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Original Article

Physiology and production of sugar-apple under water stress and application of proline

Fisiologia e produção de pinheira sob estresse hídrico e aplicação de prolina

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Abstract

The objective of this study was to evaluate the physiology and production of sugar-apple as a function of irrigation intervals and foliar application of proline under the conditions of Paraíba's semi-arid region. A randomized block design was laid out in a 4 × 2 factorial scheme, with treatments resulting from the combination of four irrigation intervals (1, 4, 8 and 12 days) and two concentrations of proline (0 and 10 mM), with four replicates, and the plot consisted of four usable plants. Increase in irrigation intervals reduced the gas exchange of sugar-apple plants at 298 days after transplanting. Exogenous application of proline at concentration of 10 mM increased contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids and fruit fresh mass in plants grown under 12-day irrigation intervals.

Keywords: water stress, osmoregulation, *Annona squamosa L.*

Resumo

Objetivou-se com este trabalho avaliar a fisiologia e a produção da pinheira em função dos turnos de rega e aplicação foliar de prolina em condições do semiárido Paraibano. Foi utilizado o delineamento de blocos casualizados em esquema fatorial 4 × 2, cujos tratamentos resultam da combinação de quatro turno de rega (1, 4, 8 e 12 dias) e duas concentrações de prolina (0 e 10 mM), com quatro repetições, cuja a parcela foi constituída de quatro plantas úteis. O incremento nos turnos de rega reduziu as trocas gasosas das plantas de pinheira, aos 298 dias após o transplantio. A aplicação de prolina na concentração de 10 mM aumentou o extravasamento de eletrólitos no limbo foliar, a condutância estomática e diminuiu a concentração interna de CO_2 das plantas de pinheira. A aplicação exógena de prolina na concentração de 10 mM aumentou os teores de clorofila a, *b*, total e carotenoides e a massa fresca de frutos nas plantas cultivadas sob turno de rega de 12 dias.

Palavras-chave: estresse hídrico, osmorregulação, *Anonna squamosa L.*

1. Introduction

Sugar-apple (*Annona squamosa* L.), also known as sweetsop, is a plant originating in the Antilles and is included in the group of Annonaceae species, fruit trees of great economic importance. The great interest in its cultivation is due to the high prices of both pulp and fruit, with great export potential (Braga Sobrinho, 2014), and because it has medicinal properties, being a source of vitamins, fibers and minerals, acting against bacterial infections and nervous disorders, besides having antidepressant action (Ferreira et al., 2021).

Brazil stands out in both production and commercialization of sugar-apple fruit, which can be consumed fresh or in processed products, contributing to the regional economy, through the generation of employment and income (Oliveira et al., 2016). About 97% of the entire sugar-apple producing area is located in the Northeast region, with the state of Bahia as the largest producer. Also in this scenario, Brazilian production exceeded 8.72 thousand tons in the 2017 season, which generated over R$ 20 million (IBGE, 2022).

Obstacles to the expansion of production areas in the Brazilian semi-arid region include rainfall irregularity and high evapotranspiration rate, which results in limitations to adequate water availability during the crop cycle, leading to production losses and changes in fruit quality (Lima et al., 2015; Capitulino et al., 2022; Pinheiro et al., 2022a). The sugar-apple is tolerant to conditions of water deficiency (Fernandes et al., 2022), however, the sensitivity of plants to stress varies according to the stages of development (Ferreira et al., 2022).

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Under water stress conditions, plants typically have inhibited growth due to the decrease in cell turgor caused by the reduction in water absorption. Water stress also causes stomatal closure, limiting transpiration and interfering with photosynthesis and production of photoassimilates and causing paralysis of protein biosynthesis (Peloso et al., 2017; Soares et al., 2020). On the other hand, under water stress conditions, plants invest in greater root architecture and more suberized epidermis (Karlova et al., 2021). Under conditions of high temperature, combined with low water availability, plant respiration rates increase, making biological and metabolic processes unstable, in addition to hindering nutrient absorption due to the decrease in translocation processes (Bárzana and Carvajal, 2020; Soares et al., 2023).

It should be considered that in the last decade research with the use of elicitors has been conducted with the purpose of mitigating the effects of abiotic stresses, such as water and salt stresses (Souza et al., 2021; Lima et al., 2016). Among the substances, proline stands out with foliar application. Proline is an osmolyte that contributes to the maintenance of osmotic homeostasis, provides the gradient of conduction for water absorption, maintenance of cell turgor by osmotic adjustment and redox metabolism to remove excessive levels of reactive oxygen species (ROS) and restore cell redox balance, as well as protecting cellular machinery from osmotic stress and oxidative damage (Ghosh et al., 2021).

Several studies have demonstrated the beneficial effect of proline application under conditions of abiotic stresses, as reported for tobacco plants by Cacefo (2020), who observed that the exogenous application of proline at 10 mmol L^{-1} reduced the negative effects of water deficit on photosynthetic activity and promoted a higher biomass accumulation in the genotypes studied. In this context, the objective of this study was to evaluate the physiology and production of sugar-apple as a function of irrigation intervals and foliar application of proline.

2. Material and Methods

The experiment was conducted from January to November 2021 under field conditions at the 'Rolando Enrique Rivas Castellón' Experimental Farm, belonging to the Center for Sciences and Agri-Food Technology - CCTA of the Federal University of Campina Grande - UFCG, in the municipality of São Domingos, Paraíba, Brazil, located by the coordinates: 06º48'50" S latitude and 37º56'31" W longitude, and altitude of 190 m. It has an average annual rainfall of 700 mm. The data of maximum and minimum temperature, relative humidity and rainfall during the experimental period are presented in Figure 1.

