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Efficiency of melatonin as a mitigating agent for saline stress in Formosa papaya in hydroponic systems

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Abstract

Due to the significant impact of salinity on agricultural production, researchers in many countries have been conducting studies using various management strategies or salinity stress mitigators across different crops. The aim of this study was to evaluate the efficiency of melatonin as a mitigating agent against salt stress in papaya plants. A split-plot experimental design was used, with the main plots represented by two hydroponic systems (Floating and hydroponics using sand as a substrate), and the subplots represented by five combinations of salt stress and melatonin application (C1–2.0 dS m^{-1} + 0 µM melatonin, C2–5.0 dS m^{-1} + 0 µM melatonin, C3–5.0 dS m^{-1} + 50 µM melatonin, C4–5.0 dS m^{-1} + 100 µM melatonin, C5–5.0 dS m^{-1} + 200 µM melatonin). The plants were collected and evaluated 50 days after transplanting for the following variables: plant height, stem diameter, root length, root volume, number of leaves, leaf area, leaf dry mass, stem dry mass, root dry mass, and total dry mass. Data analysis showed that the floating system promoted greater tolerance of papaya plants to salinity. On the other hand, in the sand substrate system, a deleterious effect of salt stress on plant growth was observed. Melatonin application at concentrations starting from 50 µM reduced the negative effects of salinity on the plants.

1. Introduction

Formosa papaya (*Carica papaya* L.), a member of the Caricaceae family, is one of the most commercially significant fruits in tropical and subtropical regions. Its fruits are rich in antioxidants such as vitamins A, C, and E, as well as essential minerals (Cao et al. 2024; Chaijan et al. 2024). In addition, papaya contains high concentrations of papain, a proteolytic enzyme that helps break down proteins, enhances nutrient absorption, promotes platelet formation, and supports wound healing and inflammation reduction (Babalola et al. 2024).

It is one of the most commercially important fruits in tropical and subtropical regions, standing out for its widely recognized nutritional and therapeutic properties (Sekeli et al. 2018; Singh et al. 2020). Brazil stands out internationally as the second-largest producer of papaya, second only to India. In 2022, Brazil exported a total of 39.8 thousand tons of papaya, accounting for 9.7% of the global total (FAOSTAT 2024).

A survey conducted by Amaral & Navoni (2023) found that the electrical conductivity of groundwater in the semi-arid region of Northeastern Brazil ranges from 1.004 to 15.069 dS m^{-1}, with an average of 3.515 dS m^{-1}. According to the authors, approximately half of the groundwater samples are classified as Class III, indicating fair quality. These waters require specific usage conditions, such as selecting crops that are tolerant to high salinity levels.

Papaya is classified as moderately sensitive to salinity, with a threshold salinity range between 2.0 and 4.0 dS m^{-1} (Sousa et al. 2019). Studies have shown that papaya plants exposed to salt stress exhibit significant changes in both photosynthetic processes and growth (Dias et al. 2020; Sá et al. 2020;

Targino et al. 2023). The harmful effects of salt stress on plants occur because it induces changes in plant metabolism. High salt concentrations in the root zone of sensitive plants reduce water uptake and consequently lead to stomatal closure. This limits CO_2 absorption, restricts photosynthesis, inhibits cell division, and ultimately reduces plant growth (Atta et al. 2023; Balasubramaniam et al. 2023; Xiao et al. 2023; Khan et al. 2025).

Given this reality, it is essential to adopt technical strategies that allow the use of saline water with minimal impact on crops. One such strategy is the exogenous application of substances capable of stimulating plant growth and enhancing plant responses to adverse conditions. Melatonin (N-acetyl-5-methoxytryptamine) has been increasingly recognized as a hormone present in plants, which has led to extensive research on its role and effects in plant physiology and its protective functions against both biotic and abiotic stresses (Akram et al. 2021; Eisa et al. 2023).

Melatonin has been shown to be effective in promoting physiological, biochemical, and molecular mechanisms in response to abiotic stressors. Thus, it strengthens physiological and biochemical responses, such as the antioxidant system, providing greater tolerance to plants when exposed to abiotic stresses, such as high temperatures (Tiryaki et al. 2012; Lv et al. 2025).

Scientific studies indicate that melatonin plays a crucial role in defense against various types of stress. The exogenous use of this molecule is effective in combating stressors such as water deficit, salinity, chemical and thermal stresses, which induce the production of reactive oxygen species (ROS) (Wang et al. 2023; Xiao et al. 2023) Furthermore, melatonin is involved in developmental transition processes affected by stress, such as flowering, fruiting, and senescence (Meng et al. 2014; Antoniou et al. 2017).

In addition to its role in the antioxidant defense system, melatonin influences gene expression in response to stress (Wei et al. 2015; Zhang et al. 2015). Wei et al. (2015) reported that melatonin induced the expression of genes related to cell division, photosynthesis, carbohydrate metabolism, fatty acid biosynthesis, and ascorbate metabolism in soybean. The ability of melatonin to modulate the expression of several antioxidant defense genes in the photosynthetic apparatus, in the delay of leaf senescence, and in the induction of genes related to cell division, among other aspects, has been observed in several studies (Arnao et al. 2019; Song et al. 2022; Xu et al. 2024).

In light of this, the present study was conducted with the aim of evaluating the effectiveness of melatonin as a mitigating agent against salt stress in papaya plants during the early growth stage under two hydroponic systems.

2. Material and Methods

The experiment was carried out December 2019 and February 2022, in a greenhouse belonging to the Department of Agrarian and Forestry Sciences of Federal Rural University of the Semi-Arid (UFERSA), Mossoró city, Rio Grande do Norte state, Brazil (5° 11' S, 37º 20' W and altitude of 18 m). The region's climate is classified, according to Köppen, as BSwh' (hot and dry), characterized by irregular rainfall, with

an annual average of 673.9 mm, an average temperature of 27°C and an average relative humidity of 68.9% (Lima et al. 2022). During the experiment, climatic data were recorded using a thermo-hygrometer installed inside the greenhouse. The maximum, minimum, and average recorded temperatures were 42.58°C, 21.80°C, and 35.70°C, respectively. The maximum, minimum, and average relative humidity values were 87.78%, 29.51%, and 47.32%, respectively.

The greenhouse is protected by a tunnel-shaped arched cover, measuring 7.0 meters in width, 18.0 meters in length, and 3.0 meters in height. It is covered with a transparent low-density polyethylene film, 0.15 mm thick. The structure is made of galvanized steel, and the side and front walls are covered with 50% shade mesh.

The design used was a split-plot design, with the plots represented by two hydroponic systems (floating and hydroponic using sand as substrate), and the subplots represented by five combinations of saline stress and melatonin application (C1–2.0 dS m^{-1} + 0 µM melatonin, C2–5.0 dS m^{-1} + 0 µM melatonin, C3–5.0 dS m^{-1} + 50 µM melatonin, C4–5.0 dS m^{-1} + 100 µM melatonin, C5–5.0 dS m^{-1} + 200 µM melatonin).

For both hydroponic systems, a nutrient solution containing the following fertilizer concentrations, in g per 1000 L^{-1}, was used: 750 g of calcium nitrate (15% of N and 20% of CaO); 500 g of potassium nitrate (13% of N and 43% of K_2O); 150 g of monoammonium phosphate (11% of N and 61% of P_2O_5); 400 g of magnesium sulfate (10% of Mg and 17% of S) (Furlani et al. 1999). For micronutrients, the commercial products Dripsol Micro Rexene Equilíbrio and Dripsol Micro Ferro Q48 (Iron chelate Q48 EDDHA 6%, Dripsol SQM Vitas®) were used at a dose of 30 g 1000 L^{-1}.

The preparation of the nutritional solution used water collected from the UFERSA supply system, with the following chemical characterization: pH = 7.55; EC = 0.56 dS m-1, Ca^{2+} = 0.85 mmol L^{-1}, Mg^{2+} = 1.22 mmol L^{-1}, K^{+} = 0.35 mmol L^{-1}, Na^{+} = 3.82 mmol L^{-1}, Cl- = 2.45 mmol L^{-1}, HCO_3^{-} = 3.15 mmol L^{-1}, CO_3^{2-} = 0.55 mmol L^{-1}, and Sodium adsorption ratio (SAR) = 3.09 (mmol L^{-1})$^{0.5}$. To adjust the pH of the nutrient solutions to 6.0 (± 0.5), 0.1 M HCl or 0.1 M NaOH solutions were applied. Papaya cultivar Fomosa Tainung No. 1 seedlings were produced in PVC trays using coconut fiber-based substrate, placing three seeds in each cell and then thinning, leaving the most vigorous plant in each cell. After thinning, the seedlings were fertigated with a nutrient solution using a capillary irrigation system. Transplanting was carried out 30 days after thinning, placing one seedling per pot.

