Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252026v3915114rc

Scientific Article

In vitro salt stress in *Physalis angulata*: morphophysiological and biochemical aspects

Estresse salino *in vitro* em *Physalis angulata*: aspectos morfofisiológicos e bioquímicos

Isabela S. C. Conceição[1]* , Gabriela Torres-Silva[1] ,Vitor O. dos Santos[1] , Marilza N. do Nascimento[1] , Alone Lima-Brito[1]

[1]Postgraduate Program in Plant Genetic Resources, Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brazil.

ABSTRACT - Salt stress, prevalent in arid and semi-arid regions, triggers agricultural problems that impair plant growth and development. *Physalis angulata* belongs to the Solanaceae family, which includes plants moderately sensitive to salinity. *In vitro* cultivation serves as a viable alternative for assessing responses to salt stress, as it allows control of environmental conditions. Given this context, the aim of this study was to evaluate the impact of salt stress on the growth, chlorophyll indices and biochemical parameters of *P. angulata* grown *in vitro*. Seedlings of *P. angulata* were germinated on half-strength MS medium; ten days later, the seedlings were transferred to medium of the same composition supplemented with NaCl at concentrations of 0, 25, 50, 75, and 100 mM. Morphological, physiological and biochemical analyses were conducted after three cultivation periods: 10, 20, and 30 days. The results demonstrate that salt stress compromises the growth and cell homeostasis of *P. angulata*. The survival of all plants throughout the cultivation period suggests that the species has some capacity to survive under saline conditions, albeit with a significant reduction in morphophysiological variables, particularly in treatments with higher salinity levels and longer exposure times. Biochemical analyses revealed significant differences among treatments in reducing sugars, total soluble sugars, proteins, and amino acids; notably, proline accumulation increased in proportion to stress intensity, indicating its role as a biochemical marker of stress.

Keywords: Cutleaf Groundcherry. NaCl. Proline.

RESUMO - O estresse salino, ocorrente em regiões áridas e semiáridas, desencadeia problemas agrícolas que prejudicam o crescimento e o desenvolvimento das plantas. A espécie *Physalis angulata* pertence à família Solanaceae, que engloba plantas moderadamente sensíveis à salinidade. Para verificar as respostas ao estresse salino, o cultivo *in vitro* apresenta-se como uma alternativa, uma vez que proporciona o controle das condições ambientais. Diante disso, esse trabalho teve como objetivo avaliar o impacto do estresse salino sobre o crescimento, índices de clorofila e compostos bioquímicos de *P. angulata* cultivada *in vitro*. Assim, sementes de *P. angulata* foram inoculadas em meio de cultura MS ½, e aos dez dias as plantas foram transferidas para meio de mesma composição acrescido de NaCl nas concentrações de 0, 25, 50, 75 e 100 mM. Após três períodos de cultivo, 10, 20 e 30 dias, foram realizadas análises morfofisiológicas e bioquímicas. Os resultados demonstram que o estresse salino compromete o crescimento e a homeostase celular de *P. angulata*. A sobrevivência de todas as plantas durante o cultivo sugere que a espécie apresenta certa capacidade de sobrevivência sob salinidade, embora com redução significativa das variáveis morfofisiológicas, especialmente nos tratamentos com maior salinidade e maior tempo de exposição ao estresse. As análises bioquímicas revelaram diferenças significativas entre os tratamentos para teor de açúcares redutores e solúveis totais, proteínas e aminoácidos, com destaque para o acúmulo de prolina, que apresentou incremento associado à intensidade do estresse, indicando sua atuação como marcador bioquímico de estresse.

Palavras-chave: Fisális. NaCl. Prolina.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

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Received for publication in: November 10, 2025.
Accepted in: September 18, 2026.

Editor in Chief: Aurélio Paes Barros Júnior
Section Editor: João Everthon da Silva Ribeiro

Data Availability: The data that support the findings of this study can be made available, upon reasonable request, from the corresponding author.

***Corresponding author**:
<isabelauefs@gmail.com>

INTRODUCTION

High levels of soil salinity constitute a recurring agricultural problem in several regions of the world, especially in arid and semi-arid areas (CASTRO; SANTOS, 2020). According to FAO (2024) estimates, approximately 1.381 billion hectares of soil worldwide are affected by salinity, representing about 10.7% of the Earth's surface. This scenario represents a significant threat to global agricultural productivity, making the study of salt stress a priority for food security, since it directly interferes with water absorption by roots due to the reduction of osmotic potential in the root environment (SILVA et al., 2018).

Plants use three main strategies to cope with stress: escape, which consists of accelerating their life cycle; avoidance, which is related to minimizing water loss and maximizing its absorption through morphological adaptations such as stomatal closure and reduction of leaf area; and metabolic adjustments associated with stress. They involve the activation of antioxidant systems, maintaining cell turgor and protecting cell structures under low water potentials (KALRA; GOEL; ELIAS, 2024).

