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## Leaf gas exchanges and production of kale under $\text{Ca}(\text{NO}_3)_2$ concentrations in salinized nutrient solution<sup>1</sup>

### Trocas gasosas e produção em couve folha sob concentrações de $\text{Ca}(\text{NO}_3)_2$ em solução nutritiva salinizada

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#### HIGHLIGHTS:

Salt stress decreases the photosynthetic process and yield of kale, regardless of the concentration of  $\text{Ca}(\text{NO}_3)_2$ .

Adequate calcium (between 1,000 and 1,300  $\text{mg L}^{-1}$ ) nutrition reduces salinity effect on gas exchanges.

Excess of  $\text{Ca}(\text{NO}_3)_2$  (1,875  $\text{mg L}^{-1}$ ) increases the osmotic effect on gas exchanges, except the internal carbon concentration.

**ABSTRACT:** Adequate mineral supplementation can be a strategy to enable the use of brackish water in the production of vegetables. This study intended to evaluate the effect of calcium nitrate concentrations on leaf gas exchanges and yield of kale (*Brassica oleracea* L.) fertigated with salinized nutrient solutions. The experiment was conducted in a randomized block experimental design (4 + 1), with four replicates. Four nutrient solutions prepared in brackish water (6.0  $\text{dS m}^{-1}$ ) containing four concentrations of  $\text{Ca}(\text{NO}_3)_2$  [(750, 1,125, 1,500, and 1,875  $\text{mg L}^{-1}$ )] and a control treatment (standard nutrient solution using low-salinity water, 0.5  $\text{dS m}^{-1}$  (750  $\text{mg L}^{-1}$  of  $\text{Ca}(\text{NO}_3)_2$ )) were used in the study. The following analyses were performed: leaf gas exchanges, leaf area, and fresh matter yield. The standard nutrient solution promoted higher values for photosynthetic rate (13.06  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance (0.19  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), transpiration (2.76  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), instantaneous water use efficiency (4.73  $\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ), instantaneous carboxylation efficiency (0.053  $\text{mmol CO}_2 \text{ mol}^{-1} \text{ CO}_2$ ), leaf area (2.78  $\text{cm}^2$  per plant), and leaf fresh matter yield (2.64 kg per plant). The  $\text{Ca}(\text{NO}_3)_2$  not nullified but mitigated the deleterious effect of salt stress on leaf gas exchanges, except for kale yield (leaf fresh matter).

**Key words:** *Brassica oleracea* L., soilless, salt stress, calcium

**RESUMO:** A suplementação mineral adequada pode ser uma estratégia para viabilizar o uso de água salobra na produção de hortaliças. Este estudo teve como objetivo avaliar o efeito das concentrações de nitrato de cálcio nas trocas gasosas foliares e na produção de couve (*Brassica oleracea* L.) fertigada com soluções nutritivas salinizadas. O experimento foi conduzido em delineamento experimental em blocos casualizados (4 + 1), com quatro repetições. Foram utilizadas cinco soluções nutritivas, sendo quatro foram preparadas em água salobra (6,0  $\text{dS m}^{-1}$ ) contendo quatro concentrações de  $\text{Ca}(\text{NO}_3)_2$  [(750, 1.125, 1.500 e 1.875  $\text{mg L}^{-1}$ )] e um tratamento controle (solução nutritiva padrão usando água de baixa salinidade, 0,5  $\text{dS m}^{-1}$  (750  $\text{mg L}^{-1}$  de  $\text{Ca}(\text{NO}_3)_2$ )). As seguintes análises foram realizadas: trocas gasosas foliares, área foliar e produção de matéria fresca. A solução nutritiva padrão forneceu maiores valores para taxa fotossintética (13,06  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), condutância estomática (0,19  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), transpiração (2,76  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), eficiência instantânea do uso da água (4,73  $\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ), eficiência instantânea de carboxilação (0,053  $\text{mmol CO}_2 \text{ mol}^{-1} \text{ CO}_2$ ), área foliar (2,78  $\text{cm}^2$  por planta) e produtividade de matéria fresca folhas (2,64 kg por planta). O  $\text{Ca}(\text{NO}_3)_2$  não anulou, mas atenuou o efeito deletério do estresse salino nas trocas gasosas foliares, com exceção da produção da couve (massa fresca de folhas).