The floating system used plastic pots with a capacity of 10 dm^3. Each pot was filled with a spherical Styrofoam plate with a diameter 1.0 cm smaller than the internal diameter of the pot. A circular hole with a diameter of 4 cm was drilled in each plate, into which a 50 mL Styrofoam cup with a 2 cm diameter hole was placed for the plant roots to pass. A drainage and collection system for the nutrient solution was installed in each pot.

The irrigation system consisted of a plastic reservoir with a capacity of 100 L of nutrient solution, which supplied four pots. A self-ventilated Metalcorte/Eberle circulation pump, model EBD250076 (driven by a single-phase motor, 210 V voltage, 60 Hz frequency) was installed in each reservoir. The system was programmed to operate in a closed mode, so that the nutrient solution was not lost over time, and circulated between the pots and the reservoir.

Nutrient solution circulation was controlled using an analog timer, adopting a 15-minute circulation schedule interspersed every 15 minutes from 5:00 a.m. to 6:00 p.m. During the night, the interval between circulations was two hours, with each event lasting 15 minutes (Martinez et al. 2021).

For the hydroponic system using substrate, plastic pots similar to those used in the floating system were used. Washed medium-textured sand was used as substrate, with 10 dm^3 of it placed in each pot. The irrigation system consisted of five 500-liter reservoirs, one for each nutrient solution. A Metalcorte/Eberle EBD250076 self-ventilated electric circulation pump (driven by a single-phase motor, 210 V voltage, 60 Hz frequency, typically used in washing machines) was used, as well as 16-mm diameter low-density polyethylene hoses, into which spaghetti-type microtubes with an internal diameter of 1.5 mm and 10 cm in length were inserted.

Irrigation was controlled by a digital timer, adjusting the duration of each irrigation throughout the crop cycle. During the day, six fertigation events were carried out, the first at 6 a.m., and the others at 2-hour intervals and each event lasting 1.0 minute from transplanting to 15 days after and 2.0 minutes from 16th to Harvest.

For both hydroponic systems, melatonin was applied using a 5 L sprayer, using an adjuvant to apply a sufficient volume to each plant to ensure complete leaf wetting.

Gas exchange was analyzed 48 days after transplanting, between 8 a.m. and 10 a.m. on a day with total cloudiness (ideal climatic conditions), on nondetached and expanded leaves, free from pest and disease attacks, located in the middle third of the plants. Portable infrared gas analyzer (IRGA) (model GFS–3000, Walz, Effeltrich, Germany). The stomatal conductance (gs) (mol of CO_2 m^{-2} s^{-1}), transpiration rate (E) (mmol of H_2O m^{-2} s^{-1}), net CO_2 assimilation rate (A) (µmol CO_2 m^{-2} s^{-1}), and the internal concentration of CO_2 (Ci) (µmol CO_2 m^{-2} s^{-1}) were analyzed.In addition, the following data were calculated: instantaneous water use efficiency (WUE) (A/E) [(µmol CO_2 m^{-2} s^{-1})/(mmol H_2O m^{-2} s^{-1})], and carboxylation efficiency (iCE) (A/Ci) [(µmol CO_2 m^{-2} s^{-}1)/(µmol CO_2 m^{-2} s^{-1})]. The following configurations were used in the analyses: an 8 cm² leaf chamber, a chamber temperature of 25°C, a CO_2 concentration of 400 ppm, an airflow of 750 µmol min^{-1}, and a photosynthetically active photon density of 1200 µmol m^{-2} s^{-1}. The evaluations were conducted Two leaves per plant were analyzed.

The plants were collected 50 days after transplanting and evaluated for the following variables: stem diameter (SD), plant height (PH), number of leaves (NL), leaf area (LA), root volume (RV), root length (RL), leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (TDM).

Stem diameter (SD), determined using a digital caliper, measuring 2 cm at the level of the floating polystyrene and the substrate, with the results expressed in millimeters.

Plant height (PH), measured using a measuring tape, from the plant collar to the apex, with the results expressed in centimeters.

Number of leaves (NL), determined by directly counting the leaves of each plant.

LA was determined by the disc method (Souza et al. 2012), using a dial and collecting 10 discs (4.9 cm^2) from each plant. The samples, discs and leaves were then placed in paper bags and dehydrated in a forced air circulation oven at 65 ºC until they reached constant weight. LA was determined according to Eq. 1:

$$\mathrm{LA} = \frac{(\mathrm{LMD} \times \mathrm{DA})}{\mathrm{DDM}}$$

1

where:

LA – leaf area, cm^2 per plant

LDM - leaf dry mass, g;

DA - leaf disc area, cm^2; and,

DDM – leaf disc dry mass, g.

RV was determined by measuring the displacement of the water column in a graduated beaker filled with a known volume of water (2000 mL). The results were expressed in cm^3.

RL was measured using a tape measure, considering the distance from the base of the root to its root cap, and was expressed in centimeters.

LDM, SDM and RDM were determined after the samples were dehydrated in an oven with forced air circulation at a temperature of 65°C until they reached constant mass. The samples were then weighed on an analytical balance (0.01 g), and the results were expressed in g per plant. Total dry mass (TDM) was determined by the sum of LDM + SDM + RDM.

Before statistical analysis, the data was subjected to the Shapiro-Wilk test for normality and Bartlett test for homogeneity. The data obtained were subjected to ANOVA using the F test ($p \leq 0.05$ and $p \leq 0.01$). The decomposition was performed when a significant interaction was detected between the studied factors. The effect of the applied treatments was analyzed using the means comparison test (Tukey, $p \leq 0.05$). The statistical software Sisvar 5.3 (Ferreira 2019) was used for the statistical analyses.

3. Results and Discussion

With regard to gas exchange, a significant response was observed for interaction between the cultivation system and combinations of salinity and melatonin for the variables transpiration (E), CO_2 assimilation rate (A), stomatal conductance (gs), intracellular CO_2 concentration (Ci), and carboxylation efficiency (iCE) at a 1% probability level, while water use efficiency (WUE) was affected at a 5% probability level (Table 1).

Table 1
Summary of variance analysis for gas exchange of papaya grown in two hydroponic systems (floating and substrate) and subjected to combinations of salinity and melatonin

Source of variations	DF	Mean squares					
		E	A	gs	Ci	WUE	iCE
Blocks	3	0.09*	2.76^{ns}	261.85^{ns}	636.65^{ns}	0.0009^{ns}	0.0003^{ns}
Systems (S)	1	0.006^{ns}	18.83*	12517.54**	6443.83*	0.010**	0.001*
Error 1	3	0.006	1.73	236.8	238.05	0.0001	0.00004
Combinations (C)	4	0.36**	64.62**	4390.77**	1010.48**	0.0016**	0.002**
S × C	4	0.66**	32.49**	15773.29**	3901.62**	0.0028**	0.0007*
Error 2	24	0.06	1.19	156.53	136.42	0.0001	0.0001
CV1 (%)		4.34	8.96	10.51	7.14	11.24	10.05
CV2(%)		13.27	7.44	8.54	5.4	12.38	12.23

DF - degree of freedom. CV – coefficient of variation. ns – non-significant; * - significant at $p \leq 0.05$; ** - Significant at $p \leq 0.01$; E - leaf transpiration (E); A- CO_2 assimilation rate; gs - stomatal conductance; Ci - intracellular CO_2 concentration; WUE - water use efficiency; iCE - carboxylation efficiency.

Leaf transpiration was affected by the systems in combinations C1 and C4, with the floating system being 41.2% higher in C1 and the semi-hydroponic system with substrate being 64.7% higher in C4. Regarding the effect of the salinity and melatonin combinations, it was found that salinity in the absence of melatonin did not affect transpiration. However, when a dose of 100 µM melatonin was applied in saline conditions (C4), it caused a 52.4% reduction in transpiration for plants grown in the floating system. There was no effect of salinity or melatonin application on the semi-hydroponic system (Fig. 1A).