The treatments consisted of the combination of four irrigation intervals - INT (1, 4, 8 and 12 days) and two proline concentrations - PRO (0 and 10 mM), distributed in randomized blocks, arranged in a 4 × 2 factorial scheme, with four replicates, and the plot consisted of four usable plants. Proline concentrations were established based on a study conducted by Lima et al. (2016).

Soil tillage was carried out with harrowing, aiming at breaking up clods and leveling the soil, followed by demarcation, installation of irrigation system and then collection of soil samples from the 0-30 cm layer, whose physical and chemical characteristics (Table 1) were determined according to the methodology of Teixeira et al. (2017).

Sowing was performed in plastic bags with dimensions of 15 × 20 cm, filled with a mixture of 84:15:1 (volume basis) of soil, sand and aged bovine manure, respectively. The soil was autoclaved to eliminate the main microorganisms capable of causing diseases in plants. Two seeds were sown in each bag to obtain the seedlings.

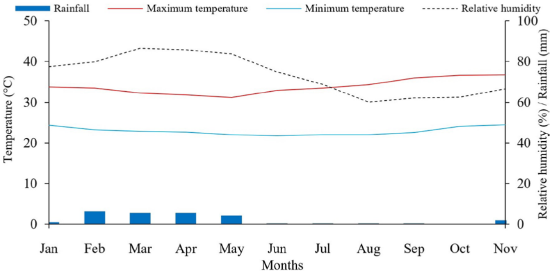

Figure 1. Data of maximum and minimum air temperature, relative air humidity and rainfall during the conduction period of the experiment.

Table 1. Chemical and physical attributes of the soil used in the experiment before the application of the treatments.

Chemical attributes								
pH (H_2O)	**OM**	**P**	**K^+**	**Na^+**	**Ca^{2+}**	**Mg^{2+}**	**Al^{3+}**	**H^+**
(1:2.5)	**g kg^{-1}**	**(mg kg^{-1})**	**...$cmol_c$ kg^{-1} ....**					
6.67	15.60	72.30	0.17	0.10	4.37	3.70	0.48	0.04
..........Chemical attributes............				...........................Physical attributes...........................				
EC_{se}	CEC	SAR	ESP	Particle-size fraction (g kg^{-1})			Moisture (dag kg^{-1})	
(dS m^{-1})	$cmol_c$ kg^{-1}	(mmol L^{-1})$^{0.5}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
0.46	8.86	0.73	1.13	758.80	197.80	43.40	13.27	4.98

pH – Hydrogen potential, OM – Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; Al^{3+}+H^+ extracted with 0.5 M CaOAc at pH 7.0; EC_{se} - Electrical conductivity of saturation extract; CEC - Cation exchange capacity; SAR - Sodium adsorption ratio of saturation extract; ESP - Exchangeable sodium percentage; [1,2] referring to the moisture contents in the soil corresponding to field capacity and permanent wilting point.

After emergence of seedlings, thinning was performed, leaving only the most vigorous plant when they were 10 cm tall. Prior to transplanting the seedlings, plowing was carried out followed by harrowing, aiming at breaking up soil clods and leveling the area. Subsequently, the holes were manually opened with the aid of a post hole digger, with spacing of 3 m between rows and 3 m between plants. The holes were 40 × 40 × 40 cm.

After opening the holes, fertilization was carried out with 10 L of bovine manure and 40, 60 and 60 g plant^{-1} of N, P_2O_5 and K_2O, respectively, as recommended by Silva and Silva (1997). Nitrogen and potassium fertilizations were performed monthly, using urea (45% N) and potassium chloride (60% K_2O) as sources of nitrogen and potassium, respectively.

Micronutrient application was performed fortnightly using Dripsol® micro (Mg^{2+} = 1.1%; B = 0.85%; Cu (Cu-EDTA) = 0.5%; Fe (Fe-EDTA) = 3.4%; Mn (Mn-EDTA) = 3.2%; Mo = 0.05%; Zn = 4.2%; 70% of chelating agent EDTA) at the concentration of 1 g L^{-1}, via foliar spraying using 2 g of fertilizer per liter. The application was carried out using a backpack sprayer (Jacto – Jacto XP®) with capacity of 12 L, working pressure (maximum) of 88 psi (6 bar) and JD 12P nozzle.

For the proline concentration of 10 mm L^{-1}, this amino acid was diluted in distilled water at ratio of 1.1376 g L^{-1} and exogenously applied using a backpack sprayer, with average solution volume of 400 mL per plant. The application was carried out fortnightly at 5:00 p.m. The product Haiten®, a non-ionic adhesive spreader, was used to break the surface tension of the water and obtain better results in the spraying.

The irrigation system used was localized drip irrigation, with 32-mm-diameter PVC pipes in the main line and 16-mm-diameter low-density polyethylene tubes in the lateral lines, using drippers with flow rate of 10 L h^{-1}. Two pressure-compensating drippers (model GA 10 Grapa) were installed in each plant, each at a distance of 15 cm from the stem. From 30 days after transplantation the plants were irrigated daily, in the morning, with public-supply water, according to the irrigation interval adopted, and the reference evapotranspiration was estimated based on the method of Hargreaves and Samani (1982) and Bernardo et al. (2013), obtained by Equation 1:

$$ETo = 0.0023 \times Qo \times \left(Tmax - Tmin\right)^{0.5}) \times \left(Tavg + 17.8\right) \quad (1)$$

Where:
ETo – reference evapotranspiration, mm d^{-1}; and
Tmax – Maximum air temperature (°C);
Tmin – Minimum air temperature (°C);
Tavg – Average air temperature (°C);
Q_o – Extraterrestrial solar irradiance (mm day^{-1}) of equivalent evaporation;

From ETo and Kc data, ETc was determined according to Bernardo et al. (2013), using Equation 2:

$$ETc = ETo \times Kc \quad (2)$$

Where:
ETc – Crop evapotranspiration, mm d^{-1}; and
ETo – Reference evapotranspiration, mm d^{-1}; and
Kc – Crop coefficient, dimensionless.