**Palavras-chave:** *Brassica oleracea* L., cultivo sem solo, estresse salino, nitrato de cálcio

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## INTRODUCTION

Due to the impending scarcity of water resources, the use of saline water in food production is a major challenge for researchers, especially in the production of leafy vegetables, which are sensitive to salt stress (Soares et al., 2020; Souza et al., 2020).

Kale (*Brassica oleracea* L. var. *acephala* D.C.) crop is classified as moderately sensitive to salt stress, showing a salinity threshold of 1.8 dS m<sup>-1</sup> for the electrical conductivity of the saturation extract (Ayers & Westcot, 1999). However, the tolerance of plants to salinity can vary according to cultivation system, among other factors. Hydroponic cultivation allows greater tolerance to salinity, because of the energy reorganization resulting from the minimization of the matric potential of system (Soares et al., 2020; Navarro et al., 2022).

High concentrations of Na<sup>+</sup> (sodium) and Cl<sup>-</sup> (chloride) ions in the cell cytoplasm can inactivate enzymes as well as metabolites, reducing photosynthesis, stomatal conductance, transpiration, and internal carbon concentration, resulting in a decrease in the water-use efficiency (Souza et al., 2020; Silva et al., 2021).

Some studies have shown that adequate calcium nutrition mitigates effects of salt stress on plant physiology (Tanveer et al., 2020; Ahmed et al., 2021; Karagöz & Dursun, 2021). Ca<sup>2+</sup> is secondary messenger, involved in the regulation of physiological processes of development and in responses to stress. This ion increases plant tolerance to salt stress because it improves water balance, Na<sup>+</sup> secretion, and cell membrane integrity (Tanveer et al., 2020; Ahmed et al., 2021).

Considering the antagonistic interaction between Ca<sup>2+</sup> and Na<sup>+</sup>, enrichment of the cultivation medium with Ca<sup>2+</sup> can be a strategy to mitigate the effect of salt stress on plants. In light of the above, this study was conducted to evaluate the effect of calcium nitrate concentrations on leaf gas exchanges and production of kale cultivated in a hydroponic system with salinized nutrient solutions.

## MATERIAL AND METHODS

The experiment was conducted from June to October 2019 in a protected environment (5° 12' 48" S, 37° 18' 44" W, at an altitude of 37 m a.s.l.), at the Universidade Federal Rural do Semi-Árido (UFERSA), in the municipality of Mossoró in the state of Rio Grande do Norte, Brazil.

During the experiment, daily data on maximum (T<sub>max</sub>), mean (T<sub>mean</sub>), and minimum (T<sub>min</sub>) temperature, maximum (RH<sub>max</sub>), mean (RH<sub>mean</sub>), and minimum (RH<sub>min</sub>) relative humidity of air were collected using an automatic weather station (Campbell Scientific Inc. model CR1000), installed inside the greenhouse. There were variations from 25.0 to 28.0 °C for T<sub>min</sub>, 26.0 to 29.0 °C for T<sub>mean</sub>, 27.0 to 30.0 for T<sub>max</sub>, 44 to 68% for RH<sub>min</sub>, 48 to 72% for RH<sub>mean</sub>, and 51 to 76% for RH<sub>max</sub>.

The adopted experimental design was randomized blocks, in a 4 + 1 scheme, with four replicates and four plants per plot. Kale was grown under two levels of electrical conductivity of water (EC<sub>w</sub>) used for the preparations of the nutrient solutions:

0.5 dS m<sup>-1</sup> - control (low-salinity water obtained from the local supply system) and 6.0 dS m<sup>-1</sup>, obtained by addition of NaCl. In the other three treatments the cultivation was performed only with salinity (0.6 dS m<sup>-1</sup>), but with different concentrations of calcium nitrate (S2 - 750, S3 - 1,125, S4 - 1,500, and S5 - 1,875 mg L<sup>-1</sup>). 750 mg L<sup>-1</sup> of calcium nitrate was used in the first two treatments. Each experimental unit was composed of three 5 dm<sup>3</sup> capacity pots containing one plant, totaling 60 plants.