Analyzing plant responses to salt stress is fundamental to understanding the physiological mechanisms involved, providing support for genetic improvement programs aimed at increasing the plants' ability to survive salinity (NASCIMENTO et al., 2024). One of the markers of these responses is the endogenous accumulation of free amino acids, especially proline, whose content

increases significantly in plants under stress, becoming a valuable parameter for the selection of resistant genotypes (LIMA et al., 2004).

Species belonging to the Solanaceae family have been the subject of scientific interest, as they exhibit moderate sensitivity to salinity (ALTAF et al., 2023). In this context, *Physalis angulata* stands out as a relevant alternative for studies aimed at characterizing its morphophysiological and biochemical behavior under salt stress conditions. *P. angulata* is notable for its nutritional and economic importance, due to its edible fruits rich in vitamins, minerals, and antioxidants, and for its medicinal potential, attributed to the presence of bioactive compounds such as flavonoids, alkaloids, saponins, and steroids, which confer anti-inflammatory, antioxidant, antimicrobial, and antitumor properties (PILLAI et al., 2022).

Plant tissue culture presents itself as an efficient experimental approach for stress studies in plants, since it allows the standardization of cultivation conditions, minimizing the influence of environmental factors and ensuring greater reliability of the results, in addition to enabling short-term analysis of plant stress response and clonal propagation of genotypes with high genetic fidelity (CELIKLI; AKKELLE; ONUS, 2017; TORRES-SILVA et al., 2025). For the genus *Physalis*, two *in vitro* studies on salt stress are reported, which investigated the deleterious effects of NaCl accumulation (CELIKLI; AKKELLE; ONUS, 2017) and the mitigating effect of silicon against this stress (REZENDE et al., 2018), both in *P. peruviana*.

Given the factors presented, this work aims to analyze the effects of salt stress on *P. angulata* cultivated *in vitro*, under different salt concentrations and different cultivation periods. Changes in growth variables, chlorophyll index, and biochemical compounds were evaluated. The central hypothesis is that increasing the NaCl concentration reduces growth and alters the osmoprotective metabolism of *P. angulata*, leading to a reduction in chlorophyll indices and adjustments in sugar and protein content as part of the ability to survive salt stress.

MATERIALS AND METHODS

In vitro establishment

Seeds of *P. angulata* were obtained from plants cultivated at the Unidade Experimental Horto Florestal (UNEHF) of the Universidade Estadual de Feira de Santana (UEFS), located in the municipality of Feira de Santana under the geographic coordinates 12.268611° S and 38.939167° W (datum SIRGAS 2000). The samples were disinfected in 70% ethanol for 30 seconds followed by a NaOCl solution (2.5% active chlorine) with a drop of neutral detergent for 10 minutes and washed with three rinses in sterile distilled water. *In vitro* establishment was performed in MS culture medium (MURASHIGE; SKOOG, 1962) with half the salt concentrations (MS ½), supplemented with 30 g L^{-1} sucrose and 8 g L^{-1} agar (ROMO-PAZ et al., 2020).

Stress induction

Ten days after seed inoculation, plants approximately 15 mm long were transferred whole to MS ½ culture medium supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar, and NaCl concentrations (0, 25, 50, 75, and 100 mM) (CELIKLI; AKKELLE; ONUS, 2017), and evaluated at 10, 20, and 30 days of cultivation. The experimental design was completely randomized, with a 3 x 5 factorial arrangement (three culture periods x five salt concentrations), totaling fifteen treatments. Each treatment consisted of ten replicates, with each replicate consisting of the average of three tubes, totaling 30 individuals/plants per treatment. Each test tube containing one plant was considered an experimental unit. Evaluations were performed on independent plants for each period due to the destructive nature of the analyses. For the evaluations carried out 30 days after inoculation, one sample was added per replicate, resulting in 40 plants per treatment.

Culture conditions

The cultures were carried out in test tubes (150 x 15 mm) sealed with polyvinyl chloride film containing 10 mL of culture medium and one plant per tube. The medium had its pH adjusted to 5.80 ± 0.01 and was subsequently sterilized in an autoclave for 15 minutes at 121 °C under a total pressure of 1.0 atm. The experiments were conducted in the Plant Tissue Culture Laboratory (LCTV), located at UNEHF of UEFS, and maintained in a growth chamber under fluorescent light with a photon flux density of 60 µmol m^{-2} s^{-1} and a photoperiod of 16 h of light and 8 h of darkness at a temperature of 25 ± 2 °C. The tubes were arranged on metal shelves uniformly distributed throughout the room.

Morphophysiological assessments

For each culture period, the following growth variables were evaluated: shoot length (cm) (SL) and root length (RL), using a millimeter ruler; number of green leaves (NGL) by direct counting; as well as chlorophyll *a*, chlorophyll *b* and total chlorophyll indices using a portable chlorophyll meter (ClorofiLOG ® CFL2060, Falker).

