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Original Article

Salt stress and calcium nitrate in arugula in soilless cultivation using substrate[1]

Estresse salino e nitrato de cálcio em rúcula em cultivo sem solo usando substrato

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HIGHLIGHTS:

Salinized nutrient solution reduces the yield of arugula grown in substrate.
Gas exchange of arugula was negatively affected by saline water.
Extra addition of calcium nitrate in 50% minimizes deleterious effects of salinity.

ABSTRACT: Due to the important functions of calcium in plant physiology, supplementation of this nutrient may be a viable strategy to increase the tolerance of plants to salinity. Thus, the objective in this study was to evaluate the gas exchange and growth of broad-leaf arugula cultivars cultivated in coconut fiber subjected to salt stress. Four nutrient solutions [(S1 - standard nutrient solution (electrical conductivity of water - ECw of 0.5 dS m^{-1}; S2 - nutrient solution using saline water with NaCl at 3.5 dS m^{-1}; S3 - S2 enriched with $Ca(NO_3)_2$ at 50%; S4 - S2 enriched with $Ca(NO_3)_2$ at 100%, obtaining values of 2.3, 5.1, 5.5, and 5.9 dS m^{-1}, for S1, S2, S3, and S4, respectively], and two cultivars of arugula (Donatella and Gigante Folha Larga), arranged in a 2 × 4 factorial scheme, with three replicates, were studied. Plants were harvested 40 days after sowing and evaluated for the following variables: plant height, number of leaves, leaf area, total fresh mass, total dry mass, specific leaf area, leaf succulence, relative chlorophyll index, stomatal conductance, CO_2 assimilation rate, transpiration rate, internal CO_2 concentration, instantaneous water use efficiency, and intrinsic carboxylation efficiency. The cultivar Gigante Folha Larga was more tolerant to salinity of nutrient solution. The use of salinized nutrient solution negatively affected arugula growth and physiology, but the extra addition of $Ca(NO_3)_2$ reduced the harmful effects of salinity. Extra addition of 50% of $Ca(NO_3)_2$ in the nutrient solution is recommended to reduce the effect of salt stress.

Key words: *Eruca sativa*, hydroponics, salinity, soilless cultivation

RESUMO: Devido às importantes funções do cálcio na fisiologia vegetal, a suplementação deste nutriente pode ser uma estratégia viável para aumentar a tolerância das plantas à salinidade. Assim, o objetivo deste estudo foi avaliar as trocas gasosas e o crescimento de cultivares de rúcula de folhas largas cultivadas em fibra de coco submetidas ao estresse salino. Quatro soluções nutritivas [(S1-solução nutritiva padrão (condutividade elétrica da água - CEa de 0,5 dS m^{-1}; S2- solução nutritiva utilizando água salina com NaCl a 3,5 dS m^{-1}; S3-S2 enriquecida com $Ca(NO_3)_2$ a 50%; S4-S2 enriquecido com $Ca(NO_3)_2$ a 100%, obtendo valores de 2,3, 5,1, 5,5 e 5,9 dS m^{-1}, para S1, S2, S3 e S4, respectivamente], e duas cultivares de rúcula. (Donatella e Gigante Folha Larga), dispostas em esquema fatorial 2 × 4, com três repetições, foram estudadas. As plantas foram colhidas 40 dias após a semeadura e avaliadas quanto às seguintes variáveis: altura de planta, número de folhas, área foliar, massa fresca total, massa seca total, área foliar específica, suculência foliar, índice relativo de clorofila, condutância estomática, taxa de assimilação de CO_2, taxa de transpiração, concentração interna de CO_2 e eficiência instantânea do uso da água e eficiência intrínseca de carboxilação. A cultivar Gigante Folha Larga foi mais tolerante à salinidade da solução nutritiva. O uso de solução nutritiva salinizada afetou negativamente o crescimento e a fisiologia da rúcula, mas a adição extra de $Ca(NO_3)_2$ reduziu os efeitos nocivos da salinidade. Recomenda-se adição extra de 50% de $Ca(NO_3)_2$ na solução nutritiva para reduzir o efeito do estresse salino.

Palavras-chave: *Eruca sativa*, hidroponia, salinidade, cultivo sem solo

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Introduction

Arugula (*Eruca sativa*) is a leafy vegetable of the family Brassicaceae (Zafar-Pashanezhad et al., 2020). In Brazil, the tender leaves are most consumed in the form of salad, mainly in the Center-South region (Salles et al., 2017). Its leaves are rich sources of glucosinolates (Bell et al., 2020), which have important function of protective effects of these compounds against cardiovascular diseases, neurodegeneration, diabetes, and several other inflammatory disorders (Connolly et al., 2021).

