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Ecophysiology of soursop seedlings fertilized with calcium silicate under different irrigation frequencies

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Abstract

Propose: The soursop tree (*Annona muricata* L.) is a tropical fruit tree cultivated in the semi-arid region of Brazil; however, it is sensitive to water deficit. The application of calcium silicate can promote plant growth and development under water stress. Therefore, the objective was to evaluate the ecophysiological and biochemical changes in soursop seedlings fertilized with calcium silicate under different irrigation frequencies.

Methods: The experiment was conducted in a greenhouse using a randomized block design with a 4 × 2 factorial scheme and four replications. The treatments consisted of four irrigation frequencies: TR1 – daily irrigation; TR2 – irrigation every two days; TR3 – irrigation every four days; TR4 – irrigation every six days, combined with the presence or absence of calcium silicate ($CaSiO_3$), applied via soil, at a dose of 3.5 g $plant^{-1}$.

Results: Soursop seedlings subjected to TR3 associated with $CaSiO_3$ showed improved gas exchange, including transpiration, stomatal conductance, and photosynthetic rate. Fertilization with $CaSiO_3$ regulated proline synthesis, increased leaf biomass and silicon and calcium content in leaf tissues, conferring greater tolerance to water deficit. Treatments TR2 and TR3 were similar to TR1 in plant height, number of leaves, leaf area, biomass accumulation, chlorophyll *a*fluorescence, chlorophyll content, and total soluble sugars, whereas TR4 significantly reduced these variables, regardless of $CaSiO_3$ fertilization.

Conclusions: The application of $CaSiO_3$ under moderate stress (TR3) increases soursop seedlings' tolerance to deficit irrigation regimes.

Highlights

Calcium silicate (CaSiO) alleviates the effects of moderate water deficit in soursop seedlings.

A six-day irrigation interval severely suppresses height, leaf emission, and biomass accumulation in soursop seedlings.

A four-day irrigation frequency combined with (CaSiO) enhances leaf gas exchange parameters in soursop seedlings.

1. Introduction

The soursop tree (*Annona muricata* L.) is a tropical fruit tree native to Central and South America. It is currently cultivated worldwide, including in Africa and Southeast Asia, and particularly in the semi-arid region of Brazil [1, 2]. This crop, formerly neglected or underutilized, has great medicinal potential and contributes to food security due to the high nutritional and caloric value of its fruits [3, 4]. Its fruits, when ripe, are consumed fresh and, after processing, are used to produce juices, jams, ice cream, sweets, nectars, yogurts, syrups, drinks, and shakes [5, 4]. Furthermore, the fruits, leaves, seeds, and stems exhibit important biological and pharmacological activities, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, anti-arthritic, hypotensive, anti-inflammatory, antidiabetic, antiparasitic, and pesticidal properties [1, 3, 6].

In Brazil, soursop is widely cultivated in the Northeast region, where it helps supplement family farmers' incomes. In this context, the fruit sector plays a crucial role in the economy of the semi-arid Northeast, both by supplying local and regional markets and by leveraging its export potential. The Northeast region accounts for approximately 27% of national fruit production, positioning it as one of the country's main fruit-growing centers [7]. However, soursops require adequate water availability to grow and sustain good yields, and their growth, development, and productivity are affected under water-deficit conditions [5, 3, 8]. Thus, producing seedlings of the species in the semi-arid areas of Brazil is challenging, as rainfall events are concentrated in a short, irregular period, making it highly relevant to understand this dynamic for agricultural management [9].

In semi-arid conditions, farmers are often compelled to adopt deficit irrigation due to water scarcity. This practice is a potential strategy to help crops withstand mild or moderate water deficits, although it impairs productivity [10, 11]. Water deficit is one of the main abiotic stresses in agriculture, affecting about 30% of the world's cultivated area [12]. This stress can severely affect the morphological, physiological, biochemical, and molecular attributes of the plant, reducing photosynthetic capacity and stomatal conductance, which increases the internal concentration of CO_2 and biomass production [13–16]. Furthermore, it causes excessive production of reactive oxygen species (ROS) [15], a decrease in the plant's water potential and relative water content in tissues, and physiological drought [17]. These effects impair plant growth, development, productivity, and quality [13, 16], thereby compromising agricultural sustainability and causing food shortages [18]. In this context, management strategies to increase plant tolerance to water deficit have been extensively studied [19].

In this context, deficit irrigation emerges not only as a consequence of water scarcity but also as a rational water management strategy, aiming to mitigate the impacts of water stress on crop performance. This practice is a smart and common strategy for reducing water consumption and improving sustainable agricultural management [20, 21]. Experimental evidence indicates that deficit irrigation, a technique that intentionally reduces water supply below the crop's total water needs, has high potential for water savings and is being progressively adopted in regions worldwide [22]. According to Li et al. [23], deficit irrigation is the controlled use of limited water resources to maintain stable productivity, with irrigation frequency being crucial for plant growth and productivity [24]. In recent years, studies have shown that deficit irrigation, defined as reduced irrigation frequency, can offer significant benefits under limited water availability [25, 26, 22].

However, even with rational irrigation management, plants remain subject to water stress, especially in the early stages of development, which drives the search for complementary mitigation strategies. In this context, the use of calcium silicate ($CaSiO_3$) has proven to be a promising agent for mitigating abiotic stresses, such as water deficit [27, 28, 12]. Silicon (Si) maintains leaf water potential, water content, and photosynthetic pigment content; promotes osmotic adjustments and reduces oxidative stress [29, 30]; and regulates gas exchange, photochemical yields, and growth [31, 14]. Furthermore, Si increases nutrient uptake and biomass production [29, 32].

While calcium (Ca^{2+}) plays a fundamental role in signal transduction in response to environmental stress [33], Malyukova et al. [34] indicated that the influx and cytoplasmic levels of Ca^{2+} are important for abscisic acid (ABA) transduction in guard cells. At the same time, ABA can regulate stomatal opening in these cells. Ca^{2+} also plays an essential role in maintaining the stability and integrity of cellular structure [35], thereby favoring photosynthetic capacity, alleviating oxidative damage, and promoting osmolyte accumulation [36, 33]

Previous studies report that the application of calcium silicate increases drought tolerance in various species, including *Oryza sativa* [37], *Saccharum officinarum* [38], *Cucumis sativus* L. [39], and *Zea mays* L. [40]. However, the benefits of Si and Ca depend on soil water availability, environmental conditions, and species-specific physiological characteristics [33, 41].

Therefore, although the beneficial effects of silicon and calcium are known in several species, the interaction between these elements, as demonstrated by the application of calcium silicate to soursop seedlings subjected to varying irrigation frequencies, remains underexplored. A gap remains regarding how this fertilization influences osmotic adjustment and the efficiency of the photosynthetic apparatus when the interval between waterings is extended. Thus, it is hypothesized that the application of $CaSiO_3$ maintains stomatal function and osmotic balance under moderate water stress, promoting greater physiological efficiency in soursop seedlings. Therefore, the objective was to evaluate the ecophysiological and biochemical changes in soursop seedlings fertilized with calcium silicate under different irrigation frequencies.

2. Materials and Methods

2.1 Location and Characterization of the Experimental Area

The experiment was conducted in a greenhouse in the seedling production sector of the Department of Agronomic and Forestry Sciences at the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró, Rio Grande do Norte State, Brazil (5°12'14.2"S and 37°19'29.3"W, with an average altitude of 18 m), from September to December 2023. The greenhouse is protected by an arched tunnel-type roof, measuring 7.0 m wide, 18.0 m long, and 3.0 m high, covered with a transparent, low-density polyethylene sheet, 0.15 mm thick. The structure is made of galvanized steel, and the side and front walls are made of 50% shade cloth.

The region's climate is of the BSwh type (hot and dry), with an average precipitation of 555 mm year⁻¹, an average annual temperature of 27.8°C, and an average relative humidity of 68.9% [42, 43]. The climatic data for temperature and relative humidity during the experiment were recorded using a digital thermometer-hygrometer (SH-122, J-Prolab, Brazil) and are presented in Fig. 1.

2.2 Plant Material, Experimental Design, and Treatments

The experimental design used was a randomized block design, in a 4 × 2 factorial scheme, with four replications and one seedling per replication, totaling 32 experimental units. The first factor consisted of four irrigation frequencies: TR1 – daily irrigation; TR2 – every two days; TR3 – every four days; and TR4 – every six days. The second factor corresponded to the presence or absence of calcium silicate ($CaSiO_3$) applied to the soil at a dose of 3.5 g plant⁻¹. The irrigation schedules were based on the studies of Fredrick, Bamalo, and Chima [44] and Yisau et al. [3], and the fertilization with $CaSiO_3$ followed the recommendations of Souza et al. [45].