The relative water content (RWC) was evaluated at the end of the experiment at 30 days, according to the methodology of Slatyer and Shmueli (1967). This was done by measuring the fresh mass (FM) of healthy leaves, turgid mass (TM) after immersion in water for 24 hours, and dry mass (DM) after 72 hours in a forced-air oven at 40 ºC. RWC was determined by the equation: RWC = [(FM - DM) / (TM - DM)] × 100.

Also, at the end of the experiment, the percentage of absolute membrane integrity (PAMI) of cell membranes was obtained by measuring the free electrical conductivity (FC) of healthy leaves submerged in water for 24 hours, and the total electrical conductivity (TC) after heating in a water bath (80 ºC) for 1 hour. PAMI was calculated using the equation: PAMI = [1- (FC / TC)] ×100, according to the methodology described by Vasquez-Tello et al. (1990). For both analyses, one sample from each replicate was used, totaling 10 samples per treatment.

Biochemical analyses

Biochemical analyses were performed at the

Germination Laboratory (LAGER), located at UNEHF, UEFS. For the preparation of the extracts, 600 mg of fresh leaf mass and 12 mL of phosphate buffer solution (pH 7.0) were used per treatment. This material was divided into three biological replicates of approximately 200 mg each, with each replicate homogenized separately in 4 mL of buffer. These samples were then centrifuged at 12000 rpm for 15 minutes at 4 °C. The supernatant was collected in a microtube and stored at -80 °C for later analysis. For the analysis of proline content, each biological replicate of leaf tissue was macerated in the presence of 3% sulfosalicylic acid as an extractive solution, following the methodology proposed by Bates, Waldren, and Teare (1973).

For the quantification of soluble protein content, determined colorimetrically by the Bradford method (1976), bovine serum albumin (BSA) was used as a standard; reducing sugars were determined by the dinitrosalicylic acid (DNS) method (MILLER, 1959), using glucose as a standard; total soluble sugars were analyzed by the anthrone method (YEMM; WILLIS, 1954), with glucose as a standard; and for the quantification of amino acids, the Ninhydrin method according to Yemm and Cocking (1955) was used. All biochemical analyses were performed in technical triplicate, and the average of these readings was considered as the biological replicate value. The three biological values were used in the ANOVA. Absorbance readings were performed using quartz cuvettes in a double-beam spectrophotometer (Biospectro SP-220®) at a wavelength of λ= 595 nm for proteins, λ= 540 nm for reducing sugars, λ= 620 nm for total soluble sugars, λ= 570 nm for amino acids and λ= 520 nm for proline.

Statistical analysis

Statistical analyses were performed using SISVAR software version 5.6 (FERREIRA, 2019). The assumptions of normality (Shapiro-Wilk) and homogeneity of variances (Bartlett) were previously verified. In the case of the protein variable, the adjusted residuals were inspected to verify the ANOVA assumptions, which were met. For the regression model, it fitted the variables SL and PAMI, with a high coefficient of determination (R^2), while the other variables were subjected to Tukey's test for comparison of means ($p < 0.05$). The variables total chlorophyll and amino acids were subjected to logarithmic transformation to meet the ANOVA assumptions, and for these variables, multiple comparison tests (Tukey) were performed on the transformed data, while the means presented correspond to the original values. When a significant interaction was found between salt concentration and culture period, the interaction was further analyzed by comparing the concentrations within each period and the periods within each concentration, using Tukey's test.

RESULTS AND DISCUSSION

Morphophysiological analyses

All plants remained alive throughout the experimental period; however, a significant reduction in growth variables, shoot and root lengths, was observed over the days of the experiment, showing that salt stress negatively affected plant growth, even without compromising their viability (Figure 1).