Several studies have already been conducted to evaluate the effect of salinity on arugula cultivated in NFT (Nutrient Film Technique) system (Yang et al., 2021), or in soilless cultivation using substrate as growth media (Cordeiro et al., 2019). The results presented by these studies show that salt stress can reduce plant development, as well as causing changes in the physiological process regarding gas exchange, a fact observed by other authors with research on leafy vegetables, such as chicory (Silva et al., 2020) and kale (Silva et al., 2023a).

In part, the reduction of yield in crops under salt stress is due to the nutritional imbalance caused by excessive accumulation of Na^+ and Cl^-, which may damage the photosynthetic machinery, gas exchange, and photosynthetic pigments, results clearly related to oxidative stress generated by the saline growth conditions (Morton et al., 2019; Silva et al., 2023b).

Considered a secondary messenger, Ca^{2+} is involved in the regulation of physiological processes of development and in responses to stress (Hameed et al., 2021). Furthermore, exogenous Ca^{2+} increases the activity of enzymes associated with carbon reactions in photosynthesis, reducing the absorption of Na^+ ions and reducing the deleterious effect of salt stress on the photosynthetic capacity of plants (Roy et al., 2019; Silva et al., 2023b).

Given the above, the objective of this study was to evaluate the gas exchange and growth of broad-leaf arugula cultivars cultivated in coconut fiber subjected to salt stress.

Material and Methods

The experiment was conducted from August to September 2019, in a greenhouse located in the experimental area of the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid Region, in Mossoró, Rio Grande do Norte (5° 11' 31" S, 37° 20' 40" W, at an altitude of 18 m), Brazil. The greenhouse (7.0-m-wide and 18.0-m-long) was installed in metal structure, with a ceiling height of 4.0 m and arched cover with transparent plastic film to protect plants from the action of ultraviolet rays and direct sunlight. The greenhouse was protected on the sides by anti-aphid screen.

During the experiment, daily data were collected on the maximum (Tmax), mean (Tmean), and minimum (Tmin) temperature (Figure 1A), and maximum (RHmax), mean (RHmean), and minimum (RHmin) relative humidity of the air (Figure 1B), using an automatic weather station (Campbell Scientific Inc, model CR1000), installed inside the greenhouse. The temperature varied from 25.5 to 28.1 °C for Tmin, 26.3 to 29.9 °C for Tmean and 27.2 to 29.9 °C for Tmax; the relative humidity varied from 44.7 to 67.1% for RHmin, 47.9 to 67.2% for RHmean and 51.1 to 70.3% for RHmax.

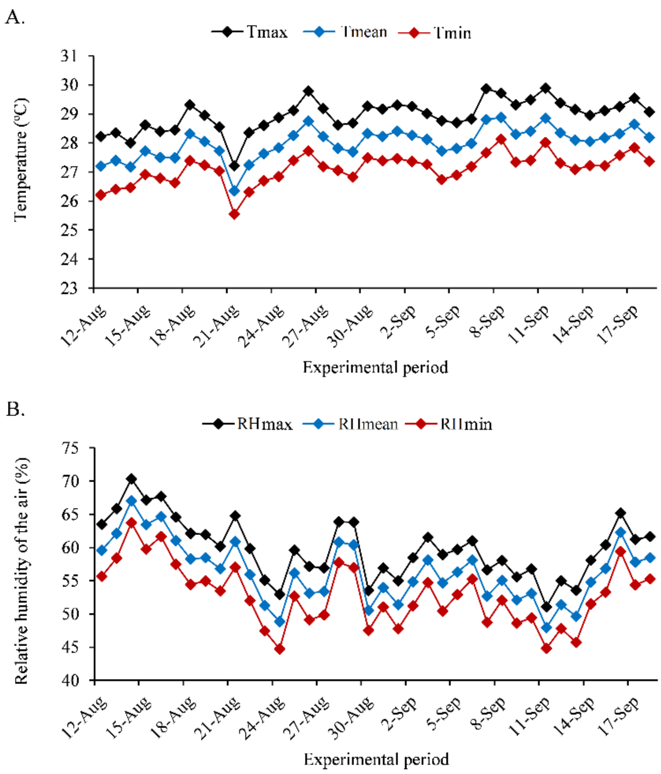

Figure 1. Climate data for temperature (A) and relative humidity of the air (B) during the experiment