Seeds of *A. muricata* L. (cultivar Morada), which exhibit high productivity, large fruits, good health, and physical quality, as described by Pinto and Silva [46] and by São José et al. [47], were obtained from mature fruits with good phytosanitary appearance, from trees located in the rural area of Mossoró, Rio Grande do Norte, Brazil. The seeds were manually extracted, washed in running water to remove pulp residues, and dried in the shade on paper for 7 days. Sowing was carried out in 2 dm^3 plastic bags filled with sand and Tropstrato® commercial substrate at a 2:1 (v/v) ratio, using three seeds at a depth of 1.5 cm, with thinning performed 20 days after emergence, retaining only one seedling per bag. The commercial substrate Tropstrato®, composed of single superphosphate, potassium nitrate, peat, vermiculite, and pine bark, presents the following chemical attributes: pH in $CaCl_2$ = 5.75; P = 65.70 mg dm^{-3}; K^+ = 1.60 $cmol_c$ dm^{-3}; Ca^{2+} = 23.80 $cmol_c$ dm^{-3}; Mg^{2+} = 12.40 $cmol_c$ dm^{-3}; Al^{3+} = 0.00 $cmol_c$ dm^{-3}; H + Al = 4.20 $cmol_c$ dm^{-3}; Sum of bases = 37.80 $cmol_c$ dm^{-3}; Cation Exchange Capacity = 42.10 $cmol_c$ dm^{-3} and Base Saturation = 89.8%, according to the manufacturer's data.

One hundred and thirty days after planting, *A. muricata* L. seedlings were transplanted into 5 L plastic pots, spaced 1.0 m apart between rows and 0.5 m between plants. The containers were filled with a substrate composed of sand and Tropstrato® at a 2:1 (v/v) ratio, and the mass of each pot was recorded for standardization. The seedlings were irrigated daily until the water retention capacity (WRC), corresponding to 100% soil moisture, was reached over 30 days of acclimatization before the application of the treatments. The chemical properties of the substrate at the beginning and end of the experiment are detailed in Table 1.

Table 1
Chemical characteristics of the substrate before and at the end of the experiment

Initial characterization													
	pH	**EC**	**O.M**	**P**	**K^+**	**Na^+**	**Ca^{2+}**	**Mg^{2+}**	**SB**	**CEC**	**Si**	**V**	**ESP**
	(H_2O) 1:25	**(dS m^{-1}) 1:25**	**(g kg^{-1})**	**(mg dm^{-3})**	**.....$cmol_c$ dm^{-3}.....**						**(mg kg^{-1})**	**..... (%).....**	
	4.82	1.0	27.84	214.31	0.58	1.09	5.60	2.60	9.87	9,87	83.68	100	11
Characterization after treatments													
TR1 absence $CaSiO_3$	5.91	0.70	8.89	177.70	0.58	2.14	3.70	1.30	7.71	7.71	125.30	100	28
TR2 absence $CaSiO_3$	5.51	1.08	11.21	245.00	0.55	2.05	4.70	2.00	9.30	9.30	104.60	100	22
TR3 absence $CaSiO_3$	5.46	0.81	11.99	256.90	0.58	1.82	4.30	2.20	8.90	8.90	98.90	100	20
TR4 absence $CaSiO_3$	5.33	0.83	11.60	284.20	0.71	1.55	4.50	1.30	8.05	8.05	76.75	100	19
TR1 Presence $CaSiO_3$	5.45	0.91	7.35	325.93	0.50	2.00	4.60	1.60	8.70	8.70	98.05	100	23
TR2 Presence $CaSiO_3$	5.44	0.84	17.01	243.30	0.55	1.91	4.40	1.30	8.16	8.16	89.67	100	23
TR3 Presence $CaSiO_3$	5.40	0.80	18.56	293.60	0.63	1.82	4.20	1.60	8.25	8.25	101.60	100	22
TR4 Presence $CaSiO_3$	5.48	0.67	17.79	250.95	0.58	1.73	4.20	2.00	8.50	8.50	109.90	100	20

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. Al^{3+}, (H + Al) and m: 0, P, K^+, Na^+: Mehlich 1 extractor; Al^{3+}, Ca^{2+}, Mg^{2+}: 1.0 mol L^{-1} KCl extractor; EC: electrical conductivity; SB: sum of bases; CEC: cation exchange capacity; O.M.: organic matter; ESP: exchangeable sodium percentage

2.3 Irrigation and fertilization management

Thirty days after transplanting the seedlings (DAT), irrigation was initiated, according to the irrigation schedule established for each treatment, using the local water supply (Table 2). Irrigation was carried out manually with a graduated cylinder, once a day in the afternoon. During this process, the water retention capacity (WRC) of the substrate was maintained at 100% using the gravimetric method [48].

Table 2
Chemical attributes of the local water supply are used for irrigating soursop seedlings

pH	EC	K^+	Na^+	Mg^{2+}	Ca^{2+}	Cl^-	CO_3^{2-}	HCO_3^-	SAR	Si
H_2O	dS m^{-1}	---------- $mmol_c$ L^{-1} ----------								mg L^{-1}
7.50	0.58	0.26	4.59	1.46	0.74	2.40	0.10	3.50	4.4	5.00

pH (H_2O): Hydrogen ion potential in water; EC: Electrical conductivity; K^+: potassium; Na^+: sodium; Mg^{2+}: magnesium; Ca^{2+}: calcium; Cl: chlorine; CO_3^{2}: carbonate; HCO_3: bicarbonate; SAR: sodium adsorption ratio; Si: silicon

To accurately determine the required water volume, all pots were weighed every 30 days. Subsequently, the pots were placed at maximum saturation and covered with plastic at the top opening to prevent water evaporation. After 24 h, the pots were drained of excess water, indicating that the substrate had reached maximum WRC, and were weighed again. Two reference pots were selected per treatment and served as the basis for calculating the volume of water to be applied during each irrigation event, enabling precise adjustments to maintain the target WRC, following the methodology adapted from Souza et al. [48]

The applied volume (VI) per pot was calculated as the difference between the average weights of the reference pots (PVr1 and PVr2) and their weights before the irrigation event (PVra1 and PVra2), as given by Eq. 1.

$$\mathrm{VI} = \left(\frac{PVr1 + PVr2}{2}\right) - \left(\frac{PVra1 + PVra2}{2}\right)$$

1

Where:

VI = Volume of water to be applied in the irrigation event (L);

PVr1 = Weight of reference pot 1 (kg);

PVr2 = Weight of reference pot 2 (kg);

PVra1 = Weight of reference pot before irrigation event 1 (kg);

PVra2 = Weight of reference pot before irrigation event 2 (kg).

The total volume of irrigation applied to the soursop seedlings across treatments was quantified throughout the experimental period (Table 3).

Table 3
Total irrigation volume in soursop seedlings fertilized with calcium silicate at different irrigation frequencies at 120 days

Irrigation volume (L plant^{-1})		
	Fertilizing	
Irrigation frequencies	Absence $CaSiO_3$	Presence $CaSiO_3$
TR1	20.258	18.736
TR2	17.602	16.829
TR3	13.433	11.899
TR4	9.624	9.573

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$

Calcium silicate ($CaSiO_3$), composed of 10–25% calcium oxide (CaO) and 75–90% silicon dioxide (SiO_2), was applied to the soil around the stem of soursop seedlings at 30 DAT, at a dosage of 3.5 g per seedling, as recommended by Souza et al. [45]. Fertilization with nitrogen, potassium, and phosphorus (NPK) was split into 12 applications and supplied weekly via fertigation, corresponding to 50% of the recommended rates of 100 mg N, 300 mg P_2O_5, and 150 mg K_2O per dm^{-3} of soil, as recommended by Novais, Neves, and Barros [49]. For NPK fertilization, 400 mg of urea (45% N), 1,250 mg of monoammonium phosphate (MAP, 12% N and 50% P_2O_5), and 650 mg of potassium chloride (KCl, 60% K_2O) were applied per pot.

