












ORIGINAL PAPER

Anatomical variation of *Capsicum chinense* leaves subjected to proline application and salt stress

Ana Carolina Bezerra¹, Marília Hortência Batista Silva Rodrigues², Thiago Jardelino Dias³, Camila Firmino de Azevedo⁴, Luana da Silva Barbosa¹, Franciscleudo Bezerra da Costa², Valéria Fernandes de Oliveira Sousa², João Henrique Barbosa da Silva¹, Vitor Araújo Targino¹, Juliane Maciel Henschel¹ & Toshik Iarley da Silva^{5*}

¹Federal University of Paraíba, Areia, Paraíba, Brazil

²Federal University of Campina Grande, Pombal, Paraíba, Brazil

³Federal University of Paraíba, Bananeiras, Paraíba, Brazil

⁴State University of Paraíba, Lagoa Seca, Paraíba, Brazil

⁵Federal University of Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil

Abstract: Plants adapt to saline conditions through various strategies, including changes in their morphology and anatomy. Thus, this study aimed to investigate the influence of salt stress and proline application on the leaf anatomy of ‘Biquinho’ pepper (*Capsicum chinense*) cultivated under salt stress conditions. Fully expanded leaves were sampled from four plants for each combination of treatments, which included five levels of irrigation water salinity (0.50, 1.30, 3.25, 5.20, and 6.00 dS m⁻¹) and five doses of proline (0.00, 2.90, 10.00, 17.09, and 20.00 mM). The sections were hand-cut transversely using a sharp blade, cleared with 1% sodium hypochlorite, stained with 10% safranin, mounted on semi-permanent slides with glycerin, and photomicrographed under a microscope. The variables analyzed included the thickness of the adaxial cuticle, thickness of the abaxial cuticle, thickness of the adaxial epidermis, thickness of the abaxial epidermis, length of the vascular bundle, diameter of the vascular bundle, thickness of the palisade parenchyma, thickness of the spongy parenchyma, leaf thickness, palisade to spongy parenchyma ratio, stomatal density of the adaxial epidermis, and stomatal density of the abaxial epidermis. Salt stress in *C. chinense* caused a reduction in abaxial cuticle thickness and an increase in adaxial epidermis thickness. The vascular bundle diameter and leaf thickness were also affected. The application of proline at a concentration of 5.35 mM increased the thickness of the adaxial cuticle. Conversely, 20 mM of proline enhanced the thickness of the abaxial epidermis, palisade to spongy parenchyma ratio, and stomatal density. These results support the notion that proline improves the leaf anatomy of peppers, potentially increasing their tolerance to salt stress.

Keywords: Salinity, amino acid, anatomy.

* Corresponding author: E-mail: toshik@ufrb.edu.br

Editors: Mairton Gomes da Silva & Petterson Costa Conceição Silva

Received in: 06 August, 2024

Accepted in: 15 October, 2024

Introduction

Peppers of the genus *Capsicum* are common in everyday life, whether fresh in dishes and salads, dried as condiments, or processed into jellies, sauces, and appetizers (Sanatombi and Rajkumari, 2020). Among the cultivated species, *Capsicum chinense* Jacq. is widely grown in various regions of Brazil (Ribeiro et al., 2018), with the 'Biquinho' genotype being particularly popular and extensively cultivated (Martinez et al., 2021). However, this crop can be affected by various abiotic stresses, including salinity (Sá et al., 2019).

Salinity stands as a significant environmental stressor for plants in arid and semi-arid regions (Al-Elwany et al., 2022). It's estimated that one-third of irrigated land globally contends with salinity, exacerbating desertification (Shaaban et al., 2022). Roughly 23% of agricultural lands and 33% of irrigated areas grapple with this issue, which worsens due to improper fertilization and irrigation practices. Projections suggest that by the mid-century, 50% of agricultural lands could fall victim to salt stress (Shahid et al., 2018; Chourasia et al., 2022).

Salinity increases the content of reactive oxygen species (ROS) in plant cells, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^\cdot), which are overproduced from chloroplasts during photosynthesis (Sachdev et al., 2021). This surge in ROS levels can cause profound or irreversible effects on tissue and organ development, often resulting in abnormal growth or plant death (Mittler, 2017; Tognetti et al., 2017). ROS interact with epigenetic modifiers and hormones, influencing plant development and stress responses (Zeng et al., 2017; Kong et al., 2018). At higher levels, ROS pose a significant threat, potentially leading to DNA damage and triggering programmed cell death (Huang et al., 2019).

Several morpho-anatomical modifications occur in leaves under salinity conditions, and these changes can indicate greater tolerance to stress. Among the most

relevant anatomical adaptations is the increase in cuticle thickness, which acts as a physical barrier against excessive water loss, protecting the plant from dehydration. Additionally, the thickening of the epidermis, especially on the adaxial side of the leaves, can also be a protective response, helping the plant minimize the excessive absorption of toxic ions. The thickness of internal tissues, such as palisade and spongy parenchyma, can indicate greater photosynthetic efficiency under adverse conditions, optimizing the use of light and nutrients. Another important aspect is stomatal density, which can influence gas exchange regulation and transpiration. Increased or optimized stomatal density can help the plant balance the need for CO_2 absorption for photosynthesis with water loss through transpiration. These anatomical changes, when present, are indicative of greater resilience and tolerance to salt stress and should be clearly discussed in the introduction, justifying the importance of the research and the impact of the results obtained.

The authors should correlate the importance of the Biquinho pepper with its potential production in saline areas, establishing it as an alternative crop that could promote socio-economic development in these regions. Highlighting this practical significance emphasizes the need for research that explores solutions for saline soil challenges.

Plants respond to saline conditions through various strategies, including ion homeostasis and compartmentalization, ion transport, osmotic adaptation, stimulation of antioxidant machinery, and polyamine biosynthesis (Freitas et al., 2019). Morphoanatomical modifications in roots, stems, and leaves, as well as alterations in the size and number of organelles such as chloroplasts, mitochondria, and peroxisomes, are crucial adaptations to cope with salinity. These changes help optimize water and nutrient use, enhance resistance to reactive oxygen species, and maintain

ionic homeostasis within cells (Acosta-Motos et al., 2017).

Given that plants can exhibit an increase in the number and size of chloroplasts and a reorganization of chloroplast membranes to optimize photosynthesis despite adverse conditions, coupled with a reduction in mesophyll cell size and an increase in intercellular space, they can enhance water use efficiency and maintain cellular turgidity (Srivastava, 2022). These morphoanatomical adaptations are crucial for plant survival and productivity in high salinity conditions.