The study was conducted in a completely randomized design, in 2 × 4 factorial arrangement, with three replicates. Treatments resulted from the combination of two broad-leaf arugula cultivars (Donatella and Gigante Folha Larga) and four nutrient solutions (S1 - standard nutrient solution, prepared in electrical conductivity of water - ECw of 0.5 dS m^{-1}; S2 - nutrient solution using saline water with NaCl at 3.5 dS m^{-1}; S3 - nutrient solution S2 enriched with calcium nitrate at 50%; S4 - nutrient solution S2 enriched with calcium nitrate at 100%). After preparing the nutrient solutions, their electrical conductivity was measured, obtaining values of 2.3, 5.1, 5.5, and 5.9 dS m^{-1}, for S1, S2, S3, and S4, respectively. The Donatella cultivar produces large, smooth, tender, dark green leaves (ISLA®). The Gigante Folha Larga cultivar has a vigorous plant with erect foliage and wide leaves, recommended for year-round cultivation (ISLA®). Thirty (30) experimental units were used, each one formed by 1.50-m-long PVC tubes (0.10-m-width and 0.10-m-depth), where 20 plants were grown. The tubes were mounted on asbestos roof tiles supported by wooden sawhorses, at 0.65 m height, spaced 0.10 m apart, with 5% slope to facilitate the collection of the excess solution, which was not used.

The standard nutrient solution was formulated according to the recommendation of Furlani et al. (1999), containing the following doses of fertilizers, in mg L^{-1}: 750 of calcium nitrate, 500 of potassium nitrate, 150 of monoammonium phosphate, and 400 of magnesium sulfate. The solutions S3 and S4 contained 1,125 and 1,500 mg L^{-1} of calcium nitrate, respectively.

A commercial compound (Rexolin®) was used as source of micronutrients, at dose of 30 g for every 1,000 L of nutrient solution. This compound has the following composition: 11.6% of potassium oxide (K_2O), 1.28% of sulfur (S), 0.86% of magnesium (Mg), 2.1% of boron (B), 2.66% of iron (Fe), 0.36% of copper (Cu), 2.48% of manganese (Mn), 0.036% of molybdenum (Mo), and 3.38% of zinc (Zn).

During the experiment, it was not necessary to adjust the pH because, after preparing the solutions and along their use, the values of pH remained within the recommended range (5.5 to 6.5).

The irrigation system comprised four PVC tanks (60 L), 16-mm-diameter lateral lines and microtube emitters with internal diameter of 0.8 mm and length of 10 cm. Nutrient solution was injected using a Metalcorte/Eberle - EBD250076 self-cooling, electric circulating pump (actuated by a single-phase motor, 210 V tension, 60 Hz frequency).

Nutrient solution application was controlled using a digital timer with capacity for eight daily programs (on/off), programmed with six irrigations: first irrigation at 07:00 a.m., second irrigation at 09:00 a.m., third irrigation at 11:00 a.m., fourth irrigation at 1:00 p.m., fifth irrigation at 3:00 p.m. and sixth irrigation at 5:00 p.m. (Cordeiro et al., 2019). Each of these events lasted 30 s. Irrigation frequency and time were sufficient to cause the nutrient solution to drain, in order to ensure that the substrate was brought to its maximum water storage capacity.

At 35 days after sowing (DAS), the relative chlorophyll index was determined using a portable chlorophyll meter (CCM-200, Opti-Science), in ten plants per plot. In addition, physiological gas exchange analyses were performed using an infrared gas analyzer (model "LCPro+" - ADC Bio Scientific Ltd.) operating with temperature control at 25 °C, irradiation of 1,200 μmol of photons m^{-2} s^{-1} and air flow rate of 200 mL min^{-1} at an atmospheric CO_2 level. The following variables of gas exchanges were evaluated: CO_2 assimilation rate (A - μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), and internal carbon concentration (Ci - μmol CO_2 mol^{-1} air). From the determination of these variables, the instantaneous water use efficiency (WUE = A/E, [(μmol CO_2 m^{-2} s^{-1})/(mmol H_2O m^{-2} s^{-1})]) and the intrinsic carboxylation efficiency (iCE = A/Ci, [(μmol CO_2 m^{-2} s^{-1})/(μmol CO_2 mol^{-1} air)]) were calculated.

At 40 DAS, a total of ten plants per plot were collected to be analyzed for the following variables: plant height (PH, in cm), number of leaves (NL), leaf area (LA, in cm^2 per plant), shoot fresh mass (SFM, in g per plant), shoot dry mass (SDM, in g per plant), specific leaf area (SLA, in cm^2 g^{-1}), and leaf succulence (LS, in g H_2O cm^{-2}). PH was measured using a graduated ruler, considering the distance between plant collar and the apex of the largest leaf. NL was determined considering only leaves with more than 70% green color and longer than 3.0 cm, disregarding yellowish and/or dried leaves.

