.29	13.15	0.57	7.02	15.49

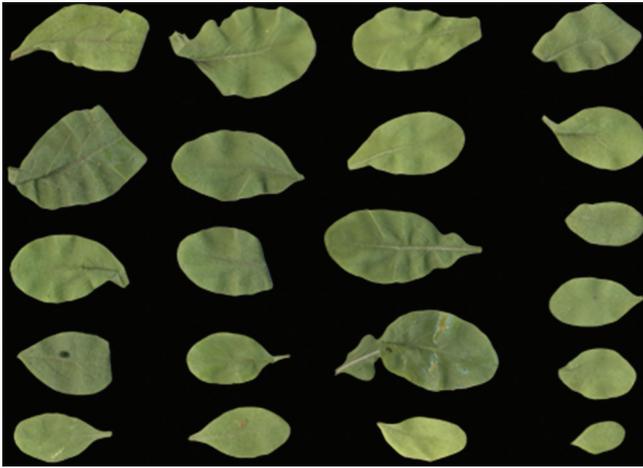
\*\*0.01 and \*0.05 level of significance by the F Test; ns: not significant. Averages with different letters in the column differed according to Student t Test ( $p \leq 0,05$ ).

light green in old leaves, and presence of purple tones in intermediate leaves, due to the accumulation of the anthocyanin pigment (Image 2).

At 20 DAT, among all the treatments evaluated, the lack of added N had significant effect reducing height, number of leaves and leaf area (Table 2 and 3).

For the arugula crop, N is the second most accumulated nutrient (Grangeiro et al., 2011). As well as other leafy green, such as lettuce, studies have shown that arugula is highly responsive to N fertilization (Purquerio et al., 2007; Affonso and Cecílio Filho, 2009; Steiner et al., 2011; Vieira Filho et al., 2017). These studies corroborate with the results obtained in this work, in which there was a drastic decrease in plant height

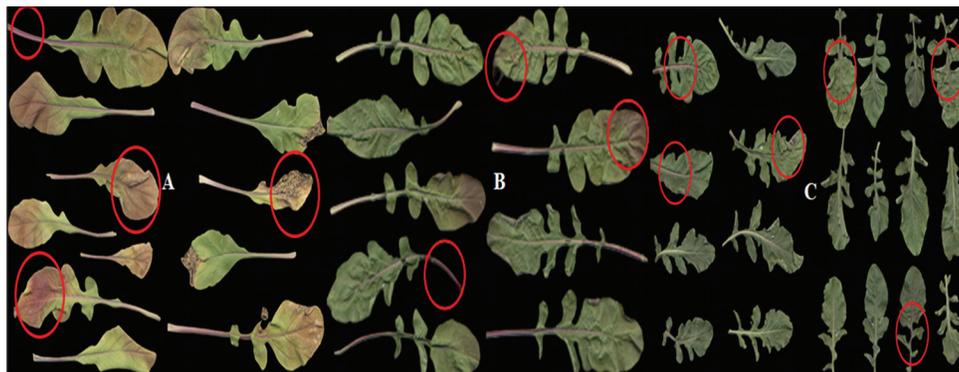
without N in nutrient solution. N- deprived plants presented 21 cm high, while plants grew with the complete nutrient solution had 33 cm high, representing an increase of 57% (Table 2).



**Image 1.** Initial chlorosis on old leaves of arugula plants at 13 days after transplanting (Phase III) as a function of nutrient solution without nitrogen.



**Image 2.** Nitrogen deficiency in old leaves (A) and intermediate leaves (B) in arugula plants at 16 days after transplanting (Phase III) as a function of nutrient solution without nitrogen.



**Image 3.** Nitrogen deficiency in old leaves (A), intermediary leaves (B) and new leaves (C) in arugula plants at 20 days (Phase III) as a function of nutrient solution without nitrogen at.

