

Mitigation of salt stress damages in *Carica papaya* L. seedlings through exogenous pretreatments of gibberellic acid and proline

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ABSTRACT

Salinity is a serious threat for global agriculture, especially in arid and semi-arid regions where its incidence leads to considerable damages in the crop growth and production. *Carica papaya* L. is currently one of the most cultivated fruit crops in the tropical and subtropical areas, and generally, papaya cultivars exhibit a moderate sensitivity to salinity, although such responses may depend on the genotypes. In the present study, papaya seedlings were subjected to salt stress (100 mM NaCl) for 41 d and to exogenous gibberellic acid (GA₃; 0.1 mM) and proline (10 mM) pretreatments to evaluate plant physiological variables linked to stress responses. Analysis of the data ($P < 0.05$) showed a general decrease of plant growth parameters induced by solely salt stress compared to control, such as stem height (47%) and thickness (33%) and plant fresh and dry mass (84% and 83%, respectively), as well as a reduction in the stomatal opening (93%), chlorophylls (40%) and carotenoids (71%) concentration. By contrast, a significant increase was found in foliar and radicular proline levels under stress (87% and 47%). Exogenous foliar GA₃ or proline respectively induced a better performance of plants under salt stress by increasing stomatal conductance (444% or 350%), stem height (142% or 144%) and plant biomass (49% or 41%) regarding solely stressed plants, and leading to pigments concentrations close to those from control plants. The results suggest that exogenous gibberellic acid and proline as growth regulator and osmo-regulator solute, respectively could increase papaya seedlings adaption against salt stress.

Key words: Photosynthetic pigments, plant growth, subtropics.

INTRODUCTION

Carica papaya L. has been considered moderately tolerant to salt stress (Elder et al., 2000; Wu and Dodge, 2005). Specifically, it has been reported that irrigation system together with salt levels influence the responses of papaya to stress (Elder et al., 2000; Wu and Dodge, 2005). Thus, Wu and Dodge (2005) reported that papaya is moderately tolerant to saline over-head irrigation water with electrical conductivity (EC) of *ca.* 1 mS·cm⁻¹ and soil EC about 2-4 mS cm⁻¹. Similar trends were indicated by Elder et al. (2000) that stated no negative effects on productivity when water irrigation EC (1.4 to 4 mS·cm⁻¹) was applied by trickle or under-tree mini-sprinkler irrigation; however, overhead irrigation induced growth decrease and leaf injuries.

The increased salinity of the substrate reduced the growth and biomass of papaya plants, and particularly the decrease of biomass accumulation following the order: leaves, roots, and stem, respectively. In addition, salinity reduced the dry weight of stem and leaves and increased that of the roots (Sá et al., 2016). Mengarda et al. (2016) reported that salt and water stress combined with high irradiance negatively affected seeds germination, and 'Caliman 01' exhibited higher

germination performance under these conditions compared to other genotypes. On the other hand, soil salinity inhibited *C. papaya* seedlings growth, being leaf area the most sensitive parameter to the imposed stress. However, the supply of bovine biofertilizers stimulated seedlings growth, although did not suppress completely the adverse effects of salinity (de Lima-Neto et al., 2016).

In general, the responses of papaya to salinity seem to depend primarily on the genotypes; however, the increasing of salt concentration in irrigation water through EC over certain threshold decreased growth, DM accumulation and plant production in most of the studied cultivars (Sá et al., 2013; Diniz et al., 2018). Salinity stress could be considered an environmental constraint for papaya expansion especially in semi-arid regions, frequently characterized by limited resources of fresh water and by high level of salinity in irrigation water and in the soil.