LA was determined by the leaf disc method (Souza et al., 2012), using a volumetric ring with internal diameter of 2.5 cm (4.9 cm^2), by collecting 20 leaf discs per plot. The leaf discs were placed in paper bags and dried in a forced air circulation oven at 65 °C until reaching constant weight. The values of disc area, disc dry mass, and leaf dry mass were used to determine plant leaf area according to Eq. 1:

$$LA = \frac{DA \times LDM}{\frac{DDM}{ND}} \qquad (1)$$

Where:

LA - leaf area (cm^2 per plant);
DA - leaf disc area (cm^2);
LDM - leaf dry mass (g);
DDM - leaf disc dry mass (g); and,
ND - number of discs used in the plot.

To quantify SDM, plants were placed in previously identified paper bags and dried in a forced air circulation oven, at temperature of 65 °C until reaching a constant weight and were then weighed on precision digital scale (0.01 g).

SLA was determined by the ratio between LA and LDM (Benicasa, 2004), Eq. 2:

$$SLA = \frac{LA}{LDM} \qquad (2)$$

Where:

SLA - specific leaf area (cm^2 g^{-1});
LA - leaf area (cm^2); and,
LDM - leaf dry mass (g).

LS was determined based on the ratio between leaf water content and leaf area (Mantovani, 1999), Eq. 3:

$$LS = \frac{LFM - LDM}{LA} \qquad (3)$$

Where:

LS - leaf succulence (g H_2O cm^{-2});
LFM - leaf fresh mass (g);
LDM - leaf dry mass (g); and,
LA - leaf area (cm^2 per plant).

The data were subjected to analysis of variance by F test ($p \leq 0.05$). The means of the variables with significant effect were compared using Tukey's test ($p \leq 0.05$). Statistical analyses were performed using the computer statistical analysis system Sisvar 5.3 (Ferreira, 2019).

Results and Discussion

The interaction between nutrient solutions and arugula cultivars significantly affected ($p \leq 0.05$) the number of leaves (NL), leaf area (LA), and leaf succulence (LS), but did not significantly affect ($p > 0.05$) plant height (PH), shoot fresh mass (SFM), shoot dry mass (SDM), and specific leaf area (SLA) (Table 1).

PH, SFM, and SDM were affected only by the nutrient solutions, with losses of 17.5, 58.9, and 56.5% when plants were fertigated with salinized nutrient solution (S2), compared

Table 1. Summary of the analysis of variance for plant height (PH), number of leaves (NL), leaf area (LA), specific leaf area (SLA), leaf succulence (LS), shoot fresh mass (SFM), and shoot dry mass (SDM), of arugula cultivars cultivated in coconut fiber with different nutrient solutions (NS)

SV	Mean squares						
	PH	NL	LA	SLA	LS	SFM	SDM
NS	27.99**	13.58*	51778.51**	1841.40ns	0.0000071*	795.59**	3.57**
Cultivars	0.071^{ns}	7.78*	18891.92*	1648.8 ^{ns}	0.000011*	33.18^{ns}	0.13^{ns}
NS × Cultivars	2.73^{ns}	11.56**	30547.69**	3661.38^{ns}	0.000015*	23.50^{ns}	0.11^{ns}
Residual	4.27	1.12	3083.83	1281.46	0.0000021	18.04	0.13
CV (%)	7.51	12.96	16.31	22.60	14.63	13.12	16.12

SV - Source of variations, CV - Coefficient of variation, ns - Non-significant; * - Significant at $p \leq 0.05$; ** - significant at $p \leq 0.01$.

to the values obtained in the standard nutrient solution (S1). Furthermore, the enrichment of the salinized nutrient solution with 50% of calcium nitrate (S3) reduced the effect of salinity on these variables, despite the reductions of 9.1, 36.8, and 35.3% in the PH, SFM and SDM, respectively (Table 2).

These results are consistent with those reported by other authors, who also found reduction in arugula when subjected to salt stress, either in soilless cultivation using substrate as growth media (Cordeiro et al., 2019) or in NFT hydroponic cultivation (Yang et al., 2021). Such behavior may be attributed to the increase in the concentration of salts in the substrate, which negatively affect the physiological process, reducing water absorption by roots, inhibiting meristematic activity, cell elongation, and consequently reducing plant growth and development (Santos et al., 2022).

Enrichment with calcium nitrate promoted higher tolerance to salinity, corroborating in part the results presented by Silva et al. (2023a), in studies with kale. According to these authors, the addition of $Ca(NO_3)_2$ in the saline nutrient solution reduced the effect of salinity on Mg absorption and the effect of NaCl addition on kale production. Such increase in tolerance may be attributed to the greater availability of NO_3^- and Ca^{2+} ions, reducing the toxic effect of Cl^- and Na^+ ions on the plants (Seifikalhor et al., 2019).