The water used to prepare the standard nutrient solution came from the local supply system, whose chemical analysis showed the following characteristics: pH = 7.30, EC = 0.50 dS m<sup>-1</sup>, Ca<sup>2+</sup> = 3.10, Mg<sup>2+</sup> = 1.10, K<sup>+</sup> = 0.30, Na<sup>+</sup> = 2.30, Cl<sup>-</sup> = 1.80, HCO<sub>3</sub><sup>-</sup> = 3.00, and CO<sub>3</sub><sup>2-</sup> = 0.20 (mmol<sub>c</sub> L<sup>-1</sup>).

The standard nutrient solution adopted was the one recommended by Furlani et al. (1999) for macronutrients, with the following fertilizer concentrations in mg L<sup>-1</sup>: 750-calcium nitrate, 500-potassium nitrate, 150-monoammonium phosphate, and 400-magnesium sulfate.

Micronutrients were supplied using a commercial compound namely Rexolin® (Yara Brazil S.A., Porto Alegre), containing the following composition: 2.1% boron (B), 2.66% iron (Fe), 0.36% copper (Cu), 2.48% manganese (Mn), 0.036% molybdenum (Mo), and 3.38% zinc (Zn); besides 11.6% potassium oxide (K<sub>2</sub>O), 1.28% sulphur (S), and 0.86% magnesium (Mg). The dose applied was as indicated by the manufacturer (30 g of the compound for the preparation of 1,000 L of nutrient solution). In order to adjust the pH of the solution, between 6.0 and 6.5, solutions of 0.1 mol L<sup>-1</sup> of KOH or HCl were applied. After preparing the nutrient solutions, their electrical conductivity was measured, obtaining 2.29, 7.48, 8.14, 8.29, and 8.64 dS m<sup>-1</sup>, for S1, S2, S3, S4, and S5, respectively.

The sowing of the kale cv. Manteiga (Feltrin® Sementes, Farroupilha, Brazil) was carried in polystyrene trays with 128 cells, using coconut fiber substrate. After emergence, thinning was performed, leaving one seedling per cell. Transplanting into pots filled with substrate and washed sand (2:1, weight basis) was done when the seedlings reached four true leaves, at 35 days after sowing.

The irrigation system was of the drip type with recirculation of the nutrient solution (closed system), where the excess of nutrient solution was returned to the reservoir by gravity. For each nutrient solution, an independent irrigation system was used, composed of polyvinyl chloride (PVC) reservoir (210 L), lateral lines of flexible hoses (16 mm), and microtube emitters (spaghetti) 10 cm long, with a mean flow rate of 3.5 L h<sup>-1</sup>. During the experiment, neither the electrical conductivity nor the pH of the nutrient solutions was monitored or controlled. When the volume of nutrient solution reached the minimum level for suction by the motor pumps, the residual solution was discarded. Then the reservoir was washed and filled with a new nutrient solution.

The control of irrigation was done using a digital timer and adjusting the duration of each event throughout the crop cycle, as follows: 1 min from transplanting (DAT) to 30 DAT, 2 min from 30 DAT to 45 DAT, and 3 min from this time until the end of the experiment. The water consumption of the plants was not measured; however, in all irrigations, the substrate moisture

was raised up to the maximum water holding capacity, based on the observation of drainage in the pots.

At 47 DAT, before the first harvest, physiological gas exchange analyses were performed using an infrared gas analyzer, model "LCPro +<sup>®</sup>" - ADC Bio Scientific Ltd. operating with temperature control at 25 °C, irradiation of 1,200 μmol of photons m<sup>-2</sup> s<sup>-1</sup> and air flow rate of 200 mL min<sup>-1</sup> at an atmospheric CO<sub>2</sub> level. The following variables of gas exchanges were evaluated: net photosynthesis (A - μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (gs - mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration (E - mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), and internal carbon concentration (Ci - [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)/(mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>]). From the determination of these variables, the instantaneous water use efficiency (WUEi = A/E, mmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O) and the instantaneous carboxylation efficiency (CEi = A/Ci, mmol [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)/(mmol CO<sub>2</sub> mol<sup>-1</sup>)<sup>-1</sup>]) were calculated.