2.4 Variables analyzed

Growth and biomass production

120 days after treatment application, the soursop seedlings were evaluated for plant height (PH), stem diameter (SD), root volume (RV), number of leaves (NL), and leaf area (LA). PH was measured from the base to the insertion of the apical bud using a graduated tape measure (cm). SD was obtained at the substrate level using a digital caliper (mm). RV (cm^3) was determined by the water displacement method, in which the root system, previously washed and dried, was immersed in a graduated cylinder filled with a known volume of water; the increase in water volume was then recorded, as described by Böhm [50]. NL was determined by direct counting of fully expanded leaves. The LA (cm^2) was determined after collecting leaves from each plant, which were digitized using a flatbed scanner (Xpress SL-C480FW, Samsung Electronics, South Korea) at 600 dpi. The images obtained were subsequently processed and analyzed using ImageJ® software.

After growth analysis, the seedlings were collected and separated into leaves, stems, and roots, and washed in running water. The plant material was then placed in Kraft paper bags and heated in a forced-air oven at 65°C until a constant weight was reached after 72 hours. Subsequently, the samples were weighed on an analytical balance with a precision of 0.0001 g to determine the dry mass of each seedling part. The results were expressed in g.

Leaf gas exchange and chlorophyll *a* fluorescence

115 days after treatment application, leaf gas exchange measurements were performed using an infrared gas analyzer (IRGA; GFS–3000, Walz, Effeltrich, Germany). The equipment was programmed with an impeller speed of 7; a reading area of 8 cm^2; a cuvette temperature of 25°C; a CO_2 concentration of 400 ppm; a flux rate of 750 µmol min^{-1}; and a photon flux density of 1,200 µmol m^{-2} s^{-1}. Measurements were taken between 7:00 and 11:00 on the third fully expanded leaf, located

in the upper middle third of each plant. The parameters included: CO_2 assimilation rate (A_N - μmol m^{-2} s^{-1}), transpiration (E - mmol of H_2O m^{-2} s^{-1}), stomatal conductance (gs - mol of H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci - μmol CO_2 mol^{-1} of air), leaf temperature (Tl - °C), and vapor pressure deficit (VPD - kPa). From these data, water-use efficiency (WUE = A_N/E), intrinsic water-use efficiency (iWUE = A_N/gs), and carboxylation efficiency (A_N/Ci) were calculated.

Chlorophyll *a* fluorescence analysis was performed between 7:00 and 11:00 on the same leaves used for gas exchange measurements. The leaves were subjected to 30 min of pre-adaptation to darkness using leaf tweezers. Subsequently, using a portable fluorometer (OS-30p, Opti-Sciences Chlorophyll Fluorometer, United States), the initial fluorescence (F0) and maximum fluorescence (Fm) values were obtained. Based on these data, the variable fluorescence (Fv), the potential quantum efficiency of photosystem II (Fv/Fm), the primary maximum efficiency of photosystem II (Fv/F0), and the basal quantum efficiency of the non-photochemical process of photosystem II (F0/Fm) were calculated [51].

Photosynthetic pigments

Chlorophyll *a*, *b*, and total indices were determined at 120 DAT (days after transplanting) in the morning, on the middle third of the third fully expanded leaf from the seedling apex, using a portable chlorophyll meter (ClorofiLOG® CFL 1030, Falker, Brazil). The results were expressed as Falker Chlorophyll Index (FCI) [52].

Osmotic adjustment

At 113 days DAT, fresh leaves from the middle third of the seedlings were collected for analysis of total soluble sugars and proline. To prepare the crude extracts, 150 mg of fresh leaves from three replicates in each treatment were placed in Eppendorf tubes containing 1 mL of 80% alcohol for extraction. The tubes were placed in the digester for 2 min to grind the tissue completely, and the supernatant was collected. The supernatant was then centrifuged at 10,000 rpm for 5 min at 10°C to obtain a clear supernatant, stored at -80°C, and subsequently used for biochemical analyses.

Total soluble sugars (TSS) were determined by the anthrone method [53] and expressed in mg TSS g^{-1} MF. The proline (PRO) content was determined in the supernatant using the method described by Bates, Waldren, and Teare [54], and the results were expressed as mol PRO g^{-1} MF.

Leaf content of K, Ca, and Si

The dry biomass of the leaves was ground in a Wiley stainless-steel mill and stored in hermetically sealed containers for the determination of potassium (K) and calcium (Ca) concentrations using the sulfuric acid digestion method [55]. For this, 0.4 g of the dry material was transferred to digestion tubes, to which 2 mL of hydrogen peroxide (H_2O_2 – 30%), 4 mL of sulfuric acid (H_2SO_4 – 98%), and 1 g of the salt mixture were added. The samples were then slowly heated to 350°C (approximately 2 h) in the digestion block to obtain a light greenish liquid, and heating was continued until a colorless liquid was achieved (approximately 4 h). After cooling, the mixture was diluted with 50 mL of deionized water in a collection bottle using a graduated cylinder, and the extract was properly stored. The K concentration was determined by flame photometry after diluting 1 mL of the extract with 19 mL of deionized water.

For calcium determination, 1 mL of the diluted extract was transferred to test tubes, to which 19 mL of deionized water was added. Then, 1 mL of this new dilution was transferred to another test tube, to which 4 mL of lanthanum solution was added. The Ca concentration was quantified by atomic absorption spectrophotometry [55]. The Si concentration was determined at the Soil and Plant Analysis Laboratory (LASP) in Petrolina, Pernambuco, Brazil, according to the methodology of Furlani and Gallo [56]. The samples were dried, ground, and subjected to alkaline digestion to solubilize the silicon. Subsequently, the element was quantified using a colorimetric method, with reading in a spectrophotometer.

Seedling quality was assessed based on plant height (PH), stem diameter (SD), total dry mass (TDM), shoot dry mass (SDM), and root dry mass (RDM). Dickson's quality index (DQI) was calculated according to Dickson et al. [57], as shown in

Eq. 2.

$$DQI = \frac{TDM}{SD} \times \frac{SDM}{RDM} \quad (2)$$

Drought tolerance (DT) was evaluated by the total dry mass of plants subjected to water deficit (Absence of $CaSiO_3$ and Presence of $CaSiO_3$) during the different irrigation shifts (DMd) and by the total dry mass of the control treatment (TR1Absence of $CaSiO_3$ or TR1Presence of $CaSiO_3$) (DMc), and calculated using the formula proposed by Fischer et al. [58], as shown in Eq. 3.

$$DT = \frac{DMd}{DMc} \quad (3)$$

The drought sensitivity index (DSI) was calculated using the equation by Ali and El-Sadek [59], with the same parameters as in Eq. 4.

$$DSI = \frac{DMc - DMd}{DMc} \quad (4)$$

2.5 Statistical analysis

The data obtained were subjected to the Shapiro-Wilk normality test ($p \geq 0.05$) [60]. Subsequently, they were subjected to analysis of variance (ANOVA) using the F test ($p \leq 0.05$). In cases of significance for the isolated factors and their interaction, the Student's t-test ($p \leq 0.05$) was applied to the fertilization factor, and the Tukey test ($p \leq 0.05$) to the irrigation schedule factor, using the statistical software SISVAR®, version 5.3 [61].

3. Results

3.1 Growth

Analysis of variance indicated no significant interaction ($p > 0.05$) between irrigation frequencies and fertilization with calcium silicate ($CaSiO_3$) for stem diameter (SD), plant height (PH), number of leaves (NL), leaf area (LA), and root volume (RV) of soursop seedlings (Table 4 and Fig. 2). However, irrigation frequency significantly influenced PH, NL, LA, and RV ($p \leq 0.05$), whereas SD did not differ significantly between irrigation schedules ($p > 0.05$) (Table 4). Fertilization with $CaSiO_3$, in turn, did not show a significant effect on any of the growth variables analyzed ($p > 0.05$; Table 4).