Table 2. Mean values of plant height (PH), shoot fresh mass (SFM), shoot dry mass (SDM), and specific leaf area (SLA) of arugula cultivars cultivated in coconut fiber with different nutrient solutions (NS)

NS	PH (cm)	SFM (g per plant)	SDM (g per plant)	SLA (cm^2 g^{-1} LDM)
S1	30.17 a	47.47 a	3.29 a	171.53 a
S2	24.90 b	19.52 c	1.43 c	174.52 a
S3	27.42 ab	30.00 b	2.13 b	140.79 a
S4	27.81 ab	32.47 b	2.18 b	145.03 a
Cultivars				
Donatella	27.63 a	31.19 a	2.18 a	166.26 a
Gigante Folha Larga	27.52 a	33.54 a	2.33 a	149.68 a

S1 - standard nutrient solution; S2 - nutrient solution using saline water with NaCl at 3.5 dS m^{-1}; S3 - nutrient solution S2 enriched with calcium nitrate at 50%; S4 - nutrient solution S2 enriched with calcium nitrate at 100%. Means followed by the same letter in the columns do not differ by Tukey's test ($p \leq 0.05$).

The NL differed between the cultivars only when plants were subjected to the standard nutrient solution, when the cultivar Gigante Folha Larga was 47.5% superior. There was no effect of nutrient solution on this variable in the cultivar Donatella, although there was a 22.2% reduction in the salinized solution (S2). On the other hand, the salt stress had significant effect on the cultivar Gigante Folha Larga, which showed reduction of 46.2% under the solution S2. However, nutrient solution enrichment with 50% of calcium nitrate caused increase in leaf production, although it did not differ from the solution S2, leading to increments of 38.2 and 31.6% in the cultivars Donatella and Gigante Folha Larga, respectively (Table 3).

Leaf area differed between the cultivars only when subjected to the standard nutrient solution; the cultivar Donatella was 61.26% better than the cultivar Gigante Folha Larga (Table 3).

The use of the saline solutions led to a significant reduction of 46.6% in LA for the cultivar Donatella, which was not affected by the addition of calcium nitrate in the solution. For the cultivar Gigante Larga, there was a reduction of 39.31% when the solution S2 was used, compared to the standard nutrient solution, but the effect of salinity on its leaf area was reduced by the 100% addition of calcium nitrate (Table 3).

Leaf area reduction is an important adaptive mechanism of plants cultivated under excessive salts and water stress because, under these conditions, it is important to reduce transpiration, consequently reducing the transport of Na^+ and Cl^- ions in the xylem and, concomitantly, conserving water in plant tissues (Santos et al., 2022).

The cultivar Gigante Folha Larga outperformed Donatella in terms of LS by 61.4 and 60.1%, when both were fertigated by the solutions S1 and S2, respectively, and did not differ in the other solutions. For both cultivars, the use of salinized water (S2) reduced leaf succulence, but the cultivars responded differently to the extra addition of calcium nitrate, with an increase for the cultivar Donatella (Table 3).

Table 3. Mean values of number of leaves (NL), leaf area (LA), and leaf succulence (LS) of arugula cultivars cultivated in coconut fiber with different nutrient solutions (NS)

NS	NL		LA (cm^2 per plant)		LS (mg H_2O cm^2)	
	Donatella	Gigante Folha Larga	Donatella	Gigante Folha Larga	Donatella	Gigante Folha Larga
S1	7.98 Ba	11.77 Aa	588.39 Aa	364.86 Ba	7.45 Bab	12.03 Aa
S2	6.20 Aa	6.33 Ab	313.83 Ab	221.43 Ab	5.70 Bb	9.13 Ab
S3	8.57 Aa	8.33 Ab	280.55 Ab	326.95 Aab	10.39 Aa	8.39 Ab
S4	8.17 Aa	8.07 Ab	268.34 Ab	359.79 Aa	9.93 Aa	9.40 Aab

S1 - standard nutrient solution; S2 - nutrient solution using saline water with NaCl at 3.5 dS m^{-1}; S3 - nutrient solution S2 enriched with calcium nitrate at 50%; S4 - nutrient solution S2 enriched with calcium nitrate at 100%. Means followed by the same letter in the columns do not differ by Tukey's test ($p \leq 0.05$). Means followed by the same uppercase letter in the rows, and lowercase letters in the column, do not differ from each other using the Tukey test ($p \leq 0.05$).