Six leaf harvests (50, 57, 64, 71, 78, and 87 DAT) were carried out, harvesting leaves with a main leaf blade length greater than 20 cm, leaving five leaves per plant (Trani et al., 2015). After the harvests, the leaf area (m<sup>2</sup> per plant) and leaf fresh matter yield (g per plant) were determined. For statistical analysis, data on leaf area and fresh mass yield related to the accumulation obtained in the six harvests were considered.

The leaf area (LA) was determined by the product between the number of harvested leaves (NL) and the leaf blade area. The leaf blade area was obtained through linear measurements of the leaf blade length (LBL) and leaf blade width (LBW) (LA = (0.82012 + 0.71913 × (LBL × LBW)), R<sup>2</sup> = 0.98), according to Marcolini et al. (2005) for kale. Leaf area values were obtained in cm<sup>2</sup> and multiplied by the factor 0.0001 to convert to m<sup>2</sup>.

The data obtained were subjected to the Shapiro-Wilk normality test and, if normal, to analysis of variance and F-test (p ≤ 0.05); variables that showed significant responses were analyzed by regression analysis, in order to evaluate the effect of Ca(NO<sub>3</sub>)<sub>2</sub> concentrations under saline conditions. Dunnett's test was used to compare the effects between the salt solution with different calcium concentrations and the control (standard nutrient solution). The statistical analyses were performed using the SISVAR statistical software (Ferreira, 2019).

## RESULTS AND DISCUSSION

All variables related to gas exchange (stomatal conductance (gs), internal carbon concentration (Ci), instantaneous water use efficiency (WUEi), photosynthetic rate (A), transpiration (E), and instantaneous carboxylation efficiency (CEi)) were affected by calcium nitrate concentrations. Calcium nitrate concentrations did not affect (p > 0.05) the leaf area (LA) or leaf fresh matter yield (LFMY) variables. There was a significant contrast between the control treatment (standard nutrient solution) and calcium nitrate concentrations for all variables analyzed, at levels of p ≤ 0.05 for Ci and p ≤ 0.01 for the other variables (Table 1).

Except for Ci, the use of saline nutrient solution reduced the other variables analyzed, regardless of the Ca(NO<sub>3</sub>)<sub>2</sub> concentrations used. When comparing the values obtained in the standard nutrient solution with those obtained in the saline solution at the same concentration (750 mg L<sup>-1</sup>) of Ca(NO<sub>3</sub>)<sub>2</sub>, there were reductions of 38.67, 26.32, 15.86, 45.28, 49.28, and 53.79%, for the variables A, gs, E, WUEi, CEi, LA, and LFMY, respectively (Table 1).

As observed in Table 1, the concentrations of Ca<sup>2+</sup> were not efficient to reduce the effect of salinity on the production of kale. This fact occurred because, despite the increase in the availability of Ca<sup>2+</sup> resulting in less absorption of Na<sup>+</sup>, high concentrations of the fertilizer increase the electrical conductivity of the nutrient solution, so that the plants were not able to overcome the osmotic effects associated with the increase in the total concentration of salt (Guimarães et al., 2012).

Still in relation to Table 1, it appears that, among these variables, only WUEi was benefited by the increase in the concentration of Ca(NO<sub>3</sub>)<sub>2</sub> at the concentration of 1,125 mg L<sup>-1</sup>, with no significant difference between this and the standard nutrient solution.

There was no effect of salinity on Ci, but the use of Ca(NO<sub>3</sub>)<sub>2</sub> at concentrations of 1,125 and 1,500 mg L<sup>-1</sup> in saline nutrient solution increased Ci by 13.44 and 10.25%, respectively (Table 1).