Table 4
F-test, Tukey test, and Student's t-test were performed for the variables: stem diameter (SD), plant height (PH), number of leaves (NL), leaf area (LA), and root volume (RV) in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)					
SV	SD (mm)	PH (cm)	NL	LA (cm^2/planta)	RV (cm^3)
Blocks	0.5754^{ns}	0.6648^{ns}	0.6766^{ns}	0.7472^{ns}	0.0969^{ns}
TR	0.1810^{ns}	0.0026^{*}	0.0060^{*}	0.0000^{*}	0.0001^{*}
$CaSiO_3$	0.2663^{ns}	0.8391^{ns}	0.0891^{ns}	0.8950^{ns}	0.7111^{ns}
TR x $CaSiO_3$	0.6678^{ns}	0.3993^{ns}	0.9580^{ns}	0.1335^{ns}	0.2094^{ns}
TR (days)	Tukey's test (SE, n = 8)				
TR1	2.23 ± 0.19 a	41.25 ± 1.68 a	31.38 ± 1.72 a	1113.37 ± 34.39 a	26.25 ± 1.83 ab
TR2	2.20 ± 0.34 a	38.28 ± 2.30 a	25.88 ± 1.41 ab	961.30 ± 45.81 ab	27.50 ± 2.50 a
TR3	1.85 ± 0.15 a	34.88 ± 1.10 ab	28.88 ± 1.79 ab	904.41 ± 39.14 b	20.00 ± 0.00 bc
TR4	1.56 ± 0.19 a	29.09 ± 2.44 b	22.63 ± 1.24 b	597.28 ± 50.82 c	15.00 ± 1.89 c
Fertilizing	Student's t-test (SE, n = 16)				
Absence $CaSiO_3$	1.83 ± 0.15 A	35.66 ± 2.15 A	25.75 ± 1.30 A	891.24 ± 66.69 A	22.50 ± 2.14 A
Presence $CaSiO_3$	2.10 ± 0.18 A	36.08 ± 1.29 A	28.63 ± 1.32 A	896.94 ± 44.39 A	21.88 ± 1.36 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. FV = Source of variation. * and ^{ns} = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

PH, NL, LA, and RV were reduced by 29.48%, 27.88%, 46.35%, and 42.86%, respectively, in treatment TR4 compared with TR1, regardless of $CaSiO_3$ fertilization (Table 4). There was no significant difference between treatments TR1 and TR2 for the variables PH, NL, and RV; however, no significant difference was observed for LA.

3.2 Leaf gas exchange

There was a significant interaction between irrigation frequencies and fertilization with $CaSiO_3$ for transpiration (*E*), stomatal conductance (*gs*), and CO_2 assimilation rate (A_N) ($p \leq 0.05$) (Table 5). *E*, *gs*, and A_N in treatment TR3, with the presence of $CaSiO_3$, increased by 81.05%, 66.48%, and 38.84%, respectively, compared to seedlings without $CaSiO_3$ (Table 5). The highest *E*, *gs*, and A_N in seedlings with $CaSiO_3$ occurred in TR3, with increases of 142.25%, 122.31%, and 68.44%, respectively, compared to the lowest performance observed in TR1 (Table 5).

Table 5
F-test, Tukey test, and Student's t-test were performed for the variables: transpiration (*E*), stomatal conductance (*gs*), and CO_2 assimilation rate (A_N) in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)						
SV	*E* (mmol de (H_2O) m^{-2} s^{-1})		*gs* (mol de H_2O m^{-2} s^{-1})		A_N (µmol (CO_2) m^{-2} s^{-1})	
Blocks	0.0671^{ns}		0.1045^{ns}		0.1145^{ns}	
TR	0.0259^{*}		0.0313^{*}		0.1013^{ns}	
$CaSiO_3$	0.4194^{ns}		0.3973^{ns}		0.4995^{ns}	
TR x $CaSiO_3$	0.0115^{*}		0.0218^{*}		0.0445^{*}	
Tukey and Student t-test (SE, n = 4)						
TR (days)	Absence $CaSiO_3$	Presence $CaSiO_3$	Absence $CaSiO_3$	Presence $CaSiO_3$	Absence $CaSiO_3$	Presence $CaSiO_3$
TR1	1.13 ± 0.22 aA	0.71 ± 0.12 bA	50.75 ± 7.69 aA	33.93 ± 3.25 bA	9.28 ± 1.10 aA	6.94 ± 1.32 bA
TR2	1.02 ± 0.13 aA	1.07 ± 0.27 bA	47.97 ± 5.97 aA	50.49 ± 13.38 abA	9.14 ± 1.09 aA	9.33 ± 0.84 abA
TR3	0.95 ± 0.06 aB	1.72 ± 0.22 aA	45.31 ± 5.33 aB	75.43 ± 8.04 aA	8.42 ± 0.82 aB	11.69 ± 0.51 aA
TR4	0.83 ± 0.21 aA	0.81 ± 0.09 bA	39.30 ± 7.65 aA	40.35 ± 2.17 bA	7.65 ± 1.30 aA	8.30 ± 0.39 abA

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ^{ns} = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate.

Vapor pressure deficit (VPD), internal CO_2 concentration (*Ci*), intrinsic water use efficiency (iWUE), instantaneous water use efficiency (WUE), instantaneous carboxylation efficiency (*EiCi*), and leaf temperature (*LT*) were not significantly influenced by the interaction between irrigation frequency and $CaSiO_3$ fertilization, nor individually ($p > 0.05$) (Table 6).

Table 6
F-test, Tukey test, and Student's t-test were performed for the variables: vapor pressure deficit (VPD), internal CO_2 concentration (*Ci*), intrinsic water use efficiency (iWUE), instantaneous water use efficiency (WUE), instantaneous carboxylation efficiency (*EiCi*), and leaf temperature (*LT*) in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)						
SV	VPD (kPa)	*Ci* (µmol CO_2 mol air^{-1})	iWUE (A_N/gs)	WUE (A_N/E)	Ei*Ci*	*LT* (°C)
Blocks	0.1011^{ns}	0.6439^{ns}	0.6640^{ns}	0.1491^{ns}	0.3176^{ns}	0.0243^{*}
TR	0.4833^{ns}	0.2639^{ns}	0.2341^{ns}	0.0510^{ns}	0.3903^{ns}	0.2197^{ns}
$CaSiO_3$	0.7947^{ns}	0.8390^{ns}	0.8805^{ns}	0.7998^{ns}	0.1143^{ns}	0.9470^{ns}
TR x $CaSiO_3$	0.6356^{ns}	0.4144^{ns}	0.3959^{ns}	0.2087^{ns}	0.4329^{ns}	0.4163^{ns}
TR (days)	Tukey's test (SE, n = 8)					
TR1	21.20 ± 0.96 a	81.14 ± 17.11 a	0.1927 ± 0.01 a	9.13 ± 0.42 a	0.2683 ± 0.14 a	29.33 ± 0.45 a
TR2	21.44 ± 0.60 a	71.99 ± 18.71 a	0.1979 ± 0.01 a	9.27 ± 0.61 a	0.2285 ± 0.07 a	29.50 ± 0.25 a
TR3	21.95 ± 0.61 a	107.04 ± 13.45 a	0.1731 ± 0.17 a	7.94 ± 0.49 a	0.1030 ± 0.01 a	29.99 ± 0.21 a
TR4	20.35 ± 0.77 a	65.18 ± 6.66 a	0.2013 ± 0.00 a	10.04 ± 0.57 a	0.1320 ± 0.02 a	29.07 ± 0.41 a
Fertilizing	Student's t-test (SE, n = 16)					
Absence $CaSiO_3$	21.33 ± 0.55 A	82.91 ± 0.59 A	0.1904 ± 0.00 A	9.03 A ± 0.35	0.1202 ± 0.02 A	29.46 ± 0.24A
Presence $CaSiO_3$	21.14 ± 0.52 A	79.76 ± 0.55 A	0.1920 ± 0.01 A	9.16 A ± 0.46	0.2457 ± 0.08 A	29.48 ± 0.26 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. FV = Source of variation. * and ns = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

3.3 Photosynthetic pigments

There was no significant interaction between irrigation schedules and $CaSiO_3$ fertilization for chlorophylls *a* and *b* and total content ($p > 0.05$) (Table 7). Chlorophyll *a, b*, and total content were affected only by irrigation frequency, regardless of $CaSiO_3$ fertilization, with reductions of 11.81%, 27.49%, and 17.18%, respectively, in treatment TR4 compared to TR1 (Table 7). The results also show that there was no significant difference in chlorophyll *a, b*, and total content among the soursop seedling treatments TR1, TR2, and TR3 (Table 7).