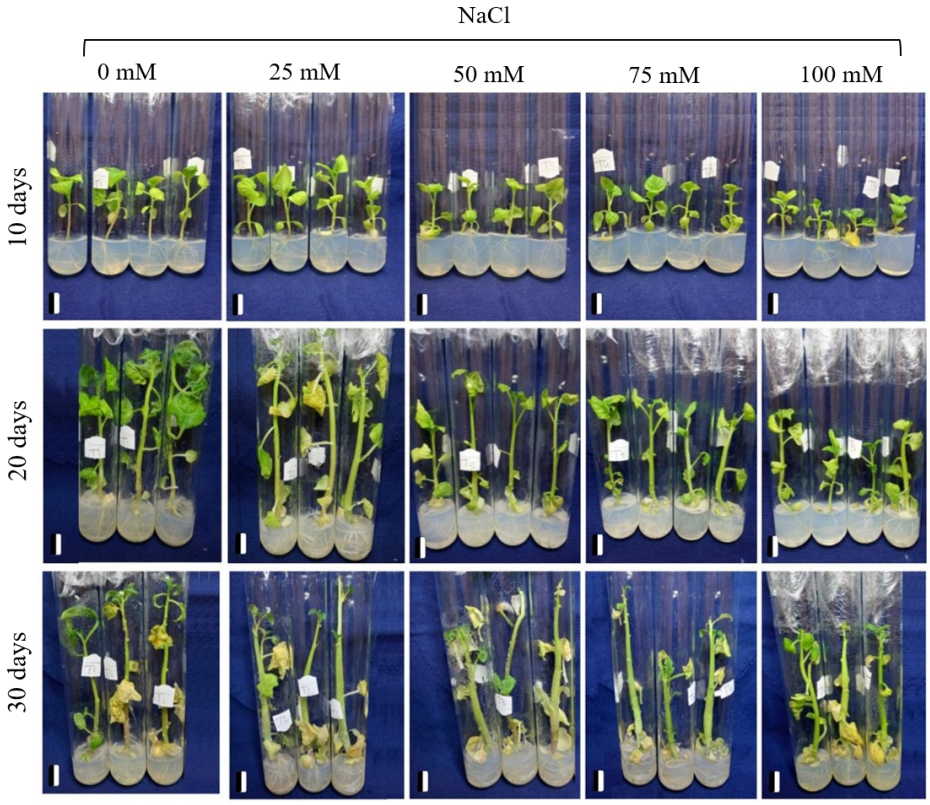


Figure 1. *Physalis angulata* plants subjected to salt stress *in vitro* over 30 days. Bar = 1 cm.

The analysis of variance (Table 1) indicated that the NGL was significantly influenced by both the culture period and the salt concentration individually. The other variables showed a significant response to the interaction between the factors, demonstrating that the salt concentration and the time of exposure to stress modulated, in an integrated way, both the responses of the pigments and the lengths.

Table 1. ANOVA results for the variables shoot length (SL), root length (RL), number of green leaves (NGL), chlorophyll *a* (C *a*), chlorophyll *b* (C *b*) and total chlorophyll (TC). CP = culture period; SC = salt concentration.

Sources of variation	DF	Mean squares					
		SL	RL	NGL	C *a*	C *b*	TC
CP	2	338.180**	234.338**	51.306**	47.828**	13.052**	0.157**
SC	4	78.086**	3.401**	13.656**	3.511 ^{ns}	1.167 ^{ns}	0.007 ^{ns}
SC x CP	8	18.128**	2.051**	2.081 ^{ns}	28.857**	1.577*	0.062**
Residual	135	1.034	0.614	1.101	8.275	0.614	0.016
CV(%)		19.87	14.68	20.47	12.80	16.75	3.93

**Significant at 1% probability level; *Significant at 5% probability level; ns = not significant.

According to statistical analyses, the SL variable showed a significant decreasing linear behavior in response to the increase in salt concentration throughout the experiment, in the three culture periods evaluated (Figure 2).

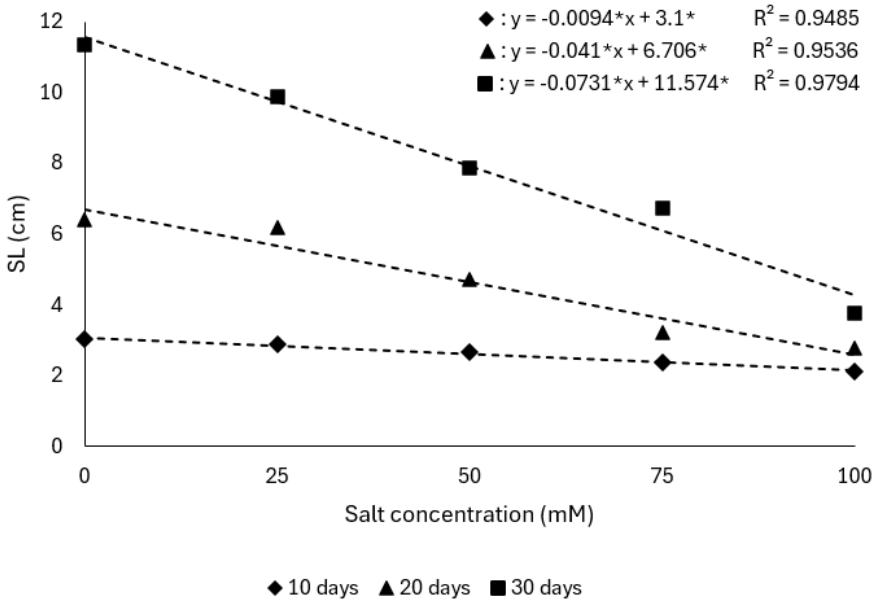


Figure 2. Shoot length (SL) of *Physalis angulata* plants subjected to *in vitro* salt stress over 30 days. *Significant at 5% probability level.

