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Rocket plants in response to nitrogen concentration in nutrient solution

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ABSTRACT

Nitrogen (N) is the second most accumulated nutrient in rocket. This nutrient greatly affects growth, productivity and quality of the vegetable. Rocket is the second most widely grown leafy vegetable in hydroponic system; however, no studies on how N concentration in nutrient solution affects this crop can be found in literature. We studied four concentrations (79.2; 118.8; 158.4 and 237.6 mg L^{-1} of N) in a randomized block design with five replicates. Maximum number of leaves, leaf area, dry mass and productivity of rocket cv. 'Folha larga' were obtained with the highest N concentration. The rocket quality, evaluated by the nitrate content, was maximum with 210.2 mg L^{-1} of N and its value in the concentration which maximized productivity is in the acceptable range for vegetables; so, it is recommended to grow rocket with 237.6 mg L^{-1} of N in the nutrient solution.

Keywords: *Eruca sativa*, soil-free cultivation, hydroponics, leafy vegetables, nitrate.

RESUMO

Resposta da rúcula à concentração de nitrogênio na solução nutritiva

O nitrogênio (N) é o segundo nutriente mais acumulado pela rúcula, o qual afeta sobremaneira o crescimento, produtividade e qualidade desta hortaliça. A rúcula é a segunda hortaliça folhosa mais cultivada em hidroponia; porém, não há estudos sobre como a concentração do N na solução nutritiva a afeta, tendo sido este o objetivo do presente estudo. Quatro concentrações (79,2; 118,8; 158,4 e 237,6 mg L^{-1} de N) foram avaliadas em delineamento experimental de blocos casualizados, com cinco repetições. Máximos número de folhas, área foliar, massa seca e produtividade da rúcula 'Folha larga' foram obtidos com a maior concentração de N. A qualidade da rúcula, avaliada pelo teor de nitrato, foi máxima com 210,2 mg L^{-1} de N. Seu valor na concentração que maximizou a produtividade encontra-se na faixa aceitável para hortaliças; de modo que se recomenda o cultivo da rúcula com 237,6 mg L^{-1} de N na solução nutritiva.

Palavras-chave: *Eruca sativa*, cultivo sem solo, hidroponia, hortaliça folhosa, nitrato.

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Rocket (*Eruca sativa*) is a very popular leafy vegetable, consumed *in natura*, in salads or in pizzas (Aguiar *et al*., 2014; Schiattone *et al*., 2018).

The growing area has expanded and the number of hydroponic growers has increased, as the market demand has grown. Considering the leafy vegetables, rocket is the second most cultivated under hydroponic system, being lettuce the first one (Matos *et al*., 2016). However, for a successful cultivation in hydroponic system, an appropriate nutrient solution and management of this solution are necessary, since they directly influence in production and quality of hydroponic crops (Genuncio *et al*., 2011; Guerra *et al*., 2011). For leafy vegetables cultivation, the nutrient solutions must have very high nitrogen (N) concentrations (Urlić *et al*., 2017) and, unfortunately, we cannot find in literature one nutrient solution which would be established for the nutritional demand of rocket. In general, the nutrient solution recommended for other leafy vegetables is used, as proposed by Furlani (1998) and Faquin & Furlani (1999).

Considering the nutrients, N is an extremely important one, as it is the second most accumulated nutrient in rocket (Grangeiro *et al*., 2011), showing effect on growing and productivity, as well as on quality (Petropoulos *et al*., 2016). Researches about N for the rocket crop under soil-free cultivation system are still scarce, but studies on field conditions are common, though (Steiner *et al*., 2011; Yoruk *et al*., 2018; Barros Júnior *et al*., 2020; Silva *et al*., 2020).

On the other hand, high N availability can increase rocket productivity, but can also affect negatively the quality of this crop. The vegetables produced under this system, using concentrated N nutrient solutions, can accumulate nitrate (NO_3^-) in excess in leaves (Andriolo, 2020). However, the accumulation of NO_3^- depends on several factors, such as management, amount and type of nitrogen fertilizers and light intensity (Faquin, 2008). N availability is one of the main factors affecting and contributing to increase NO_3^- mainly in the leaves of the plants. Since leaf is the edible part of rocket, a special monitoring of N is essential in order to avoid consumer intoxication. This compost is associated to a possible formation of carcinogenic and mutagenic compounds in the human body (Ranasinghe & Marapana, 2018;

Moazeni *et al*., 2020).