Reference evapotranspiration (ETo) was determined daily from climatic data collected at the São Gonçalo Weather Station, located in the municipality of Sousa - PB, and the data were used to determine ETo by the Penman-Monteith method.

At 298 days after transplanting (DAT), gas exchange, contents of photosynthetic pigments, electrolyte leakage and relative water content in leaf blade were also evaluated. Gas exchange was measured based on the CO_2 assimilation rate - *A* (μmol CO_2 m^{-2} s^{-1}), transpiration - *E* (mmol H_2O m^{-2} s^{-1}), stomatal conductance - *gs* (mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration - *Ci* (μmol CO_2 m^{-2} s^{-1}) with a portable infrared carbon dioxide analyzer (IRGA), "LCPro+" model from ADC BioScientific Ltda. These data were then used to quantify water use efficiency - *WUE* (A/E) [(μmol CO_2 m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] and instantaneous carboxylation efficiency - *A/Ci* [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-2} s^{-1}]$^{-1}$. Readings were performed between 6:30 and 10:00 a.m., on the third fully expanded leaf counted from the apical bud under natural conditions of air temperature, CO_2 concentration and using an artificial source of radiation established through the photosynthetic light-response curve, to obtain the photosynthetic light saturation point (Fernandes et al., 2021).

Chlorophyll and carotenoid contents were determined using the methodology of Arnon (1949), with chlorophyll extraction performed in containers with 8 mL of 80% acetone, and a leaf disc with known weight and area of 2.8 cm^2 collected from the third leaf of the stem apex, which were kept in the dark and in a refrigerator for 48 hours. The chlorophyll and carotenoid contents in the solutions were determined by spectrophotometer at absorbance wavelengths (ABS) (470, 647 and 663), according to Equations 3, 4, 5 and 6:

$$\text{Chl a} = \left(12.21 \times ABS_{663}\right) - \left(2.81 \times ABS_{646}\right) \quad (3)$$

$$\text{Chl b} = \left(20.13 \times ABS_{646}\right) - \left(5.03 \times ABS_{663}\right) \quad (4)$$

$$\text{Chl T} = \left(7.15 \times ABS_{663}\right) + \left(18.71 \times ABS_{646}\right) \quad (5)$$

$$\text{Car} = \left(\left(1000 \times ABS_{470}\right) - \left(1.82 \times \text{Chl a}\right) - \left(85.02 \times \text{Chl b}\right)\right)/198 \quad (6)$$

The values obtained for chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents in the leaves were expressed in mg g^{-1} of fresh mass.

Electrolyte leakage in the leaf blade was obtained according to Scotti-Campos et al. (2013). For this, 10 leaf discs with area of 113 mm^2 were collected from the 3rd leaf of the stem apex, placed in beakers with 50 mL of bidistilled water, and closed hermetically with aluminum foil. The beakers were kept at 25 ºC for 24 hours, and then the initial electrical conductivity (Ci) was determined. Subsequently, the beakers were taken to the oven with forced air ventilation and subjected to a temperature of 80 ºC for 90 minutes, when the final electrical conductivity (Cf) was determined, thus obtaining the percentage of electrolyte leakage according to Equation 7:

$$\%EL = \frac{Ci}{Cf} \times 100 \quad (7)$$

Where: %EL = Electrolyte leakage in the leaf blade;
Ci= initial electrical conductivity (dS m^{-1});
Cf= final electrical conductivity (dS m^{-1}).

Relative water content was determined according to the methodology described by Weatherley (1950). In this methodology, to obtain the fresh mass, discs were collected from the 3rd leaf of the stem apex and immediately weighed on a scale with precision of 0.001 g; to determine the turgid weight of the discs (TW), these were immersed in distilled water for 24 hours, dried and weighed, recording the values referring to the weight. Dry weight was obtained after drying these discs in an oven. Relative water content was calculated using Equation 8:

$$RWC(\%)=\frac{(FW\text{-}DW)}{(TW\text{-}DW)}\times 100 \quad (8)$$

Where:

RWC = Relative water content (%);

FW, DW and TW = fresh weight, dry weight and turgid weight of leaves, respectively.

Harvest was carried out from May to June 2022, using morphophysiological criteria as the ideal point of harvest, such as carpel interspace (Pereira et al., 2010). Fruit fresh mass was obtained by weighing all fruits and calculating the average according to the treatments applied. The number of fruits per plant was determined by counting all fruits obtained per plant.

The data obtained were evaluated by analysis of variance Polynomial regression analysis ($p<0.05$) was performed for irrigation interval and Tukey test ($p<0.05$) was performed for proline concentrations, using the statistical program SISVAR - ESAL version 5.6 (Ferreira, 2019).

3. Results and Discussion

There was no significant effect of the interaction between the factors (INT × PRO) for intercellular CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*) and CO_2 assimilation rate (*A*) of sugar-apple plants, at 298 days after transplantation (DAT) (Table 2). The irrigation intervals significantly affected all variables measured. The proline concentrations significantly influenced only the intercellular CO_2 concentration (*Ci*) and stomatal conductance (*gs*) of sugar-apple plants.