Gibberellins (GAs) are now well-known as plant growth regulators involved in seed germination, stem and internode elongation, leaf expansion, flower and fruit growth (Ubeda-Tomás et al., 2009; Nelissen et al., 2012; Taiz et al., 2014). Salt stress seems to affect plant growth through the reduction of GAs synthesis; however, exogenous GAs addition could revert such decrease and induce several physiological processes such as leaf area enlargement, cell division and/or elongation, photosynthetic rate, and upregulation of invertase activity (Saeidi-Sar et al., 2013; Niharika et al., 2021). In addition, exogenous GAs have been able to enhance the performance of seeds (Nimir et al., 2020) and plants under salt stress conditions (Aziz Khan et al., 2012; Iqbal and Ashraf, 2013; Xu et al., 2016). Moreover, Sá et al. (2020) reported that the application of GAs or GAs and auxin mitigate the effects of salt stress on papaya seedlings. Gibberellins improved plant growth in several species under salt stress conditions (Tuna et al., 2008; Maggio et al., 2010; Nasri et al., 2012; Kandil et al., 2014), and increased water use efficiency and stomatal conductance (g_s) under moderate salinity (Maggio et al., 2010). Furthermore, it has been reported that GAs alleviate the negative impacts induced by salt excess through the increase of chlorophyll (chl) content, N use efficiency, nitrate reductase activity, and the absorption of mineral nutrient resulting in an increase of yield (Criado et al., 2017; Niharika et al., 2021). In flax plants subjected to salt stress, Khan et al. (2010) reported that the mixture of gibberellic acid (GA_3) and calcium chloride increased the accumulation of glycine betaine and proline, and consequently improved the osmotic adjustment. Tuna et al. (2008) reported that exogenous GA_3 maintained the membrane permeability and the macro- and micro-nutrient concentrations by increasing proline content under salt stress.

Proline is considered an osmolyte and a signal molecule that has been involved in several plant stress responses through the preservation of cell turgor, stabilization of the membranes, and by scavenging the reactive oxygen species (ROS) and maintaining redox balance in plants (Ashraf and Foolad, 2007; Hayat et al., 2012; Li et al., 2014; Meena et al., 2019). The effects of exogenous proline supplementation have been explored in several crops and most of the results showed a favorable response against salt or drought stress. Proline supply increased the plant height, number of roots, nitrate content, nitrate reductase and glutamine synthetase activities in roots of rice under salt stress. Consequently, the ability of proline to mitigate salt stress injuries appear to be linked to the N assimilation activities (Teh et al., 2016). In addition, exogenous proline increased gas exchange parameters, water use efficiency and antioxidant mechanisms under salt stress (Butt et al., 2020). In bean, exogenous proline mitigated the oxidative stress induced under salinity by increasing the concentrations of endogenous proline, photosynthetic pigments, ascorbic acid, mineral nutrients, and enhancing the activity of antioxidant enzymes (Abdelhamid et al., 2013). Under K deficiency, Zhang et al. (2020) suggested that exogenous proline could improve the crop quantity and quality of lettuce by the regulation of biochemical processes.

It is important to note that the effectiveness of foliar proline is dependent on the plant species, the stage of development, and the concentration of the osmolyte (Ashraf and Foolad, 2007). For instance, it has been reported that higher concentration of proline (25 mM) was more effective than lower one (12.5 mM) in increasing plant growth characteristics, photosynthetic pigments, indole acetic acid (IAA), and proline contents in quinoa plants under drought stress (Elewa et al., 2017). On the other hand, the *proline resistant 1-1* (*pre1-1*) mutant of *Arabidopsis thaliana* accumulated less proline than the wild type when cultivated in a medium with proline. In contrast, salt stress decreased the uptake of proline in *pre1-1* mutants; however, increased its content in the wild type, suggesting that plants can increase proline level by the *amino acid permease 1* (*AtAAP1*, At1g58360) mediated the uptake of proline from the medium (Wang et al., 2017).

In this study we investigated the responses of *C. papaya* plants to salt stress conditions with special emphasis into plant growth and physiological variables associated to the mechanisms of stress tolerance. In addition, we assessed the effectiveness of exogenous GA_3 and proline pretreatments on papaya responses the stress condition.

MATERIALS AND METHODS

Plant material and experimental conditions

Carica papaya L. 'Sweet Mary' seedlings were used in this study to elucidate its responses to salt stress conditions. This genotype is characterized by a great agronomic potential and suitable organoleptic properties in tropical and subtropical climates. Two-month-old papaya seedlings were grown under greenhouse in plastic pots (5 L) containing universal potting soil (Leader German Uniplus, Arber Horticulture, Alezio, Italy). Two weeks before the onset of the experiments, plants were fertilized with 20 g per pot of granular fertilizer (19-9-10 NPK + 2 MgO + trace elements, Osmocote (Pro 5-6M, ICL Specialty Fertilizers, Murcia, Spain). During the experimental period temperature in the greenhouse oscillated between 20 and 30 °C and relative humidity between 60% and 85%. Control and treated plants were irrigated at field capacity between two and three times a week. The entire experiment was repeated twice in two consecutive seasons (2019 and 2020) under the same conditions finding similar results, and finally data for one of the experiments were presented herein.