Among the leafy vegetables, the production of rocket to be used in ready-to-eat salads is increasing in order to meet the market demand; nevertheless, being rocket known as a nitrate hyperaccumulating species, great attention is needed, especially, in the precise management of nitrogen fertilization (Mola *et al*., 2019). Thus, Cavarianni *et al*. (2008) studied nitrogen concentrations in nutrient solution (60.8 - 243.5 mg L^{-1}) and verified a linear response in rocket leaf nitrate content (3378 mg kg^{-1}), when N concentration in nutrient solution increases, which can compromise the quality of the vegetable.

Given the above, this study aimed to evaluate the effect of N concentration in the nutrient solution regarding productivity and quality of rocket grown under hydroponic system.

MATERIAL AND METHODS

Time and location

The experiment was carried out from July 3 to August 16, 2018, in nutrient film technique (NFT) hydroponic system, at UNESP, Jaboticabal campus, São Paulo, Brazil (21°15'22"S, 48°15'58"W, 615 m altitude).

The region climate is classified as Aw according to Köppen-Geiger. Tropical climate: rainy and hot summer, dry winter, mild temperatures, considering the average temperature of the coldest month above 18ºC (André & Garcia, 2015). During the experiment, the maximum, average and minimum temperature were 36.4ºC, 28.5ºC and 21.5ºC, maximum, average and minimum relative humidity were 64.7%, 38.6% and 13.1%, respectively, solar average and maximum radiation were 16.1 and 726.3 MJ m^{-2}, respectively. The data were obtained in an agrometeorological microstation installed inside the greenhouse at FCAV-UNESP, Jaboticabal-SP.

Treatments and experimental design

We evaluated four treatments related to nitrogen concentration in the nutrient solution: 79.2; 118.8; 158.4 and 237.6 mg L^{-1}. The experimental design used was randomized blocks with five replicates. The experimental unit consisted of a bench with five 1.3 m-long channels. The useful area of the experimental unit for data collection corresponded to the three central channels of the bench, excluding the first and last plant of each channel.

Installation and conduction of the experiment

Rocket cultivar 'Folha Larga' was sown on July 3 in phenolic foam boards measuring 2 x 2 x 2 cm, previously washed under running water for approximately 10 minutes, in order to clean any kind of residue. During germination, these boards were kept in a greenhouse, under sprinkler irrigation system, being irrigated with pure water (Phase I).

Eight days after sowing (8 DAS), when the seedlings showed expanded cotyledons, the phenolic foam cells were individualized and seedlings taken to the polypropylene channels, 5-cm wide, in NFT system (Phase II).

The channels denominated "nursery", had 5% slope. The nutrient solution supply was intermittent, alternating 15 minutes with circulation and 15 minutes without circulation (6 a.m. to 6 p.m.). This period corresponded to the initial growing of the plants, before being taken to definite channels. In this phase, the complete nutrient solution proposed by Furlani (1998) was used: concentrations of 750 [$Ca(NO_3)_2$]; 500 ([KNO_3); 150 ($NH_4H_2PO_4$) and 400 ($MgSO_4$) g 1000 L^{-1}. Micronutrients were applied in concentrations, recommended by the author: 1.76 (H_3BO_3); 0.15 ($CuSO_4$); 1.5 ($MnSO_4$); 0.30 ($ZnSO_4$) and 0.10 $(NH_4)_6Mo_7O_{24}$ g L^{-1} and 60 mL of Fe for 1000 L^{-1}.

At 24 DAS, after showing three fully expanded leaves, the seedlings were transplanted into the final growing channels, 10-cm diameter and 5% slope (Phase III), spacing 0.25 m between channels and 0.05 m between plants. We used the nutrient solution proposed by Furlani (1998), with modifications to meet N concentrations in the nutrient solution (Table 1).

Micronutrients were applied in the concentrations recommended by Furlani (1998): 1.8 g L^{-1} H_3BO_3; 0.1 g L^{-1} $CuSO_4$; 1.5 g L^{-1} $MnSO_4$; 0.11 g L^{-1} $(NH_4)_6Mo_7O_{24}$; 0.30 g L^{-1} $ZnSO_4$ and 60 mL 1000 L^{-1} of iron (Fe).

