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Salicylic Acid Applied via Irrigation Enhances Young *Carica papaya* L. Plant Performance under Water Deficit

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Abstract: Generally, water deficit induces a negative impact on vegetative growth and physiological and biochemical processes in *Carica papaya* L. However, the effects of drought magnitude and duration may be dependent on the ability of the genotype to activate mechanisms of defense against the imposed stress. Thus, the purpose of this research was to investigate the effectiveness of adding salicylic acid (SA) to the root system via irrigation against water stress. To assess the morphological and physiological responses of papaya to drought stress, seedlings were exposed to a regulated deficit irrigation system combined with the addition of SA to their irrigation water for 44 days. Results showed that water shortage inhibited papaya growth through the reduction in functional leaf number (27%), fresh (13%) and dry weights (17%), and stem width (9%). Moreover, water scarcity significantly decreased stomatal conductance (48%) and chlorophyll content (21%) and increased proline production (31%). Nevertheless, the exogenous application of SA relieved the effects of water stress on these characteristics, yielding similar values to those from control plants. Therefore, these findings prove the effectiveness of SA applied via irrigation in alleviating papaya damage under water deficit by preserving growth, stomatal conductance, photosynthetic pigments, and proline levels.

Keywords: biomass; leaves; photosynthetic pigments; proline; stomatal conductance

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1. Introduction

Environmental stresses are a worldwide concern for agriculture, and water stress is one of the main factors directly involved in impairing crop production, because of the decline of water resources. In several plant species, drought effects induce photosynthetic apparatus damage, membrane instability, and cell wall alterations, and they reduce leaf water relations, water potential, photosynthetic activity, and photosynthetic pigments [1–6]. Moreover, drought increases malondialdehyde (MDA), membrane injury, and reactive oxygen species (ROS), leading to photooxidative and structural damage [3,5,6]. Generally, it is accepted that stomatal closure is a key strategy to minimize transpiration and leaf water loss and maintain cell turgidity. In this context, drought-avoiding species close stomata at water potentials higher than their turgor loss point, while drought-tolerant ones keep stomatal conductance at lower water potentials than their turgor loss point, leading to major dehydration tolerance [7]. Furthermore, the resistance of plants to drought appears to depend on timely stomatal closure [8]. Although drought stress signals detected by the leaves induce them to close stomata, this response is clearly sensitive to soil moisture depletion and occurs earlier than leaf mesophyll turgor pressure reduction [9,10]. Severe drought induces stomatal closure and inhibits solute accumulation and protein synthesis [11]. In

hybrid *Pennisetum*, short-term drought stress reduces net CO_2 assimilation and leaf pigment concentration, and such a decrease in photosynthesis seems to result through the stomatal and non-stomatal limitations. In addition, the changes in chlorophyll fluorescence prove that drought harms structural stability and alters the balance between electrons at the acceptor and donor sides of Photosystem II [12]. In an experimental system using oat x maize hybrids, short-term drought reduced chlorophyll and carotenoid contents and slightly changed chlorophyll fluorescence parameters [13]. Gori et al. (2021) [14] proposed that carotenoids contribute to controlling and countering the photooxidative stress, and their antioxidant role is noticeable in plants subjected to drought, especially when climatic conditions impair the light stress. Zahra et al. (2023) [15] reviewed plant photosynthetic responses under drought stress and highlighted the role of stomatal closure in disturbing the photosynthetic apparatus, significantly decreasing electron transport rates and pigment concentrations under drought conditions. In addition, the review referred to the positive role of chloroplast signaling in sustaining the plant's photosynthetic function.

In many species, plants enhance the content of soluble sugars, proline, free amino acids, and phenolic compounds, and they increase enzymatic and non-enzymatic antioxidant activities [1,5,16,17], leading to improved osmotic adjustment [5,18,19]. For instance, in soybean (Glycine max L. Merrill), drought dissimilarly increases the concentrations of proline, total soluble solids (TSSs), and MDA depending on the stages of growth, flowering, and cultivars [20].