These results are similar to those presented by Pessoa et al. (2023), who researched lettuce in this same cultivation system and also observed a positive effect of Ca^{2+} on leaf succulence in plants subjected to salt stress. The succulence allows the regulation of salt concentration in leaf tissues, allowing hydration of the leaves under conditions of low water availability, and depends directly on the absorption, transport, and accumulation of ions in the leaf tissues, which may contribute to reducing the effect of salt stress (Liu et al., 2024). The accumulation of sodium ions promotes an increase in mesophyll cells and leaf succulence, which enables additional water storage and mitigating losses (Oliveira et al., 2023).

For gas exchange, the interaction between nutrient solutions and arugula cultivars affected ($p \leq 0.05$) the relative chlorophyll index (RCI), transpiration rate (E), and intrinsic carboxylation efficiency (iCE). Stomatal conductance (gs), CO_2 assimilation rate (A), and instantaneous water use efficiency (WUE) were affected by the interaction between nutrient solutions and arugula cultivars ($p < 0.01$). There was no significant effect of the applied treatments ($p > 0.05$) on internal CO_2 concentration (Ci) (Table 4).

As previously presented, the treatments applied did not affect the internal CO_2 concentration (Ci), whether from the addition of NaCl or the addition of calcium nitrate to saline nutrient solution, and the overall mean was 209.36 μmol CO_2 mol^{-1} air. Under salt stress, there is a reduction in stomatal conductance and, as a result, water use efficiency tends to increase, that is, the plant absorbs more CO_2 per unit of water transpired, as stomatal closure limits transpiration more than the internal CO_2 concentration (Santos et al., 2022), which explains why E has decreased and there has been no response for Ci.

These results are partially similar to those obtained by Tzortzakis (2009), who worked with lettuce and chicory under saline conditions and foliar application of calcium nitrate, and found no significant response for internal CO_2 concentration. Similar results were also observed by Silva et al. (2023a) in kale, which did not show a significant effect of salt stress or calcium nitrate on Ci.

On the other hand, there was interaction between nutrient solutions and arugula cultivars for relative chlorophyll index (RCI), stomatal conductance (gs), transpiration rate (E), CO_2 assimilation rate (A), instantaneous water use efficiency (WUE), and intrinsic carboxylation efficiency (iCE) (Table 5).

The relative chlorophyll index (RCI) differed between the cultivars in the solutions S1 and S2, and Gigante Folha Larga was 66.7% superior to Donatella in the solution S1, but 20.5% inferior in the solution S2 (Table 5). The effect of the nutrient solutions on RCI varied according to the cultivar. For Donatella, the RCI increased with the use of salinized nutrient solution and addition of calcium nitrate, whereas for Gigante Folha Larga the use of salinized solution reduced the RCI, but this index increased with the enrichment using calcium nitrate (Table 5).

Increase of chlorophyll content in arugula cultivated under saline conditions has also been observed by Yang et al. (2021). The reduction in the chlorophyll index in plants subjected to salinity occurs because salt stress reduces the absorption of water and minerals due to the osmotic effect, by plant cells, reducing the chlorophyll content and affecting the function of the pigment-protein complex, thus reducing the absorption and conversion of chloroplasts to light energy (Guo et al., 2021). On the other hand, applying calcium increased RCI in

Table 4. Analysis of variance for internal CO_2 concentration (Ci), relative chlorophyll index (RCI), stomatal conductance (gs), transpiration rate (E), CO_2 assimilation rate (A), instantaneous water use efficiency (WUE), and intrinsic carboxylation efficiency (iCE) in arugula cultivars cultivated in coconut fiber with different nutrient solutions (NS)

SV	Mean squares						
	Ci	RCI	gs	E	A	WUE	iCE
NS	946.36 ns	81.84*	26846.71**	1.01*	35.44*	16.75**	0.0028**
Cultivars	545.87 ns	75.42*	18686.58*	0.09*	27,67*	4.65*	0.0012*
NS × Cultivars	1420.61 ns	101.74*	18531.91**	1.15*	52,32**	10.81**	0.0016*
Residual	620.69	24.63	2754.36	0.21	12,54	0.55	0.0008
CV (%)	10.45	17.22	13.65	14.74	16.36	9.19	18.44

SV - Source of variations, CV - Coefficient of variation, ns - Non-significant; * - Significant at $p \leq 0.05$; ** - significant at $p \leq 0.01$.