Salt stress triggers physiological and morphoanatomical effects that manifest from the cellular level to the development of the organism as a whole. One of the first impacts observed is the limitation of vegetative growth, evidenced in this study by the reduction in SL. This direct effect on cell expansion and meristematic division results from the decrease in water potential, hindering the absorption of water and essential nutrients (CID; TEIXEIRA, 2017). A similar result was documented in *Physalis peruviana* (CELIKLI; AKKELLE; ONUS, 2017), which showed reduced growth under high salt levels.

The comparison between the means demonstrated that the effect of salinity compromises RL at the highest salt concentration at 10 days of cultivation; with the progression of culture time, at 20 days, the effect of salinity compromising RL was recorded from a concentration of 50 mM (Table 2). The effect of salinity on NGL was observed in plants cultivated with 100 mM NaCl at 20 and 30 days of cultivation, which demonstrates that the leaves are sensitive to longer periods of stress at high NaCl concentrations.

Table 2. Root length and number of green leaves of *Physalis angulata* plants subjected to *in vitro* salt stress over 30 days. CP = culture period.

CP	Salt concentration (mM)				
	0	25	50	75	100
	Root Length				
10	2.9 ± 0.2 Ca	3.0 ± 0.1 Ca	2.9 ± 0.1 Ca	2.5 ± 0.1 Cab	2.2 ± 0.1 Cb
20	6.8 ± 0.3 Ba	6.0 ± 0.2 Bab	5.4 ± 0.2 Bbc	5.8 ± 0.3 Bb	4.9 ± 0.4 Bc
30	7.8 ± 0.2 Aa	6.8 ± 0.3 Aab	6.8 ± 0.1 Aab	6.7 ± 0.1 Aab	6.6 ± 0.3 Ab
	Number of green leaves				
10	4 ± 0.2 Ba	4 ± 0.1 Ba	4 ± 0.2 Ba	4 ± 0.2 Ba	4 ± 0.2 Ba
20	6 ± 0.1 Aa	6 ± 0.1 Aa	6 ± 0.2 Aa	6 ± 0.2 Aa	4 ± 0.4 Ab
30	7 ± 0.3 Aa	6 ± 0.3 Aa	6 ± 0.4 Aa	6 ± 0.3 Aa	4 ± 0.9 Ab

Values indicate mean ± standard error. Means followed by the same letters, uppercase in columns and lowercase in rows, do not differ from each other by Tukey's test at a 5% probability of error.

Associated with growth restriction, salt stress also compromises cellular stability and functionality due to the accumulation of toxic ions such as Na^+ and Cl^-. This accumulation triggers symptoms such as chlorosis and necrosis, leading to a reduction in the number of green leaves and premature senescence (CID; TEIXEIRA, 2017).

The C *a* index showed a significant reduction as early as 10 days of cultivation at the highest concentrations (75 and 100 mM), and at 30 days at all salt concentrations. For C *b*, the impairment due to salinity became evident from 20 days onwards, at concentrations of 75 and 100 mM; and at 30 days, in treatments with salt concentrations from 50 mM onwards (Table 3).

Table 3. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll index of *Physalis angulata* plants subjected to *in vitro* salt stress over 30 days. CP = culture period.

CP	Salt concentration (mM)				
	0	25	50	75	100
	Chlorophyll *a*				
10	23.0 ± 0.9 Ba	22.9 ± 1.4 Aa	22.5 ± 0.5 Aa	20.6±0.8 Bb	19.9±0.6 Bb
20	23.4 ± 0.8 Ba	23.2 ± 0.7 Aa	23.3 ± 0.7 Aa	22.1±0.6 Aab	22.0 ± 0.5 Aab
30	24.1±0.7 Aa	21.1±0.6 Bb	20.9±0.7 Bb	20.7±0.3 Bb	20.6±0.8 Bb
	Chlorophyll *b*				
10	4.5±0.1 Ba	4.4±0.2 Ba	4.4±0.2 Ba	4.1±0.2 Ba	4.0±0.3 Ba
20	5.4±0.3 Aa	5.3 ± 0.2 Aa	5.3±0.1 Aa	4.9±0.2 Ab	4.7±0.1 Ab
30	5.1±0.3 ABa	5.0 ± 0.2 Aa	4.1±0.2 Bb	4.2±0.1 Bb	4.1±0.2 Bb
	Total chlorophyll				
10	27.5 ± 0.9 Aa	27.3 ± 1.5 Aa	26.9±0.7 ABa	24.7±1.0 Bab	23.9 ± 0.7 Bb
20	28.8 ± 1.0 Aa	28.5 ± 0.9 Aa	28.6 ± 0.8 Aa	27.0 ± 0.7 Ab	26.7±0.5 Ab
30	29.2 ± 0.9 Aa	26.1±0.7 Ab	25.0 ± 0.9 Bb	24.9±0.4 Bb	24.7±0.9 Bb

Values expressed as FCI (Falker Chlorophyll Index). Values indicate mean ± standard error. Means followed by the same letters, uppercase in columns and lowercase in rows, do not differ from each other by Tukey's test at a 5% probability of error.