The role of proline as a compatible solute and its accumulation in the cytoplasm to protect cells against dehydration is well recognized in numerous species. The increase in proline levels has been associated with the maintenance of low water potentials, cell turgor, membrane and protein structure, and stability by retrieving the ROS [21–25].

Salicylic acid as a signal molecule is involved in various plant responses under drought and other stress conditions [26–29]. The role of SA in the regulation of plant growth, photosynthesis, plant metabolism, and other physiological processes is currently evident [30–34]. It is also considered to be a plant hormone involved in the modulation of growth, development, stomata function, respiration, membrane integrity, enzyme synthesis, and several plant responses against many environmental stresses [32,35–43]. However, the adequate concentrations required to reach an optimal plant growth response under environmental stress conditions are not completely established [43] since these depend on many variables, such as species, genotypes, stress intensity, etc. In horticultural species, foliar application of SA increases the leaf relative water content (RWC), membrane stability index, chlorophyll content, and yield in *Cucurbita pepo* L. plants under non-irrigation and irrigation at 50% of their water requirement [44]. In the same way, exogenous foliar SA decreases MDA, hydrogen peroxide (H_2O_2), ion leakage, catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) and moderates the increase in carbohydrate and fatty acids in plants under water deficit conditions [44]. In addition, the applications of SA through seed priming maintain photosynthetic efficiency and plant biomass, halt nodule senescence, and decrease oxidative stress, triggering an enhancement of productivity in chickpeas under salt stress [45]. On the other hand, drought stress increases the concentrations of proline, total soluble carbohydrates, and phenolic compounds, whereas the application of foliar SA increases all these variables under both full-irrigation and deficit-irrigation circumstances, leading to an improvement of osmotic adjustment, RWC, and yield in milk thistle plants under stress conditions [46].

Papaya is a plant that requires an appropriate water supply to ensure optimal growth and development, and consequently greater productivity, at least in subtropical and semi-arid conditions. Unfortunately, the limited water resources entailed by climate change could be a serious menace for this crop and other species. The detrimental effects of drought or water deficit on plant growth and physiological and biochemical processes in *Carica papaya* L. have been reported in earlier and recent experimental systems [47–51]. Leaf senescence and subsequent abscission are the most perceptible injuries induced by water stress, especially in young papaya plants; however, drought also affects other plant

growth parameters, such as leaf and root biomass and stem growth expressed as height and perimeter [52–56]. The decrease in the shoot–root ratio, growth, development, and yield is apparent in other species [57,58]. It has been reported that drought-tolerant papaya plants exhibit more stem height and thickness, crown width, and functional leaves with respect to drought-affected ones [53]. RWC and leaf gas exchange variables, such as net CO_2 assimilation, transpiration rate, and stomatal conductance, considerably decrease under drought conditions and depend overall on water stress extent [51,54–56]. As reported above, many experimental systems investigated the role of foliar SA application in plant abiotic stress responses such as drought; however, its involvement in those responses when applied via irrigation is not currently known. In addition, the effects of water stress magnitude and extent may be dependent on the capacity of each genotype to activate the mechanisms of defense to face the stress situation. Therefore, the aim of this study was to explore the effectiveness of SA supply directly to the root system under deficit irrigation conditions. The effects of the exogenous SA on morphological and physiological responses in young papaya plants were assessed.