Table 7
F-test, Tukey test, and Student's t-test for the variables: chlorophyll *a, b*, and total in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)			
SV	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll Total
Blocks	0.1935^{ns}	0.2394^{ns}	0.2178^{ns}
TR	0.0017^{*}	0.0057^{*}	0.0032^{*}
$CaSiO_3$	0.7160^{ns}	0.8577^{ns}	0.7939^{ns}
TR x $CaSiO_3$	0.8836^{ns}	0.9159^{ns}	0.9051^{ns}
TR (days)	Tukey's test (SE, n = 8)		
TR1	30.31 ± 0.51 a	15.79 ± 0.63 a	46.10 ± 1.12 a
TR2	30.34 ± 0.73 a	15.43 ± 1.07 a	45.77 ± 1.79 a
TR3	29.21 ± 0.49 ab	14.45 ± 0.67 ab	43.66 ± 1.16 ab
TR4	26.73 ± 0.72 b	11.45 ± 0.77 b	38.18 ± 1.49 b
Fertilizing	Student's t-test (SE, n = 16)		
Absence $CaSiO_3$	29.03 ± 0.59 A	14.20 ± 0.73 A	43.23 ± 1.31 A
Presence $CaSiO_3$	29.26 ± 0.55 A	14.35 ± 0.68 A	43.61 ± 1.22 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. FV = Source of variation. * and ns = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

3.4 Chlorophyll *a* fluorescence

Statistical analysis revealed no significant interaction between irrigation and $CaSiO_3$ fertilization schedules ($p > 0.05$), as well as no significant isolated effects ($p > 0.05$) for maximum fluorescence (Fm), variable fluorescence (Fv), maximum quantum efficiency of photosystem II (PSII) (*Fv/Fm*), maximum primary efficiency of photosystem II (PSII) (Fv/F_0), and basal quantum efficiency of the non-photochemical process of photosystem II (PSII) (F_0/Fm) (Table 8). For initial fluorescence (F_0), there was a significant difference between irrigation frequencies ($p \leq 0.05$, Table 8).

Table 8
F-test, Tukey test, and Student's t-test for the variables: initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), maximum quantum efficiency of photosystem II (PSII) (*Fv/Fm*), maximum primary efficiency of photosystem II (PSII) (Fv/F_0), basal quantum efficiency of the non-photochemical process of photosystem II (PSII) (F_0/Fm), in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)						
SV	F_0	F_m	F_v	F_v/F_m	F_v/F_0	F_0/F_m
Blocks	0.0020^{ns}	0.0235^{*}	0.0847^{ns}	0.4956^{ns}	0.6928^{ns}	0.7202^{ns}
TR	0.0062^{*}	0.1715^{ns}	0.4248^{ns}	0.2193^{ns}	0.5794^{ns}	0.5658^{ns}
$CaSiO_3$	0.9371^{ns}	0.8900^{ns}	0.8664^{ns}	0.8937^{ns}	0.8762^{ns}	0.8985^{ns}
TR x $CaSiO_3$	0.3063^{ns}	0.6559^{ns}	0.8093^{ns}	0.6971^{ns}	0.9405^{ns}	0.9114^{ns}
TR (days)	Tukey's test (SE, n = 8)					
TR1	95.75 ± 3.67 b	419.00 ± 16.77 a	323.25 ± 14.57 a	0.7708 ± 0.01 a	3.39 ± 0.13 a	0.2294 ± 0.01 a
TR2	93.87 ± 3.64 b	417.00 ± 15.88 a	323.13 ± 12.74 a	0.7741 ± 0.00 a	3.45 ± 0.08 a	0.2254 ± 0.00 a
TR3	99.88 ± 3.11 ab	423.75 ± 21.41 a	323.88 ± 19.83 a	0.7566 ± 0.01 a	3.24 ± 0.17 a	0.2380 ± 0.01 a
TR4	110.25 ± 5.25 a	461.88 ± 14.31 a	351.63 ± 9.90 a	0.7611 ± 0.01 a	3.21 ± 0.09 a	0.2381 ± 0.01 a
Fertilizing	Student's t-test (SE, n = 16)					
Absence $CaSiO_3$	100.06 ± 3.55 A	429.31 ± 12.50 A	329.25 ± 9.81 A	0.7661 ± 0.00 A	3.31 ± 0.08 A	0.2332 ± 0.00 A
Presence $CaSiO_3$	99.81 ± 2.77 A	431.50 ± 12.94 A	331.69 ± 11.20 A	0.7652 ± 0.00 A	3.33 ± 0.09 A	0.2323 ± 0.00 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. FV = Source of variation. * and ns = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

The F_0 in soursop seedlings increased by 15.14% in treatment TR4 compared with TR1, regardless of $CaSiO_3$ fertilization (Table 8). However, there were no significant differences between TR1, TR2, and TR3, indicating that, regardless of irrigation frequencies, soursop seedlings show similar performance in F_0, except for TR4.

3.5 Biomass accumulation

There was no significant interaction between irrigation frequencies and $CaSiO_3$ fertilization for leaf dry mass (LDM), stem dry mass (SDM), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), and the root dry mass/shoot dry mass ratio (RDMR) ($p > 0.05$, Table 9).

For the LSM of soursop seedlings, the isolated factors, irrigation schedules ($p \leq 0.05$) and CaSiO3 fertilization ($p \leq 0.05$), showed a significant effect (Table 9). For SDM, RDM, and TDM, the significant effect was observed only for irrigation schedules ($p \leq 0.05$; Table 9).

Table 9
F-test, Tukey test, and Student's t-test were performed for the variables: leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), total dry mass (TDM), and root dry mass/shoot dry mass ratio (RDMR) in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

	F-test (p-value)				
SV	**LDM (g)**	**SDM (g)**	**RDM (g)**	**TDM (g)**	**RDMR**
Blocks	0.5314^{ns}	0.5936^{ns}	0.2141^{ns}	0.3752^{ns}	0.2929^{ns}
TR	0.0000^{*}	0.0109^{*}	0.0044^{*}	0.0001^{*}	0.5988^{ns}
$CaSiO_3$	0.0292^{*}	0.4731^{ns}	0.8615^{ns}	0.2363^{ns}	0.1727^{ns}
TR x $CaSiO_3$	0.3539^{ns}	0.9161^{ns}	0.4322^{ns}	0.8568^{ns}	0.5372^{ns}
TR (days)	Tukey's test (SE, n = 8)				
TR1	6.06 ± 0.30 a	6.71 ± 0.39 a	4.85 ± 0.27 a	17.62 ± 0.68 a	0.38 ± 0.01 a
TR2	4.58 ± 0.30 b	5.93 ± 0.56 ab	4.11 ± 0.36 ab	14.62 ± 1.12 ab	0.39 ± 0.02 a
TR3	4.51 ± 0.26 b	5.42 ± 0.39 ab	3.85 ± 0.27 ab	13.78 ± 0.81 bc	0.39 ± 0.02 a
TR4	3.07 ± 0.32 c	4.43 ± 0.20 b	3.16 ± 0.25 b	10.68 ± 0.53 c	0.42 ± 0.03 a
Fertilizing	Student's t-test (SE, n = 16)				
Absence $CaSiO_3$	4.23 ± 0.34 B	5.47 ± 0.35 A	3.97 ± 0.27 A	13.67 ± 0.88 A	0.41 ± 0.41 A
Presence $CaSiO_3$	4.88 ± 0.31 A	5.78 ± 0.34 A	4.02 ± 0.24 A	14.68 ± 0.80 A	0.38 ± 0.38 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. FV = Source of variation. * and ns = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

The leaf dry mass (LDM) of soursop irrigated every six days (TR4) decreased by 49.33% compared to seedlings irrigated daily (TR1), regardless of $CaSiO_3$ supply (Table 9). The presence of $CaSiO_3$ resulted in a 15.37% increase in LDM compared with seedlings without $CaSiO_3$, regardless of irrigation frequencies (Table 9).

The stem dry mass (SDM), root dry mass (RDM), and total dry mass (TDM) were reduced by irrigation schedules by 33.98%, 34.85%, and 39.39%, respectively, in TR4 compared to TR1, regardless of $CaSiO_3$ supply (Table 9). The total dry mass (TDM) showed similar behavior between TR1 and TR2 (Table 9).

3.6 Seedling quality indices and stress tolerance

There was an isolated effect of irrigation frequencies on the Dickson Quality Index (DQI) ($p \leq 0.05$, Table 10). The Dickson Quality Index (DQI) was reduced by 18.03% in treatment TR4 compared to TR1, regardless of fertilization with $CaSiO_3$ (Table 10). However, treatments TR1, TR2, and TR3 did not differ significantly from one another (Table 10).