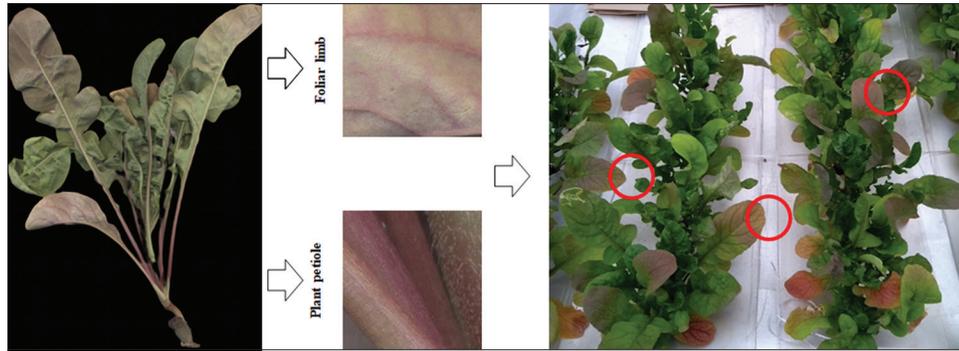
N deficiency reduced the emission and leaf expansion of arugula plants. Plants N-deplete presented 12 leaves per plant while plants grown in the complete nutrient solution had 16 leaves per plant, representing an increment of 33% (Table 2). –N-deplete plant had leaf area to 253 cm<sup>2</sup> per plant<sup>-1</sup>, while in the complete nutrient solution had 718.7 cm<sup>2</sup> of leaves per plant (Table 3).

Therefore, it is observed the effect of absence of N is largely linked to the emission of leaves, but the biggest problem is in the photosynthetically active leaf expansion, compared to the complete nutrient solution, observed increase of 184% in the leaf area. In general, the lower development of plants with N deficiency is explained by its role in plant metabolism, since this nutrient is present in all proteins and nucleic acids of the plant and has a great importance on division and cell expansion (Malavolta and Moraes, 2007).

At 20 DAT the symptoms of N-depletion were visually much more evident (severe) in the old leaves, followed by the intermediate and then new ones (Image 3).

Chlorosis followed by necrosis was observed in the old leaves, the intermediates showed only chlorosis, while the new ones presented a slight concentration of anthocyanins in the main vein. With the progress of the deficiency, there was a reduction in the growth, development, and increase in the presence of anthocyanins in the whole plant (Image 4).

This happens because the flavonoids found in vacuoles, represented mainly by anthocyanins, which are vacuolar pigments and in the extracellular environment, provide photoprotection, which, in addition to other functions in plants, protects photosynthetic machinery against UV-B radiation, altering the intensity and quality of light that reaches chloroplast (Steyn et al., 2002; Chemler et al., 2008; Hernández et al., 2008). With this, some species under N deficiency may overproduce it, giving the leaves a slightly purplish coloration (Römheld, 2012; Taiz et al., 2017). This



**Image 4.** Symptoms of nitrogen deficiency in arugula plants at 20 days after transplanting (Phase III) as a function of nutrient solution without nitrogen.

occurs because the deficiency of the nutrient causes the inhibition of carbohydrate synthesis, increasing sugar levels, which stimulates the synthesis of anthocyanin (Bergmann, 1992; Marschner, 1995; Gazula et al., 2007).

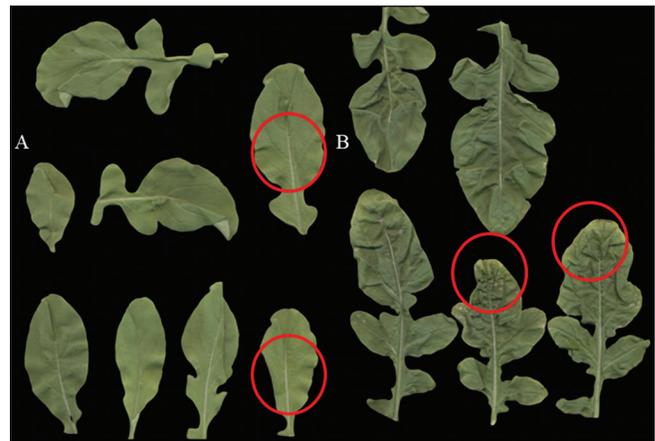
According to the results, N-depletion in relation to the other omissions caused a greater impact on crop yield, giving it prominence for being a nutrient with importance in the production and quality of leafy vegetables. With this, investigating the distribution of N at 20 days in Phase III (Table 3), it was verified that the N contents decrease proportionally in the following order NL > IL > OL. A high distribution of N in the plant occurs, due to its high mobility in the leaf tissue and as a characteristic the first symptoms occur in the old leaves, confirmed by the contents obtained in the old, intermediate and new leaves.

The arugula shows an initial phase of slow growth, which lasts up to about two-thirds of the cycle, with rapid accumulation of dry matter and, consequently, nutrients. The nutritional demand speed up as the plant approaches the end of the cycle to the harvest and therefore the symptoms of nutritional deficiencies are more evident in this period (Grangeiro et al., 2011).