2. Materials and Methods

2.1. Plant Material and Experimental Characteristics

Carica papaya L. seedlings 'Red Joy' were provided by *La Cosma* Nursery (Tenerife, Canary Islands). This cultivar exhibits a great agronomical organoleptic potential in these subtropical locations, as observed by the growers. The average single fruit weight (kg) and total soluble solids (°Brix) are 1.3 and 12, respectively (https://knownyouseed.com/product/red-joy/, accessed on 30 May 2023). After acclimation, three-month-old papaya seedlings were transplanted under greenhouse conditions to 5 L plastic pots using a universal substrate (Profi-Substrat, Gramoflor, Valencia, Spain, https://www.sumipla.es/producto/gramoflor-containersubstrat-lfc/, accessed on 1 June 2023. Before transplanting, we added and mixed 50 g of granular fertilizer (ProTurf®, N, P, K fertilizer with Ca and Mg: 12-5-20 + 2 CaO + MgO) into each potting substrate. The experiment was performed over 44 days between October and December 2021 and repeated at the same period in 2022 (see Figures S1 and S2), obtaining similar results. During that period, greenhouse temperatures oscillated between 8.6 and 30.2 °C, with a daily average temperature of 16.1 ± 0.2 °C, expressed as the mean daily value ± standard error. Relative humidity ranged from 43.9% to 94.5%, with a daily average value of 80.4 ± 0.7%, expressed as indicated above.

2.2. Water Stress Conditions and Treatments

Fifty-four plants were randomly distributed following a Latin square experimental design with three rows and blocks, each of those containing six plants per treatment, resulting in 18 plants per treatment. The three established treatments were performed as follows: (a) control, fully watered plants (1 L of water per plant, 100% of required irrigation applied three times a week); (b) water stress, deficit-irrigated plants (1 L of water per plant and week, 33% of needed watering); (c) water stress + SA, deficit irrigated plants (1 L of water containing 100 mg SA per plant and week). SA was provided by Sigma-Aldrich, Madrid, Spain.

2.3. Soil Moisture Measurements

Soil water content was measured on three dates (0, 22, and 44 days after treatment (DAT)) before the application of treatments, by insertion of an ML2x Theta Probe soil moisture sensor (Delta-T Devices Ltd., Burwell, Cambridge, UK) into the substrate.

2.4. Growth Measurements and Sampling

Plant growth was evaluated by measuring the plant stem thickness and leaf number at the onset, middle, and end of the experimental period (0, 22, and 44 DAT, respectively), as well as by determining the average single leaf fresh weight (FW) and dry weight (DW) per plant at the end of the trial. Only non-senescent, healthy, and completely extended

leaves were considered for these determinations. Fresh leaves were weighed after sampling, immediately frozen with liquid nitrogen and stored at −20 °C for further analyses. The DWs of leaves were determined after the lyophilization of leaf tissues.

2.5. Stomatal Conductance

A steady-state porometer (SC-1 Leaf Porometer, Meter Group Inc., Pullman, Washington, DC, USA) was employed to measure stomatal conductance on the third leaf from the plant apex. During the measurement period (between 09:00 and 10:30 a.m.), the temperature within the leaf chamber was 24.1 ± 0.2 °C, and percentages of moisture ranged from 61.8 ± 0.4 to 68.9 ± 0.4%, expressed as mean ± standard errors in all cases.

2.6. Photosynthetic Pigment Determination

Samples of 0.2 g (m_0) frozen ground leaves in 5 mL (V_0) 8/2 acetone/H_2O solution were stirred with a homogenizer (T 25 digital Ultra-Turrax, IKA-Werke, Staufen, Germany) at 10,000 rpm for 1 min. The aqueous extracts were centrifuged at 4500 rpm and 4 °C for 30 min, and the supernatants were carefully transferred to spectrophotometer cuvettes. Absorbances were successively read at λ = 663, 647, and 470 nm, and the concentrations of chlorophyll a (Chl a) and b (Chl b), total chlorophylls (Chl t), and carotenoids (Car) were respectively obtained by the following equations adapted from Lichtenthaler and Buschmann [58]:

$$\text{Chl a } (\text{mg}\cdot\text{g}^{-1} \text{ frozen sample}) = (12.25\cdot A_{\lambda = 663\text{ nm}} - 2.79\cdot A_{\lambda = 647\text{ nm}})\cdot V_0/(m_0\cdot 1000),$$
$$\text{Chl b } (\text{mg}\cdot\text{g}^{-1} \text{ frozen sample}) = (21.50\cdot A_{\lambda = 647\text{ nm}} - 5.10\cdot A_{\lambda = 663\text{ nm}})\cdot V_0/(m_0\cdot 1000),$$
$$\text{Chl t } (\text{mg}\cdot\text{g}^{-1} \text{ frozen sample}) = \text{Chl a} + \text{Chl b},$$
$$\text{Car } (\text{mg}\cdot\text{g}^{-1} \text{ dry sample}) = (5.05\cdot A_{\lambda = 470\text{ nm}} + 2.08\cdot A_{\lambda = 663\text{ nm}} - 9.21\cdot A_{\lambda = 647\text{ nm}})\cdot V_0/(m_0\cdot 1000).$$