Salt stress and chemical treatments

To evaluate the influence of salinity stress and the pretreatments of chemical compounds with the aim to alleviate the impact of salt damages on papaya plants, we used a total of 72 plants distributed into a randomized complete block design with three blocks and six plants per block and treatment. Then, the following four treatments were established: (a) control, plants were irrigated with fresh water; (b) salt stress, plants were irrigated with enriched water solution by the addition of 100 mM NaCl; (c) salt stress + gibberellic acid (GA₃), plants pretreated with GA₃ (0.1 mM) and irrigated with salt stress (see treatment b); and (d) salt stress + proline (10 mM), plants pretreated with proline and watered with salt stress (see treatment b).

The period of salt stress imposition was performed considering the established typical symptoms of salt stress, such as leaf necrosis and especially leaf abscission. Thus, treatments were ceased, and the experimental period was finalized when plants lost approximately 50% of their functional leaves. Finally, these conditions were achieved 41 d after the beginning of the salt application. Concerning chemical treatments, proline and GA₃ (Sigma-Aldrich, Madrid, Spain) were applied during 2 wk prior to the onset of salt imposition with a frequency of three treatments a week through foliar spray, ensuring a complete wetting of the plants in each application. Proline and GA₃ were dissolved in 5% (v/v) aqueous ethanol and water, respectively. To ensure an effective foliar moistening, few drops of 0.05% Tween-20 were added to each solution. In addition, control and salt-stressed plants (treatment b, see above) were parallelly sprayed by distilled water with 0.05% Tween-20.

Growth measurements and sampling

Plant growth was determined as stem height and thickness, leaf number, and plant fresh and dry mass. Stem growth and leaf number were measured periodically during the experimental period. Only functional leaves (completely extended and healthy, discarding senescent ones) were recorded. Plant tissues sampling was performed both at onset and at the end of the trial, recording fresh mass of leaves, stem, and roots immediately after sampling. Additionally, at the end of the experimental period plants organs were separately frozen in liquid nitrogen, lyophilized, triturated and weighted to get their dry mass. Leaves thus obtained were employed for photosynthetic pigments and proline determination, as well as dry root tissues for proline determination, as described below.

Stomatal conductance

Stomatal conductance was measured with a steady-state porometer (SC-1 Leaf Porometer, Meter Group Inc., Pullman, Washington, USA) on fully expanded leaves, generally among the 3rd and the 5th leaf counting from plant apex. Values were normally recorded between 08:30 and 11:30 h, being registered within the leaf chamber temperatures of 27.6 ± 0.1 °C and percentages of moisture ranging from $58.7 \pm 0.3\%$ to $71.1 \pm 0.3\%$.

Photosynthetic pigments determination

A sample of 0.05 g (m_0) of lyophilized ground leaves in 5 mL (V_0) 80% v/v acetone aqueous solution was stirred by means of a homogenizer (T 25 digital Ultra-Turrax, IKA-Werke, Staufen, Germany) at 10000 rpm for 1 min. The extracts were

centrifuged at 4500 rpm and 4 °C for 30 min, and then the supernatants were carefully aspirated to spectrophotometer cuvettes. Absorbances were successively read at $\lambda = 663, 645$ and 480 nm, and the concentration of chlorophylls a (Chl a), b (Chl b), total chlorophylls (Chl t) and carotenoids were respectively obtained by the following equations adapted from (Kumari et al., 2018):

$$\begin{aligned} \text{Chl a (mg g}^{-1} \text{ dry sample)} &= (12.70 A_{\lambda=663 \text{ nm}} - 2.69 A_{\lambda=645 \text{ nm}}) V_0 / (m_0 1000) \\ \text{Chl b (mg g}^{-1} \text{ dry sample)} &= (22.90 A_{\lambda=645 \text{ nm}} - 4.68 A_{\lambda=663 \text{ nm}}) V_0 / (m_0 1000) \\ \text{Chl t (mg g}^{-1} \text{ dry sample)} &= C_{\text{chl a}} + C_{\text{chl b}} \\ \text{Carotenoids (}\mu\text{g g}^{-1} \text{ dry sample)} &= (A_{\lambda=480 \text{ nm}} + 0.114 A_{\lambda=663 \text{ nm}} + A_{\lambda=645 \text{ nm}} - 0.638) V_0 / m_0. \end{aligned}$$