Table 10
F-test, Tukey test, and Student's t-test were performed on the Dickson Quality Index (DQI) of soursop seedlings fertilized with calcium silicate under different irrigation frequencies, measured 120 days after transplanting

	F-test (p-value)
SV	**DQI**
Blocks	0.4574^{ns}
TR	0.0416^{*}
$CaSiO_3$	0.0739^{ns}
TR x $CaSiO_3$	0.5530^{ns}
TR (days)	Tukey's test (SE, n = 8)
TR1	4.27 ± 0.18 a
TR2	4.06 ± 0.20 ab
TR3	3.91 ± 0.16 ab
TR4	3.50 ± 0.21 b
Fertilizing	Student's t-test (SE, n = 16)
Absence $CaSiO_3$	3.76 ± 0.14 A
Presence $CaSiO_3$	4.11 ± 0.14 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ^{ns} = Significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

The interaction ($p \leq 0.05$) between irrigation schedules and fertilization with $CaSiO_3$ was significant for drought tolerance efficiency (DTE) and drought sensitivity index (DSI) in soursop seedlings (Table 11). Water deficit decreased drought tolerance efficiency (DTE) in irrigation frequencies (Table 11). In treatments TR2, TR3, and TR4, the presence of $CaSiO_3$ resulted in increases of 4.94%, 1.28%, and 20%, respectively, compared to seedlings without $CaSiO_3$ (Table 11). The lowest DTE in seedlings with $CaSiO_3$ was observed in TR4, resulting in a 22.35% reduction compared to the highest value in treatment TR2 (Table 11).

Table 11
F-test, Tukey test, and Student's t-test were performed for the variables: drought tolerance efficiency (DTE) and drought sensitivity index (DSI) in soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)				
SV	DTE		DSI	
Blocks	1.0000 ^{ns}		0.0000*	
TR	0.0000*		0.0000*	
$CaSiO_3$	0.0000*		0.0000*	
TR x $CaSiO_3$	0.0000*		0.0000*	
Tukey and Student t-test (SE, n = 4)				
TR (days)	Absence $CaSiO_3$	Presence $CaSiO_3$	Absence $CaSiO_3$	Presence $CaSiO_3$
TR1	-	-	-	-
TR2	0.81 ± 0.00 aB	0.85 ± 0.00 aA	0.19 ± 0.00 aA	0.19 ± 0.00 abA
TR3	0.78 ± 0.00 aB	0.79 ± 0.00 bA	0.22 ± 0.00 bA	0.21 ± 0.00 bB
TR4	0.55 ± 0.00 aB	0.66 ± 0.00 cA	0.45 ± 0.00 cA	0.34 ± 0.00 cB

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ^{ns} = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

The drought sensitivity index (DSI) increased with water deficit, regardless of $CaSiO_3$ supply (Table 11). The presence of $CaSiO_3$ reduced the DSI by 4.54% and 24.44% in treatments TR3 and TR4, respectively, compared to seedlings without $CaSiO_3$ (Table 11). The highest DSI values, both in the absence and presence of $CaSiO_3$, occurred in TR4, with increases of 136.84% and 78.95%, respectively, compared to the lowest DSI observed in TR2 (Table 11).

3.7 Osmotic adjustment

There was no significant interaction between irrigation schedules and $CaSiO_3$ fertilization on total soluble sugar (TSS) content ($p > 0.05$) (Table 12). There was only an isolated effect ($p \leq 0.05$) on the irrigation frequency (Table 12). Treatment TR4 resulted in a 27.50% increase in TSS compared to TR1 (Table 12). It is observed that treatment TR2 did not differ from TR1, TR3, and TR4, indicating that seedlings can be irrigated every two days without exhibiting water stress in relation to TSS.

Table 12
F-test, Tukey's test, and Student's t-test were performed on the variable total soluble sugar (TSS) content in leaf tissues of soursop seedlings fertilized with calcium silicate under different irrigation frequencies, measured 120 days after transplanting

F-test (p-value)	
SV	TSS (mg TSS g FM^{-1})
Blocks	0.0253*
TR	0.0053*
$CaSiO_3$	0.1073ns
TR x $CaSiO_3$	0.0585ns
TR (days)	Tukey's test (SE, n = 8)
TR1	28.04 ± 1.62 b
TR2	32.57 ± 2.39 ab
TR3	34.02 ± 1.27 a
TR4	35.75 ± 1.48 a
Fertilizing	Student's t-test (SE, n = 16)
Absence $CaSiO_3$	31.43 ± 1.19 A
Presence $CaSiO_3$	33.77 ± 1.53 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ns = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

The interaction between irrigation frequencies and fertilization with $CaSiO_3$ was significant for proline (PRO) ($p \leq 0.05$) (Table 13). The PRO content increased by 192.30% in seedlings without $CaSiO_3$ in TR3, compared to seedlings with $CaSiO_3$ (Table 13). In the absence of $CaSiO_3$, the greatest accumulation of PRO in seedlings was observed in TR3, with an increase of 329.03% compared to the lowest accumulation recorded in TR2 (Table 13). The presence of $CaSiO_3$ resulted in the greatest accumulation of PRO in TR3 and TR4, of 50.41% and 192.56%, respectively, compared to the lowest accumulation observed in TR1 (Table 13).

Table 13
F-test, Tukey test, and Student's t-test for the variable proline content (PRO) in the leaf tissues of soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)		
SV	PRO (mol PRO g MF^{-1})	
Blocks	0.2594	
TR	0.0003*	
$CaSiO_3$	0.0310*	
TR x $CaSiO_3$	0.0057*	
Tukey's test (SE, n = 4)		
TR (days)	Absence $CaSiO_3$	Presence $CaSiO_3$
TR1	3.65 ± 0.49 bA	2.42 ± 0.79 bA
TR2	2.48 ± 0.39 bA	2.63 ± 0.58 bA
TR3	10. 64 ± 2.43 aA	3.64 ± 0.85 abB
TR4	6.07 ± 0.12 bA	7.08 ± 1.36 aA

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ^{ns} = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

3.8 Leaf content of K, Ca, and Si

There was no significant effect of the interaction between the factors studied, isolated or in combination, on the potassium (K) content ($p > 0.05$) (Table 14).

Table 14
F-test, Tukey test, and Student's t-test for the variable potassium (K) content in the leaf tissues of soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)	
SV	K (g kg^{-1})
Blocks	0.2609^{ns}
TR	0.0567^{ns}
$CaSiO_3$	0.9960^{ns}
TR x $CaSiO_3$	0.7766^{ns}
TR (days)	Tukey's test (SE, n = 8)
TR1	13.45 ± 0.36 a
TR2	13.22 ± 0.85 a
TR3	11.85 ± 0.35 a
TR4	11.24 ± 0.68 a
Fertilizing	Student's t-test (SE, n = 16)
Absence $CaSiO_3$	12.44 ± 0.42 A
Presence $CaSiO_3$	12.44 ± 0.53 A

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ^{ns} = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

The interaction between irrigation frequencies and fertilization with $CaSiO_3$ was significant for silicon (Si) and calcium (Ca) contents ($p \leq 0.05$) (Table 15).

Table 15
F-test, Tukey test, and Student's t-test were performed for the variables silicon (Si) and calcium (Ca) content in leaf tissues of soursop seedlings fertilized with calcium silicate under different irrigation frequencies, 120 days after transplanting

F-test (p-value)				
SV	Si (g kg^{-1})	Ca (g kg^{-1})		
Blocks	0.4979^{ns}	0.0713^{ns}		
TR	0.0000^{*}	0.2647^{ns}		
$CaSiO_3$	0.0000^{*}	0.0006^{*}		
TR x $CaSiO_3$	0.0000^{*}	0.0342^{*}		
Tukey's test (SE, n = 4)				
TR (days)	Absence $CaSiO_3$	Presence $CaSiO_3$	Absence $CaSiO_3$	Presence $CaSiO_3$
TR1	8.57 ± 0.33 aA	9.01 ± 0.25 bA	1.73 ± 0.20 aA	1.69 ± 0.14 bA
TR2	9.05 ± 0.36 aA	9.76 ± 0.40 bA	1.63 ± 0.16 aA	1.86 ± 0.10 abA
TR3	8.58 ± 0.11 aB	14.73 ± 0.10 aA	1.63 ± 0.14 aB	2.32 ± 0.26 aA
TR4	8.31 ± 0.31 aA	8.73 ± 0.27 bA	1.49 ± 0.12 aB	2.27 ± 0.10 abA

TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$. SV = Source of variation. * and ^{ns} = significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively, according to the F-test. Means followed by the same letter in the column do not differ from each other by Tukey's test and Student's t-test ($p > 0.05$). ***Means ± standard deviation. TR: irrigation frequency; $CaSiO_3$: calcium silicate

Silicon (Si) content increased by 71.68% in treatment TR3, in which seedlings were fertilized with $CaSiO_3$, compared to the absence of CaSiO3 (Table 15). The highest Si content in seedlings with $CaSiO_3$ was observed in TR3, which corresponds to an increase of 68.72% compared to the lowest content observed in TR4 (Table 15).