The decrease in A of kale under salt stress corroborates the results already shown by Souza et al. (2020), who also found

**Table 1.** Summary of the F-test and mean values for net photosynthesis (A), stomatal conductance (gs), transpiration (E), internal carbon concentration (Ci), instantaneous water-use efficiency (WUEi), instantaneous carboxylation efficiency (CEi), leaf area (LA), and leaf fresh matter yield (LFMY) in kale cv. Manteiga subjected to standard nutrient solution and salinized nutrient solutions enriched with calcium nitrate

SV	F test								
	A	gs	E	Ci	WUEi	CEi	LA	LFMY	
Block	ns	ns	ns	**	**	*	ns	ns	
Calcium nitrate	*	**	*	**	**	*	ns	ns	
Calcium nitrate x Control	**	**	**	**	**	**	**	**	
CV (%)	14.13	15.17	12.87	7.21	5.51	16.85	13.22	10.10	
Nutrient solutions	Mean values								
	A	gs	E	Ci	WUEi	CEi	LA	LFMY	
S1	13.06	0.19	2.76	246.25	4.73	0.053	2.78	2.64	
S2	8.01 <sup>#</sup>	0.14 <sup>#</sup>	1.95 <sup>#</sup>	225.25	3.98 <sup>#</sup>	0.029 <sup>#</sup>	1.41 <sup>#</sup>	1.22 <sup>#</sup>	
S3	8.75 <sup>#</sup>	0.16 <sup>#</sup>	2.12 <sup>#</sup>	279.35 <sup>#</sup>	4.48	0.035 <sup>#</sup>	1.44 <sup>#</sup>	1.15 <sup>#</sup>	
S4	8.88 <sup>#</sup>	0.13 <sup>#</sup>	2.32 <sup>#</sup>	271.50 <sup>#</sup>	3.87 <sup>#</sup>	0.033 <sup>#</sup>	1.50 <sup>#</sup>	1.15 <sup>#</sup>	
S5	6.85 <sup>#</sup>	0.11 <sup>#</sup>	2.02 <sup>#</sup>	266.32	3.28 <sup>#</sup>	0.026 <sup>#</sup>	1.23 <sup>#</sup>	1.05 <sup>#</sup>	

SV - Sources of variation; DF - Degree of freedom; S1 - Standard nutrient solution (750 mg L<sup>-1</sup>); S2 - Saline nutrient solution (750 mg L<sup>-1</sup>); S3 - Saline nutrient solution (1,125 mg L<sup>-1</sup>); S4 - Saline nutrient solution (1,500 mg L<sup>-1</sup>); S5 - Saline nutrient solution (1,875 mg L<sup>-1</sup>); ns; \*; \*\* - Not significant, significant at p ≤ 0.05 and p ≤ 0.01, respectively by F test. # - significantly different from control treatment, by Dunnett's test, p ≤ 0.05. A - μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>; gs - mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>; E - mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>; Ci - mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>; WUEi - mmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O; CEi - mmol CO<sub>2</sub> mol<sup>-1</sup> CO<sub>2</sub>; LA - m<sup>2</sup> per plant; LFMY - kg per plant.

a decrease in photosynthetic activity in species of the same botanical family as kale under salt stress.

Salinity affects photosynthetic activity due to the accumulation of  $\text{Na}^+$  and/or  $\text{Cl}^-$  ions in chloroplasts, which affect the biochemical and photochemical processes involved in photosynthesis. In addition, salt stress decreases  $\text{CO}_2$  uptake because of salt stress, causing the closure of stomata, reducing the photosynthetic process (Silva et al., 2021).

In a study conducted by Ahmed et al. (2021) with *Limonium stocksii*, the authors found that the application of  $\text{CaCl}_2$  increased the photosynthetic activity of the plants subjected to salt stress. Salachna et al. (2017), working with *Brassica oleracea* var. *Sabellica*, known as kale, also observed a reduction in stomatal conductance in plants subjected to salt stress. 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).

The decreased transpiration in plants under salt stress is related to the closure of stomata in response to osmotic stress caused by the increased salinity (Mastrogiannidou et al., 2016).