Proline determination

All chemicals were purchased from Sigma-Aldrich (Madrid, Spain) and used as received without further purification. Lyophilized ground tissues (0.05 g) in 5 mL 3% w/v sulfosalicylic acid aqueous solution were stirred by means of a homogenizer (T 25 digital Ultra-Turrax) at 10000 rpm for 1 min. The extracts were centrifuged at 4500 rpm and 4 °C for 45 min, and then proline determination was performed following the procedure of Bates et al. (1973). Briefly, 1 mL supernatant was mixed with 2 mL 50% w/v ninhydrin solution in glacial acetic acid. The resulting mixture was heated in a water bath at 100 °C for 1 h, and then was cooled into an ice bath for 15 min. Absorbance was read in the organic phase at $\lambda = 520$ nm through a Genova Plus Spectrophotometer (Jenway, Bibby Scientific, Chelmsford, UK). Proline concentration was calculated by interpolation using a standard curve prepared with commercial proline as previously reported (Bates et al., 1973; Mahouachi et al., 2012).

Statistical analyses

Statistical analyses were performed using the software SPSS Statistics version 25 for Windows (IBM Corporation, Armonk, New York, USA). Variables with a normal distribution (according to Shapiro-Wilk test) and homogeneity of variance (according to Levene test) were subjected to ANOVA, and *post hoc* Duncan test was used for mean separation. For variables with a non-normal distribution of the residuals or non-homogeneity of the variance, non-parametric Kruskal-Wallis *H* test was performed, and such cases are properly indicated in figure legends. The significance level for all tests was set at $P < 0.05$.

RESULTS

Plant growth

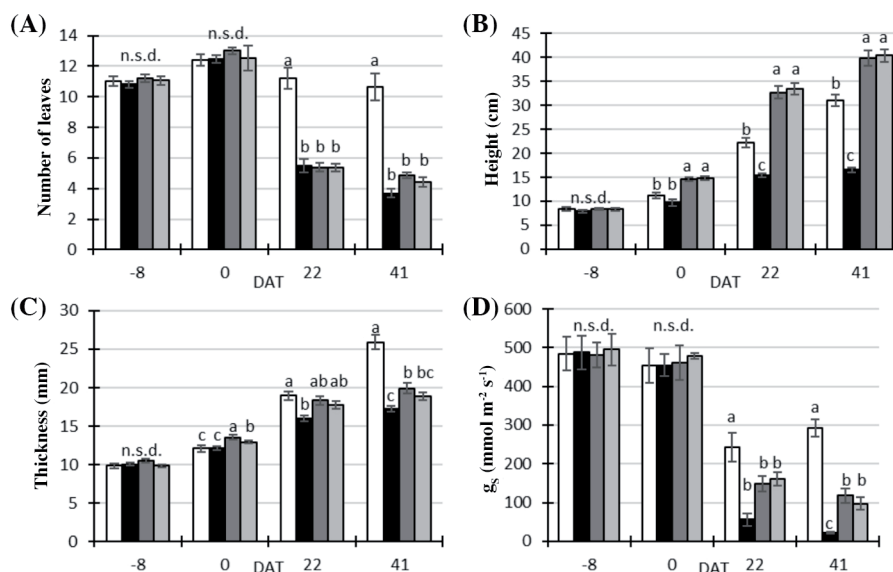
The functional leaf number oscillated between 11 and 12 leaves per plant in control plants along the experimental period (Figure 1A). The application of salinity treatments decreased the leaf number about 50% 22 d after treatment (DAT) in all the salt-stressed plants, regardless of whether there was foliar pretreatment or not. At the end of the experiment (41 DAT), the decrease of leaf number induced by NaCl-stress reached 65%; however, in plants pretreated with exogenous GA₃ and proline such decrease was relatively smaller, achieving 54% and 58% compared to control, respectively.