Table 5. Mean values of relative chlorophyll index (RCI), stomatal conductance (gs), transpiration rate (E), CO_2 assimilation rate (A), instantaneous water use efficiency (WUE), and intrinsic carboxylation efficiency (iCE) in two arugula cultivars fertigated with different nutrient solutions

NS	RCI		gs		E	
	Donatella	Gigante Folha Larga	Donatella	Gigante Folha Larga	Donatella	Gigante Folha Larga
S1	24.84 Bc	41.43 Aa	0.33 Aa	0.16 Bb	2.38 Aab	1.88 Bab
S2	36.60 Ab	29.12 Bb	0.15 Ab	0.17 Ab	3.27 Aa	2.36 Bab
S3	39.98 Aab	38.14 Aa	0.14 Ab	0.21 Aab	1.82 Bb	2.82 Aa
S4	46.27 Aa	42.59 Aa	0.36 Aa	0.27 Ba	1.94 Ab	1.73 Ab
	A		**WUE**		**iCE**	
	Donatella	Gigante Folha Larga	Donatella	Gigante Folha Larga	Donatella	Gigante Folha Larga
S1	18.52 Aab	18.41 Aa	7.76 Bb	9.69 Aa	0.085 Aab	0.092 Aa
S2	13.56 Bb	18.99 Aa	4.20 Bc	8.03 Aab	0.054 Ab	0.096 Aa
S3	15.09 Aab	19.57 Aa	8.29 Ab	7.05 Ab	0.076 Aab	0.092 Aa
S4	21.35 Aa	13.32 Bb	11.03 Aa	7.85 Bb	0.124 Aa	0.067 Ba

S1 - Standard nutrient solution; S2 - Nutrient solution using saline water with NaCl at 3.5 dS m^{-1}; S3 - Nutrient solution S2 enriched with calcium nitrate at 50%; S4 - Nutrient solution S2 enriched with calcium nitrate at 100%. Means followed by the same letter in the columns do not differ by Tukey's test ($p \leq 0.05$). Means followed by the same uppercase letter in the rows, and lowercase letters in the column, do not differ from each other using the Tukey's test ($p \leq 0.05$). gs - mol H_2O m^{-2} s^{-1}; E - mmol H_2O m^{-2} s^{-1}; A - μmol CO_2 m^{-2} s^{-1}; WUE - [(μmol CO_2 m^{-2} s^{-1})/(mmol H_2O m^{-2} s^{-1})]; iCE - [(μmol CO_2 m^{-2} s^{-1})/(μmol CO_2 mol^{-1} air)].

salt-stressed plants, by inhibiting the accumulation of H_2O_2, thereby avoiding oxidative damage in plants (Li et al., 2022).

Both arugula cultivars differed with respect to stomatal conductance (gs) in the nutrient solutions S1 and S4. The cultivar Donatella outperformed Gigante Folha Larga by 103.86 and 29.89% in the solutions S1 and S4, respectively (Table 5).

Regarding the effects of the nutrient solutions on stomatal conductance, it was observed that the salinized nutrient solution (S2) reduced gs by 51.33% in the cultivar Donatella, but the deleterious effect of salinity on this variable was nullified when plants were fertilized with the salinized nutrient solution enriched with 100% of $Ca(NO_3)_2$ (S4). For the cultivar Gigante Folha Larga, there was no effect of salinity on gs, but this variable increased with the enrichment of the nutrient solution using calcium nitrate, mainly with the extra addition of 100% (S4) (Table 5).

The reduction in stomatal conductance in response to salt stress is a consequence of a decrease in leaf water potential, leading to loss of turgor (Dantas et al., 2021; Sousa et al., 2022). Reduction in gs in plants subjected to salt stress was also observed by Silva et al. (2023a), in a study with the cabbage crop. These authors also observed an increase in gs in response to increased Ca availability.

Higher stomatal conductance is critical for plant growth because it could enhance CO_2 supply, thus improving net CO_2 assimilation rate (Yang et al., 2021). Ca^{2+} acts in activation mechanisms underlying stomatal closure during immune signaling and suggests specificity in Ca^{2+} influx mechanisms in response to different stresses.

For transpiration rate (E), the cultivar Donatella outperformed Gigante Folha Larga in the nutrient solutions S1 and S2, respectively. By contrast, the cultivar Gigante Folha Larga was 54.94% superior in the solution S3 (Table 5).

In relation to the effect of the nutrient solutions on transpiration rate, it was observed that there was no significant effect of the use of salinized solution (S2), but the addition of $Ca(NO_3)_2$ (S3 and S4) reduced this variable in the cultivar Donatella. On the other hand, the cultivar Gigante Folha Larga had increased transpiration rate when subjected to the salinized nutrient solution enriched with 100% of $Ca(NO_3)_2$ (S4) (Table 5).