The application of compatible osmolytes, such as proline, can also lead to modifications, as proline contributes to maintaining the integrity of mitochondria and peroxisomes, thereby aiding plants in managing oxidative damage caused by excess ROS produced under high salinity conditions (Srivastava, 2022). Proline acts as a compatible protein hydrotrope, enhancing the solubility of hydrophobic compounds (Butt et al., 2020), supports cytoplasmic acidosis, and maintains the NADP⁺/NADPH ratios necessary for cellular metabolism. The application of proline can improve water use efficiency and plant water relations under salt stress by inhibiting water efflux and stabilizing cellular membranes. Furthermore, foliar application of proline enhances stomatal conductance, transpiration, photosynthetic rates, and water use efficiency (Kaya and Tuna, 2006).

Soil salinity presents a significant challenge to agriculture, particularly for solanaceous crops like *C. chinense*, adversely affecting plant growth and development. One noticeable consequence of this stress is observed in leaf anatomy, where the thickness of the cuticle and epidermis is influenced, compromising the plants' resilience to stress and cellular integrity. This underscores the necessity to explore mitigating agents to alleviate these detrimental effects, such as proline.

Although proline is recognized for its role in regulating plant growth,

development, and physiology in response to salt stress, the understanding of its specific effects on leaf anatomy, particularly in 'Biquinho' pepper plants, remains limited. While previous research, such as that by Butt et al. (2016), has primarily focused on the metabolic and physiological aspects of proline, there is a significant gap in understanding how it directly influences the production and deposition of anatomical components essential for the acclimatization of 'Biquinho' pepper plants to salt stress.

These adaptations play a crucial role in water conservation within plant tissues under stress conditions. From this perspective, this study tests the hypothesis that the exogenous application of proline to 'Biquinho' pepper plants cultivated under salt stress results in anatomical modifications in the leaves that contribute to enhanced salt stress resistance by stabilizing cellular structure and improving water use efficiency.

Given the increasing salinization of agricultural lands, the cultivation of salinity-tolerant crops like the 'Biquinho' pepper offers a promising alternative. The increasing salinity stress not only threatens crop yield but also exacerbates socio-economic challenges in affected areas, emphasizing the importance of research focused on enhancing the resilience of crops. Additionally, the physiological acclimations of the 'Biquinho' pepper to saline conditions, including changes in leaf anatomy, are critical to its survival and productivity. Understanding these adaptations can provide insights into effective farming practices and contribute to the sustainability of agriculture in saline-prone areas. By promoting the cultivation of the 'Biquinho' pepper, it can provide smallholder farmers with viable options that enhance food security and economic stability, highlighting the practical significance of this research. Therefore, this study aimed to investigate the influence of salt stress and proline application on the leaf anatomy of 'Biquinho' pepper (*Capsicum*

chinense) cultivated under salt stress conditions.

Materials and Methods

Experiment site

The experiment was conducted in a protected environment at the Center for Agricultural Sciences (CCA), belonging to the Federal University of Paraíba (UFPB), in Areia (6° 57' 42" S, 35° 41' 43" W, 573 m), Paraíba, Brazil, with a climate classified as As', characterized by hot and dry summers and rainfall in the winter. The structure was covered with a polyethylene material, which is known for its high light transmittance, typically around 80-90%. This covering allows a significant amount of direct and diffused light to enter the greenhouse, promoting optimal photosynthetic conditions for plant growth. During the experiment, it was observed that approximately 60% of the light entering the greenhouse was direct sunlight, while about 40% was diffused light. The average temperature and relative humidity observed during the experiment was 31.7°C and 57.8%, respectively. Data were recorded daily (Figure 1) using a digital thermo-hygrometer model AK28 (AKSO®, São Leopoldo, RS, Brazil) installed within the protected environment.

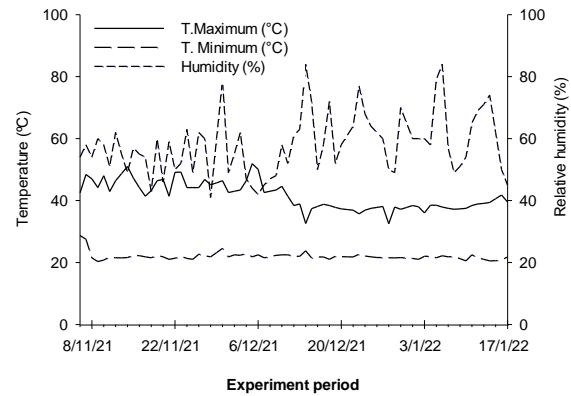


Figure 1: Maximal and minimal temperatures, and relative humidity monitored within the protected environment throughout the experimental period.

Experimental design

The treatments were distributed in randomized blocks using the Box central composite design (Mateus et al., 2001; Tekindal et al., 2014), combining electrical conductivity of irrigation water (ECw) and proline concentrations (PRO). The minimum ($-\alpha$) and maximum (α) values ranged from 0.5 to 6.0 dS m⁻¹ for ECw and from 0 to 20 mM for proline, totaling nine treatments, with four replicates and four plants per replicate (Table 1).

Table 1: Treatments generated by the Box central composite design

Treatments	Electrical conductivity (dS m ⁻¹)	Proline (mM)
1	0.50	10.00
2	1.30	2.90
3	1.30	17.09
4	3.25	0.00
5	3.25	10.00
6	3.25	20.00
7	5.20	2.90
8	5.20	17.09
9	6.00	10.00

Plant material

Seeds of 'Biquinho' pepper (*Capsicum chinense*) subcategory Pimenta Doce (Feltrin® Sementes, Farroupilha, RS, Brazil) were sown in plastic trays with 200 cells, filled with commercial substrate

Mecplant® (MecPrec Ind. e Com. LTDA., Telêmaco Borba, PR, Brazil) and three seeds per cell were placed. After emergence, the seedlings were transplanted into polyethylene plastic pots with a volumetric capacity of 5.0 dm³, filled with

4.5 kg of soil, cattle manure, and fertilized according to soil analysis (Table 2) and recommendations for bell pepper cultivation (Cavalcanti, 2008). Irrigation

was carried out daily with the volume established by the field capacity method.

Table 2: Chemical attributes of the substrate used in the experiment

pH	P	K ⁺	Na ⁺	H ⁺ +Al ³⁺	Al ³⁺	Ca ²⁺	Mg ²⁺	SB	CEC	V	OM
H ₂ O (1:2.5)	---mg dm ⁻³ --					-----cmol _c dm ⁻³ -----				%	g kg ⁻¹
5.97	45.68	202.0	0.51	9.9	0.5	1.4	1.4	3.31	13.21	25.05	8.16

pH – soil solution hydrogen potential; P – phosphorus; K⁺ – potassium; Na⁺ – sodium; H⁺+Al³⁺ – potential soil acidity; Al³⁺ – aluminum; Ca²⁺ – calcium; Mg²⁺ – magnesium; SB – sum of bases (Ca²⁺ + Mg²⁺ + Na⁺); CEC – cation exchange capacity = [SB + (H⁺ + Al³⁺)]; V – base saturation = (SB/CEC) x 100; OM – organic matter.