Stem height varied between 8 and 31 cm from the onset until the end of the trial in control plants (Figure 1B). Plants only treated with NaCl experienced a decrease of stem height over 31% 22 DAT; in contrast, plants pretreated with GA₃ and proline showed an increase of stem height around 47% and 50%, respectively, compared to control. In the same way, the decrease of stem height in salt-stressed plants was prominent 41 DAT (47% with respect to control) while pretreated-salt-stressed plants with GA₃ and proline were respectively 142% and 144% higher than only salinized ones (Figures 1B and 2).

Concerning stem thickness, salinity stress reduced this variable in approximately 15% (22 DAT) and 33% (41 DAT), compared to control (Figures 1C and 2). The exogenous pretreatment of GA₃ significantly improved stem thickness about 15% with respect to plants solely treated with NaCl.

Plant fresh weight was determined at the initial and the end of the experimental system (Table 1). Sal stress greatly reduced leaf fresh mass per plant in presence or absence of exogenous pretreatments (92%-94% compared to control, 41 DAT). The effects of NaCl in root biomass followed the same trend as well as the leaves with the exception that the decrease was relatively minor (82% compared to control). In addition, salinity decreased stem fresh weight about 80% with respect to control; however, such decrease only reached 64% and 66% in plants pretreated with GA₃ and proline,

Figure 1. Number of leaves (A), stem height (B), stem thickness (C), and stomatal conductance (g_s ; D) in ‘Sweet Mary’ *Carica papaya* plants.

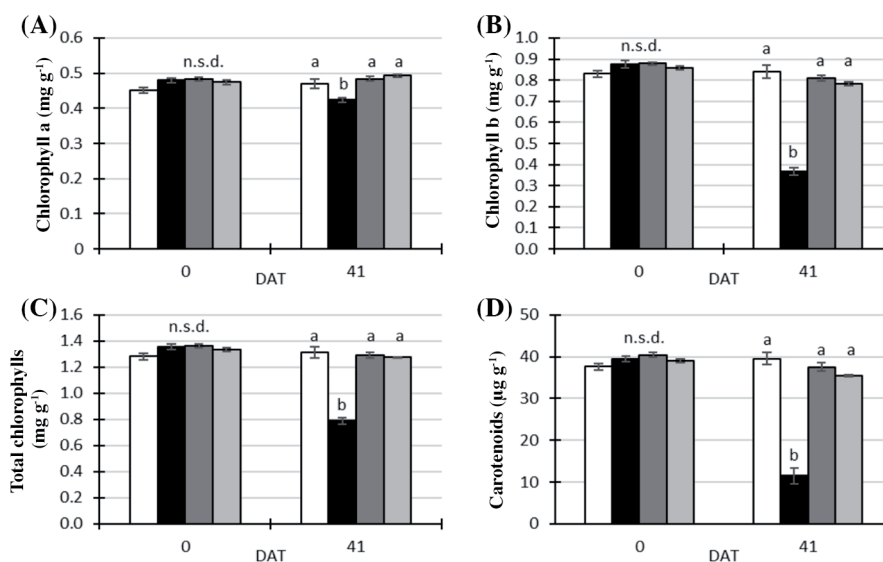


Data are means \pm standard errors based on measurements of plants (six from each block, i.e., $n = 18$ per treatment) subjected to control watering (□), 100 mM NaCl stress (■), 100 mM NaCl stress plus 0.1 mM gibberellic acid (GA_3) (▨), and 100 mM NaCl stress plus 10 mM proline (▩) for 41 d.

For each graphic and date, different letters indicate significant differences ($P < 0.05$) according to ANOVA and Duncan test, except for number of leaves at DAT 0, thickness at DAT -8, and g_s at DAT 22, in which Kruskal-Wallis H test was performed.

DAT: Days after treatment; n.s.d.: nonsignificant differences.

Figure 2. Initial (DAT 0) and final (DAT 41) concentration of chlorophylls a (A), b (B), sum of a and b (C) and carotenoids (D) in ‘Sweet Mary’ *Carica papaya* plants.