The solution was pumped continuously by submerged pumps (one per plot) (Chosen® brand, model Power Head CX-300) at 1000 L h^{-1} flow. The irrigation system was controlled by an electronic timer, activated from 7 a.m. to 6 p.m. (15 minutes with circulations and 15 minutes without circulation). The pH was measured daily, using a pHep®+ pocket-sized pHmeter and the electrical conductivity (CE) with a DIST4 digital conductivity meter. The pH was kept from 5.5 to 6.5, using sodium hydroxide or hydrochloric acid, in order to increase or reduce pH, respectively. The nutrient solution was renewed when CE of the treatments reached 70% of the initial CE (dS m^{-1}).

Evaluated traits

To evaluate agronomic traits of the rocket, five sets of plants were collected randomly in the useful area of each plot. Height, number of leaves, leaf area, shoot dry mass, leaf nitrogen content, productivity and nitrate content were evaluated, 20 days after the beginning of Phase III. Plant height was measured using a measuring tape (0.5 cm above the plant collar to the highest leaf), the leaves were counted and leaf area was obtained with the aid of the leaf area integrator LI-COR 3100. To obtain shoot dry mass, plants were washed and dried in an oven with forced air circulation at 65-70ºC until reaching constant mass, then they were weighed using a semi-analytical scale (two decimal places).

Fresh mass was obtained right after harvest, between 6 and 7 a.m. Productivity was estimated by the product of fresh mass and planting density (80 plants m^{-2}). Shoot dry mass was used to evaluate N leaf content, according to Miyazawa *et al*. (2009) method.

To determine the nitrate content of the shoot part of rocket, six plants were collected randomly in the useful area of each plot, between 5:30 and 6:30 hours. Nitrate was determined according to Mantovani *et al*. (2005): 0.2 g of leaf dry matter and 20 mL deionized water was placed in 50 mL Falcon tubes. The tubes remained in water bath at

60±5°C for one hour, being agitated every 15 minutes. The samples were filtered through fast filter paper. The authors took 5 mL of this extract and added 20 mL deionized water and 0.2 g MgO. Ammonium was converted into ammonia through distillation and the distillate was discarded. Then, we added, in this same extract, 0.4 g Devarda's alloy for the reduction of nitrate to ammonium which, in an alkaline medium, was converted into ammonia, which was entrained by vapors and collected in a beaker containing 10 mL of 20 g L^{-1} H_3BO_3. Afterwards, we quantified N in the form of ammonium using an automatic titrator (848 Titrino plus®) with standard solution of H_2SO_4 0.00263 mol L^{-1}. The nitrate content obtained in the leaf dry mass was converted to mg of NO_3^- per kg of fresh mass, considering 5% of fresh mass in the rocket.

Statistical analysis

Obtained data were submitted to variance analysis (F test, $p<0.05$). Polynomial regression analysis for N concentration in nutrient solution was performed. An equation with the highest level of significance and determination coefficient was used. The authors used AgroEstat statistical software (Barbosa & Maldonado Júnior, 2015).

RESULTS AND DISCUSSION

N concentration in the nutrient solution influenced all the evaluated variables (Table 2). Number of leaves, leaf area and shoot dry mass positively responded to the increase of N concentration in the nutrient solution, using linear equation adjustment, whereas plant height showed the maximum value (37.7 cm) with 229.5 mg L^{-1} of N (Figure 1). The maximum values for number of leaves, leaf area, shoot dry mass and height were 18% (+3 leaves per plant), 54% (+258.3 cm^2), 49% (+1.3 g) and 23% (+7 cm) higher, respectively, comparing with plants grown with lower N concentration in the nutrient solution (79.2 mg L^{-1}).

In this study, this effect was verified in rocket, since the increase of N availability in the nutrient solution reflected in an increase of N content in the leaf and, consequently, in biometric traits (height, leaf area, shoot dry mass), productivity and quality. The results corroborate the information that rocket is very responsive to nitrogen fertilization (Barros Júnior *et al*., 2011; Grangeiro *et al*., 2011; Steiner *et al*., 2011; Benett *et al*., 2015, 2019; Vieira Filho *et al*., 2017; Silva *et al*., 2020).

The highest N content in the leaf was 28.2 g kg^{-1}, reached using 186 mg L^{-1} of N in the nutrient solution (Figure 2A). However, this leaf content did not represent the value of N concentration related to the maximum productivity. This productivity responded linearly and positively to the increase of N availability in the nutrient solution, maximum (3.89 kg m^{-2}) with 27 g kg^{-1} of leaf N concentration (Figure 2A), an increase of 35% in the crop productivity in relation to plants grown in a nutrient solution with 79.2 mg L^{-1} of N, with a content of 23.6 g kg^{-1} leaf N concentration.