There was an increase in calcium (Ca) content of 42.33% and 52.35% in treatments TR3 and TR4, respectively, in seedlings with $CaSiO_3$, compared to those without $CaSiO_3$ (Table 15). In the presence of $CaSiO_3$, the highest Ca content in seedlings was observed in TR3, with an increase of 37.28% compared to the lowest content recorded in TR1 (Table 15).

4. Discussion

Water stress is one of the main limiting factors in agricultural production, profoundly affecting plant growth, development, and metabolism, especially in TR4, irrigated every 6 days. The impacts are even more severe in perennial species of high economic value, such as soursop (*A. muricata* L.), which, in this study, showed reductions in growth variables, including height, number of leaves, leaf area, and root volume [44, 3, 8, 16]. The application of $CaSiO_3$ significantly improves the CO_2 assimilation rate, stomatal conductance, transpiration, leaf biomass, modulation of proline synthesis, and leaf silicon and calcium content.

The positive effects observed with the application of $CaSiO_3$ are mainly because silicon is an important element for some fruit crops, since, when deposited below the cuticle, it modulates several physiological activities, reducing excessive water loss and maintaining stable stomatal opening [12]. In this way, the species maintained active cellular metabolism, even with less water available. Maintaining cellular hydration favors physiological efficiency and plant resistance to various

stresses [62, 12, 63]. In addition, calcium contributes to membrane stability and the maintenance of cell turgor, which enhances photosynthesis and transpiration under water stress [64].

Our results demonstrate that daily irrigation (TR1), every two days (TR2), and every four days (TR3) yield similar growth performance, biomass, initial fluorescence (F_0), total soluble sugars, and seedling quality index, regardless of $CaSiO_3$ fertilization. This finding indicates that moderate deficit irrigation every four days (TR3) does not compromise soursop seedling development, confirming that irrigation scheduling is an important component of irrigated and sustainable agriculture, aiming to improve water use efficiency by applying the right amount of water at the right time [65].

These results represent an important strategy for the semi-arid region of Brazil, since rainfall irregularity limits agricultural production, including fruit species, and irrigation is an input that maintains the economic and social viability of the production system [66]. However, under severe stress every six days (TR4), seedling growth decreased, since soursop requires adequate water availability [5, 8] and, in very dry soils with an arid moisture regime, growth is severely restricted [4]. Under these conditions, there is a reduction in cell turgor pressure, which causes less cell division and development [67, 16], contributing to the limitation of new leaf expansion, abscission, wilting, senescence, and reduction of leaf area [13, 3, 68].

Under severe stress conditions (TR4), seedlings likely also divert metabolic energy towards osmotic acclimation and the synthesis of compatible solutes, such as total soluble sugars, which accumulate in the leaves, mainly in the cytoplasm, as mechanisms to mitigate the adverse effects of water stress [13, 69, 12]. This results in lower availability of energy resources for growth and biomass production [70], also compromising the quality of soursop seedlings [8].

Water stress also increased F0, indicating that, when electron flow through photosystem II (PSII) is obstructed, the plastoquinone acceptor (QA−) cannot be fully oxidized under stress, thereby damaging PSII and reducing the species' photosynthetic efficiency [71, 51]. Furthermore, the reduction in chlorophyll content indicates that drought is directly associated with chlorophyll degradation, primarily due to excessive formation of reactive oxygen species in chloroplasts [72–74]. These alterations interfere with CO_2 assimilation rates and lead to reductions in growth, biomass, and the quality of soursop seedlings.

On the other hand, the application of $CaSiO_3$ proved effective in improving leaf biomass and gas exchange, increasing leaf calcium and silicon levels, and regulating proline content. The increase in leaf biomass may be related to the incorporation of silicon into plant structures, especially in leaves, which require less energy than the synthesis of organic compounds because of the high permeability of lipid bilayers [75]. Thus, the increase in silicon levels in plant tissues can partially replace carbon-based components, thereby reinforcing cell walls, conferring greater rigidity and resistance to desiccation, and increasing accumulated biomass [76]. Therefore, the increase in leaf biomass in fertilized seedlings indicates that the application of $CaSiO_3$ partially reduced the growth inhibition caused by drought. The research results corroborate studies that report that Triticum plants fertilized with silicon increased shoot dry mass when exposed to water stress, compared to the unfertilized control [13]. These results reinforce the role of silicon as a strategic element in mitigating the negative impacts of water limitation on plant development [12].

Regarding the application of $CaSiO_3$, our results indicate that under moderate water-deficit conditions (TR3), it significantly improves transpiration (*E*), stomatal conductance (*gs*), and CO_2 assimilation rate (A_N) in soursop seedlings. These results can be attributed to Si deposits, in the form of amorphous silica ($SiO_2 \cdot nH_2O$) in the cell wall, which form a double layer of cuticular silica, providing better osmotic adjustment, maintaining cell turgor, and increasing photosynthetic capacity [77, 78]. The accumulation of Si in the leaf epidermis can help minimize oxidative damage, protecting cell membranes and the photosynthetic apparatus [79].

The effect of Si on transpiration rates varies according to the severity of the stress. Under moderate stress conditions, Si can increase water absorption by the roots, thereby increasing transpiration rates and, consequently, plant growth under

drought stress [29]. However, our research observed continued growth up to TR3. The results corroborate recent research indicating that A_N, *gs*, and *E* reached higher values than those of the control seedlings when treated with Si, which demonstrates the adaptation of the studied species [80, 38, 30]. Calcium also acts as an essential second messenger, amplifying signals and modulating target signaling proteins, culminating in the activation of physiological responses and the potentiation of antioxidant mechanisms, thereby increasing plants' tolerance to drought [80, 81].

The accumulation of proline in stressed plants indicates a defense mechanism to maintain intracellular osmotic pressure [19, 18], as it is an antioxidant and participates in cell signaling [35, 29]. In the present study, the significant reduction in proline content in seedlings treated with calcium silicate under moderate stress (TR3) suggests that calcium silicate may indicate relief from stress-induced damage [83]. This result may be related to foliar gas exchange and the benefits of silicon and calcium in regulating water loss through foliar transpiration, depending on the root water *status*, which would lead to greater water transport to the leaves, reflecting an increase in osmotic potential, reducing plant stress and, consequently, proline concentration [29, 84]. This osmotic adjustment is believed to be critical for protecting cellular structures and maintaining membrane and protein integrity, preventing cell damage and ensuring continuous metabolic activity [30, 85]. Similar results were observed in *Phoenix dactylifera* L. [83].; *Oryza sativa* L. [86]., and *Triticum aestivum* L. [84].

Increased transpiration in TR3 led to higher concentrations of Si and Ca in seedlings fertilized with $CaSiO_3$. This result relates to the elements' primary absorption mechanism, which occurs predominantly via mass flow. In this process, Si is absorbed as silicic acid at the root level and, once inserted into the xylem vessels, is transported to the shoots and leaves through the transpiration stream [87]. Calcium, in turn, shows similar absorption, and its increase confirms that $CaSiO_3$ acts as a direct source of the nutrient, releasing Ca^{2+} into the soil as it solubilizes. Under water-deficit conditions, the cytosolic accumulation of Ca^{2+} plays a central role in signal transduction, activating various metabolic and molecular pathways involved in stress adaptation [88].

Under moderate stress conditions (TR3), the use of $CaSiO_3$ demonstrates that seedling producers, researchers, or farmers can adopt fertilization as a sustainable strategy in semi-arid regions. This practice allows for longer irrigation intervals without compromising foliar gas exchange performance and mineral nutrition, thereby increasing the drought tolerance of soursop seedlings and reducing their water sensitivity. For the future, we recommend further studies on this species, particularly focused on evaluating different $CaSiO_3$ dosages, to expand knowledge of its agronomic potential.

Soursop seedlings irrigated daily, every two days, and every four days showed similar results for growth, photosynthetic pigments, biomass accumulation, chlorophyll *a* fluorescence, and total soluble sugars, demonstrating that irrigation management every four days is viable in a water-deficient environment. Therefore, irrigation every six days, which indicates a severe water deficit, restricts seedling growth and development. Applying $CaSiO_3$ during the 4-day irrigation interval is an effective strategy to mitigate the impacts of deficit irrigation on soursop seedlings, thereby improving gas exchange, leaf biomass, proline accumulation, and silicon and calcium absorption. These effects increased the seedlings' tolerance to water deficits, optimizing water use and promoting more sustainable production.