The increase in Ci with elevated  $\text{Ca}^{2+}$  concentrations in plants subjected to salt stress was also observed by Ahmed et al. (2021). For He et al. (2018), exogenous calcium improved the photosynthetic capacity by enhancing the carbon assimilation capacity of leaves and by regulating stomatal movement under stress. The internal  $\text{CO}_2$  concentration is commonly related to stomatal dynamics since stomatal closure hinders  $\text{CO}_2$  influx and decreases its concentration in the substomatal chamber (Navarro et al., 2022; Silva et al., 2022). According to Fernandes et al. (2010), this type of behavior demonstrates that the reduction of the photosynthetic process in the saline treatment is due not only to the reduction of stomatal opening, but also to damage to the cellular structure responsible for  $\text{CO}_2$  assimilation, is possibly caused by a reduction in the osmotic-water potential and accumulation of ions outside the range that plants tolerate.

The decrease in CEi occurred because the deleterious effect of salt stress was greater on photosynthesis compared to internal carbon concentration. As salt stress becomes more severe, dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired and carboxylation efficiency is compromised (Velooso et al., 2022).

The decrease in leaf development and, consequently, in kale yield in response to salt stress has also been observed in the literature (Karagöz & Dursun, 2021; Šamec et al., 2021; Zeiner et al., 2022), as well as by other authors working with other Brassicaceae, such as pak choi (*Brassica campestris* var. *Chinensis* L.) (Ding et al., 2018), broccoli (*Brassica oleracea* L. var. *Italica*) (Rios et al., 2020), and cauliflower (*Brassica oleracea* var. *botrytis* L.) (Soares et al., 2020). In cauliflower, Soares et al. (2020) observed a decrease in the accumulation of fresh mass in response to an increase in salinity.

When analyzing the effect of calcium fertigation, it appears that all variables reflecting gas exchange were affected in a quadratic way by the increase in  $\text{Ca}(\text{NO}_3)_2$  concentrations, with higher values occurring at levels of 1.235, 1.084, 1.369, 1.391, 1.147, and 1.254, leading to  $9.11 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ,  $2.24 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ,  $267.23 \mu\text{mol CO}_2 \text{ mol}^{-1}$ ,

$4.35 [(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})/(\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})^{-1}]$ , and  $0.035 [(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})/(\text{mmol CO}_2 \text{ mol}^{-1})^{-1}]$ , for A (Figure 1A), gs (Figure 1B), E (Figure 1C), Ci (Figure 1D), WUEi (Figure 1E), and CEi (Figure 1F), respectively.

When comparing these values with those obtained at the lowest concentration of  $\text{Ca}(\text{NO}_3)_2$ , the greatest gains were obtained in the variables A (14.59%), E (16.67%), Ci (20.35%), and CEi (20.69%). On the other hand, excessive concentrations of  $\text{Ca}(\text{NO}_3)_2$  caused reductions in these variables, mainly for A, gs, WUEi, and CEi, with losses of 20.64 (Figure 1A), 26.67% (Figure 1B), 21.61% (Figure 1E), and 22.86% (Figure 1F), respectively, compared to the values obtained at the lowest concentration of  $\text{Ca}(\text{NO}_3)_2$ .

Despite that, the increase in  $\text{Ca}(\text{NO}_3)_2$  concentrations did not nullify the deleterious effect of salt stress on the analyzed variables, confirming the results presented by Ahmed et al. (2021), who observed that the application of  $\text{Ca}^{2+}$  ( $\text{CaCl}_2$ ) did not improve photosynthetic gas exchange of *Limonium stocksii* under saline conditions. However, the data presented show that, depending on the analyzed variable, kale subjected to salt stress responded positively to calcium fertigation. These results indicate that adequate  $\text{Ca}^{2+}$  nutrition can be an efficient strategy to decrease the deleterious effect of salt stress on plants, thus confirming the results reported by other authors (Ahmed et al., 2021).