2.7. Proline Production

Fresh leaf tissues were triturated in liquid nitrogen, then 0.1 g FW was extracted into 5 mL of 3% *w*/*v* sulfosalicylic acid (Sigma-Aldrich, Madrid, Spain) and homogenized using a T 25 digital Ultra-Turrax (IKA-Werke, Staufen, Germany). The mixture was centrifuged at 4500 rpm and 4 °C for 45 min, and proline production was determined according to previously established procedures [56,59]. In brief, 1 mL of the supernatant was mixed with 2 mL of glacial acetic acid and ninhydrin reagent (Sigma-Aldrich, Madrid, Spain) in a 1:1 (*v*:*v*) ratio. The reaction of the mixture was performed in a water bath at 100 °C for 1 h and afterwards cooled in ice for ca. 15 min. Absorbance was measured in the organic phase at λ = 520 nm by means of a Genova Plus Spectrophotometer (Jenway, Bibby Scientific, Chelmsford, UK). A standard curve was performed with commercial proline (Sigma-Aldrich, Madrid, Spain) to determine its final concentration in the samples.

2.8. Statistical Analyses

Data representation was conducted with Excel 2019 version 1808 (Microsoft Corporation, Redmond, Washington, DC, USA), and statistical analyses were performed with SPSS Statistics version 26.0.0.0 for Windows (IBM Corporation, Armonk, New York, NY, USA). Parameters that showed a normal distribution according to the Lilliefors-corrected Kolmogorov–Smirnov test and homoscedasticity according to the Levene test were submitted to analysis of variance (ANOVA) to establish the significance level at $p < 0.05$, 0.01, or 0.001, as specified in each case; the post-hoc Duncan test was chosen for detection of mean groups. Correlations were established based on Pearson correlation coefficients at the significance levels indicated in each case.

3. Results

3.1. Soil Moisture

In well-irrigated plants, substrate moisture recorded water contents between 26 and 36% (Figure 1). The applied deficit irrigation caused a decrease in soil water content which

reached levels around 14% and 18.4% at 22 and 44 DAT, respectively. As expected, similar moisture values were measured in plants subjected to water stress (WS) + SA.

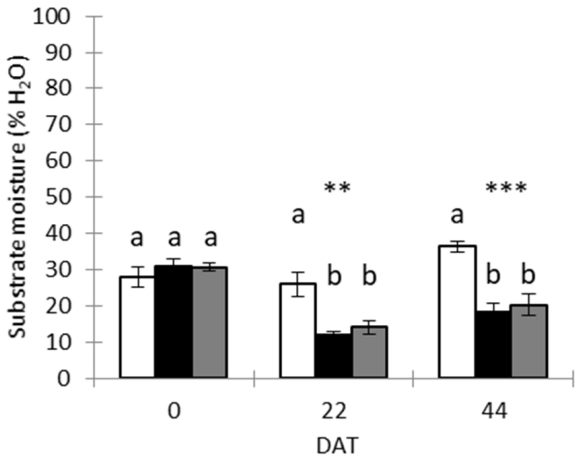

Figure 1. Evolution of water content in soil substrate during experiment. Data are means ± standard errors based on measurements of substrates (six from each block, n = 18 per treatment) for those plants subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. For each date, distinct letters denote significant differences at the significance level indicated by asterisks, i.e., $p < 0.01$ (**) or $p < 0.001$ (***), based on the ANOVA test. DAT = days after treatment.