C1–2.0 dS m^{-1} + 0 µM melatonin. C2–5.0 dS m^{-1} + 0 µM melatonin. C3–5.0 dS m^{-1} + 50 µM melatonin. C4–5.0 dS m^{-1} + 100 µM melatonin. C5–5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase

in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n = 4).

Figure 1. Leaf transpiration (A), CO_2 assimiliation rate (B), stomatal conductance (C), and internal CO_2 concentration (D) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

In a study conducted by Targino et al. [12] on salt stress and humic acid in papaya seedlings, the authors observed an increase in transpiration at higher salt levels. These authors suggest that this occurred because the soil water content was sufficient to keep the stomata open and prevent plant dehydration. As two hydroponic systems were used in the present study, which make it easier for plants to absorb water and nutrients, this may explain the absence of salinity on plant transpiration.

For CO_2 assimilation rate, there was a difference between the cultivation systems only in combinations C3 and C5. In combination C3, the floating system was 25.8% higher, while the semi-hydroponic system was 26.0% higher than the floating system in combination C5 (Fig. 1B). The salinity of the nutrient solution (C2) did not affect photosynthesis for either system. However, in the floating system, the application of melatonin at the highest dosage (C5) resulted in a 29.0% reduction in photosynthesis. For the semi-hydroponic system, combinations C3 resulted in an average reduction of 12% (Fig. 1B). Souza et al. (2024) observed a reduction in CO_2 assimilation rate for salinity above 4 dS m^{-1}. The absence of a significant response in the present study may be attributed to the cultivation system used, given that the hydroponic system provides greater plant tolerance to salinity.

It was found that there was a significant difference in stomatal conductance between the cultivation systems in combinations C1 and C2, with the semi-hydroponic system being 102.3% higher in the absence of salt stress (C1), while the floating system was 113.4% higher in the saline condition and without melatonin application (C2) (Fig. 1C). Regarding the effect of salinity combinations with melatonin application, varied responses were observed according to the cultivation system used. In the floating system, the addition of NaCl to the nutrient solution (C2) resulted in a 123.7% increase in gs of the plant, compared to the values obtained in the standard nutrient solution (C1). However, foliar application of melatonin reduced gs. For the semi-hydroponic system, salinity reduced gs by 48.2%, but foliar application of melatonin reduced the effect of salt stress on this variable.

Na^+ and Cl^- ions in the nutrient solution reduce the plants' ability to absorb water, while the decline in stomatal conductance is an adaptive response that reduces water loss through transpiration, thus protecting plants from dehydration (Sharipova et al. 2022). Stomata are channels for the exchange of water and gases between plants and the external environment, and the size, number, and regulatory function of stomata are related to physiological processes such as transpiration (Kang et al. 2025).

NaCl reduces the level of CO_2 in the mesophyll, causing changes in leaf photochemistry and carbon metabolism, which directly affect plant photosynthesis (Wang et al. 2024). Under salt stress, the length

and width of stomata and the width of plant pore diameter decrease, and stomatal density increases (Liao et al. 2023).

Melatonin acts by increasing plant tolerance to salinity by increasing the length, width, and opening of stomata, thereby increasing stomatal efficiency (Khan et al. 2019; Dai et al. 2020). Melatonin treatment can increase the size and opening of large pores and reduce stomatal density, promoting leaf blade expansion and reducing stomatal density, thereby increasing the photosynthetic capacity of plants (Jiang et al. 2021).

There was a significant difference in intercellular CO_2 concentration between the cultivation systems (substrate and floating) in combinations C1 and C2, in which the substrate system was superior to the floating system by 83.4 and 80.0%, respectively (Fig. 1D). Regarding the effect of salinity on Ci, it was found that the addition of NaCl to the nutrient solution increased Ci by 69.5% in plants grown in the floating system. On the other hand, in the semi-hydroponic system, salt stress reduced Ci by 14.7%, with the greatest effect when plants were subjected to salt stress and received the highest dose of melatonin (C5), with a loss of 19.9%.

The reduction in Ci in stressed plants is due to the closure of stomata, which regulate the sub-stomatal concentration of CO_2 during gas exchange (Takahashi et al. 2025). Thus, Ci reflects the availability of substrate for photosynthesis and can indicate whether stomatal closure is restricting photosynthetic activity (Pereira et al. 2020). Saline stress can cause disturbances in the plant related to reduced stomatal conductance and, consequently, reduced CO_2 diffusion and photosynthetic rate, impairing the entire process of plant growth and development (Balasubramaniam et al. 2023). According to Ancín et al. (2024), the decrease in Ci could lead to a drop in A due to the reduction in CO_2 concentration for RuBisCO activity. However, lower Ci values also stimulate stomatal opening, allowing greater CO_2 influx into the sub-stomatal cavity, which tends to balance CO_2 consumption and intake, keeping Ci approximately constant (Takahashi et al. 2025).

According to Jiang et al. (2021), melatonin can protect the chloroplast structure and stability of photosynthetic organs to a certain extent, maintain a high level of chlorophyll, increase the ability of leaves to capture light energy, promote photosynthesis, and effectively alleviate the inhibitory effect of leaf photosynthesis under salt stress.

Regarding water use efficiency (WUE), regarding the effect of salinity combinations with melatonin on WUE, different responses were observed for each cultivation system. Were significant differences between the cultivation systems for the combinations C1, C2, C3 and C5. The floating system was superior by 101.38% and 56.27% in combinations C1 and C3, respectively. On the other hand, the semi-hydroponic system was superior by 113.61% and 83.87% in combinations C2 and C5, respectively. Saline stress (C2) reduced WUE by 49.18% in the floating system; however, the application of melatonin at a dose of 50 µM (C3) inhibited the effect of saline stress on WUE.

C1–2.0 dS m^{-1} + 0 µM melatonin. C2–5.0 dS m^{-1} + 0 µM melatonin. C3–5.0 dS m^{-1} + 50 µM melatonin. C4–5.0 dS m^{-1} + 100 µM melatonin. C5–5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n = 4).

Figure 2. Water use efficiemcy (A) and instantaneous carboxylation efficiency (B) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

In contraste, in the semi-hydroponic system, saline stress increased WUE by 118.60% (C2), not differing from C5. plants grown in a semi-hydroponic system were more affected by salinity (C2), as they increased their water use efficiency under saline stress (Fig. 2A). Melatonin doses of 50 and 100 µM reduced salt stress, and consequently in the WUE. However, doses of 200 µM increased the effect of salt stress (Fig. 2A). The increase in WUE in response to salinity occurred because the effect of salt stress was greater on transpiration compared to photosynthesis. The reduction in WUE with melatonin application is due to the fact that the hormone reduced the effect of salinity on photosynthesis. During gas exchange, the absorption of CO_2 from the external environment promotes water loss, and in order to reduce this loss, the plant restricts the entry of CO_2 (Ren et al. 2020). This interdependence expressed by the relationship between photosynthesis and transpiration indicates water use efficiency, which relates the amount of carbon that the plant fixes per unit of water it loses (Zhao et al. 2020).

For carboxylation efficiency (EiC), there was no difference between the cultivation systems for any of the combinations of salinity and melatonin. Hydroponic systems affected EiC in treatments C1 and C3, with the floating system being superior by 60.30%, while in C4 the semi-hydroponic system was superior by 40.37% (Fig. 2B). The effect of nutrient solutions on EiC varied according to each hydroponic system. For the floating system, the highest EiC occurred under saline conditions and a 50 µM melatonin dose (C3), being 53.43% higher compared to C1. Under saline stress, the highest melatonin doses (C4-100 µM and C5-200 µM) reduced the EiC. For the semi-hydroponic system, the highest values occurred in C1 and C2, and decreased by 31.81% for the 50 µM melatonin dose (C3) (Fig. 2B).

The decrease in instantaneous carboxylation efficiency due to salt stress may be associated with metabolic restrictions in the Calvin cycle and the occurrence of non-stomatal factors that act on the photosynthetic apparatus, such as the inhibition of RuBisCO enzyme activity due to reduced availability of ATP and NADPH (Prywes et al. 2023). However, as previously presented, EiC increased in response to salinity. This behavior can be attributed to the fact that the experiment was developed in hydroponic systems, which provide plants with tolerance to salinity (Oliveira et al. 2023).