For TC, differences were observed between treatments in all evaluated periods, with emphasis on the longer time of exposure to stress (30 days), when the pigments were affected by all tested salt concentrations. The degradation of chlorophyll pigments observed in this study, evidenced by the reduction in indices with increasing salt concentrations and cultivation period, can be attributed to the greater activity of the chlorophyllase enzyme and the inhibition of the synthesis of the precursor 5-aminolevulinic acid (LIMA et al., 2004). This pattern is consistent with that observed by Celikli, Akkelle and Onus (2017), who also recorded a reduction in chlorophyll after four weeks of *in vitro* salt stress in *P. peruviana*.

According to the analysis of variance (Table 4), salt concentrations had a significant effect ($p < 0.01$) on PAMI. Regarding the RWC variable, there was no significant effect in the ANOVA.

Table 4. ANOVA results for the variables percentage of absolute membrane integrity (PAMI) and relative water content (RWC).

Sources of variation	DF	Mean squares	
		PAMI	RWC
Salt concentrations	4	7130.52**	267.60 ^{ns}
Residual	45	113.63	263.41
CV (%)		19.77	25.33

**Significant at 1% probability level. ns = not significant.

According to the statistical regression analysis, the PAMI variable showed a decreasing linear trend as a function of increasing NaCl concentration (Figure 3), characterizing lower membrane integrity in plants cultivated with higher salt concentration, reaching a significant reduction in plants in the 100 mM NaCl treatment compared to the control treatment. This result illustrates the functional impairment of cells, resulting from increased permeability of the lipid bilayer and loss of electrolytes, which impairs cellular homeostasis, selective transport, and intracellular signaling, potentially culminating in programmed cell death (LIU; JIANG; YUAN, 2024). In agreement, Hannachi et al. (2022) reported increased electrolyte leakage from two eggplant cultivars under saline conditions. The mathematical regression model did not fit the data obtained for the RWC variable.

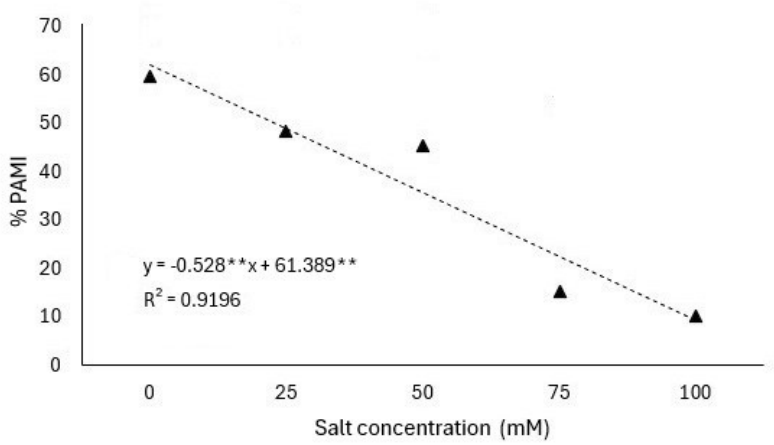


Figure 3. Percentage of Absolute Membrane Integrity (PAMI) of membranes of *Physalis angulata* plants subjected to salt stress after 30 days of *in vitro* culture. **Significant at 1% probability level.

Biochemical analyses

The analysis of variance (Table 5) showed a significant interaction between salt concentrations and culture periods for all biochemical variables.

The accumulation of RS was affected by salt stress throughout the *in vitro* culture period. At 10 days, the means obtained at the highest concentrations were significantly higher than those obtained with the control and the lowest concentration tested (25 mM), while at 20 and 30 days only the highest concentration (100 mM) showed significant superiority over the other treatments (Figure 4A). Similarly, TSS also showed this pattern, with greater accumulation in plants cultivated under 75 and 100 mM NaCl at 10 and 20 days. At 30 days, TSS production was not affected by salinity (Figure 4B).

The structural, physiological, and cellular integrity restrictions discussed earlier may have resulted in adaptive metabolic changes, highlighting the complexity of the systemic response to salt stress, as also observed biochemically with the increased production of sugars identified in treatments with higher salt concentrations. This mechanism was also found in eggplant (ALKHATIB; ABDO; MHEIDAT, 2021); however, discrepancies in the literature are reported, such as in the study by Zamljen et al. (2022), who observed a reduction in sugar content in pepper cultivars under NaCl. These differences may be related to both the genotype analyzed and the methodological conditions (exposure time, environmental conditions), which directly modulate the physiological response.

Table 5. ANOVA results for the variables reducing sugars (RS), total soluble sugars (TSS), amino acids, proteins, and proline. CP = culture period; SC = salt concentration.