Anatomical analysis

Leaf sampling for microscopic analysis was conducted at the end of the experiment (60 days after trasplanting). Ten fully expanded leaves were sampled from four plants per treatment combination. The selected materials for anatomical analyses were fixed in FAA solution (5% formaldehyde, 5% acetic acid, and 90% of 70% ethanol) for 48 h and stored in 70% ethanol (Johansen, 1940). Cross-sections were hand-cut using a sharp blade (with Styrofoam support), then cleared with 1% sodium hypochlorite, stained with 10% safranin, mounted on semi-permanent slides with glycerin, and photographed under a microscope.

Variables analyzed

For the evaluation of adaxial cuticle thickness (µm), abaxial cuticle thickness (µm), adaxial epidermis thickness (µm), abaxial epidermis thickness (µm), vascular bundle length (µm), vascular bundle diameter (µm), palisade parenchyma thickness (µm), spongy parenchyma thickness (µm), leaf thickness (µm), palisade to spongy parenchyma ratio (µm), adaxial epidermal stomatal density (µm²), and abaxial epidermal stomatal density (µm²), leaves were always taken from the same node (Figure 2).

The epidermis and cuticle thickness of the stem and leaf were determined by averaging measurements from five different sections of the same stem or leaf using the

Hayear[®] program. Stomatal counting was conducted using paradermal sections of leaves, with a base of 1 mm², using a Neubauer counting chamber.

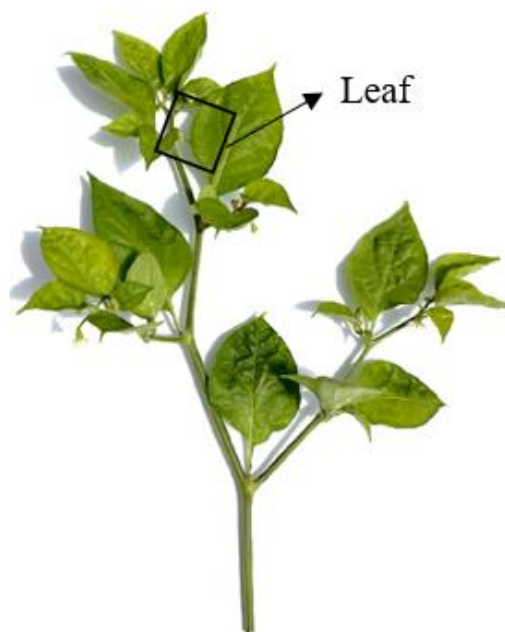


Figure 2: Location of the sections and measurements of *Capsicum chinense*.

Statistical analysis

The data were subjected to normality analysis of residuals using the Shapiro-Wilk test, homogeneity of variances using the Bartlett test, followed by analysis of variance using the F test ($p \leq 0.05$), and polynomial regression analysis up to the second degree. These analyses were conducted using the R 3.6.3 platform with the ExpDes.pt package (Ferreira et al., 2018). The response surface equations were

generated using the rsm package (Lenth, 2009) and GA (Scrucca, 2013).

Results

For the adaxial cuticle thickness of pepper leaves, maximum values of 17.97 μm were observed at a proline concentration of 0.0 mM and low ECw of 0.50 dS m^{-1} (Figures 3a and 4). In contrast, the thickness of the abaxial cuticle decreased with increasing salinity, with a reduction from 15.57 to 12.28 μm (a decrease of 21.13%) between the conductivity of 0.5 and 6.0 dS m^{-1} , without the application of proline. With proline application, a higher value of 17.91 μm was obtained in plants grown under ECw of 0.52 dS m^{-1} and sprayed with a concentration of 0.02 mM (Figures 3b and 4).

Concerning the adaxial epidermis thickness of pepper leaves, a linear increase was observed with rising water salinity. There was an increment of 5.30 μm in adaxial epidermis thickness compared to plants subjected to the lowest ECw of 0.5 dS m^{-1} , reaching a maximum value of 115 μm at ECw of 6.0 dS m^{-1} (Figures 3c and 4). Additionally, as proline doses increased, there was a 3.67% increment in adaxial epidermis thickness at the highest dose (20 mM) compared to the lowest dose utilized (Figures 3d and 4).

The thickness of the abaxial epidermis also did not show a significant interaction between the evaluated factors, exhibiting a linear reduction with increasing water salinity up to an ECw of 4.31 dS m^{-1} , where estimated minimum values of 101.09 μm were observed. From this point onwards, there was a tendency for an increase (3.40%) in thickness, reaching 104.37 μm at an ECw of 6.0 dS m^{-1} (Figures 3e and 4). On the other hand, the thickness of the abaxial epidermis under foliar proline application exhibited a quadratic behavior with increasing concentrations, reaching an estimated maximum value of 113.37 μm at a concentration of 8.59 mM, followed by a reduction of 17.37% at the highest ECw,