According to the results of the analysis of variance. it was found that there was a significant effect of the interaction between the factors cultivation systems (S) and combinations of salinity with melatonin (C) for the variables root length (RL). root volume (RV). plant height (PH). stem diameter (SD) and leaf area

(LA) at a 1% probability level. while the number of leaves (NL) was affected only by the combination of salinity and melatonin ($p \leq 0.05$) (Table 1).

Table 1
Summary of analysis of variance for root length (RL). root volume (RV), plant height (PH), stem diameter (SD), number of leaves (NL). and leaf area (LA) of papaya grown in two hydroponic systems (floating and substrate) and subjected to combinations of salinity and melatonin

Source of variations	DF	Mean squares					
		RL	RV	PH	SD	NL	LA
Block	3	19.95*	0.001^{ns}	0.005^{ns}	8.57^{ns}	1.55^{ns}	333690.91^{ns}
Systems (S)	1	275.62**	0.23**	1.48**	262.65^{ns}	0.90^{ns}	1425.64^{ns}
Error 1	3	1.24	0.003	0.006	262.60	0.90	268028.02
Combinations (C)	4	104.32**	0.014*	0.059**	42.10**	0.35*	3268147.11**
S × C	4	165.09**	0.023**	0.060**	27.18**	2.50^{ns}	2783270.67**
Error 2	24	10.56	0.004	0.013	1.99	2.22	104202.01
CV1 (%)		9.88	18.57	9.5	29.80	6.32	18.39
CV2(%)		12.25	16.22	13.86	10.21	9.95	11.46

DF - degree of freedom. CV – coefficient of variation. ns – non-significant; * - significant at $p \leq 0.05$; ** - Significant at $p \leq 0.01$

Plant height differed among the cultivation systems in combinations C1, C3, C4, and C5. in which the substrate system was superior to the floating system (Table 2). Salinity affected plant height only in plants grown in the substrate system. resulting in a 35.9% loss. However. foliar application of melatonin reduced the effect of salt stress on this variable (Fig. 1A).

C1–2.0 dS m^{-1} + 0 µM melatonin. C2–5.0 dS m^{-1} + 0 µM melatonin. C3–5.0 dS m^{-1} + 50 µM melatonin. C4–5.0 dS m^{-1} + 100 µM melatonin. C5–5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n = 4).

Figure 3. Plant height (A), stem diameter (B), root length (C), root volume (D) and leaf area (E) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

This reduction can be attributed to the difficulty in absorbing water from soil with reduced water potential due to high salt concentrations. which leads to stomatal closure and suppression of photosynthesis (Aguilar-Bautista et al. 2022).

Stem diameter was not affected by the addition of NaCl to the nutrient solution for plants grown in the floating system. However. foliar application of melatonin reduced stem diameter by 15.6%. In contrast. in plants grown in the substrate system. the addition of NaCl caused a 33.6% reduction in this variable. Furthermore. foliar application of melatonin reduced the effect of salt stress on stem diameter (Table 2). Regarding the effect of the cultivation system. a significant difference was observed only in the combination of NaCl and absence of melatonin (C2). in which the floating system was superior to the system with substrate by 60.2% (Fig. 1B).

Aguilar-Bautista et al. (2022) also observed that salinity stress reduced stem diameter. Salinity reduces growth and stem diameter in several fruit species. and its level of tolerance or sensitivity varies according to genotype. Water stress. induced by salt concentration. reduces growth not only due to its effects on CO_2 assimilation but also by reducing the rates of cell division and elongation (Borgo et al. 2025).

Root length was affected by the cultivation systems in combinations C1 and C2. in which the floating system was superior to the substrate system. Regarding the effect of the combinations of salinity and melatonin application on root length. it was found that salt addition (C2) reduced root length in both cultivation systems. with a loss of 19.3% in the floating system and 31.1% in the substrate system. It was also observed that melatonin application reduced the effect of salinity stress on root length only in plants grown in the substrate system (Fig. 1C).

For root volume. significant differences were observed between the cultivation systems in combinations C2. C3. C4. and C5. with the floating system being superior to the substrate system in these combinations. with the most significant difference in combination C2 (Table 2). For root volume. the effect of salinity was observed only in plants grown in the substrate system. with a loss of 82.1%. In this system. foliar applications of melatonin reduced the effect of salinity (Fig. 1D).

Taproot length and root volume decrease with increasing NaCl concentrations due to restrictions on cell division and elongation, mainly due to ionic toxicity in the rhizosphere (Sousa et al. 2011). Melatonin can increase the occurrence of taproots, lateral roots, and adventitious roots and increase the number, length, and density of lateral roots (Liang et al. 2017; Zhan et al. 2019). Thus, affecting the ability of plants to obtain water and nutrients under stress (Sousa et al. 2011; Barichello et al. 2020).

Regarding leaf area. significant differences were observed between the cultivation systems only in combinations C1 and C2. with the semi-hydroponic system being superior in combination C1 and the floating system being superior in combination C2 (Fig. 1E). Regarding the effect of salinity on leaf area. a significant response was observed only in plants grown in the semi-hydroponic system. with losses of 68.5% occurring in combination C2. Furthermore. melatonin reduced the effect of salinity on leaf area (Fig. 1E).

The reduction in leaf area is a plant defense mechanism closely related to gas exchange. especially regarding CO_2 absorption and water loss through transpiration, with the aim of promoting osmotic

adjustment (Zhan et al. 2019). Melatonin reduces the effect of salt stress on leaf area by improving the actions of xylem and phloem, as well as other properties that aid in the transport of water. inorganic salts. and organic substances, protecting the structural stability of leaves, which are the main photosynthetic organs of leaves (Liang et al. 2017; Zhan et al. 2019).

Leaf number was affected only by the combinations of salinity and melatonin, with a difference occurring only between the standard nutrient solution (C1) and the salinized nutrient solution with the lowest foliar dose of melatonin (C3). with a variation of 15.9% (Fig. 2).

Columns with the same letter do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n = 4).

Figure 4. Number of leaves to papaya as a function of salinity and foliar application of melatonin

Considering that a plant's leaf area is related to the number of leaves produced and the size of the leaf blade, the results obtained in this study showed that the effect of the applied treatments was more evident on leaf blade expansion than on the emission of new leaves.

Regarding biomass accumulation. a significant effect of the interaction between the cultivation system and combinations of salinity and melatonin was observed for the variables leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), and total dry matter (TDM), at a 1% probability level (Table 2).

Table 2
Summary of analysis of variance for leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

Source of variations	**DF**	**Mean squares**			
		LDM	**SDM**	**RDM**	**TDM**
Block	3	5.28^{ns}	60.34^{ns}	8.94^{ns}	104.34^{ns}
Systems (S)	1	93.94*	68.38^{ns}	62.50^{ns}	671.58^{ns}
Error 1	3	8.26	30.54	11.35	136.09
Combinations (C)	4	73.89**	309.34**	123.78**	1354.43**
S × C	4	45.83**	236.4**	181.02**	1223.58**
Error 2	24	2.08	14.79	8.32	38.84
CV1 (%)		19.22	20.45	17.16	20.25
CV2(%)		12.4	17.01	16.24	12.8

DF - degree of freedom; CV – coefficient of variation; ns – Non-significant; * - significant at $p \leq 0.05$; ** - Significant at $p \leq 0.01$.

For leaf dry mass (LDM). the system with substrate was 20.5% superior to the floating system in combination C1. On the other hand. the floating system was superior to the system with substrate in combinations C2 (206.6%) and C4 (44.2%) (Fig. 3A). Regarding the effect of salinity and melatonin combinations on LDM. it was found that there was no effect of salinity (C2) on plants grown in the floating system. However, the application of melatonin at the lowest dose under saline conditions (C3) caused a 34.6% reduction in LDM. In the system with substrate. the addition of NaCl without melatonin application (C2) resulted in a 73.67% reduction in LDM. Furthermore. it was found that foliar application of melatonin reduced the effect of salt stress on this variable. with no significant difference between doses (Fig. 3A).