Data are means \pm standard errors based on measurements of homogenous lyophilized leaf tissues from each block ($n = 3$ per treatment).

Studied plants were subjected to control watering (□), 100 mM NaCl stress (■), 100 mM NaCl stress plus 0.1 mM gibberellic acid (GA_3) (▨), and 100 mM NaCl stress plus 10 mM proline (▩) for 41 d. For each graphic and date, different letters indicate significant differences ($P < 0.05$) according to ANOVA and Duncan test.

DAT: Days after treatment; n.s.d.: nonsignificant differences.

Table 1. Fresh and dry mass of papaya organs and whole plant prior to foliar pre-treatments (DAT -8), and at the end of the experimental period (DAT 41).

	Treatment	DAT -8		DAT 41	
		Fresh mass (g)	Fresh mass (g)	Dry mass (g)	Dry mass (g)
Leaves	1	9.32 ± 1.64	86.23 ± 3.68a	17.49 ± 0.96a	
	2		5.65 ± 1.26b	0.85 ± 0.17b	
	3		7.04 ± 0.67b	1.24 ± 0.12b	
	4		5.38 ± 0.69b	0.80 ± 0.10b	
Stem	1	20.07 ± 2.68	177.08 ± 8.33a	21.22 ± 1.20a	
	2		34.32 ± 2.21c	4.58 ± 0.26c	
	3		62.81 ± 3.89b	7.77 ± 0.50b	
	4		60.20 ± 2.86b	7.32 ± 0.31b	
Roots	1	18.55 ± 2.88	127.39 ± 6.82a	13.24 ± 0.86a	
	2		22.32 ± 1.56b	3.19 ± 0.16b	
	3		22.91 ± 1.69b	3.21 ± 0.25b	
	4		22.38 ± 1.74b	3.24 ± 0.31b	
Total plant	1	47.94 ± 6.83	390.69 ± 16.33a	51.96 ± 2.69a	
	2		62.29 ± 4.58c	8.61 ± 0.53c	
	3		92.76 ± 5.83b	12.22 ± 0.78b	
	4		87.96 ± 4.87bc	11.35 ± 0.65bc	

Data are means ± standard errors based on measurements of n = 6 plants at DAT -8 and n = 18 plants per treatment at DAT 41. Plants were subjected to the following treatments: control watering (1), 100 mM NaCl stress (2), 100 mM NaCl stress plus 0.1 mM gibberellic acid (GA₃) (3), and 100 mM NaCl stress plus 10 mM proline (4). Different letters denote significant differences (P < 0.05) according to ANOVA and Duncan test. DAT: Days after treatment.

respectively. Finally, it is interesting to note that at whole plant level, the decrease of fresh mass was 84%, 76% and 77% in plants subjected to NaCl, NaCl + GA₃ and NaCl + proline, respectively in comparison with the control. Expressed differently, GA₃ pretreatment yielded to a significant increase of the fresh mass regarding solely stressed plants (49%), and proline pretreatment led to a similar improvement (41%). The same trend was found for plant dry weight in all the organs and treatments (Table 1).

Stomatal closure

Salt stress progressively decreased g_s along the experimental period reaching values around 77 and 93 mmol m⁻² s⁻¹ 22 and 41 DAT, respectively, compared to control (Figure 1D). In contrast, exogenous GA₃ pretreatments increased g_s 165% and 444% 22 and 41 DAT, respectively, with respect to exclusively salt-stressed ones. In turn, proline application similarly to GA₃ increased g_s about 188% and 350% 22 and 41 DAT, respectively. Therefore, at the end of the experiment when stressed plants registered values 93% lesser than control, plants pretreated with GA₃ and proline exhibited reductions about 60% and 67%, respectively.