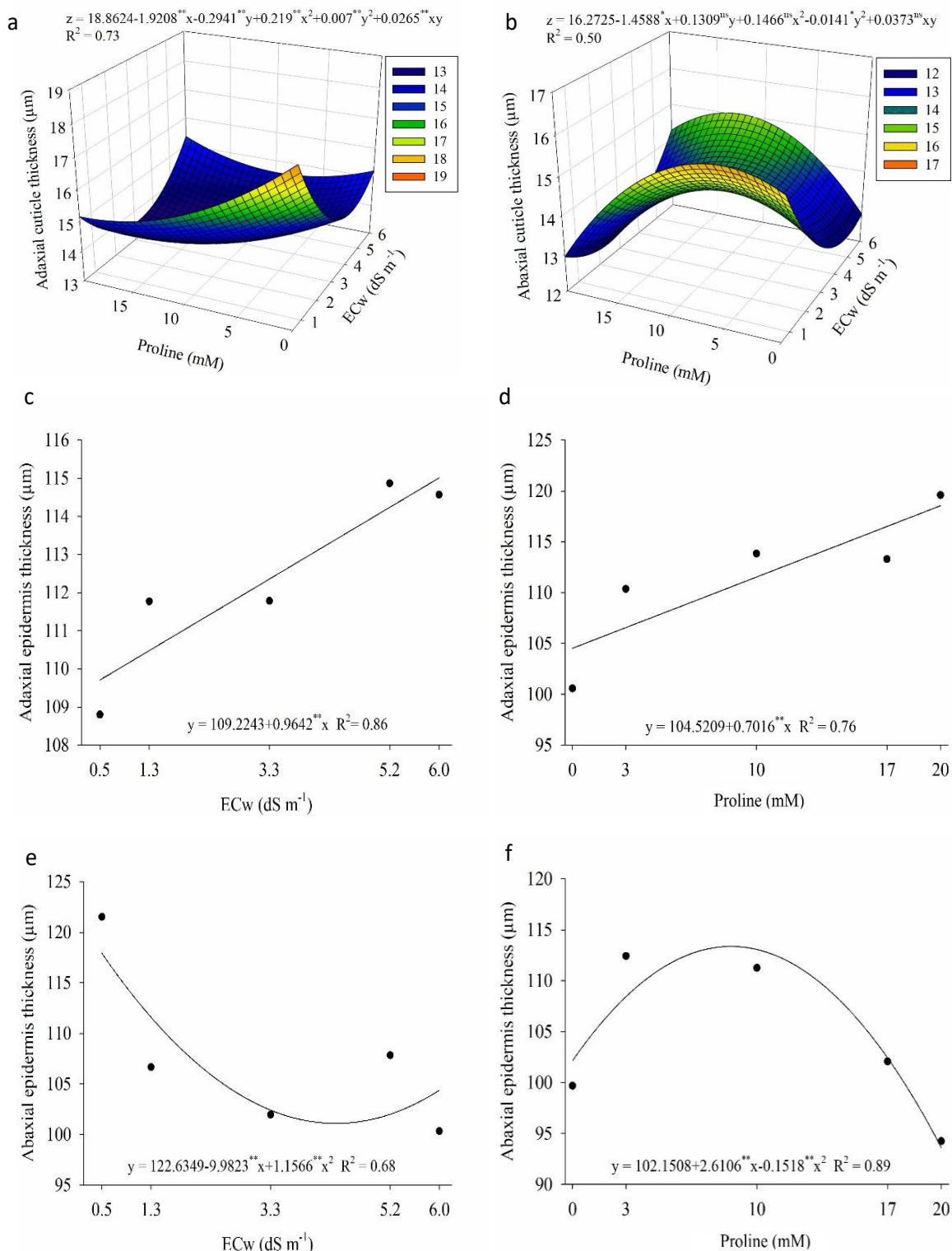
reaching a thickness of 93.64 μm (Figures 3f and 4).

The diameter of the vascular bundle in *C. chinense* leaves did not show a significant interaction between the evaluated factors, displaying a quadratic behavior in response to the increase in ECw. It reached an estimated minimum value of 246.73 μm at an ECw of 4.96 dS m^{-1} , followed by a trend of increase (Figures 5a and 6). Regarding the increase in applied proline concentration, the diameter of the vascular bundle in plants decreased linearly, showing a reduction of 15.12% at the highest concentration (20 mM) compared to plants that did not receive the mitigator (Figures 5b and 6).

The longest length of the vascular bundle in plants, measuring 910.14 μm , was observed in *C. chinense* cultivated under an ECw of 5.99 dS m^{-1} with the application of 0.05 mM of proline (Figures 5c and 6).

The greatest leaf thickness, measuring 404.39 μm , was recorded in plants cultivated under an ECw of 5.99 dS m^{-1} without proline application (0.00 mM), as shown in Figures 7a and 8. Additionally, higher values in spongy parenchyma thickness, reaching 251.76 μm , were observed under the same salinity conditions and absence of proline (Figures 7b and 8).

The thickness of the palisade parenchyma showed a reduction with increasing proline concentrations, being most significant (140.62 μm) at a concentration of 11.42 mM (Figures 7c and 8), followed by a trend of growth with increasing concentration of the mitigator. On the other hand, the ratio between palisade and spongy parenchyma was higher (0.95 μm) in plants cultivated under an ECw of 3.61 dS m^{-1} with the application of proline at a concentration of 19.99 mM (Figures 7d and 8). Regarding the highest stomatal density of the abaxial epidermis (53.29 μm^2), it was observed in plants subjected to an ECw of 0.50 dS m^{-1} and a proline concentration of 19.97 mM (Figures 9 and 10).



** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, and ns – not significant by the F-test.

Figure 3: Adaxial cuticle thickness (a), abaxial cuticle thickness (b), adaxial epidermis thickness (c and d), and abaxial epidermis thickness (e and f) of *Capsicum chinense* seedlings subjected to salt stress and proline.

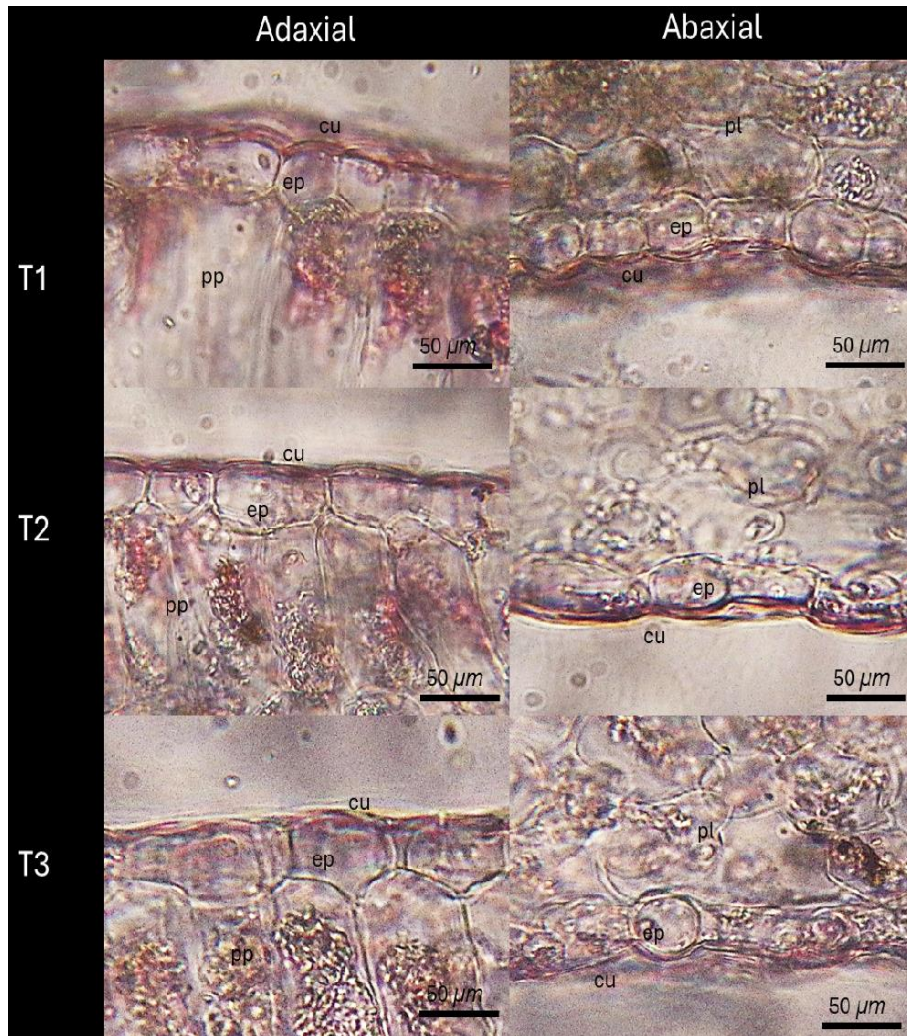


Figure 4: Thickness of the adaxial and abaxial epidermis and of the cuticle of the abaxial and adaxial leaf surface of *Capsicum chinense* subjected to salt stress and proline. T1 – ECw of 0.50 dS m^{-1} and 10.00 mM proline; T2 – ECw of 1.30 dS m^{-1} and 2.90 mM proline; T3 – ECw of 1.30 dS m^{-1} and 17.09 mM proline; ep – epidermis; cu – cuticle; pp – palisade parenchyma; pl – spongy parenchyma.