3.2. Plant Growth

The functional leaf number was similar in control plants (8.8–9.7) during the experimental period (Figure 2). Water stress and WS + SA did not alter leaf number during the first 22 DAT; however, WS decreased this parameter by around 27% compared to the control at 44 DAT. By contrast, water-stressed and SA-treated plants showed similar leaf numbers as the control.

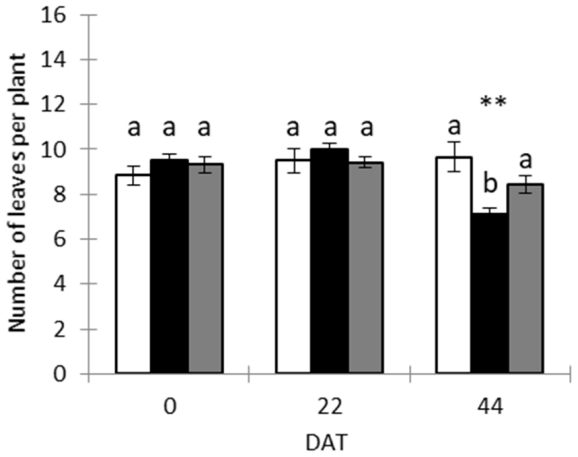

Figure 2. Functional leaf number per plant. Data are means ± standard errors based on measurements of plants (six from each block, n = 18 per treatment) subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. For each date, distinct letters denote significant differences at $p < 0.01$ (**), based on the ANOVA test. DAT = days after treatment.

On the other hand, the individual leaf FW increased from 8.7 to 13.6 g in fully irrigated plants from the start until the end of the trial, respectively (Figure 3). Nevertheless, water deficit decreased leaf FW by about 13% compared to the control, whereas the combination of water restriction and SA treatment did not alter this variable. Likewise, leaf DW displayed the same trend as FW, but water stress reduced DW by around 17% in comparison with well-watered plants (Figure 3). In addition, the stem thickness varied from 33 to 36.3 mm

at the beginning and end of the experiment, respectively, in the control plants (Figure 4). However, WS decreased this variable by around 9% at 44 DAT in comparison with the control, while WS + SA maintained stem thickness values similar to the control. At the end of the experimental period, the control plant stem thicknesses showed a positive significant correlation with dry single leaf weight (Table S1). Fresh and dry single leaf weights were significantly and positively correlated in the three treatments (Tables S1–S3) and fresh single leaf biomass with stem thickness in plants subjected to water stress plus SA (Table S3).

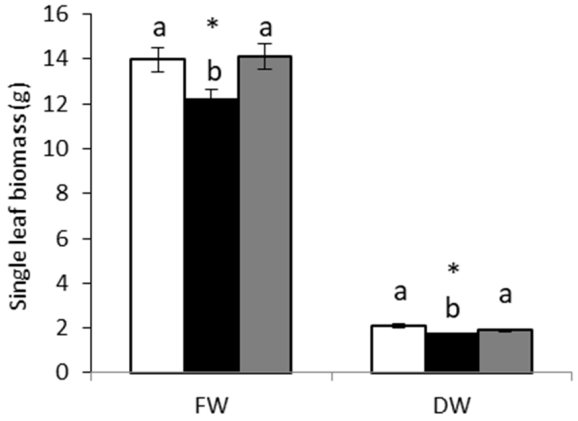

Figure 3. Leaf biomass at the end of the experimental period. Data are means ± standard errors based on measurements of plants (six from each block, n = 18 per treatment) subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. For each variable (FW or DW), distinct letters in the figure denote significant differences at $p < 0.05$ (*), based on the ANOVA test. FW: fresh weight. DW: dry weight.