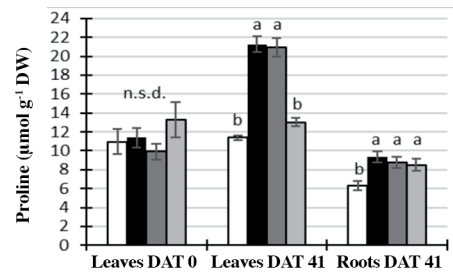
Photosynthetic pigments changes

Carotenoids and Chls concentrations were determined in papaya leaves under stress and non-stress conditions 41 DAT (Figure 2). The Chl a concentration decreased around 11% compared to control in NaCl-stressed plants (Figure 2A); however, plants exogenously pulverized by GA₃ or proline kept their Chl a concentration similar to control. Moreover, Chl b suffered a significant decrease (56% with respect to control) in plants irrigated with NaCl enriched solution, whereas its concentration was maintained like the control in plants previously treated with GA₃ or proline (Figure 2B). Altogether, only NaCl stress reduced total Chls about 40% compared to control, whilst NaCl + GA₃ and NaCl + proline conserved the same amount of total Chls as the control (Figure 2C). In this context, carotenoids concentrations showed the same pattern of change as Chls (Figure 2D). Thus, a significant reduction of this pigment was induced by NaCl treatments (71% compared to control); however, salt stress did not affect its concentrations in plants pretreated with GA₃ or proline.

Proline determination

Proline production was determined at the end of the experiment system in leaf and root tissues. In plants subjected to salt stress and those pretreated with GA₃ too, proline concentrations were 87% and 84% higher than the control (Figure 3). In contrast, the exogenous supply of proline to salt-stressed plants maintained the concentration of endogenous proline like the control 41 DAT. In addition, salt stress increased proline level in root organs about 47% compared to control, and plants pretreated with GA₃ or proline showed similar concentration of endogenous proline than only NaCl-stressed ones.

Figure 3. Initial (DAT 0) and final (DAT 41) concentration of proline in leaves and final (DAT 41) concentration of proline in roots of ‘Sweet Mary’ *Carica papaya* plants.



Data are means \pm standard errors based on measurements of homogenous lyophilized tissues from each block ($n = 3$ per treatment). Studied plants were subjected to control watering (\square), 100 mM NaCl stress (\blacksquare), 100 mM NaCl stress plus 0.1 mM gibberellic acid (GA₃) (\blacksquare), and 100 mM NaCl stress plus 10 mM proline (\blacksquare) for 41 d.

For each date, different letters indicate significant differences ($P < 0.05$) according to ANOVA and Duncan test. DAT: Days after treatment; n.s.d.: nonsignificant differences.

DISCUSSION

We have previously reported that *Carica papaya* plants respond to water stress via the abscission of the old leaves to maintain the turgor of the persisted ones through the osmotic adjustment process (Mahouachi et al., 2012). Data presented here showed a similar tendency regarding leaf number since salinity stress also has an osmotic- before the toxic-ionic component (Munns, 2002), and plants lost 65% of their functional leaves 41 DAT (Figure 1A). Although nonsignificant differences were found in leaf number when plants were sprayed by GA₃ or proline prior to the application of NaCl-stress, a relatively lesser salt damage was observed in leaf number (54% and 58% loss regarding control plants, respectively, Figure 1A). It is important to mention that papaya even under non-stress conditions detaches old leaves to regulate a balanced canopy according to its stage of growth, nutritional and water status. Plant growth expressed as stem height suffered a significant decrease since 22 until 41 DAT by the salt treatment; however, the effects of GA₃ and proline were noticeable on reverting the impact of salinity and even induced a major stem height than that of control plants (Figure 1B). Furthermore, NaCl-stress decreased stem thickness parallelly to the height, although concerning exogenous treatments only GA₃ alleviated the influence of salinity on this parameter (Figure 1C). On the other hand, the impact of salt stress resulted in a strong reduction of plant organs fresh and dry weights, being the leaves and roots the most harmed (Table 1). In contrast, the exogenous treatments of GA₃ or proline modulated the negative effect of salt toxicity on plant weight. Previously, other experimental systems using different genotypes reported compatible results with our findings, such as the reduction of growth and biomass, specifying a major decrease on leaves and roots than on the stems (Sá et al., 2016) and leaf area (Mengarda et al., 2016) induced by salinity conditions.