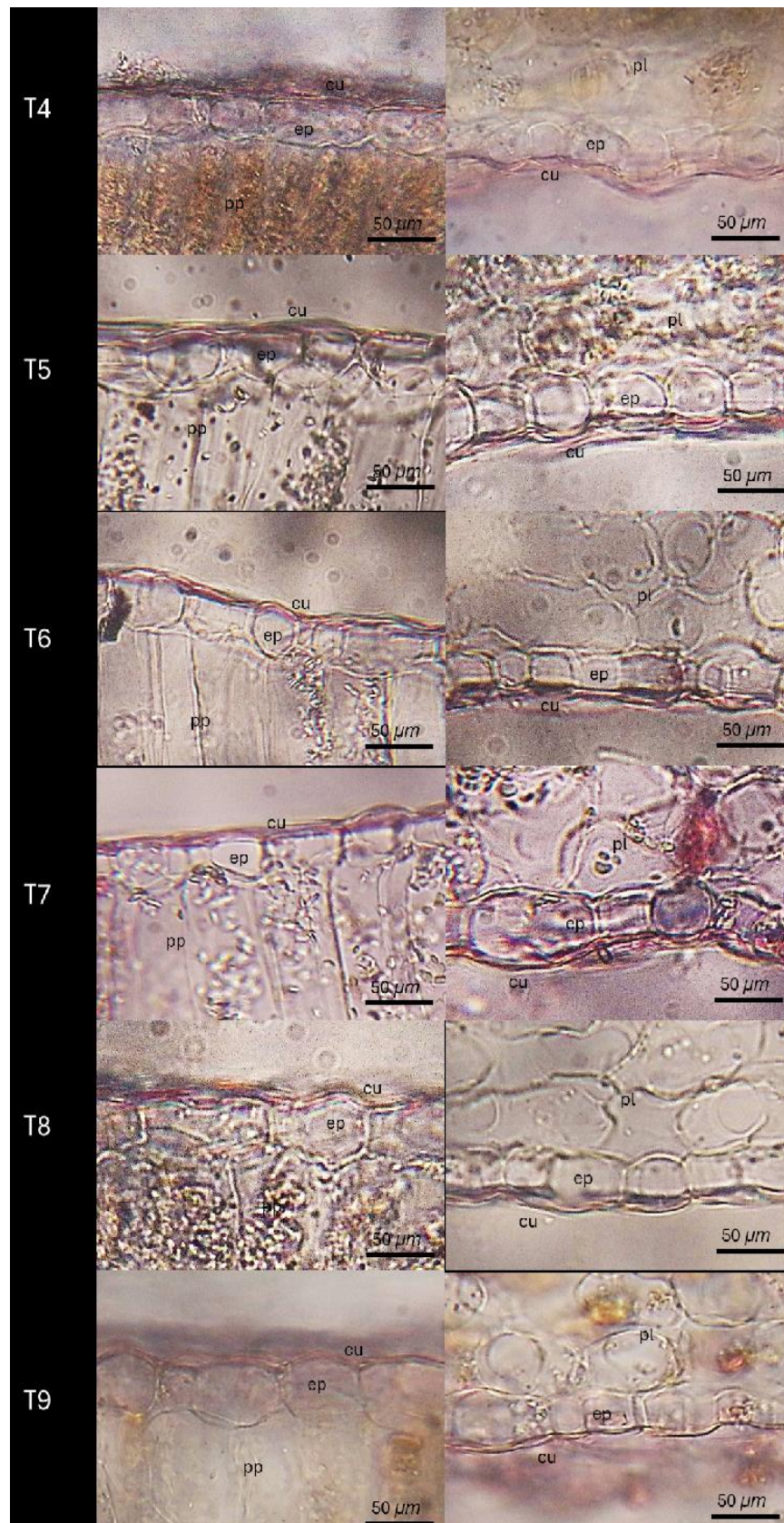
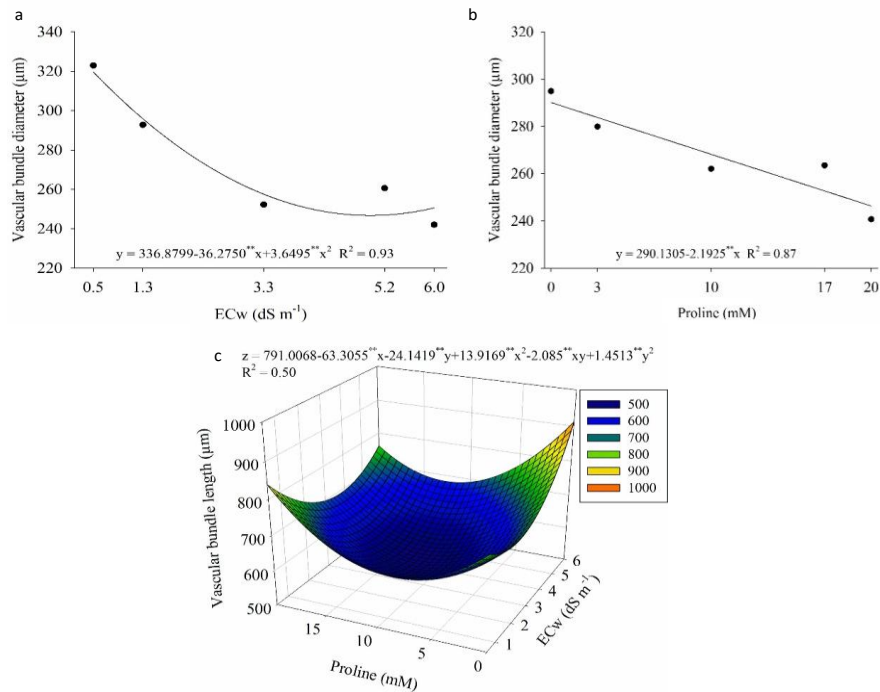


Figure 4: (Continued). T4 – ECw of 3.25 dS m⁻¹ and 0.00 mM proline; T5 – ECw of 3.25 dS m⁻¹ and 10.00 mM proline; T6 – ECw of 3.25 dS m⁻¹ and 20.00 mM proline; T7 – ECw of 5.20 dS m⁻¹ and 2.90 mM proline; T8 – ECw of 5.20 dS m⁻¹ and 17.09 mM proline; T9 – ECw of 6.00 dS m⁻¹ and 10.00 mM proline; ep – epidermis; cu – cuticle; pp – palisade parenchyma; pl – spongy parenchyma.



** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, by the F-test.

Figure 5: Vascular bundle diameter (a and b) and vascular bundle length (c) of *Capsicum chinense* subjected to salt stress and proline.

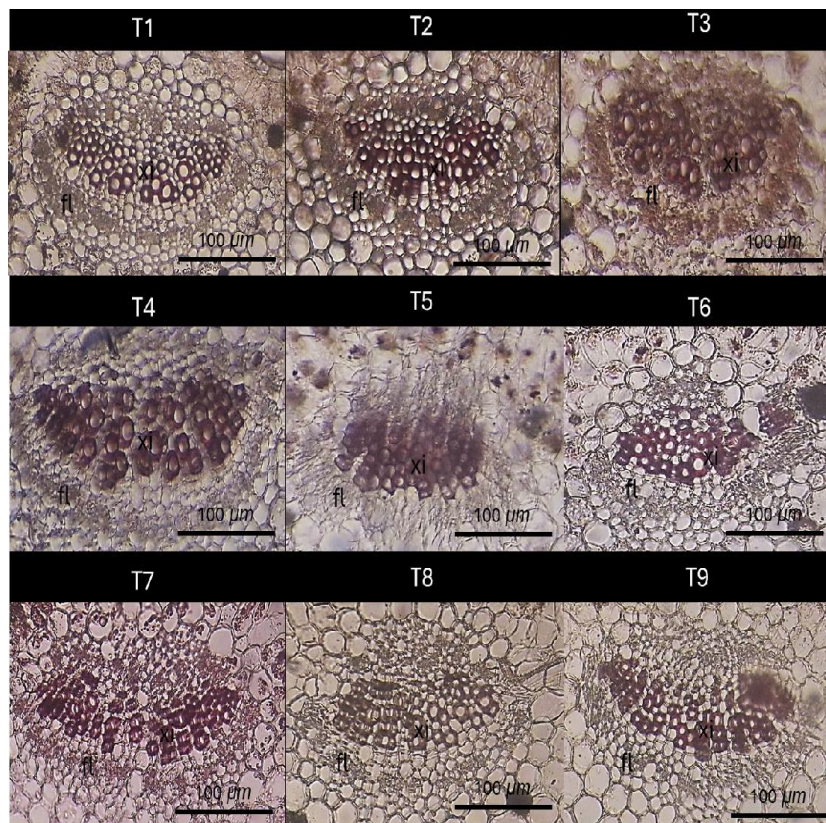
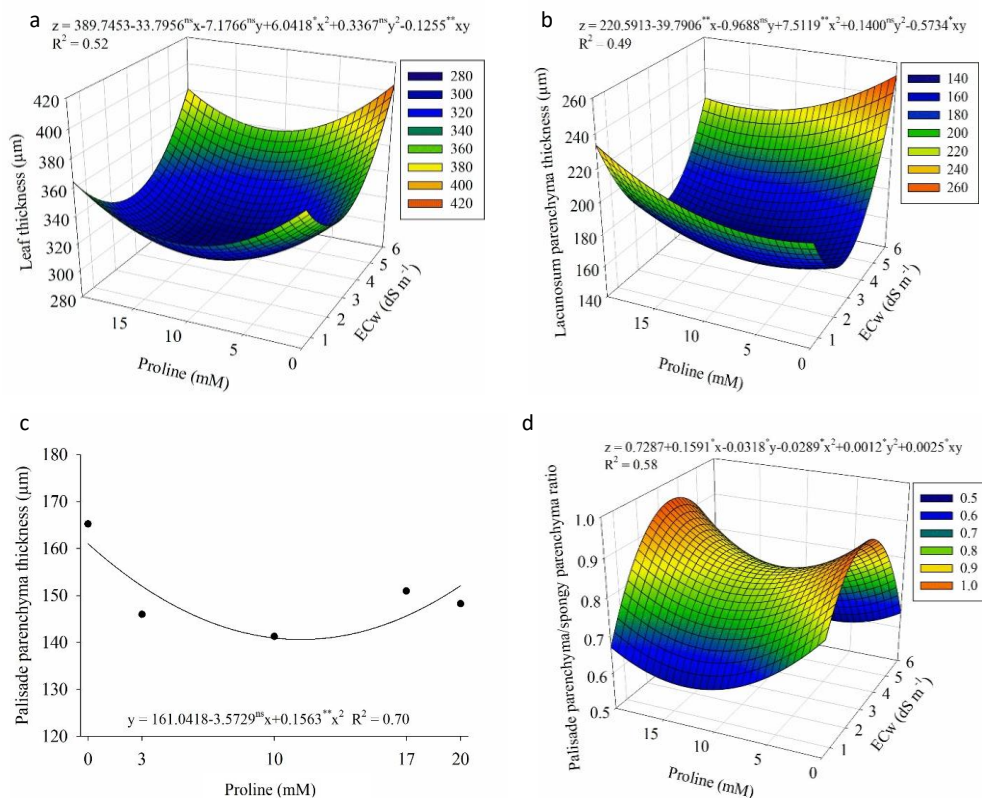


Figure 6: Vascular bundle of *Capsicum chinense* leaves subjected to salt stress and proline. T1 – ECw of 0.50 dS m^{-1} and 10.00 mM proline; T2 – ECw of 1.30 dS m^{-1} and 2.90 mM proline; T3 – ECw of 1.30 dS m^{-1} and 17.09 mM proline; T4 – ECw of 3.25 dS m^{-1} and 0.00 mM proline; T5 – ECw of 3.25 dS m^{-1} and 10.00 mM proline; T6 – ECw of 3.25 dS m^{-1} and 20.00 mM proline; T7 – ECw of 5.20 dS m^{-1} and 2.90 mM proline; T8 – ECw of 5.20 dS m^{-1} and 17.09 mM proline; T9 – ECw of 6.00 dS m^{-1} and 10.00 mM proline; xi – xylem; fl – phloem.



** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, and ns – not significant by the F-test.

Figure 7: Leaf thickness (a), lacunosum parenchyma thickness (b), palisade parenchyma thickness (c), and palisade parenchyma to spongy parenchyma ratio (d) of *Capsicum chinense* subjected to salt stress and proline.