5. Conclusions

Soursop plants subjected to water stress and silicon application showed physiological and nutritional responses that reinforced the potential of these practices as a sustainable strategy in semi-arid regions. Irrigation every two days kept the seedlings free from water stress, while intervals of up to four days were manageable under water-deficit conditions without compromising plant growth and development. On the other hand, irrigation every six days negatively affected the growth, physiology, and biochemistry of the seedlings, highlighting the limits of drought tolerance. $CaSiO_3$ increases tolerance to water deficit in soursop seedlings, mainly under moderate stress, by maintaining stomatal regulation and

osmotic balance, favoring physiological performance. Future perspectives indicate the need for further studies at different $CaSiO_3$ dosages to expand knowledge of its agronomic potential for the species.

Declarations

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-Ethical Approval

Not applicable.

-Consent to Participate

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-Authors Contributions

Conceptualization: PECA, VM, FVSS; Methodology: PECA, VM, FVSS, JESR, AMFO; Formal analysis and investigation: PECA, VM, FVSS, FNF, JESR, AMFO, JLL, DEGS, ASF, TR, MAS, GCS, RRS, LPC, ECMV; Writing – preparing the original draft: PECA, VM, FVSS, AMFO, JESR; Writing – proofreading and editing: PECA, VM, FVSS, AMFO, JESR, AGLS; Obtaining funding: VM, FVSS; Resources: VM, FVSS, ASF; Supervision: VM, FVSS, JESR.

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-Declaration of Interest Statement

We have no conflicts of interest to disclose.

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Figures

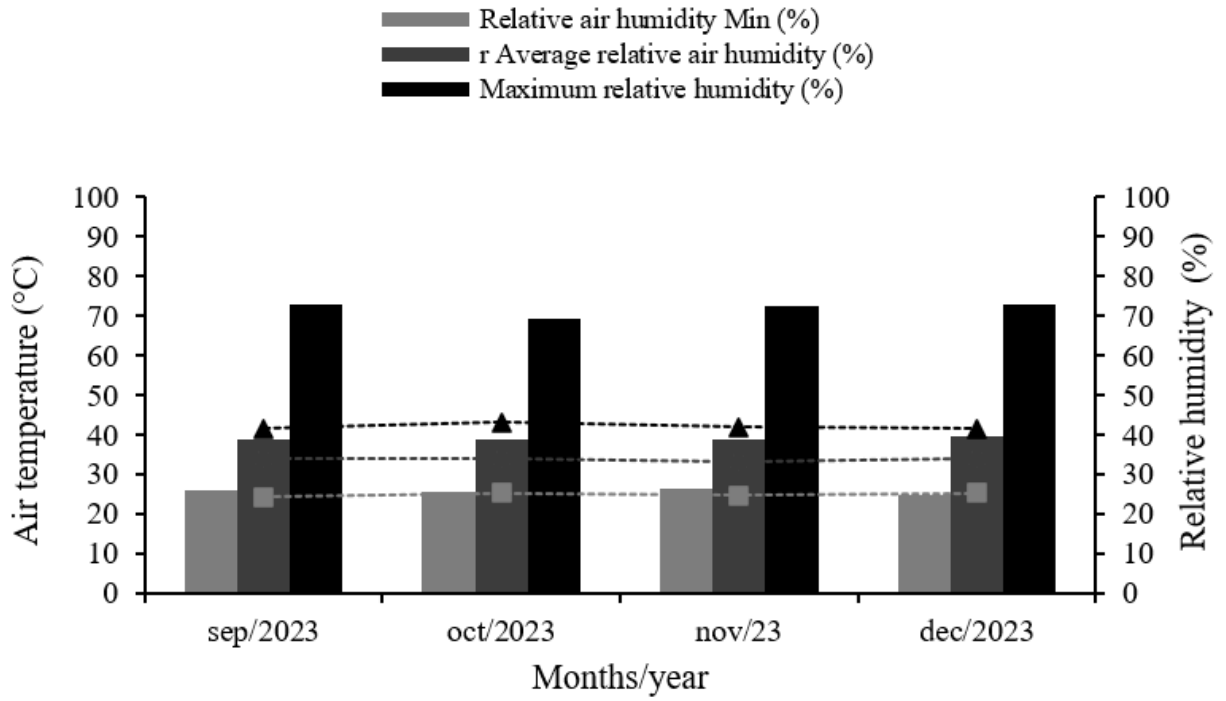


Figure 1

Temperature and relative humidity: maximum (max.), minimum (min.), and average during the experimental period

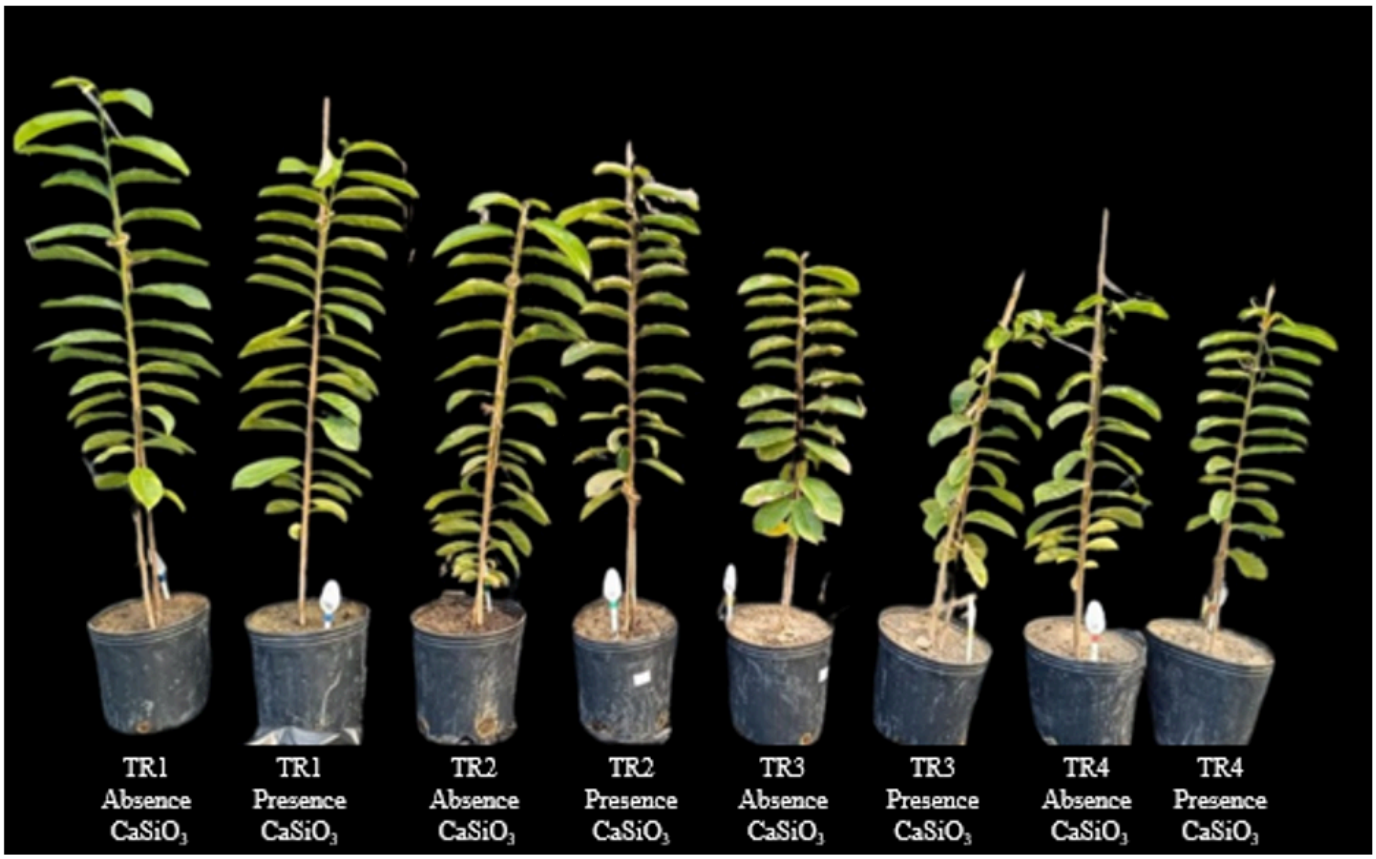


Figure 2

Soursop seedlings are fertilized with calcium silicate under different irrigation frequencies. TR1: daily irrigation; TR2: irrigation every two days; TR3: irrigation every four days; and TR4: irrigation every six days. Absence of $CaSiO_3$ and presence of $CaSiO_3$