The irrigation intervals caused linear decrease in the intercellular CO_2 concentration of sugar-apple plants (Figure 2A), with reduction of 8.93% per 4-day increment in irrigation interval. When comparing the *Ci* of plants subjected to the 12-day irrigation interval to that of plants cultivated with daily irrigation, a decrease of 25.14% (57.00 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) was observed. The decrease in internal CO_2 concentration reflects the decrease in the total water potential in the soil, which induces stomatal closure and hence reduces the entry of CO_2 into the substomatal chamber.

Table 2. Summary of the analysis of variance for intercellular CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*) and CO_2 assimilation rate (*A*) of sugar-apple plants grown under different irrigation intervals and foliar application of proline, at 298 days after transplanting.

Sources of variation	DF	Mean squares			
		Ci	E	Gs	A
Irrigation intervals (INT)	3	5546.4843**	0.6537*	0.0229**	61.9010**
Linear regression	1	15287.9045**	1.9096**	0.0555**	149.7690**
Quadratic regression	1	242.0550ns	0.0276ns	0.0128**	26.7180*
Proline (PRO)	1	3669.4602*	0.0612ns	0.0050*	3.8920ns
Interaction (INT × PRO)	3	313.8726ns	0.1706ns	0.0003ns	4.0788ns
Blocks	3	397.3831ns	0.2575ns	0.0025*	2.3342ns
Residual	21	509.6595	0.1509	0.0009	3.7831
CV (%)		11.31	12.96	12.92	10.36

DF- degrees of freedom; CV (%) - coefficient of variation. *significant at p ≤ 0.05. ** significant at p ≤ 0.01. ns not significant.

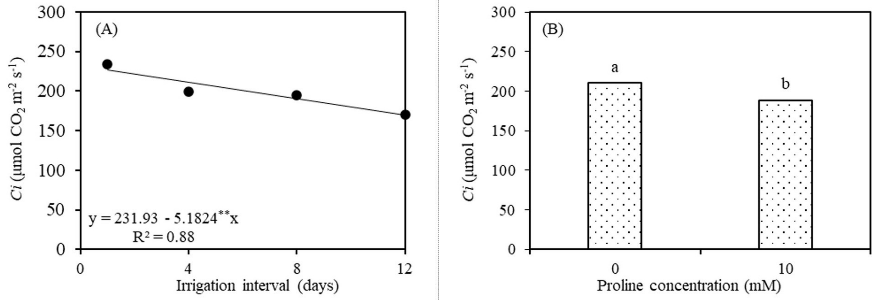

Figure 2. Intercellular CO_2 concentration - *Ci* of sugar-apple plants, as a function of irrigation intervals (A) and proline concentrations (B) of sugar-apple plants, at 298 days after transplanting. ** Significant at p ≤ 0.01 by F test. Means followed by different letters indicate significant difference between treatments by Tukey test (p ≤ 0.05).

Decrease in intercellular CO_2 concentration may also lead to a decline in photosynthesis and reductions in carboxylation efficiency and in the activity of RuBisCO and other enzymes, compromising the photosynthetic capacity and development of plants (Resende et al., 2019).

Foliar application of proline at concentration of 10 mM decreased the internal CO_2 concentration compared to plants subjected to 0 mM L^{-1} (Figure 2B). It was observed that foliar application of 10 mM proline decreased 21.42 µmol CO_2 m^{-2} s^{-1} compared to plants cultivated with 0 mM of proline. The reduction in intercellular CO_2 concentration with the application of proline may have been due to the excessive accumulation of this amino acid in the plant, because high concentrations in plant tissues, together with its exogenous supply, can cause toxic effects (Lima et al., 2016).

The increase in irrigation intervals caused a linear decrease in the transpiration of sugar-apple plants (Figure 3A), with reduction of 6.42% per 4-day increment in irrigation interval. When comparing, in relative terms, the transpiration of plants subjected to the 12-day irrigation interval to that of plants that received daily irrigation, a decrease of 0.64 mmol H_2O m^2 s^{-1} was observed. The reduction in transpiration is related to the stomatal closure that occurs due to the restriction in water availability in the soil, consequently reducing the CO_2 assimilation rate (Jacinto Júnior et al., 2019). When subjected to deficit conditions, the plant seeks to increase the efficiency in the use of available water, inducing stomatal closure and avoiding losses by transpiration (Kapoor et al., 2020).

Stomatal conductance (*gs*) was also influenced by irrigation intervals (Figure 3B), showing a linear decrease of 12.07% per 4-day increment in irrigation interval. In relative terms, a reduction of 36.20% was observed in the stomatal conductance of plants grown under the 12-day irrigation interval compared to those subjected to daily irrigation. Stomatal closure is one of the factors that contribute to the reduction in the photosynthetic rate of the plant, due to the interruption in the CO_2 flow to the carboxylation sites that directly influenced CO_2 assimilation. Stomatal conductance levels tend to decrease if the process that regulates stomatal opening and closure due to restriction in soil water absorption (Bosco et al., 2009). Silva et al. (2020a), in a study evaluating the effects of irrigation depths on sugar-apple (60, 80, 100, 120 and 140% of the actual evapotranspiration), found the maximum value of *gs* (0.17 mol of under the irrigation depth of 114.8% ETr, with decreases in stomatal conductance from this level on.

Regarding the effects of foliar application of proline on stomatal conductance (Figure 4A), it was verified that plants subjected to the concentration of 10 mM differed significantly from those that received 0 mM. When comparing the *gs* of plants subjected to a concentration of 10 mM to that found in the control treatment (0 mM), an increase of 9.75% was observed. The increase in stomatal conductance with exogenous application of proline may be associated with the beneficial effect of proline in protecting chloroplast structures (especially in photosystem II) and photosynthetic apparatus (Silva et al., 2020b).