C1–2.0 dS m^{-1} + 0 µM melatonin. C2–5.0 dS m^{-1} + 0 µM melatonin. C3–5.0 dS m^{-1} + 50 µM melatonin. C4–5.0 dS m^{-1} + 100 µM melatonin. C5–5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n = 4).

Figure 5. Leaf dry mass (A), stem dry mass (B), root dry mass (C), and total dry mass (D) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

Regarding stem dry mass (SDM), a significant difference was observed between the systems in combinations C1 and C2. with the system with substrate being 46.1% superior in combination C1, while the floating system was 64.1% superior in combination C2 (Fig. 3B). Analyzing the effect of the combinations on SDM. a similar behavior to that observed in SDM was observed. In the floating system. there was no effect of salinity (C2), but the application of melatonin at a lower dose under saline conditions (C3) caused a 29.7% reduction in SDM. For the system with substrate, salinity reduced SDM. regardless of melatonin application, with the greatest reduction (74.2%) being in the absence of melatonin (C2). However. melatonin application provided a reduction in the effect of saline stress on this variable. with no difference occurring between melatonin doses (C3, C4, and C5) (Table 4). Analyzing root dry mass (RDM). it was observed that the substrate system provided higher values in combination C1. being 87.2% higher compared to the floating system. On the other hand, the floating system was superior in combinations C2, C4, and, C5, by 381.2. 51.7. and 52.8%, respectively (Fig. 3B).

Still regarding RDM. an effect of the combinations was observed for both cultivation systems. In the floating system, a significant difference occurred only in combinations C2 and C3, with C3 being 55.9% higher. For the substrate system. the highest RDM value occurred in combination C1, and reduced by 87.1% when salt solution was used in the absence of melatonin. However, foliar application of melatonin reduced the effect of salinity on RDM. with no difference between hormone doses (Fig. 3C).

For total dry mass (TDM). a similar behavior was observed for the other dry mass variables. The substrate system was 49.3% superior in combination C1, while the floating system was superior in combinations C2 (222.2%), C4 (26.16%), and C5 (36.5%) (Fig. 3D). Analyzing the effect of salinity combinations with melatonin. different behavior was observed according to each cultivation system analyzed. In the floating system, there was no effect of salinity in the absence of melatonin on TDM. however. a significant difference was observed in combinations C3 and C4, with C4 being 38.3% superior. For the substrate system. combination C2 caused a 78.0% reduction in TDM, compared to combination C1. In the other combinations using melatonin (C3, C4, and C5), a reduction in the effect of saline stress was observed, as these combinations resulted in lower TDM losses compared to C1 (Fig. 3D).

In general, papaya plants were found to be more tolerant to salinity when grown in the floating system. This behavior can be attributed to the fact that the plant's root system is in constant contact with the nutrient solution, resulting in lower energy expenditure for the plants to absorb water and nutrients. On the other hand. plants grown in the substrate system showed significant losses in biomass accumulation. whether analyzed by individual plant parts (LDM, SDM, and RDM) or as a whole (TDM).

Several authors have observed a significant reduction in biomass accumulation by papaya plants when subjected to saline stress (Sousa et al. 2019; Targino et al. 2023). This reduction occurs due to the toxic effect of Na^+ and Cl^- ions. Plants under saline stress tend to use their reserves. which would otherwise be used for growth and development, to acclimate to the adverse conditions of high salt concentrations in the soil solution, which leads to reduced carbon assimilation and biomass gain [59].

As previously shown. melatonin was effective in reducing the effect of salinity on papaya biomass accumulation (Barichello et al. 2020). The beneficial effect of melatonin occurs because this hormone acts to protect the chloroplast structure and the stability of photosynthetic organs to some extent. maintaining a high level of chlorophyll, increasing the leaves' ability to capture light energy, promoting photosynthesis, and effectively alleviating the inhibitory effect of leaf photosynthesis under saline stress (Barichello et al. 2020). Melatonin also has antioxidant properties. aiding in the removal of reactive oxygen species (ROS). Thus, reducing oxidative stress (Zhan et al. 2019).

Li et al. (2021) observed that melatonin treatments lead to a reduction in the effects of salinity on photosynthesis and dry mass, as melatonin plays a significant role in photosynthesis and photoprotection. which could explain the dry mass gain of papaya in the system with substrate and under salt stress. Melatonin functions as a potent free radical scavenger and antioxidant. contributing to the enhancement of antioxidant systems under salt stress conditions. Thus, reducing the damage suffered by plant growth (Cen et al. 2020).

The floating hydroponic system promoted greater tolerance of papaya plants to salinity and no significant effect of melatonin application was observed in this system. The effect of nutrient solution

salinity on papaya plants was more evident in plants grown in the hydroponic system with substrate. Melatonin application at concentrations above 50µM reduces the negative effects of salinity on plants.

Principal component analysis (PCA) explained 66.41% of the total variability of the data, with 46.9% attributed to PC1 and 19.51% to PC2, indicating adequate multivariate representation of the physiological and growth variables evaluated (Fig. 6). PC1 was strongly associated with gas exchange variables, growth, and biomass variables such as total dry mass (TDM), above-ground dry mass (SDM), leaf dry mass (LDM), root dry mass (RDM), leaf area (LA), and number of leaves (NL), as well as CO_2 assimilation rate (A), stomatal conductance (gs), and transpiration rate (E).

The arrangement of these variables in the positive quadrant of PC1 suggests that this component represents an axis related to integrated physiological performance and biomass accumulation. The treatments positioned on the right side of the graph, especially those with melatonin application below 5.0 ECw, show a positive association with these variables, indicating a mitigating effect of the plant regulator under saline stress. This pattern suggests that melatonin favored the maintenance of photosynthetic activity and, consequently, phytomass accumulation, even under higher water electrical conductivity (Li et al. 2019; Hamed et al. 2025).

On the other hand, treatments positioned on the negative side of PC1, particularly under 5.0 ECw without melatonin application, were associated with lower values of these variables, showing that salt stress compromised growth and gas exchange. The reduction in A and gs, observed by vector orientation, indicates stomatal limitation and possible photosynthetic restriction induced by osmotic and ionic stress, directly reflecting a decrease in biomass production (Li et al. 2025; Zhang et al. 2025).

The treatments under 5.0 dS m^{-1} without melatonin (0 µM) were positioned on the negative side of this axis, indicating reduced photosynthetic activity and lower phytomass accumulation under saline stress. In contrast, the application of melatonin promoted a shift of the treatments to the positive quadrant of PC1, especially at doses of 100 and 200 µM, showing a direct association with higher values of total biomass and leaf area. The 100 µM dose showed strong proximity to root variables (RL and RV), suggesting stimulation of root system growth as an adaptive strategy to osmotic stress. The 200 µM dose showed a greater association with structural variables of the aerial part (SDM, TDM, and LA), indicating a greater contribution to the accumulation of total dry matter. The intermediate dose of 50 µM was positioned more centrally on the graph, indicating a moderate effect on salt stress mitigation, with partial improvement in gas exchange and growth, but inferior to the doses of 100 and 200 µM.

PC2 mainly discriminated variables related to water use efficiency (WUE), chlorophyll index (Ci), and root characteristics (RL and RV). The separation along this axis suggests differences in the physiological mechanisms of stress adjustment. Treatments positioned in the upper region of PC2 showed an association with greater root length and volume, indicating a possible adaptive strategy for substrate exploration under saline conditions. In contrast, treatments in the lower quadrant showed a greater relationship with variables such as iCE and physiological changes associated with greater stress impact.

Overall, PCA shows that the effect of melatonin was dose-dependent at 5.0 dS m^{-1}, with greater efficiency at concentrations of 100 and 200 µM, which promoted positive integration between gas exchange, root growth, and biomass accumulation. These results indicate that melatonin acted as a physiological modulator, probably contributing to the maintenance of photosynthetic activity and greater tolerance to salt stress.

Conclusions

Saline stress negatively affects growth parameters and gas exchange in papaya, but the effect of salinity depends on the cultivation system used. The floating hydroponic system promoted greater tolerance of papaya plants to salinity, and no significant effect of melatonin application was observed in this system. The effect of nutrient solution salinity on papaya plants was more evident in plants grown in the hydroponic system with substrate. Melatonin application at concentrations above 50µM reduces the negative effects of salinity on plants.

Declarations

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Ethical Approval

Not applicable.

Consent to Participate

All authors consented to participate.