Calcium helps plants to maintain relative water content and stomatal conductance, thus preventing damage due to cytoplasm dehydration (Ahmad et al., 2018). For Rashedy et al. (2022), the presence of Ca ions alleviated the toxic effects of salinity by promoting tissue growth, resulting from the role of  $\text{Ca}^{2+}$  in plant cell elongation and division, permeability of cell membrane, nitrogen metabolism, and carbohydrate transport.

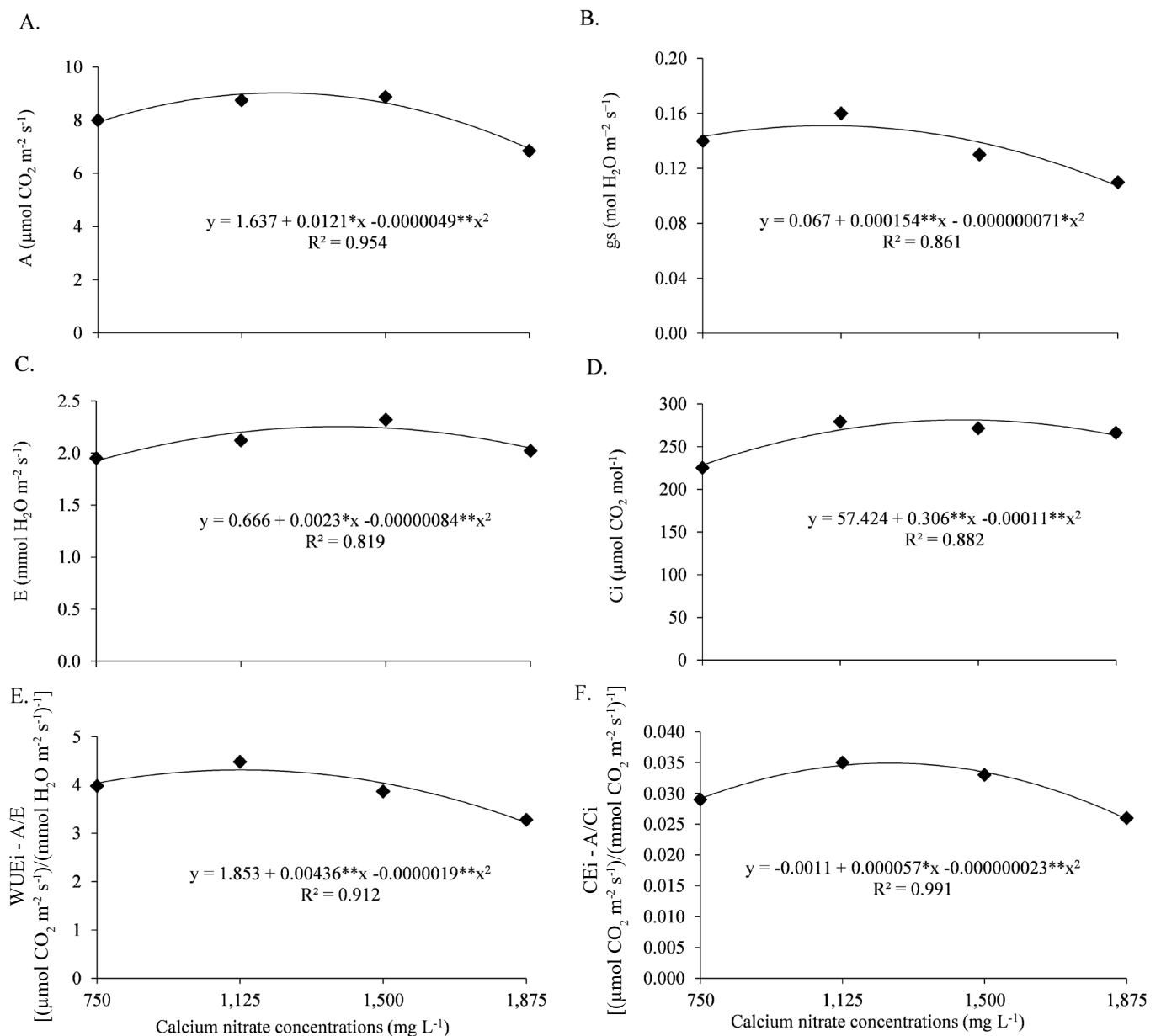
In addition,  $\text{Ca}^{2+}$  acts on stomatal movement, which influences the transpiration process, carbon assimilation, and water use efficiency. The action of this element in stomatal opening and closing indicates the triggering of different signals depending on the oscillation speed of calcium concentration in the cytoplasm (Liu et al., 2013).