The involvement of GAs in stem and internode elongation, leaf expansion and growth regulation has been extensively shown in several plant species since the early nineties (Ubeda-Tomás et al., 2009; Nelissen et al., 2012; Taiz et al., 2014). The GAs enhanced plant growth in several species under salt stress conditions (Tuna et al., 2008; Maggio et al., 2010; Nasri et al., 2012; Kandil et al., 2014). It has also been reported that the influence of salt stress on plant growth appears to be associated to the reduction of GAs synthesis, and that exogenous GAs could revert such decrease and induce cell division and/or elongation, leaf area enlargement, and photosynthetic rate (Saeidi-Sar et al., 2013; Niharika et al., 2021). In papaya the application of GAs or GAs and auxin mitigates the effects of salt stress on vegetative growth (Sá et al., 2020). Likewise, exogenous proline increased the plant height and root number in rice under salt stress (Teh et al., 2016).

Besides, proline supply increased nitrate content, nitrate reductase and glutamine synthetase activities in roots, which suggest that proline could alleviate salt stress damages through its ability to regulate the N assimilation activities (Teh et al., 2016).

In relation to g_s and photosynthetic pigments, salt stress continuously reduced g_s throughout the experimental period that reached minimum values 41 DAT, compared to control (Figure 1D). In contrast, exogenous GA₃ and proline significantly increased g_s with respect to only salt-stressed plants. Similar results were obtained in other experimental systems reporting an increase of g_s and water use efficiency induced by GAs under moderate salinity (Maggio et al., 2010). Data presented here also showed a general decrease of Chl a (Figure 2A), Chl b (Figure 2B), total Chls (Figure 2C) and carotenoids (Figure 2D) in plants subjected to salt stress; meanwhile, plants pretreated with GA₃ or proline maintained the concentrations of these pigments at control level. Taken together, the results may indicate that the exogenous treatments (GA₃ or proline) improved the stomatal movement and consequently the photosynthetic pigments concentrations leading to an enhancement of the photosynthetic machinery function under salt stress conditions. Furthermore, data may suggest that GAs as hormonal signal could stimulate cell division and enlargement and consequently promote growth, and proline as an osmoprotectant substance could improve tissue turgidity, and then both molecules contribute to the cell protection leading to a better plant performance under salt excess in the medium. Our results are concomitant with other findings which reported that GAs overcome the negative effects of salt stress by the increase of Chls concentration, N use efficiency, and the mineral nutrient assimilation, resulting subsequently in an increase of yield (Criado et al., 2017; Niharika et al., 2021).

Regarding proline production, it should be noted an increase of its level in plants watered with salt solution and those also exogenously treated with GA₃; however, the application of proline did not alter its endogenous content with respect to control. In roots, salt stress similarly increased proline amounts irrespective of exogenous pretreatments. On the other hand, results showed that the addition of GA₃ did not alter proline concentration indicating that no direct relationship between both molecules since GAs act as a plant growth promoter. Proline has been involved in the maintenance of cell turgor, the stabilization of the membranes, and by scavenging the ROS, and preserving redox balance (Ashraf and Foolad, 2007; Hayat et al., 2012; Tesfaye et al., 2014; Li et al., 2014; Meena et al., 2019). The exogenous supply of proline increased gas exchange, water use efficiency and the antioxidant activities in response to salt stress (Butt et al., 2020). Likewise, exogenous proline increased the concentrations of photosynthetic pigments, ascorbic acid, mineral nutrients, activity of antioxidant enzymes, and endogenous proline in bean under salt stress (Abdelhamid et al., 2013). The reported increase of proline level in this species relatively differs from our data in papaya where exogenous proline only raised slightly its foliar concentration, albeit a significant increase was found in the roots leading to a major production of this osmolyte at plant level. Therefore, the data presented here suggest that the supply of exogenous proline may regulate its biosynthesis under salt stress and avoid major endogenous production to face the stress impact. The potential protective mechanism of proline and the mode of action of GAs on papaya under salt stress should be explored in future experiments.

CONCLUSIONS

The present study showed that the effect of salinity led to a lessening of plant growth parameters (plant fresh mass and stem height and thickness), stomatal conductance and photosynthetic pigments concentration. However, foliar and radicular proline levels increased under salt stress. The pretreatments of gibberellic acid or proline improved the performance of papaya plants against salt stress through the increase of stomatal conductance, chlorophylls and carotenoids concentrations, plant height and biomass.

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