Sources of variation	DF	Mean squares				
		RS	TSS	Amino acids	Proteins	Proline
CP	2	0.00112**	19.76861**	0.59588**	0.00555 ^{ns}	3.20891**
SC	4	0.00004 ^{ns}	7.44864**	0.12902**	0.00314 ^{ns}	1.33506**
SC x CP	8	0.00026**	6.17558**	0.03634*	0.01938*	0.36451**
Residual	135	0.00002	0.41756	0.013643	0.00686	0.02729
CV(%)		23.18	10.86	23.91	58.99	26.89

**Significant at 1% probability level; *Significant at 5% probability level; ns = not significant.

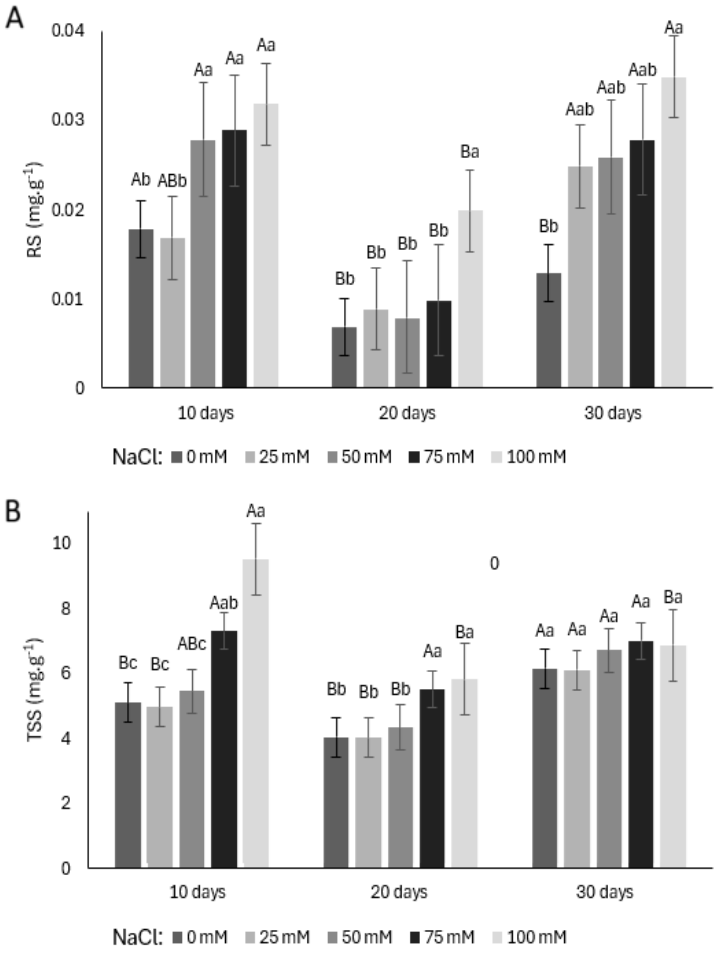


Figure 4. Contents of reducing sugar - RS (A) and total soluble sugars - TSS (B) of *Physalis angulata* plants subjected to *in vitro* salt stress over 30 days. Identical uppercase letters above bars of the same color (salt concentration) and identical lowercase letters above bars of the same group (cultivation period) do not differ from each other by Tukey's test ($p < 0.05$).

Protein content showed significantly higher means at 10 and 20 days of cultivation, at concentrations of 75 and 100 mM. At 30 days, there was no effect of salinity (Figure 5A). These differences may be associated with the expression of defense genes, synthesis of osmoprotectants, and production of antioxidant enzymes, which are essential for preserving cell integrity, neutralizing reactive oxygen species (ROS), and maintaining ionic homeostasis (SINGH et al., 2022).

The production of AA showed a reduction in its levels in the treatments subjected to the highest salt concentration in all three periods evaluated (Figure 5B). At 10 days, concentrations of 75 and 100 mM affected the AA content, and over time, this impairment occurred only at the highest salt concentration (100 mM). This pattern observed in this study demonstrates a probable metabolic prioritization of the plant in forming functional proteins at the expense of maintaining free AA under stress conditions.

The increase in salt concentration in the medium

induced a significant increase in proline levels in all three culture periods (Figure 5C). After 10 days of stress exposure, plants in the 100 mM NaCl treatment showed a major increase compared to plants in the control treatment. At 20 and 30 days, plants in the 75 and 100 mM NaCl treatments showed a large increase compared to the control, after 30 days of stress exposure.

Among amino acids, proline is considered a biochemical marker, as its increasing accumulation under conditions of higher salt stress demonstrates its function as a compatible osmolyte (LIMA et al., 2004). This amino acid contributes to osmotic adjustment, stabilization of membranes and cellular structures, maintenance of metabolic activity, and also acts in the neutralization of ROS (SINGH et al., 2022). In the present study, this pattern is confirmed in plants subjected to the highest concentration of NaCl, which showed the highest proline values in all three cultivation periods. This finding reinforces the role of proline as a bioindicator of stress, corroborating the study by Sun et al. (2025), who also found an increase in proline content in eggplant roots under salt stress.