Consent to Publish

All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no conflict of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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CRediT authorship contribution statement

BAMC: Conceptualization. Data curation. Formal analysis. Investigation. Methodology. Visualization. Validation. Writing – original draft. **VM**: Conceptualization. Investigation. Data curation. Methodology. Visualization. Validation. Project administration. Resources. Supervision. Writing – review & editing. **FAO**: Conceptualization. Investigation. Data curation. Methodology. Visualization. Validation. Project administration. Resources. Supervision. Writing – review & editing. **MJSO**: Investigation. Data curation. Methodology. Writing – review & editing. Supervision. **JCP:** Conceptualization. Investigation. Data curation. Methodology. Visualization. Validation. Project administration. Resources. Supervision. Writing – review & editing. **RAD**: Data curation. Formal analysis. Investigation. Methodology. **FFBP**: Data curation. Formal analysis. Investigation. Methodology. **AGLS**: Investigation. Data curation. Methodology. Visualization. Validation. Writing – review & editing. **VBB**: Data curation. Formal analysis. Investigation. Methodology. **FVSC**: Data curation. Formal analysis. Investigation. Methodology. **IKS**: Data curation. Formal analysis. Investigation. Methodology. **LMA**: Data curation. Formal analysis. Investigation. Methodology. **BLCL**: Investigation. Data curation. Methodology. Visualization. Validation. Writing – review & editing. **KGSC**: Data curation. Formal analysis. Investigation. Methodology. **MJVC:** Data curation. Formal analysis. Investigation. Methodology. **VLD**: Data curation. Formal analysis. Investigation. Methodology. **GEGQ**: Data curation. Formal analysis. Investigation. Methodology.

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Figures

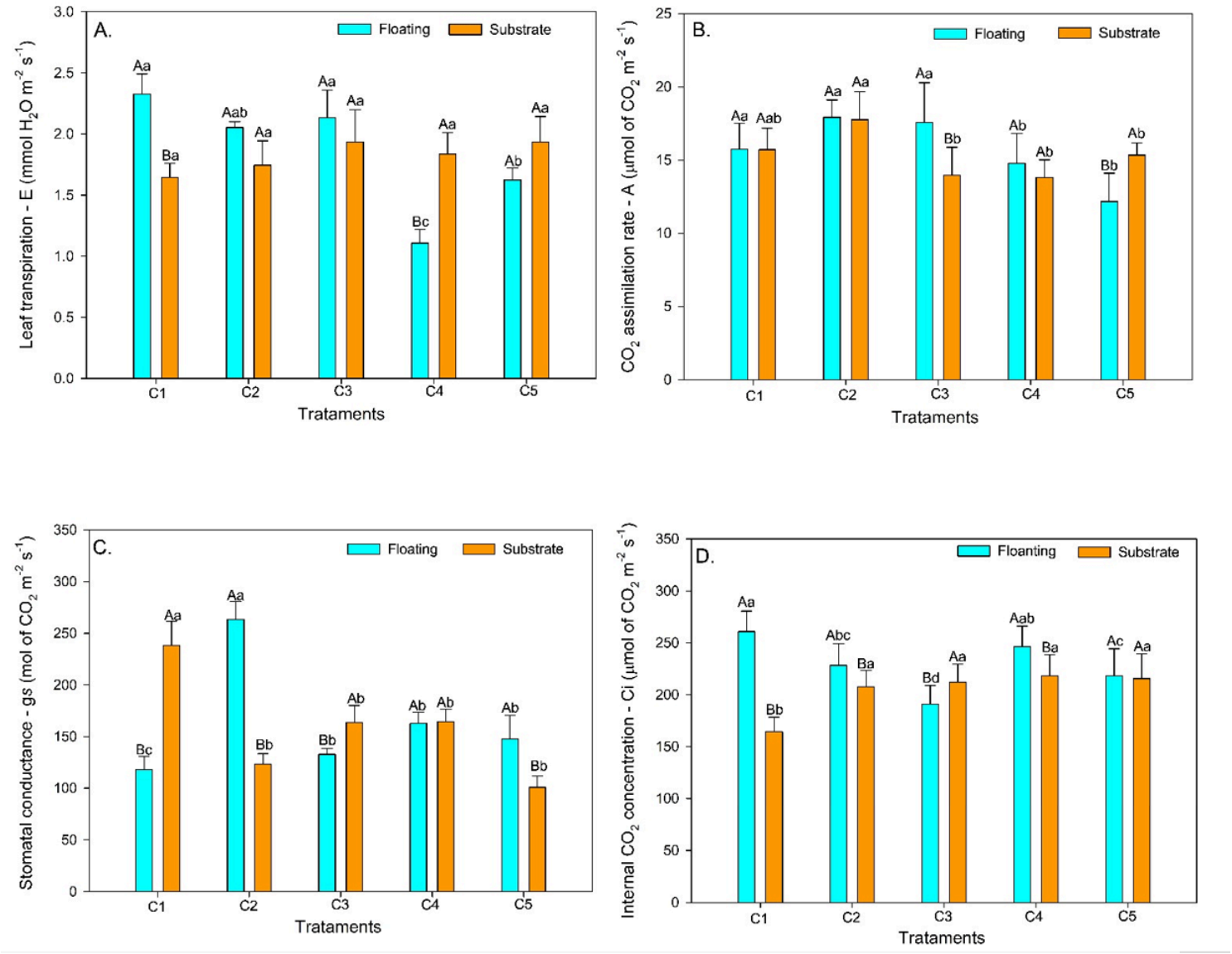


Figure 1

Leaf transpiration (A), CO_2 assimiliation rate (B), stomatal conductance (C), and internal CO_2 concentration (D) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

C1 - 2.0 dS m^{-1} + 0 µM melatonin. C2 - 5.0 dS m^{-1} + 0 µM melatonin. C3 - 5.0 dS m^{-1} + 50 µM melatonin. C4 - 5.0 dS m^{-1} + 100 µM melatonin. C5 - 5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n=4).

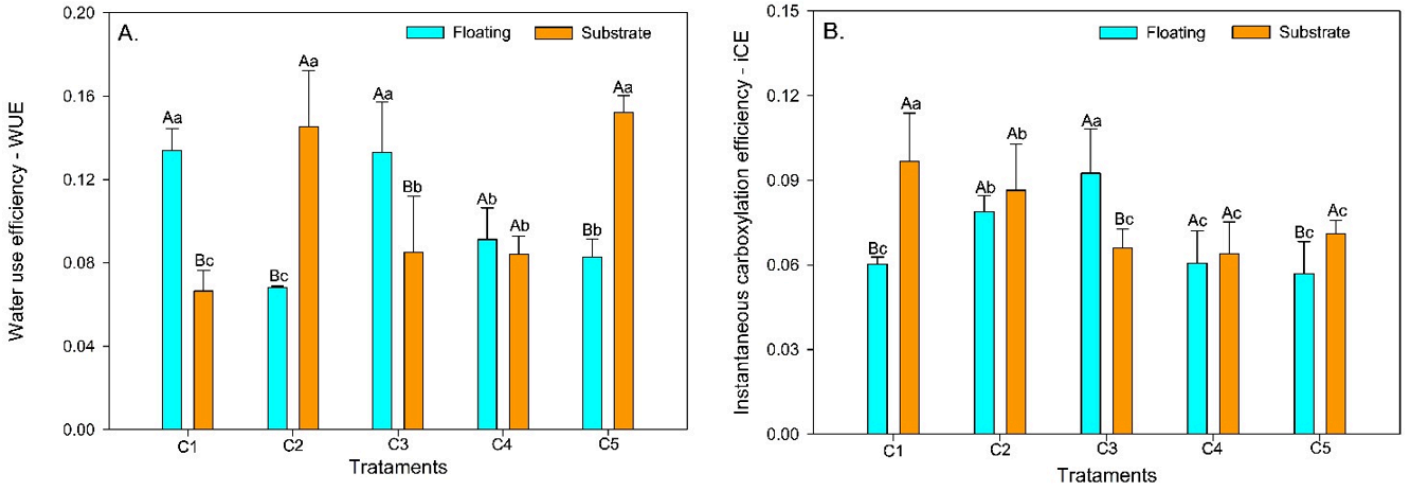


Figure 2

Water use efficiemcy (A) and instantaneous carboxylation efficiency (B) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

C1 - 2.0 dS m^{-1} + 0 µM melatonin. C2 - 5.0 dS m^{-1} + 0 µM melatonin. C3 - 5.0 dS m^{-1} + 50 µM melatonin. C4 - 5.0 dS m^{-1} + 100 µM melatonin. C5 - 5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n=4).