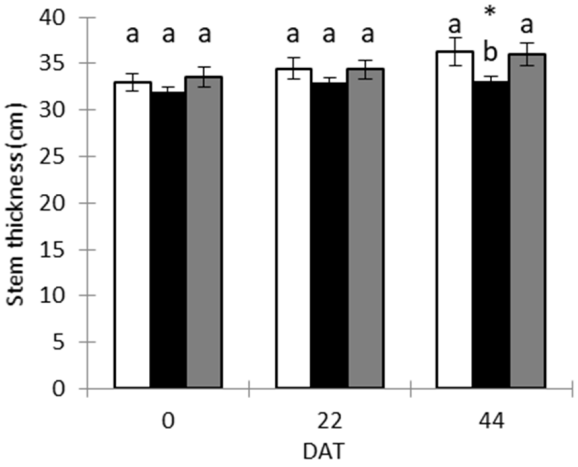

Figure 4. Plant stem thickness at the beginning, half-way point, and end of the trial. Data are means ± standard errors based on measurements of plants (six from each block, n = 18 per treatment) subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. For each variable (FW or DW), distinct letters in the figure denote significant differences at $p < 0.05$ (*), based on the ANOVA test.

3.3. Stomatal Conductance

Stomatal conductance (gs) varied between 96 and 123 mmol $m^{-2}s^{-1}$ in well-watered plants throughout the experimental period (Figure 5). Water stress as well as WS + SA reduced gs by about 34% in comparison with the control at 22 DAT; later, this decrease was enhanced by only WS (about 48%) and alleviated by WS + SA (24%) at 44 DAT.

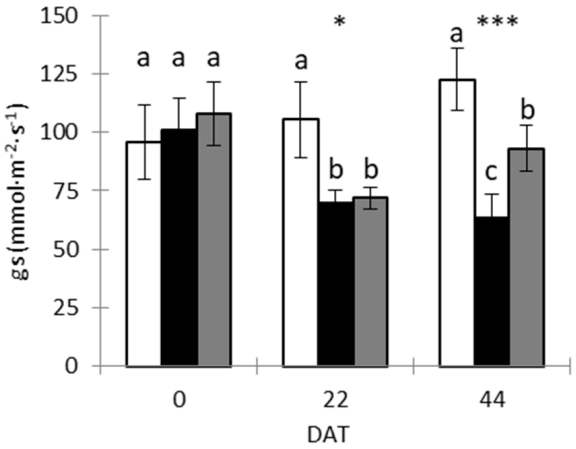

Figure 5. Stomatal conductance (gs) at the beginning, halfway point, and end of the trial. Data are means ± standard errors based on measurements of plants (six from each block, n = 18 per treatment) subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. For each date, distinct letters denote significant differences at the significance level indicated by asterisks, i.e., $p < 0.05$ (*) or $p < 0.001$ (***), based on the ANOVA test. DAT = days after treatment.

3.4. Photosynthetic Pigment Determination

Photosynthetic pigments were determined in leaf fresh tissues collected at the end of the trial (Figure 6). Chl a, Chl b, and total Chls significantly decreased under WS alone by around 21% compared to the control. Nonetheless, the supply of SA to deficit-irrigated plants improved the total Chls content, which maintained similar concentrations as the control. On the other hand, WS or WS plus SA did not alter carotenoid levels in the leaves.

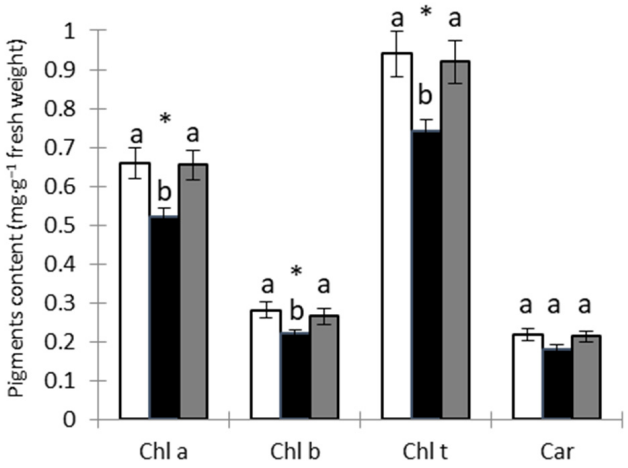

Figure 6. Leaf photosynthetic pigments at the end of the trial. Data are means ± standard errors based on measurements of frozen leaf plants (six from each block, n = 18 per treatment) subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. For each variable, distinct letters denote significant differences at $p < 0.05$ (*), based on the ANOVA test. Chl a = chlorophyll a; Chl b = chlorophyll b; Chl t = total chlorophylls; Car = carotenoids.