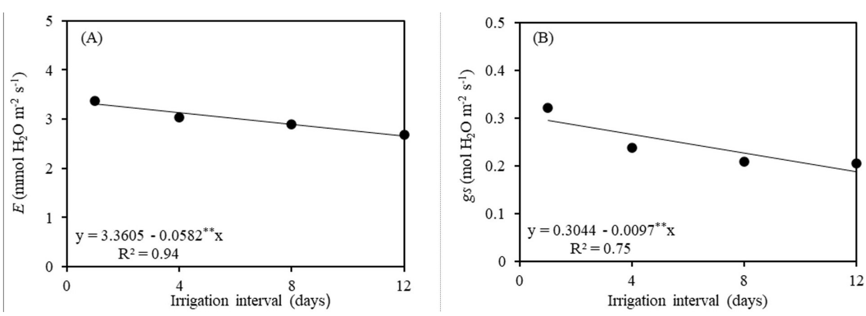

Figure 3. Transpiration - *E* (A) and stomatal conductance - *gs* (B) of sugar-apple plants, as a function of irrigation intervals, at 298 days after transplanting. ** Significant at $p \leq 0.01$ by F test.

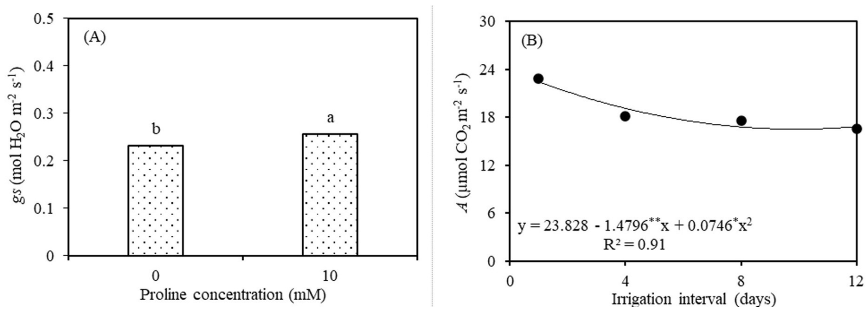

Figure 4. Stomatal conductance – *gs* (A) of sugar-apple plants as a function of proline concentrations, and CO_2 assimilation rate - *A* (B) as a function of irrigation intervals, at 298 days after transplanting. Means followed by different letters indicate a significant difference between treatments by Tukey test ($p \leq 0.05$). *, ** Significant at $p \leq 0.05$ and 0.01 by F test.

The CO_2 assimilation rate of sugar-apple plants decreased quadratically with the increase in irrigation intervals (Figure 4B), and its maximum estimated value of 22.42 µmol CO_2 m^{-2} s^{-1} was obtained under 1-day irrigation interval. Conversely, 12-day irrigation interval resulted in the estimated minimum value of 16.82 µmol CO_2 m^{-2} s^{-1}, leading to a decrease of 5.6 µmol CO_2 m^{-2} s^{-1}, when compared to the highest value found. The reduction in CO_2 assimilation rate is related to the decrease in soil water potential because, under water restriction conditions, its water potential becomes more negative, causing a reduction in *gs* to avoid excessive water loss to the atmosphere; consequently, *Ci* and *A* also decrease (Pinheiro et al., 2022b).

There were significant effects of irrigation intervals on electrolyte leakage (%EL) and relative water content (RWC) in the leaf blade of sugar-apple plants at 298 DAT (Table 3). Exogenous application of proline significantly influenced only electrolyte leakage in the leaf blade of sugar-apple plants. In turn, the interaction between the factors (INT × PRO) did not significantly affect any of the variables evaluated.

According to Figure 5A, a quadratic model fitted to the data of electrolyte leakage (%EL) in the leaf blade of sugar-apple, and the irrigation intervals of 4 and 8 days resulted in maximum values of 24.80 and 28.69%, respectively. Compared to the highest value (34.58%) estimated in plants irrigated with frequency of 12 days, there were decreases of 9.78 and 5.89 percentage points for the 4-day and 8-day irrigation intervals, respectively. Probably, a longer period without the supply of water to sugar-apple plants caused the formation of ROS such as hydrogen peroxide (H_2O_2) and singlet oxygen ($_1O^2$), which are usually produced in greater quantity when plants are under stress conditions.

Table 3. Summary of the analysis of variance for instantaneous water use efficiency (*WUEi*), instantaneous carboxylation efficiency (*CEi*), electrolyte leakage (%EL) and relative water content (%RWC) in the leaf blade of sugar-apple plants, cultivated under different irrigation intervals and foliar application of proline, at 298 days after transplantation.

Sources of variation	DF	Mean squares			
		WUEi	CEi	%EL	%RWC
Irrigation intervals (INT)	3	0.7895^{ns}	0.0001^{ns}	128.2927**	82.7886**
Linear regression	1	1.0023^{ns}	0.000023^{ns}	49.1708*	186.0347**
Quadratic regression	1	1.0907^{ns}	0.0004^{ns}	301.2285**	62.0080*
Proline (PRO)	1	0.0367^{ns}	0.0004^{ns}	69.2487*	18.4118^{ns}
Interaction (INT × PRO)	3	0.7952^{ns}	0.0001^{ns}	16.3151^{ns}	9.4041^{ns}
Blocks	3	1.8658^{ns}	0.0001^{ns}	17.4132^{ns}	3.0382^{ns}
Residual	21	1.3006	0.0003	12.9178	8.2402
CV (%)		17.93	18.76	12.05	4.07

DF - Degrees of freedom; CV (%) - Coefficient of variation. *significant at $p \leq 0.05$. ** significant at $p \leq 0.01$. ^{ns} not significant.