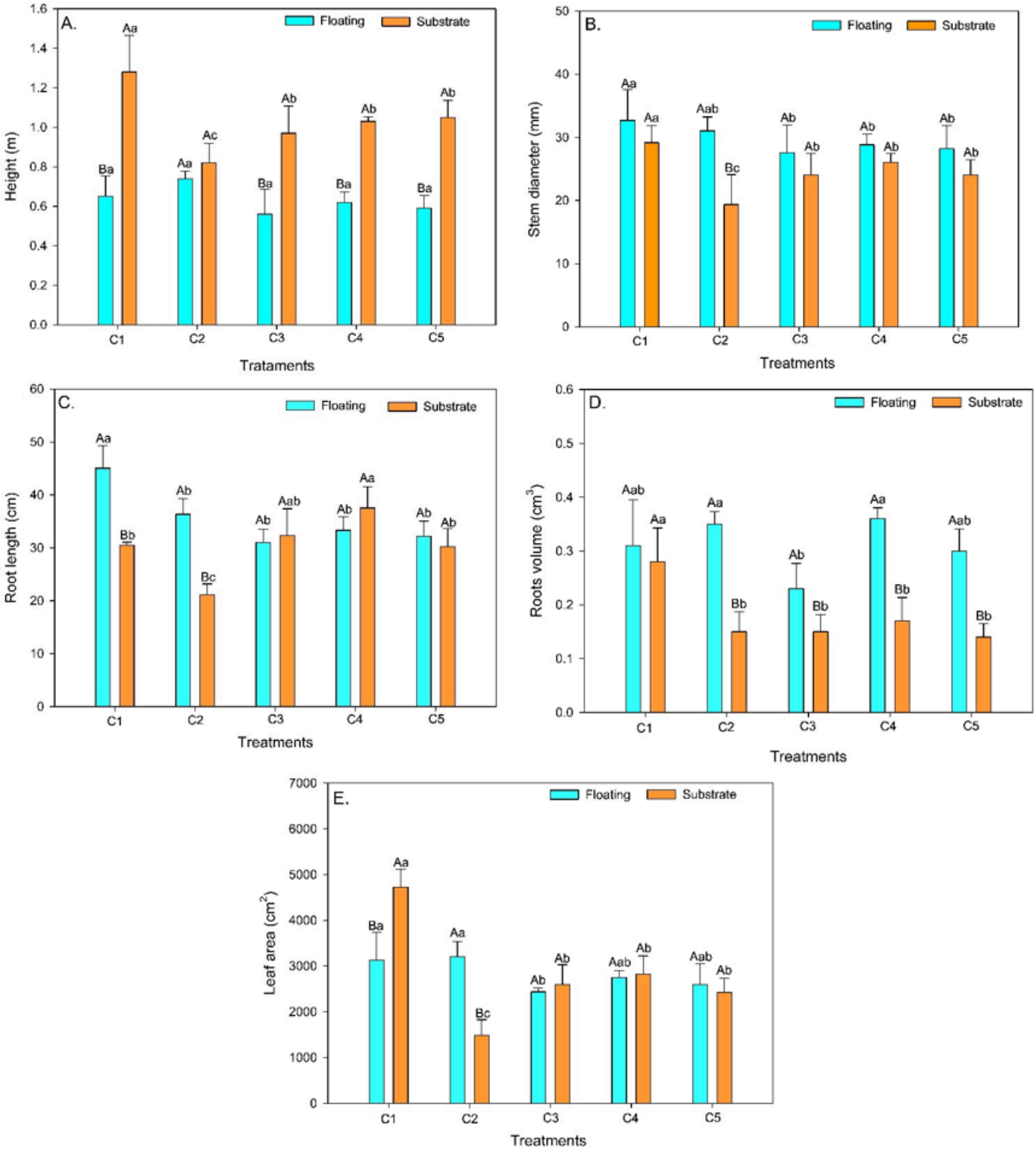


Figure 3

Plant height (A), stem diameter (B), root length (C), root volume (D) and leaf area (E) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

C1 - 2.0 dS m^{-1} + 0 µM melatonin. C2 - 5.0 dS m^{-1} + 0 µM melatonin. C3 - 5.0 dS m^{-1} + 50 µM melatonin. C4 - 5.0 dS m^{-1} + 100 µM melatonin. C5 - 5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB –

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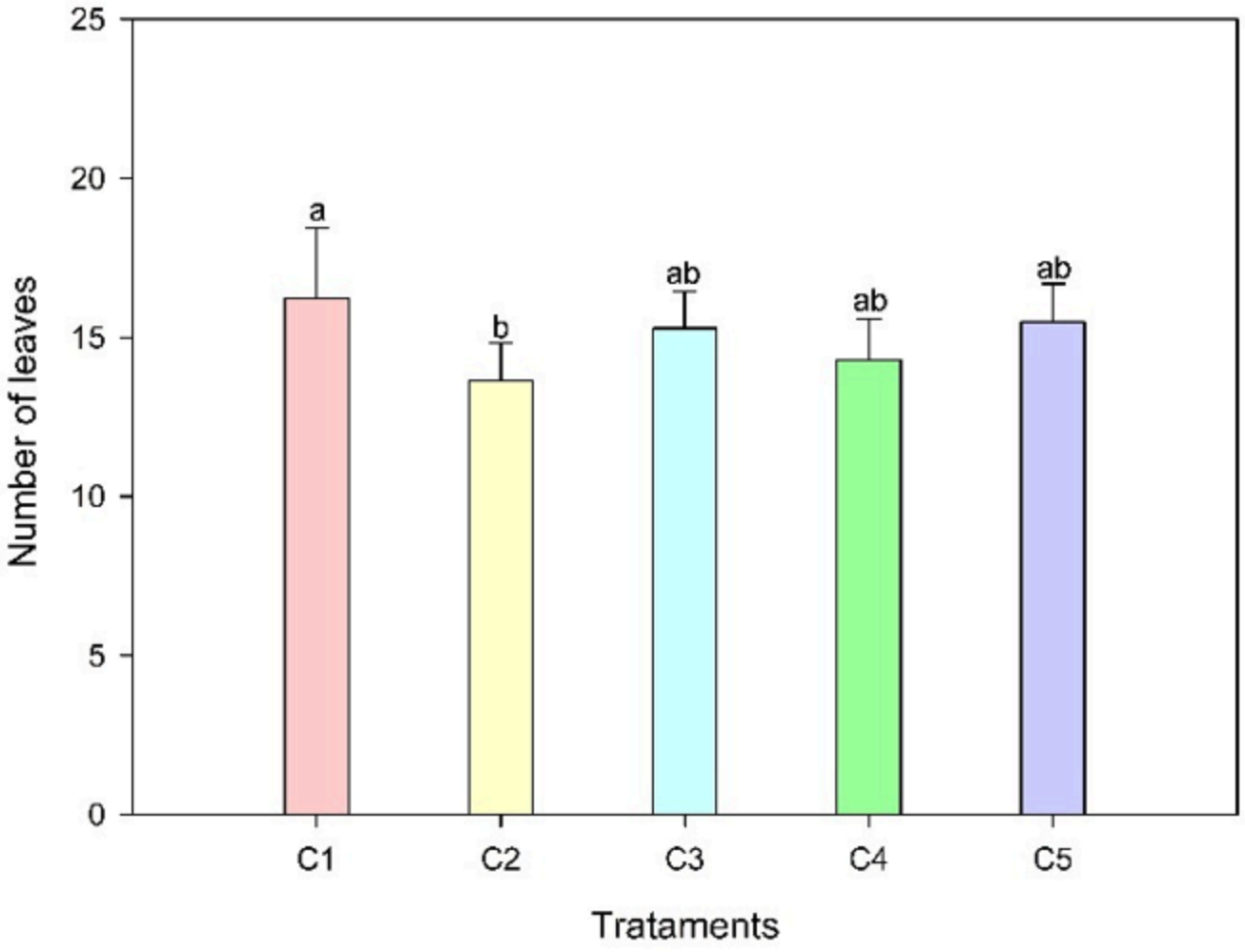


Figure 4

Number of leaves to papaya as a function of salinity and foliar application of melatonin

Columns with the same letter do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n=4).