For all treatments, Chl a and Chl b exhibited a positive significant correlation, and both were correlated with total Chls; furthermore, all Chls were positively and significantly correlated with Car except in stressed plants, which showed a positive but insignificant correlation (Tables S1–S3).

3.5. Proline Production

Foliar proline concentrations were similar at the onset as well as the end of the experiment in control plants (Figure 7). However, 44 days after the constraint of irrigation,

a significant increase in this osmolyte (31%) was determined in water-stressed plants in comparison with fully irrigated ones, and similar foliar levels of this solute were observed between control and plants treated together with SA and watering shortage.

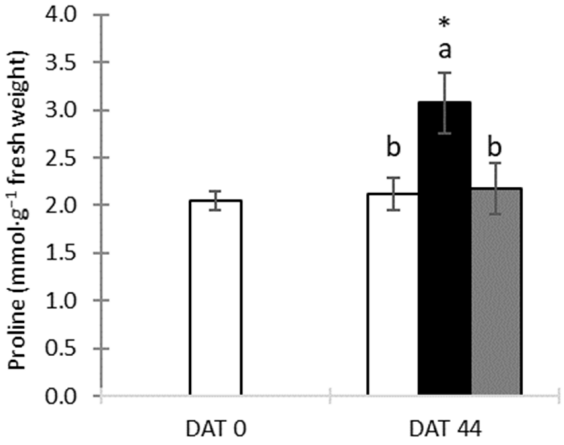

Figure 7. Foliar proline production at the end of the trial. Data are means ± standard errors based on measurements of frozen leaf plants (six from each block, n = 18 per treatment) subjected to regular watering (□), water stress (■), and water stress plus SA (■) for 44 days. Different letters denote significant differences at $p < 0.05$ (*), based on the ANOVA test.

Proline content and substrate moisture showed a significant positive correlation in stressed plants but a significant negative correlation in stressed plants treated with SA (Tables S2 and S3). Moreover, in stressed plants, proline and gs were positively and significantly correlated (Table S2).

4. Discussion

According to previous experiments, the most apparent symptom induced by watering shortage in the *Carica papaya* L. canopy (especially in young plants) is the decrease in the total leaf area per plant, induced by the progressive senescence and subsequent abscission of old leaves [52–56,60]. These responses appear to be relatively common amongst the different genotypes; however, the effects of drought magnitude and duration may be dependent on the ability of the cultivar to avoid the stress situation.

Data presented here show that the impact of WS on growth variables was more noticeable after a relatively long-term (44 DAT) compared to a short-term (22 DAT) stress and occurred parallelly to the decrease in soil moisture (Figure 1). Thus, WS decreased the functional leaf number by about 27% in comparison with the control at 44 DAT (Figure 2). Nevertheless, the addition of SA to water-stressed plants alleviated the damage of WS by maintaining leaf numbers similar to the control throughout the experimental period.

Regarding leaf biomass, water deficit reduced individual leaf FW and DW at the end of the trial (44 DAT) with respect to the control; however, the supply of SA to deficit-irrigated plants displayed similar leaf FW and DW as full-watered ones (Figure 3). In the same way, water restrictions decreased stem thickness at 44 DAT, and again the addition of SA to the watering solution kept this variable similar to the control (Figure 4).

The decrease in plant growth and development under drought conditions has also been reported in many plant species [57,58,60]; however, drought-tolerant papaya displays larger functional leaves, stem heights, and thicknesses compared to drought-susceptible plants [53]. Our data concluded that SA applied to water-stressed plants through an irrigation system enhanced leaf number (Figure 2), leaf biomass (Figure 3), and stem thickness (Figure 4). Furthermore, correlations between growth variables and soil moisture occurred at the end of the stress period (Tables S1–S3).