Nitrogen concentration which maximized rocket productivity (237.6 mg L^{-1} of N) was superior to the one obtained by Cavarianni *et al*. (2011), 104 mg L^{-1} of N (1.2 kg m^{-2}). Nevertheless, the highest productivity obtained was similar to the one verified by Purquerio *et al*. (2007), 3.0 and 3.3 kg m^{-2}, in the field and in a protected environment, respectively.

Table 1. Concentration of nutrients in the nutrient solution. Jaboticabal, UNESP, 2018.

Nutrient solution	SM	NC	NK	MAP	CC	CK	MKP	NA	AN (mL 1000L^{-1})
	(g 1000 L^{-1})								
79.2 mg L^{-1}	320	480	-	44	84	228	87	-	-
118.8 mg L^{-1}	320	600	139	76.4	-	157	48.7	-	-
158.4 mg L^{-1}	320	600	400	120	-	-	-	-	-
237.6 mg L^{-1}	320	600	292	-	-	-	135	138	68

SM = Magnesium sulfate; NC = Calcium nitrate; NK = Potassium nitrate; MAP = Phosphate monoammonium; CC = Calcium chloride; CK = Potassium chloride; MKP = Monobasic potassium phosphate; NA = Ammonium nitrate; AN = Nitric acid.

Table 2. Plant height (AP), number of leaves (NF), leaf area (AF), shoot dry mass (MSPA), leaf nitrogen content (N), productivity (PROD) and leaf nitrate content (NO_3^-) of rocket in relation to the nitrogen concentration (N) in the nutrient solution. Jaboticabal, UNESP, 2018.

N (mg L^{-1})	AP (cm)	NF (leaves plant^{-1})	AF (cm^2 plant^{-1})	MSPA (g plant^{-1})	N (g kg^{-1})	PROD (kg m^{-2})	NO_3^- (g kg^{-1})
79.2	30.73	12.30	463.56	2.34	23.7	2.74	1004.62
118.8	33.63	12.50	537.69	2.99	26.2	3.22	2083.58
158.4	35.49	13.95	655.45	3.50	27.9	3.60	3259.12
237.6	36.17	14.35	718.05	3.66	27.0	3.78	3391.06
F values	10.26**	19.28**	14.8**	5.99**	13.22**	8.39**	41.17**
CV (%)	4.99	3.93	11.49	17.28	4.29	10.65	16.03

**Significant at 1% probability ($p<0.01$).

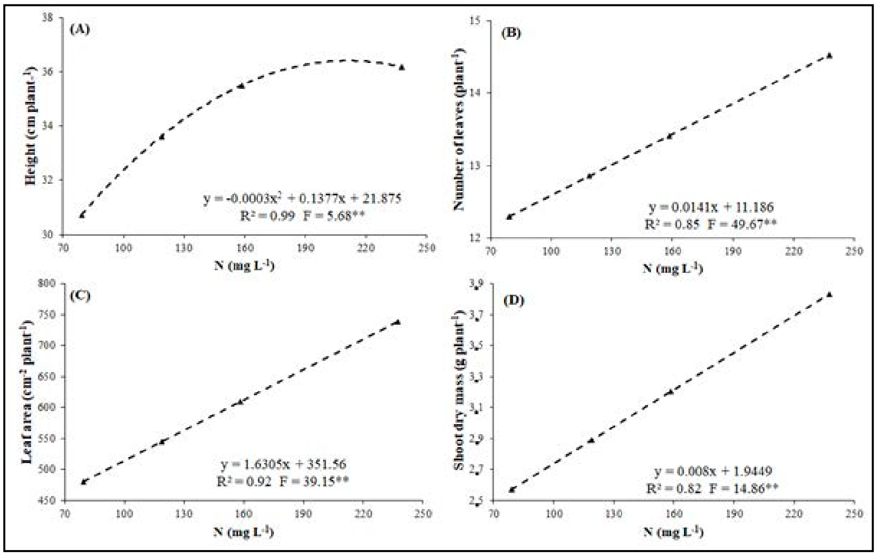

Figure 1. Height (A), number of leaves (B), leaf area (C), shoot dry mass (D) of rocket in relation to the nitrogen concentration (N) in the nutrient solution. Jaboticabal, UNESP, 2018.