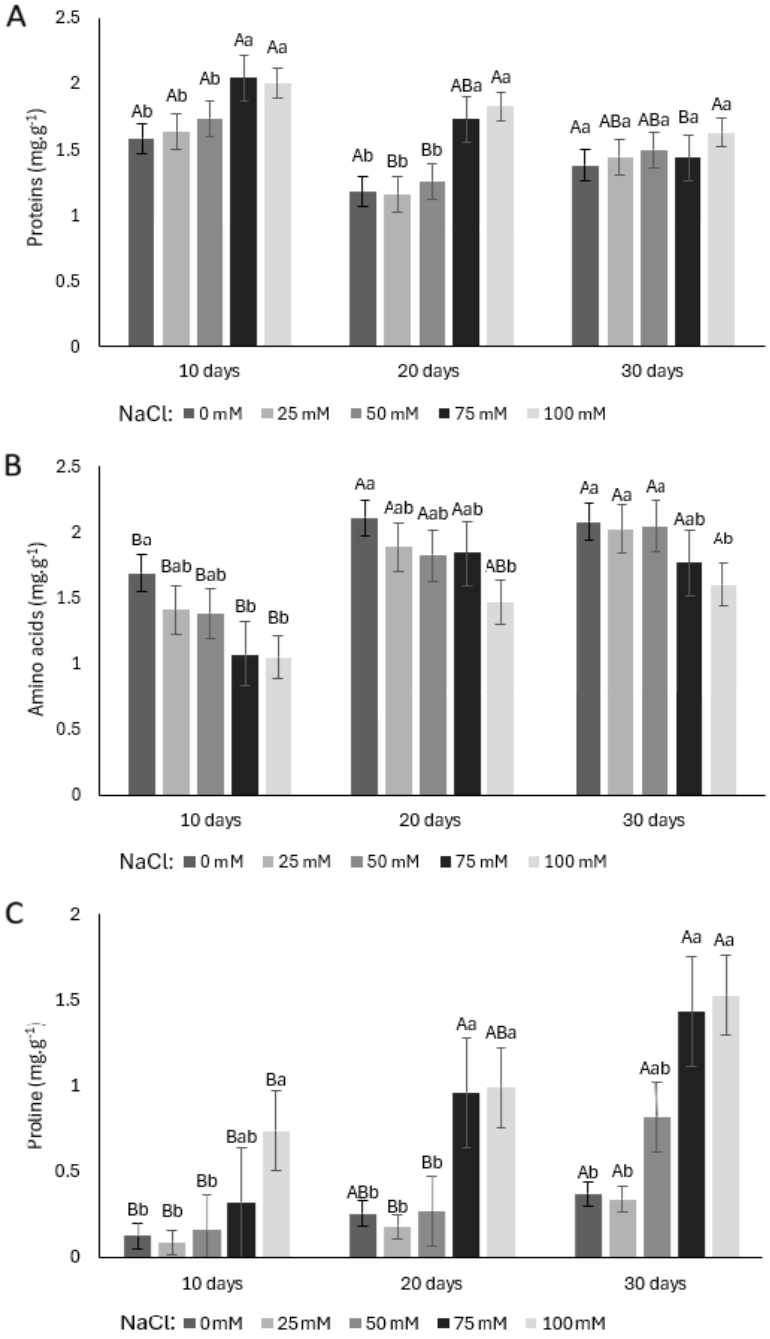


Figure 5. Protein (A), amino acid (B) and proline (C) contents of *Physalis angulata* plants subjected to *in vitro* salt stress over 30 days. Identical uppercase letters above bars of the same color (salt concentration) and identical lowercase letters above bars of the same group (cultivation period) do not differ from each other by Tukey's test ($p < 0.05$).

In an integrated manner, the results obtained reveal that salt stress impacts various physiological levels in the plant, from growth to cellular integrity and metabolic adaptations. It should also be noted that the experiments were conducted *in vitro*, a condition that, while allowing for a controlled environment, does not reproduce all environmental factors, partially limiting the extrapolation of the data.

CONCLUSION

Physalis angulata L. maintained survival up to 100 mM NaCl in the *in vitro* system, but showed alterations in growth, chlorophyll indices, and membrane integrity. Biochemical responses depended on salt concentration and exposure time, with greater proline accumulation at higher concentrations. The results characterize morphophysiological and biochemical responses to salt stress, but do not, in isolation, allow the species to be classified as tolerant.

ACKNOWLEDGEMENTS

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil (CNPq) for the financial support for this research and the Unidade Experimental Horto Florestal of the Universidade Estadual de Feira de Santana for the structural support.

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