As shown, addition of NaCl to the nutrient solution did not affect plant transpiration, but this variable was reduced in plants subjected to a higher concentration of $Ca(NO_3)_2$. Thus, it appears that the osmotic effect was more detrimental to transpiration than the isolated effect of Na^+ and Cl^- ions. Increased osmotic pressure in plants under salt stress leads to water loss and therefore causes the water potential of plants to decrease, resulting in decreased stomatal conductance and reduced transpiration rate (Guo et al., 2021).

Yang et al. (2021) also observed a reduction in transpiration in arugula subjected to a lower osmotic potential of the nutrient solution. In a study with kale, Silva et al. (2023a) found that excessive concentrations of $Ca(NO_3)_2$ reduced transpiration in plants under salt stress.

The two cultivars differed in terms of CO_2 assimilation rate (A) when subjected to the salinized nutrient solution (S2) and the salinized nutrient solution enriched with 100% of $Ca(NO_3)_2$ (S4). Gigante Folha Larga was 40.04% superior in the solution S2, whereas Donatella was 60.28% superior in the solution S4 (Table 5).

The arugula cultivars responded differently to the nutrient solutions in relation to their CO_2 assimilation rate (A). Donatella had a reduction of 26.78% in this variable (A) with the use of salinized solution (S2), but the enrichment of the saline solution with 100% of calcium nitrate (S4) was efficient in inhibiting the effect of salinity. The cultivar Gigante Folha Larga showed no reduction in this variable when the saline solution was used, but this variable decreased by 27.65% when plants were subjected to the saline solution S4 (with extra 100% of calcium nitrate) (Table 5).

Reduction in CO_2 assimilation rate in leafy vegetables in response to salt stress has also been observed by other authors, for instance in watercress (Souza et al., 2020) and kale (Silva et al., 2023a). In general, when plants are exposed to salt stress, the very first response is osmotic shock, followed by induction of stomatal closure (Hameed et al., 2021). According to these authors, stomatal closure, in turn, limits photosynthetic capacity by the restriction of CO_2 supply.

In relation to the instantaneous water use efficiency (WUE), the cultivar Gigante Folha Larga outperformed Donatella by 24.87 and 91.19% in the nutrient solutions S1 and S2, respectively. By contrast, Donatella was 40.51% more efficient in the nutrient solution S4 (Table 5).

Also for WUE, the two cultivars responded differently to the nutrient solutions used. The cultivar Donatella showed a significant reduction (45.87%) when fertigated with salinized solution (S2), but its WUE increased by 42.14% with the enrichment using 100% of $Ca(NO_3)_2$, compared to the WUE obtained in the standard nutrient solution. By contrast, the cultivar Gigante Folha Larga did not show significant effect of the salinized nutrient solution (S2), but its WUE decreased by 18.99% in the nutrient solution S4 (Table 5).

The maintenance of WUE under salt stress (S2) observed in Gigante Folha Larga indicates that, for this cultivar, the applied saline condition did not cause limitation to the efficiency of this physiological process, differently from what occurred with the cultivar Donatella. It is worth pointing out that the lower the water availability, the lower the degree of stomatal opening to reduce water loss; consequently, WUE is higher to maintain a minimum water balance and, in C3 plants, like arugula, CO_2 assimilation is limited under adverse water conditions (Santos et al., 2022).

The two cultivars of arugula differed in terms of intrinsic carboxylation efficiency (iCE) only when plants were fertigated with the salinized nutrient solution enriched with 100% of $Ca(NO_3)_2$ (S4), in which Donatella was 85.07% superior (Table 5).

The use of salinized nutrient solution (S2) reduced iCE only in the cultivar Donatella, by 36.47%. In addition, the increase in $Ca(NO_3)_2$ concentration increased its iCE. By contrast, the cultivar Gigante Folha Larga did not show significant effect of the nutrient solutions, obtaining average iCE of 0.086 (Table 5).

Intrinsic carboxylation efficiency depends on CO_2 availability in leaf mesophyll, quantity of light, temperature, and on the enzymatic activity for photosynthesis to occur. If the intercellular concentrations of CO_2 are too low, its influx into mesophyll cells is restricted, so the plant uses CO_2 from respiration to maintain a minimum level of photosynthetic rate, making it limited (Santos et al., 2022).

Conclusions

1. The cultivar Gigante Folha Larga was more tolerant to salinity of nutrient solution.

2. Both cultivars studied (Donatella and Gigante Folha Larga) were negatively affected by the increase of salinity in the nutrient solutions.

3. Nutrient solution enrichment with 50% of calcium nitrate, compared to the concentration of this fertilizer in the standard solution, was efficient in reducing the effects of salt stress on arugula plants.

4. Extra addition of calcium nitrate in the nutrient solution is a viable strategy for fertigation of arugula grown in substrate under conditions in which the use of saline water is inevitable to prepare the nutrient solution.

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