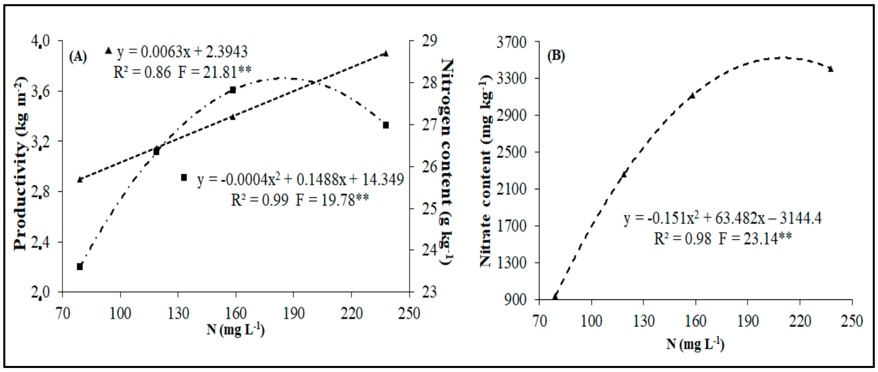

Figure 2. Nitrogen content (■) and productivity (▲) (A) and nitrate contente (B) of rocket in relation to the nitrogen concentration (N) in the nutrient solution. Jaboticabal, UNESP, 2018.

According to Trani *et al.* (2014), appropriate leaf N concentration for rocket is between 40 and 50 g kg^{-1}, which is related to a sampling period between half and two thirds of the cycle. The leaf contents used in this study were obtained in harvest and, probably, this can explain the contents lower than the appropriate rate, since no deficiency symptoms were observed, even in the lowest concentration in the nutrient solution. The highest N content in shoot dry mass was obtained using a concentration estimated of 186 mg L^{-1} whereas the highest productivity was observed with the highest N concentration in the nutrient solution. This behavior can be explained by the nutrient diluting effect. According to Maia *et al.* (2005), this happens when the relative growth rate of the dry matter is superior to the relative uptake rate of the nutrient, which is confirmed by the response of the dry mass accumulation of the rocket, provided by an increase of N concentration in the solution (Figure 1D).

Plants well nourished with N had a positive effect on rocket productivity (Figure 2A), showed a negative effect on quality, though (Figure 2B). The results are in accordance with the observed by Purquerio *et al.* (2007) and Steiner *et al.* (2011) and also that N supply is one of the main factors which promote greater nitrate accumulation (Ceylan *et al.*, 2002; Guadagnin *et al.*, 2005; Barros Júnior *et al.*, 2020). This conflict caused by nitrogen fertilization deserves attention from researchers for establishing N concentration in the nutrient solution.

In relation to linear adjustment for productivity averages (Figure 2A), the maximum value was not reached; it can be increased with an increase of N concentration in the nutrient solution. However, the limit of this concentration should not be the species' ability to maximize productivity; but, the nitrate content accumulated in rocket leaves should be taken in consideration. This compost is associated with the possibility of forming carcinogenic and mutagenic compounds in the human body (Tamme *et al*., 2010; Ahmed *et al*., 2017; Moazeni *et al*., 2020). In most diets, vegetables generally contribute more than 70% of the total nitrate ingested (Guadagnin *et al*., 2005).

Considering the nitrate content, Brazil has no legislation for the presence of nitrate in vegetables; however, the European Union established that for rocket produced from October to March and from April to September, the maximum contents should be 7000 and 6000 mg kg^{1} (fresh mass), respectively (UE, 2011). The nitrate content in the shoot fresh mass of rocket, considering concentration of 237.6 mg L^{-1} which maximized productivity, is below the limit established for the crop, offering no risk whatsoever to health.

The increase of N concentration in the nutrient solution provided greater nitrate accumulation in the rocket (Figure 2B). The maximum nitrate content (3527.70 mg kg^{-1}) in the shoot fresh mass of the crop was obtained in the concentration 210.2 mg L^{-1} of N in the nutrient solution, an increase of 277% in relation to the lowest concentration. Using 237.6 mg L^{-1} of N, dose which maximized the productivity, nitrate content was 3414.4 mg kg^{-1}.

Thus, the increase of N concentration provided higher plants, greater number of leaves, leaf area, shoot dry mass and leaf nitrogen content. Nitrate content did not reach the maximum limit established for the crop and so, the highest N concentration (237.6 mg L^{-1} of N) can be used to maximize productivity.

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