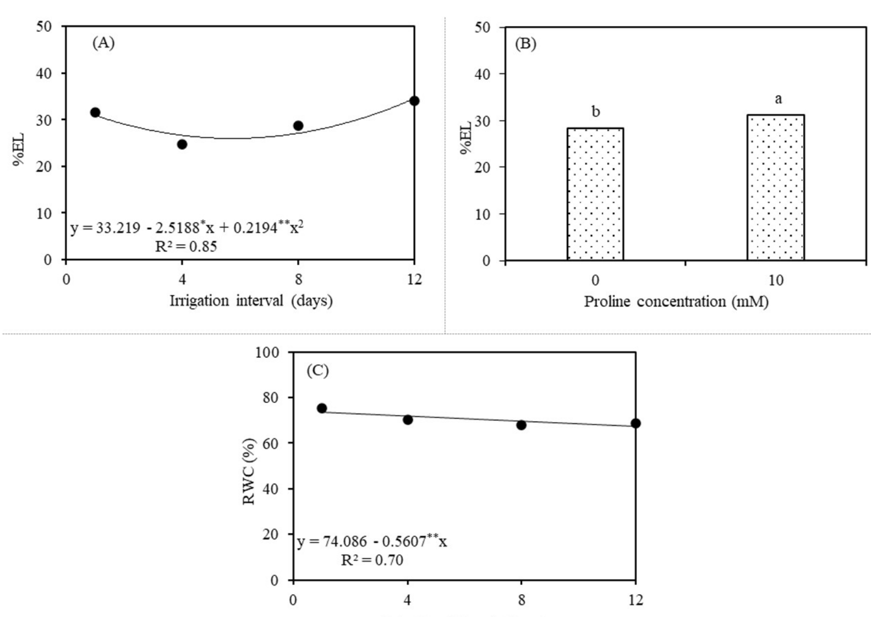

Figure 5. Electrolyte leakage - %EL (A) in the leaf blade of sugar-apple plants as a function of irrigation intervals, and proline concentrations (B) and relative water content (C) as a function of irrigation intervals, at 298 days after transplanting. Means followed by different letters indicate a significant difference between treatments by Tukey test ($p \leq 0.05$); *, ** Significant at $p \leq 0.05$ and 0.01 by F test.

These ROS cause destabilization of the cell membrane, ionic imbalance and damage to the cytoplasm, releasing ions; thus, the higher the leaked content of the cell, the greater the damage to the cell membrane (Cunha et al., 2022). Fernandes et al. (2021), in a study evaluating cell damage in sugar-apple through the combination of two levels of electrical conductivity of irrigation water (1.3 and 4.0 dS m^{-1}) and five doses of potassium (K1 - 50%, K2 - 75%, K3 - 100%, K4 - 125% and K5 - 150%), found that the increase in potassium doses caused a linear increase in the %EL of sugar-apple leaves, equal to 5.64% per 25% increment in K_2O doses.

Foliar application of proline also significantly affected electrolyte leakage in the leaf blade of sugar-apple plants. Plants subjected to a concentration of 10 mM obtained a statistically higher electrolyte leakage compared to those that received 0 mM (Figure 5B), with an increase of 2.95%. The excess of proline accumulated in the cells can cause toxicity and imbalance in biochemical processes, with consequences for cell damage, due to the destructuring of the membrane. According to Ferreira et al. (2021), the highest value of electrolyte leakage in the leaf blade obtained in sugar-apple was 18.19%, when plants were cultivated under electrical conductivity of irrigation water of 3.0 dS m^{-1}, while the lowest value was estimated in plants irrigated with water of lowest salinity (0.8 dS m^{-1}), with %EL of 14.82%.

The relative water content in the leaf blade of sugar-apple plants decreased by 13.21% with the 4-day increment in irrigation intervals (Figure 5C), with the maximum estimated value for RWC of 75.09% verified in plants cultivated with a 1-day irrigation interval. This is an indication that the water stress caused by the increase in irrigation intervals of 4, 8 and 12 days was not enough for the plants to acclimate and maintain balance in RWC, because the low water availability had a negative influence, preventing them from performing osmotic homeostasis, due to stress conditions. From the stomatal conductance data (Figure 3B), it is observed that under the high level of stress, plants tend to close their stomata to avoid excessive loss of water by transpiration; consequently, there is also a reduction in their water content.

There was a significant effect of the interaction between the factors (INT × PRO) for the chlorophyll *b* (*Chl b*) contents of sugar-apple plants grown under irrigation intervals and foliar application of proline (Table 4). Foliar application of proline significantly affected all variables analyzed in sugar-apple plants at 298 DAT.

The chlorophyll *a* contents of plants subjected to the application of proline at 10 mM were statistically higher than those of plants grown under the concentration of 0 mM (Figure 6A). When comparing in relative terms, there was an increase of 4.47 mg g^{-1} FM between plants grown with 0 and 10 mM. This is indicative that the exogenous

Table 4. Summary of the analysis of variance for chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), total chlorophyll (*Chl t*) and carotenoid (*Car*) contents of sugar-apple plants grown under irrigation intervals and foliar application of proline, at 298 days after transplanting.