#### **Potassium (-K) and magnesium (-Mg)**

Arugula growth was unaffected by the omission of K and Mg. The plant height was 30.2 and 31.96 cm for -K and -Mg, respectively, while for the complete nutrient solution it was 32.9 cm, although they did not differ statistically, represent an increase of 9 and 3% for the respective nutrients (Table 2).

It is observed that the K and Mg in the hydroponic system conditions are not very limiting in the growth of plants until the 20 days in Phase III, when compared to the complete nutrient solution, however, this reality could be different with the extension of the crop cycle under these conditions. In general, the cultivation of the arugula lasts around 40 - 45 days after sowing (Almeida et al., 2020; Purquerio



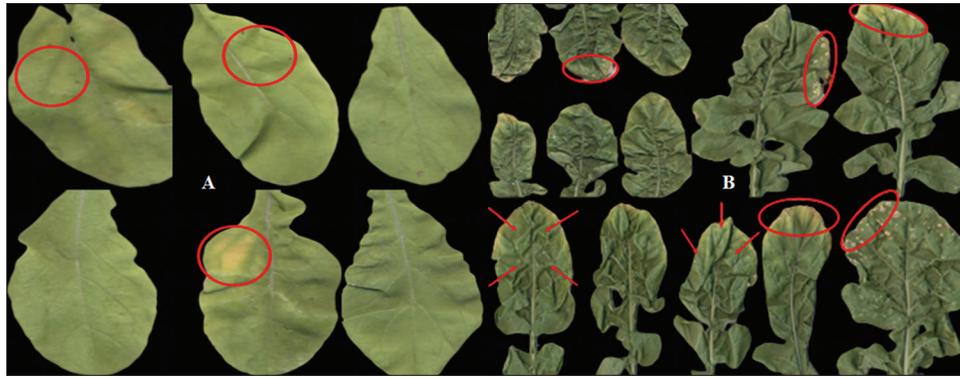
**Image 5.** Symptoms of deficiency in the old leaves (A) and intermediate leaves (B) at 18 days (Phase III) according to the nutritive solution with potassium omission..

et al., 2007; Grangeiro et al., 2011), in the hydroponic cultivation this period can increase or decrease in function of the region and climate.

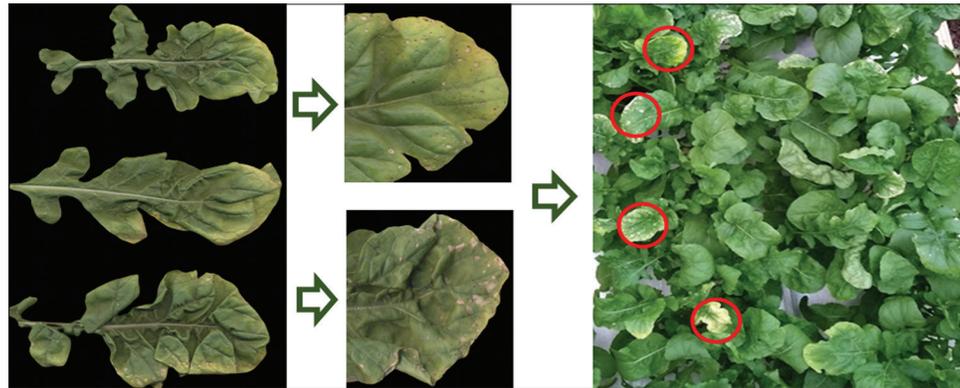
Despite there was no significant effect on arugula growth, the K plays an important role in regulating the osmotic potential of plant cells and enzymatic activation, due to this fact, its deficiency first leads to a reduction in the plant growth (Malavolta et al., 1997; Coelho et al., 2012).

According to the results, -K and -Mg had similar visual symptom. At 16 DAT, mild symptoms of deficiency were observed in the old leaves for both nutrients. At 18 DAT to -K the symptoms were moderate, presenting chlorosis in the old and intermediate leaves (Image 5).