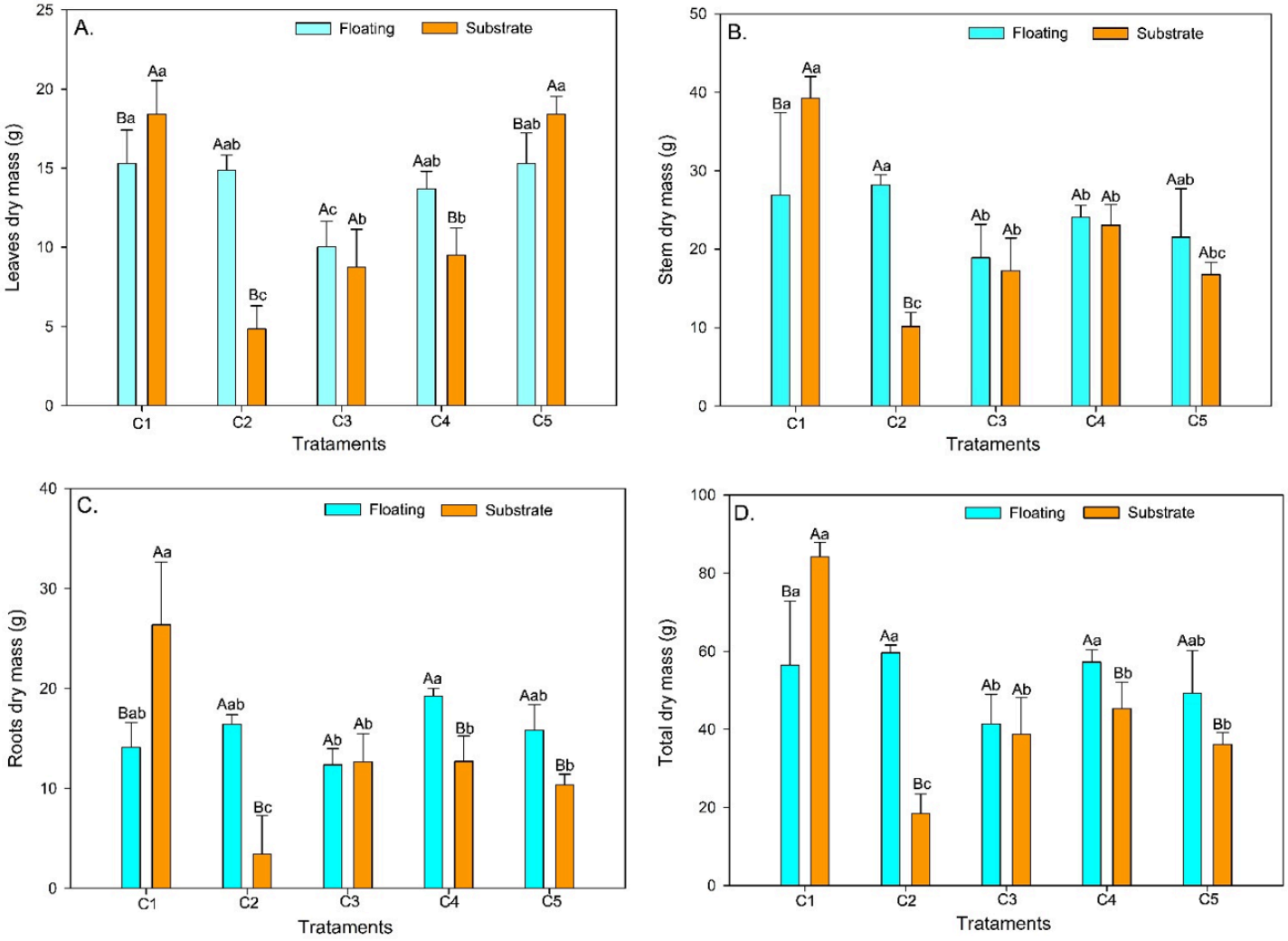


Figure 5

Leaf dry mass (A), stem dry mass (B), root dry mass (C), and total dry mass (D) of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin

C1 - 2.0 dS m^{-1} + 0 µM melatonin. C2 - 5.0 dS m^{-1} + 0 µM melatonin. C3 - 5.0 dS m^{-1} + 50 µM melatonin. C4 - 5.0 dS m^{-1} + 100 µM melatonin. C5 - 5.0 dS m^{-1} + 200 µM melatonin. FLO – floantig. SUB – hydroponics with substrate. Letters followed by the same letters. uppercase in the rows and lowercase in the columns. do not differ from each other by the Tukey test ($p \leq 0.05$). The spread above the bars represents the standard deviation from the mean (n=4).

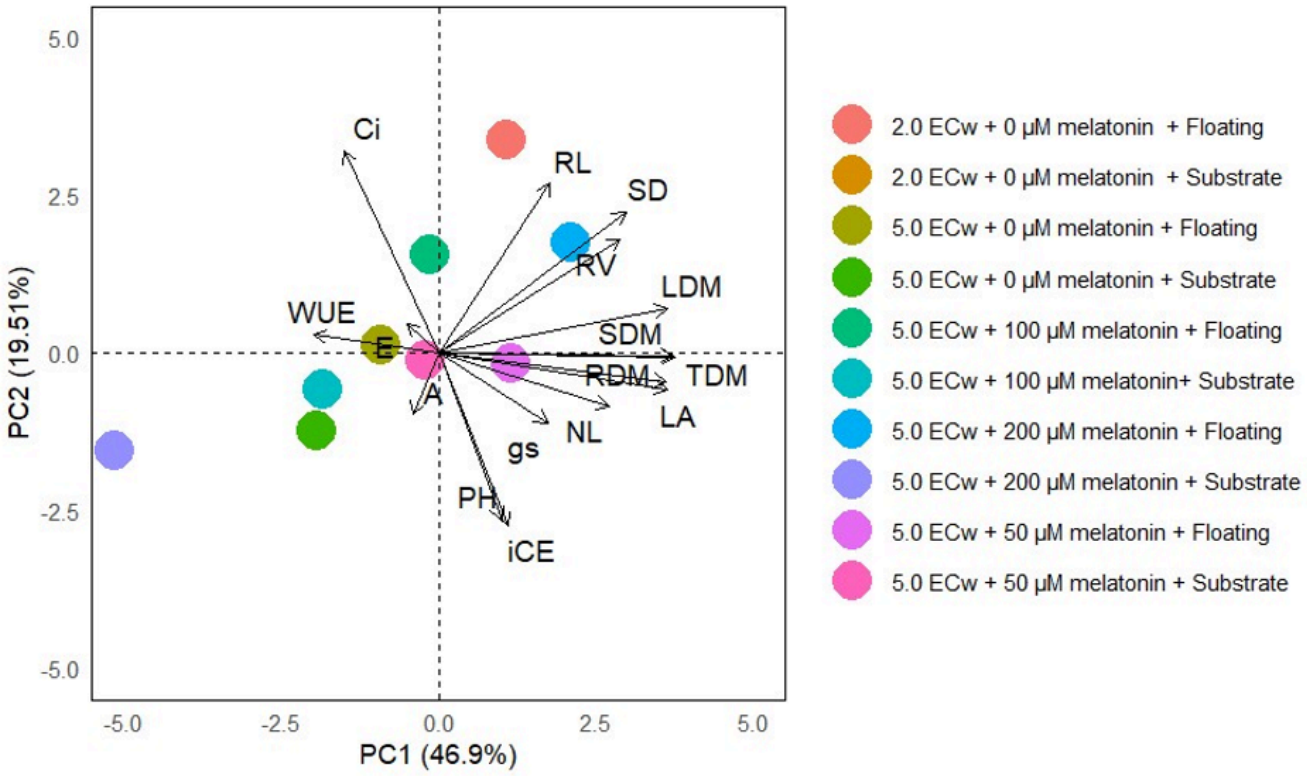


Figure 6

Principal component analysis (PCA) for gas exchange, growth, and biomass accumulation variables of papaya grown in two hydroponic systems and subjected to combinations of salinity and melatonin