Sources of variation	DF	Mean squares			
		Chl a	Chl b	Chl t	Car
Irrigation intervals (INT)	3	11.1032^{ns}	1.0794^{ns}	13.7942^{ns}	0.9692^{ns}
Linear regression	1	17.7209^{ns}	1.2757^{ns}	38.8809^{ns}	1.6656^{ns}
Quadratic regression	1	11.1344^{ns}	1.4403^{ns}	1.1141^{ns}	0.0734^{ns}
Proline (PRO)	1	160.4825^{**}	6.7243^{*}	106.7589^{*}	11.1002^{**}
Interaction (INT × PRO)	3	19.2057^{ns}	3.3004^{*}	50.9889^{ns}	1.4996^{ns}
Blocks	3	6.4881^{ns}	0.3257^{ns}	2.5162^{ns}	0.6843^{ns}
Residual	21	9.0141	0.9428	14.5424	1.1281
CV (%)	-	18.87	28.39	19.00	18.26

DF - Degrees of freedom; CV (%) - Coefficient of variation. *significant at $p \leq 0.05$. ** significant at $p \leq 0.01$. ^{ns} not significant.

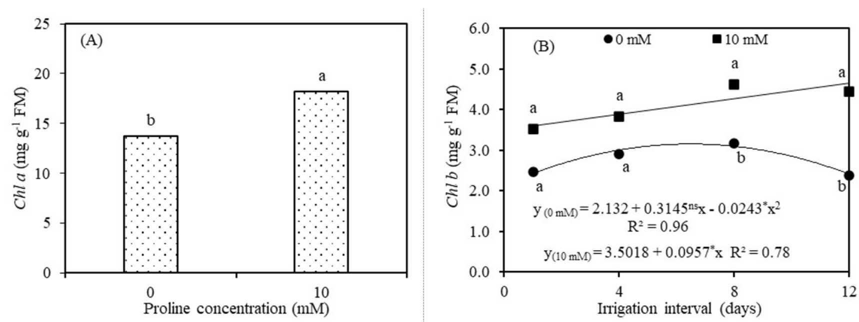

Figure 6. Chlorophyll *a* contents of sugar-apple plants under two proline concentration (A) and chlorophyll *b* contents (B) as a function of irrigation intervals, at 298 days after transplanting. Means followed by different letters indicate a significant difference between treatments by the Tukey test ($p \leq 0.05$); ^{ns}, * Not significant, significant in $p > 0.05$ and $p \leq 0.05$ by F test.

application of proline can favor the photosynthetic capacity of plants, probably because it is closely linked to the increase in the synthesis of aminolevulinic acid, a precursor molecule of chlorophyll synthesis (Merwad et al., 2018). According to these authors, foliar application of proline at 6 mM in cowpea promoted an increase in chlorophyll and carotenoid contents, besides promoting higher growth and yield of plants under water deficit conditions.

Chlorophyll *b* content was significantly influenced by the interaction between irrigation intervals and foliar application of proline (Figure 6B). Plants subjected to foliar application of proline at the concentration of 0 mM obtained the maximum estimated value for Chl *b*, 3.09 mg g^{-1} FM, under the 8-day irrigation interval. On the other hand, the application of proline at the concentration of 10 mM promoted a linear increase of 10.93% per 4-day increment in irrigation interval. When comparing the Chl *b* contents of plants subjected to foliar application of proline (10 mM) under 12-day irrigation interval to those of plants grown under 1-day irrigation interval, an increase of 1.05 mg g^{-1} FM was observed. Analysis of proline concentrations considering each irrigation interval showed that foliar application of proline at the concentration of 10 mM resulted in chlorophyll *b* contents statistically higher than those found in plants that received 0 mM under the irrigation intervals of 8 and 12 days. The role of proline in mitigating the deleterious effects of water stress on chlorophyll *b* contents may be related to its physiological function in osmotic adjustment, favoring greater absorption of water and nutrients from soil solution, besides participating as a constituent of several structuring proteins, which are necessary in the synthesis and activation of chlorophyll (Monteiro et al., 2014).

For total chlorophyll contents (Figure 7A), plants under foliar application of proline at the concentration of 10 mM differed statistically from those that received 0 mM. There was an increase of 16.68% in the total chlorophyll content of plants that received 10 mM compared to those grown without foliar application of proline (0 mM). The increase in chlorophyll synthesis as a function of proline application may be related to the effect of osmoregulation, which acts on the balance of redox reactions in stressed cells. In addition, proline is a compatible solute in the cytosol that contributes to intracellular osmotic balance, protecting cytosolic enzymes when the concentration of ions increases, thus maintaining the water potential and turgor of cells (Per et. al., 2017; Lima et al., 2016).

Regarding carotenoid contents (Figure 7B), plants subjected to 10 mM of proline obtained a significant increase in this variable, being statistically superior to those that received the proline concentration of 0 mM. When comparing the carotenoid contents of plants subjected to 10 mmol of proline to those of plants in the control treatment (0 mM), an increase of 1.18 mg g^{-1} FM was observed. Carotenoids are metabolites that act as photoprotectors in plants, because they have antioxidant activity, acting in the reduction of oxidative stress, through the acquisition of singlet oxygen that is produced in the thylakoid membranes by photosystem II, reducing damage that results from ROS (Barbosa et al., 2014).

There was a significant effect of the interaction between the factors (INT×PRO) only for fruit fresh mass (FFM) of sugar-apple plants cultivated under irrigation intervals and foliar application of proline (Table 5). It is also observed that the irrigation intervals significantly affected only the number of fruits (NFRU) of sugar-apple plants.