As far as we know, this is the first work to reveal the favorable effects of SA applied through irrigation against water deficits in papaya growth. Although further experiments

stated a link between SA and plant responses under water and salt stress conditions [26–28], most of them used this compound via foliar application. Among other responses, the application of foliar SA improves growth, plant biomass, and yield [29,44], and it delays senescence [45].

Even though the effect of water deficit was clearly established in plant growth at 44 DAT, its impact on stomatal closure occurred earlier (22 DAT), coinciding with the significant decrease in soil moisture (Figure 1), and it increased at the end of the experiment (Figure 5) parallel to the reduction in substrate water content, which may suggest that this genotype could possess a certain ability to protect the turgidity of cells once it receives the first signals of dehydration.

These results are compatible with the statement that the tolerance of plants to drought seems to be dependent on timely stomatal closure [8] and stress extent [9–12]. As is well-known, stomata movement can affect several physiological processes through the regulation of photosynthesis and water status [61]), leading to the reduction in leaf biomass (Figure 3) and the increase in leaf abscission (Figure 2).

On the other hand, the addition of SA alleviated the influence of water stress on stomatal conductance only at the end of the experimental period. This result may indicate that the supply of SA via the root system could be active under relatively severe stress or/and its action against drought is not immediate compared to foliar application; however, it is effective under relatively long-term stress. Even when applied by means of seed priming, SA sustains photosynthetic efficiency and reduces oxidative stress against salt stress [45].

Simultaneous to the changes of stomatal closure and opening under water deficit, Chl a, Chl b, and total Chls (analyzed in leaf FW) significantly decreased in water-stressed plants compared to the control (Figure 6), indicating a close relationship between these pigments and stomatal conductance. To mitigate the effects of water stress on photosynthetic pigments, the addition of SA to the stressed plants kept the concentration of all Chls variables similar to the control at 44 DAT. Nevertheless, non-changes in carotenoid levels were induced solely by water deficit or water deficit combined with SA in the collected leaves. In this regard, the positive effects of exogenous foliar SA in the enhancement of chlorophyll concentration [29], carbohydrate [44,46], and phenolic compounds [46] have been reported in several species under water shortage.

Proline accumulation and its involvement in protecting cells from dehydration and osmotic stress through the regulation of water potentials, turgor, membrane structure and stability, and various physiological processes have been reported in several species [20,22–25,57]. In the same way, the lowering of soil moisture induced by water deficit significantly increases foliar proline production (31% compared to its content in well-watered plants, Figure 7); nevertheless, the addition of SA through irrigation in water-stressed plants avoids the increased excess of the osmolyte, which remained at the control level at 44 DAT.

Overall, the data might suggest a close interaction between exogenous SA, proline production, and total Chls content, together contributing to improving the osmotic adjustment of cells under drought conditions.

5. Conclusions

The present research showed that water deficits induce a decrease in leaf number and biomass, stem girth, stomatal conductance, and chlorophyll content and increase proline production in young papaya plants. However, the addition of SA to the root system alleviates the effects of water stress on all measured variables, especially after a relatively long-term stress (44 DAT). Therefore, the exogenous application of SA seems to be useful through irrigation for increasing papaya adaption to water deficits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9101070/s1, Table S1: Pearson correlation coefficients between variables for treatment 1; Table S2: Pearson correlation coefficients between variables for treatment 2; Table S3: Pearson correlation coefficients between variables for treatment 3; Figure S1: Evolution of data recorded in season 2022; Figure S2: Final measurements from season 2022.

Author Contributions: Conceptualization, J.M.; methodology, J.M. and S.J.Á.-M.; formal analysis, J.M., A.D.M.-C., S.J.Á.-M. and A.U.-G.; investigation, J.M., A.D.M.-C., S.J.Á.-M. and A.U.-G.; data curation, J.M., A.D.M.-C., S.J.Á.-M. and A.U.-G.; writing—original draft preparation, J.M.; writing—review and editing, J.M.; visualization, J.M.; supervision, J.M.; project administration, J.M.; funding acquisition, J.M. All authors have read and agreed to the published version of the manuscript.

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