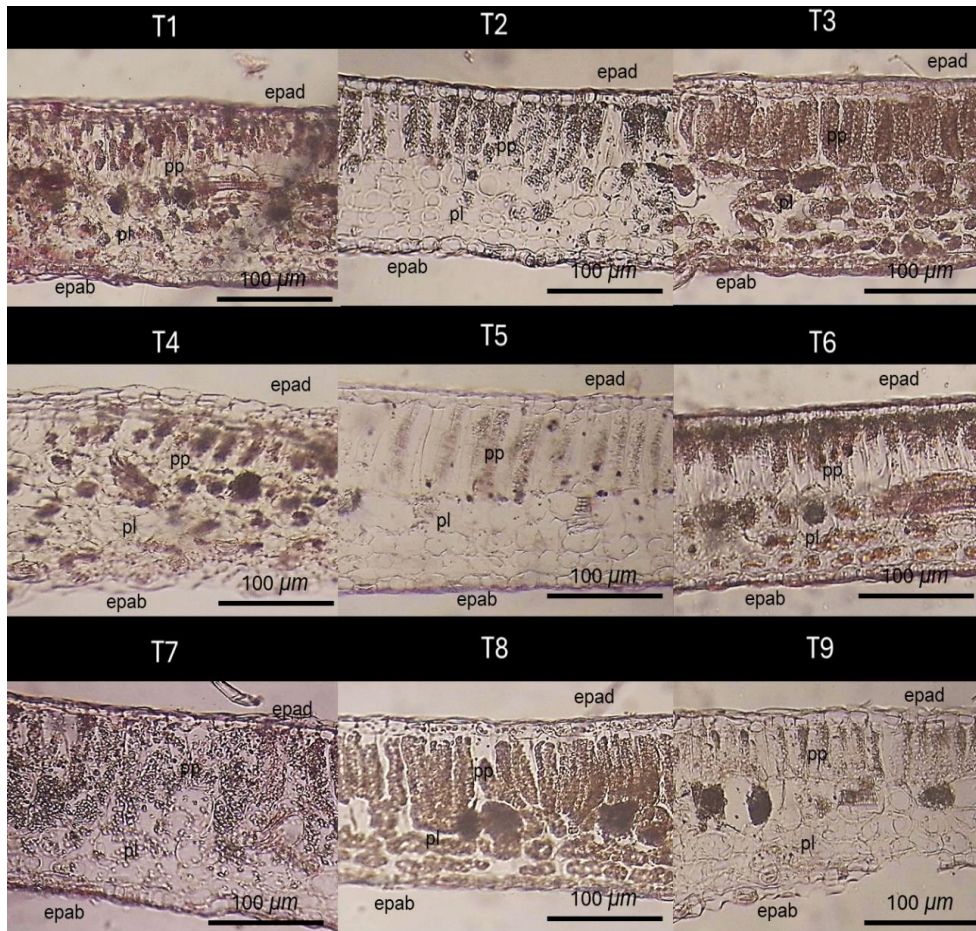
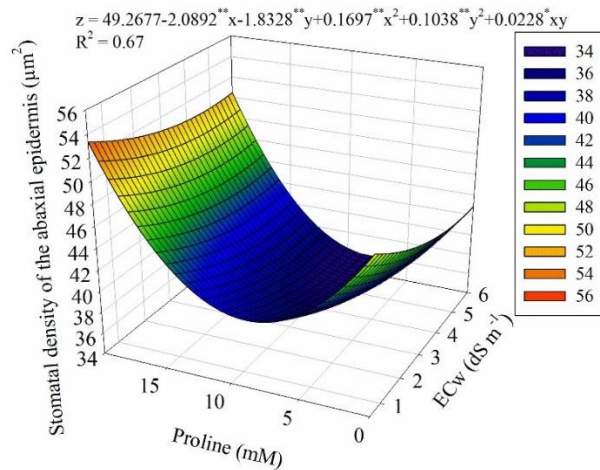


Figure 8: Cross-section showing the palisade and spongy parenchyma of *Capsicum chinense* leaves subjected to salt stress and proline. T1 – ECw of 0.50 dS m^{-1} and 10.00 mM proline; T2 – ECw of 1.30 dS m^{-1} and 2.90 mM proline; T3 – ECw of 1.30 dS m^{-1} and 17.09 mM proline; T4 – ECw of 3.25 dS m^{-1} and 0.00 mM proline; T5 – ECw of 3.25 dS m^{-1} and 10.00 mM proline; T6 – ECw of 3.25 dS m^{-1} and 20.00 mM proline; T7 – ECw of 5.20 dS m^{-1} and 2.90 mM proline; T8 – ECw of 5.20 dS m^{-1} and 17.09 mM proline; T9 – ECw of 6.00 dS m^{-1} and 10.00 mM proline; epda – adaxial epidermis; pp – palisade parenchyma; pl – spongy parenchyma; epab – abaxial epidermis.



** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, by the F-test.

Figure 9: Stomatal density of the abaxial epidermis of *Capsicum chinense* leaves subjected to salt stress and proline.