Fruit fresh mass was significantly influenced by the interaction between irrigation intervals and foliar application of proline (Figure 8A). The highest production per sugar-apple plant was obtained in the control treatment (0 mM) and under 8-day irrigation interval. On the other hand, in plants that received foliar application of 10 mM, the maximum estimated value for FFM was found under 12-day irrigation interval. In the analysis of proline concentrations considering each irrigation interval, plants grown under proline concentration of 10 mM showed higher FFM than those in the control treatment (0 mM) under the irrigation intervals of 1 and 12 days. These results suggest that proline promoted an osmotic adjustment in sugar-apple plants, because this amino acid has osmoregulation function and contributes to water absorption even at low water potential (12-day irrigation interval) and, consequently, to greater translocation of nutrients needed to maintain ionic balance in cells, which affected fruit fresh mass. In a study with bell pepper under irrigation with saline water, Lima et al. (2016) found the highest values of FFM (241.5 and 246.4 g per plant) in plants subjected to proline applications of 12 and 0 mM under irrigation with water of 0.6 and 3.0 dS m^{-1}, respectively.

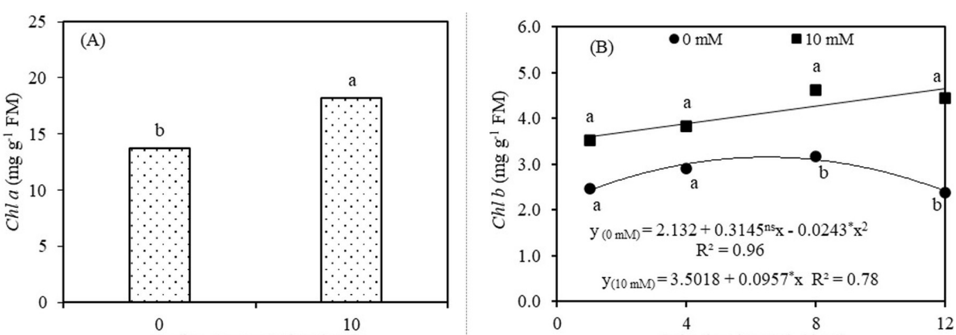

Figure 7. Total chlorophyll (A) and carotenoid (B) contents of sugar-apple plants grown under two proline concentrations at 298 days after transplanting. Means followed by different letters indicate significant difference between treatments by Tukey test ($p \leq 0.05$). ns, * Not significant, significant in $p > 0.05$ and $p \leq 0.05$ by F test.

Table 5. Summary of the analysis of variance for fruit fresh mass (FFM) and number of fruits (NFRU) of sugar-apple plants, cultivated under different irrigation intervals and foliar application of proline.

Sources of variation	DF	Mean squares	
		FFM	NFRU
Irrigation intervals (INT)	3	425.8322**	24.2005**
Linear regression	1	957.5111**	0.0015ns
Quadratic regression	1	319.4760*	60.50**
Proline (PRO)	1	2386.8867**	3.1250ns
Interaction (INT × PRO)	3	774.7614**	4.0260ns
Blocks	3	295.8257*	1.0755ns
Residual	21	72.0729	1.7124
CV (%)		6.25	16.65

DF - degrees of freedom; CV (%) - coefficient of variation. *significant at p ≤ 0.05. ** significant at p ≤ 0.01. ns not significant.

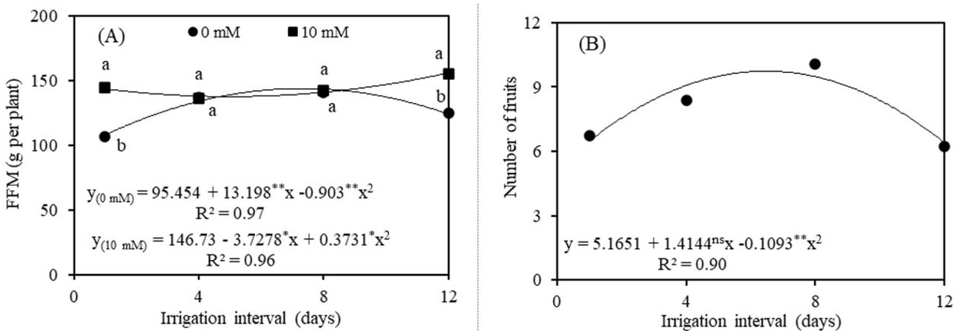

Figure 8. Fruit fresh mass - FFM of sugar-apple plants, as a function of irrigation intervals at each proline concentrations (A) and number of fruits (B) as a function of irrigation intervals, at 501 days after transplanting. ns, *, **, Not significant, p > 0.05, Significant at p ≤ 0.05 and 0.01, respectively, by F test.

The number of fruits of sugar-apple plants (Figure 8B) was statistically influenced by irrigation intervals, and the maximum estimated value of 10.06 fruits per plant was obtained under 8-day irrigation interval. On the other hand, the 12-day irrigation interval resulted in a lower estimated number of fruits (6.21 unit per plant). The irrigation interval indicated for a higher production of sugar-apple fruits is 8 days; from this value, there is a decrease in this variable, which is explained by the reduction in CO_2 assimilation rate, whose values were lower in plants irrigated at an interval of 12 days (Figure 4B), and thus a lower amount of photoassimilates available for fruit formation.

4. Conclusions

Irrigation at 12-day intervals is detrimental to gas exchange in sugar apple plants at 298 days after transplanting.

The application of proline at a concentration of 10 mM increases the fresh weight of the fruits and the levels of chlorophyll b in sugar apple plants under irrigation with intervals of up to 12 days.

The application of 10 mM of proline increases the levels of chlorophyll *a*, total chlorophyll and carotenoids and the extravasation of electrolytes in the leaf blade of sugar apple plants under 12-day irrigation.

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