The A showed a very strong correlation with the variables gs, E, WUEi, and CEi (Table 2). The gs correlated strongly with E and very strongly correlated with the variables A, WUEi, and CEi. A greater gs allows an increase in the flux of  $\text{CO}_2$  into the plant and can affect transpiration rates and, subsequently, the A process. The positive correlation between gs and E can be explained by a greater opening of the stomata, causing E to continue along with A (Burbano-Erazo et al., 2020).

The Ci was moderately correlated with WUEi and very strongly correlated with CEi. The variables WUEi and CEi showed a strong and positive correlation among themselves (Table 2), thus demonstrating that stomatal opening is efficient for these variables, since it showed a  $\text{CO}_2$  fixation in the leaf mesophyll without causing  $\text{H}_2\text{O}$  loss (Coutinho et al., 2020).

The yield of kale has a positive correlation, ranging from moderate to strong, with the variables A, gs, E, WUEi, and CEi (Table 2). Thus, the effect of the environmental conditions to which the plants are subjected on gas exchange directly affects the production of plant biomass (Navarro et al., 2022).

Although calcium nitrate reduced the effect of salinity on some gas exchange variables, this response did not occur in kale



\*,\*\* - Significant at  $p \leq 0.05$  and  $p \leq 0.01$  by F test, respectively

**Figure 1.** Net photosynthetic rate (A), stomatal conductance (B), transpiration (C), internal  $\text{CO}_2$  concentration (D), instantaneous water-use efficiency (E), and instantaneous carboxylation efficiency (F) in kale cv. Manteiga fertigated with calcium nitrate concentrations in salinized nutrient solutions

**Table 2.** Pearson's correlation between gas exchange variables and leaf fresh matter yield in kale fertigated with calcium nitrate concentrations in salinized nutrient solutions

	A	gs	E	Ci	WUEi	CEi	LFMY
A	1.00						
gs	0.91**	1.00					
E	0.95**	0.74*	1.00				
Ci	-0.19 <sup>ns</sup>	-0.20 <sup>ns</sup>	0.01 <sup>ns</sup>	1.00			
WUEi	0.83**	0.97**	0.65*	-0.11 <sup>ns</sup>	1.00		
CEi	0.99**	0.92**	0.94**	-0.13 <sup>ns</sup>	0.84**	1.00	
LFMY	0.96**	0.86**	0.88**	-0.42 <sup>ns</sup>	0.74**	0.95**	1.00

ns\*,\*\* - Not significant, significant at  $p \leq 0.05$  and  $0.01$ , respectively; 0 to 0.19 – Very weak; 0.20 to 0.39 – Weak; 0.40 to 0.69 – Moderate; 0.70 to 0.89 – Strong; 0.90 to 1.00 – Very strong

yield, indicating that under salt stress conditions with electrical conductivity above  $7.0 \text{ dS m}^{-1}$ , the supplementation with  $\text{Ca}^{2+}$  is not justified. However, the results presented demonstrate the need for more studies that allow a better understanding of calcium nutrition in vegetables under salt stress lower than that adopted in the present study.

## CONCLUSIONS

1. The use of saline water in the nutrient solution reduces the leaf gas exchange, leaf area, and leaf fresh matter yield in kale.
2.  $\text{Ca}(\text{NO}_3)_2$  concentrations ranging from  $1,000$  to  $1,300 \text{ mg L}^{-1}$  were efficient in reducing the effect of salt stress on gas exchanges.

3. The concentrations of  $\text{Ca}(\text{NO}_3)_2$  applied did not mitigate the deleterious effect of salt stress on the leaf fresh matter yield of kale.

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