At 20 days in Phase III, the plants presented symptoms with particular differences of deficiency caused by the absence of nutrients, being severe in the old leaves, and moderate in the intermediate ones. The K-deficient plants had old leaves with intense chlorosis and the intermediate ones with chlorosis followed by a slight necrosis at the edges, towards the central vein, with small whitish dots in the form of



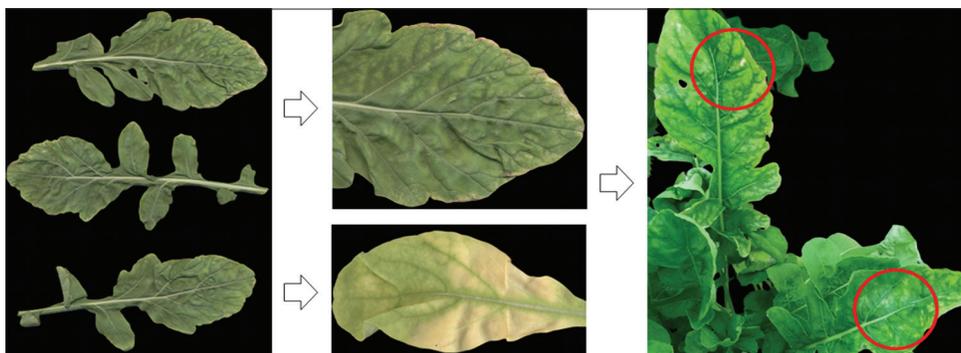
**Image 6.** Deficiency symptoms in old leaves (A) and intermediate leaves (B) at 20 days after transplanting (Phase III) as a function of nutrient solution without potassium.



**Image 7.** Symptoms of deficiency at 20 days after transplanting (Phase III) as a function of nutrient solution without potassium.



**Image 8.** Deficiency symptoms in old leaves (A) and intermediate leaves (B) at 20 days after transplanting (Phase III) as a function of nutrient solution without magnesium.



**Image 9.** Symptoms of deficiency at 20 days after transplanting (Phase III) as a function of nutrient solution without magnesium.



**Image 10.** Old (A), intermediate (B) and new (C) leaves at 20 days (Phase III), according to the complete nutritional solution.

circles distributed in the foliar limb (Images 6 and 7). The new leaves showed no symptoms of deficiency.

According to the literature, marginal chlorosis is the first characteristic symptom of K deficiency, which evolves to necrosis, occurring in the older leaves by the mobility of this element in the plant tissue. That occur due to chemical changes in deficient plants, such as increase in putrescine content, organic acid content, and lower starch sugar content in reserve organs (Malavolta et al., 1997; Malavolta 2006).

Mg symptoms were most evident at 20 DAT, with emphasis in the old and intermediate leaves. The old leaves showed more intense chlorosis compared to the intermediate leaves, where internerval chlorosis was verified from the leaf tip towards the central vein (Images 8 and 9).

It was observed that the nutrients deficiency symptoms under study depend directly on the function of the element in the plant and on the nutrient mobility in the phloem, thus they are indicators that the nutrient is in deficiency or adequate levels, and these are largely responsible for the plants green color.

The Mg has a specific function in the activation of enzymes involved in the processes of respiration, photosynthesis, and synthesis of DNA and RNA (Taiz et al., 2017). The deficiency symptoms are first visualized on the older leaves by the mobility of this nutrient in the plant tissue, characterized by interveinal chlorosis (Coelho et al., 2012). This fact is justified because the chlorophyll in the veins remains unchanged for longer periods than the chlorophyll in the foliar limb (between the veins). Although it was not the case, under severe deficiency, the whole plant becomes chlorotic (Bergmann, 1992; Römheld, 2012).

In general, an effect very close to -K and -Mg was observed in the analyzed variables. It is worth mentioning that Mg as well as N play a structural role in the chlorophyll molecule

(central atom) and based on the results -Mg did little to affect the development of the plant, however, plants under conditions of omission do not present acceptable market characteristics, which shows the effect of the nutrient on the visual quality.

The treatments caused nutritional disturbances, the leaf demonstrated anomalies caused by nutrient omissions, as the plants grown with complete nutrient solution did not present anomalies, only natural senescence, with chlorosis in the old leaves (Image 10).

## CONCLUSION

-N negatively affected the growth of the plant, while -K and -Mg affected mainly the visual quality. Symptoms of N deficiency in arugula plants were observed firstly at 10 days after transplanting (DAT) in the phase III while K and Mg at 13 DAT, with the symptoms beginning on the old leaves.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHORS CONTRIBUTIONS

The authors acted in equal proportion in the elaboration, planning, conducting, evaluating, collecting data, laboratory analysis, statistical interpretation, literature review and final writing.

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