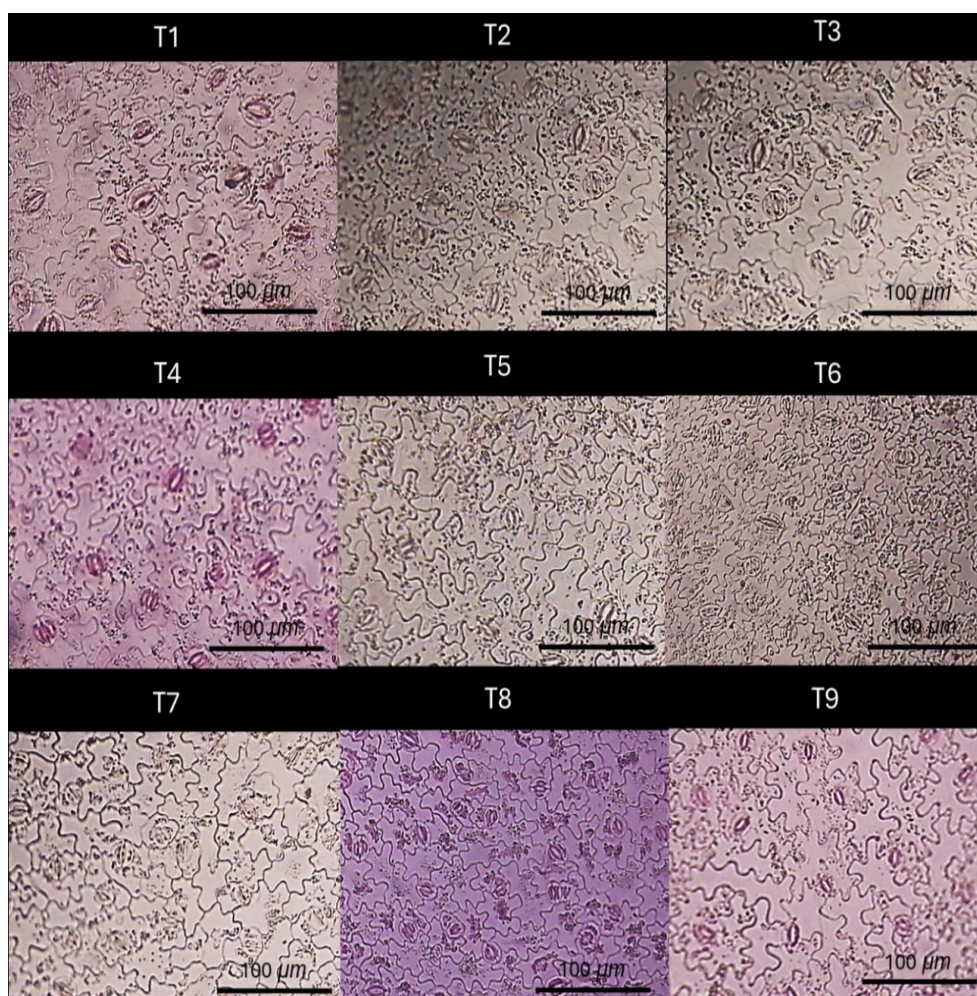


Figure 10: Stomatal density of the abaxial epidermis of *Capsicum chinense* leaves subjected to salt stress and proline. T1 – ECw of 0.50 dS m⁻¹ and 10.00 mM proline; T2 – ECw of 1.30 dS m⁻¹ and 2.90 mM proline; T3 – ECw of 1.30 dS m⁻¹ and 17.09 mM proline; T4 – ECw of 3.25 dS m⁻¹ and 0.00 mM proline; T5 – ECw of 3.25 dS m⁻¹ and 10.00 mM proline; T6 – ECw of 3.25 dS m⁻¹ and 20.00 mM proline; T7 – ECw of 5.20 dS m⁻¹ and 2.90 mM proline; T8 – ECw of 5.20 dS m⁻¹ and 17.09 mM proline; T9 – ECw of 6.00 dS m⁻¹ and 10.00 mM proline.

Discussion

For the adaxial cuticle thickness, foliar proline application resulted in improvements in pepper plants, with the highest value (15.97 μm) observed in plants subjected to a concentration of 5.35 mM and low ECw of 0.50 dS m⁻¹ (Figures 3a and 4). This outcome suggests that under salt stress conditions, plants excessively produce reactive oxygen species (ROS), causing oxidative damage to proteins, lipids, nucleic acids, and the cell's plasma membrane (Aboryia et al., 2022), which can lead to a decrease in the adaxial cuticle thickness of pepper leaves, compromising their ability to withstand stress and maintain

cellular integrity. Proline application, in turn, can act as a metabolic signal, regulating antioxidants and directly scavenging ROS, helping plant cells to survive under stress conditions (Liang et al., 2013; Fu et al., 2018), contributing to the production and deposition of cuticle components, resulting in a thicker layer.

The thickness of the abaxial cuticle decreased with increasing salinity, from 15.57 to 12.28 μm (a reduction of 21.13%) between the conductivity levels of 0.5 and 6.0 dS m⁻¹, without proline application. However, with proline application, a higher value of 17.91 μm was obtained in plants cultivated under an ECw of 0.52 dS m⁻¹ and

sprayed with a concentration of 0.02 mM (Figures 3b and 4). Plants exposed to saline conditions undergo osmotic imbalances, leading to salt accumulation in the leaves and an increase in abaxial cuticle thickness as an additional physical barrier to reduce water loss through transpiration and excessive salt entry into leaf cells (Shahid et al., 2020; Spiekerman and Devos, 2020; Feng et al., 2020).

For the thickness of the adaxial epidermis, there was no significant interaction between the evaluated factors, indicating that the analyzed factors did not jointly influence the thickness. However, water salinity showed a progressively linear effect on adaxial thickness, meaning that as salinity increased, there was also an increase in adaxial epidermis thickness, with a 5.30 μM increment compared to plants exposed to the lowest EC_w of 0.5 dS m^{-1} , reaching a maximum value of 115 μM at EC_w of 6.0 dS m^{-1} (Figures 3c and 4). With increasing proline doses, a 3.67% increase in adaxial epidermis thickness was observed at the highest dose (20 mM) compared to the lowest dose used (Figures 3d and 4).

Plants respond to salinity by adapting through hormonal regulation, changes in redox potential, epigenetic control of stress genes, and biosynthesis of osmolytes such as proline (Mansour and Hassan, 2022). Specifically, proline promotes cellular growth by increasing cell number and resulting in a greater thickness of the adaxial epidermis (Alnajjar et al., 2020).

In the thickness of the abaxial epidermis, there was also no significant interaction between the evaluated factors, showing a linear reduction with increasing water salinity up to an EC_w of 4.31 dS m^{-1} , achieving values of 101.09 μM , with a subsequent trend of increase (Figures 3e and 4). Salinity stress causes changes in plant physiological and morphological processes, such as reduction in osmotic potential, which hinders water and nutrient absorption by plants, leading to decreased cell turgor (Sousa et al., 2022; Khan et al.,

2024), and consequently, a reduction in the thickness of the abaxial epidermis.

The increase in salinity can induce the production of plant hormones, such as abscisic acid (ABA), which is the main regulator of plant stress tolerance, promoting various adaptive responses including cell thickening as a defense mechanism, helping plants enhance their resistance to salt stress (Rai et al., 2024). This explains the increase in the thickness of the abaxial epidermis after reaching an EC_w of 4.31 dS m^{-1} .

However, the thickness of the abaxial epidermis showed a quadratic response to increasing concentrations of proline, reaching an estimated maximum of 113.37 μM at a concentration of 8.59 mM, followed by a decrease at higher concentrations (Figures 3f and 4). Proline plays a crucial role in plant metabolism under stress conditions, increasing cellular respiration rate. This increase in respiration leads to ATP production, which is essential for cell division and growth. Therefore, proline supports cell expansion and thickening of the abaxial epidermis up to an optimal point, beyond which the effect diminishes, possibly due to excessive proline accumulation or other negative effects associated with high concentrations (Taha et al., 2001; Alnajjar et al., 2011).

In the diameter of the vascular bundle, a quadratic behavior was observed, decreasing with increasing EC_w. The estimated minimum value was 246.73 μM at an EC_w of 4.96 dS m^{-1} , followed by a tendency to increase (Figures 5a and 6). This reduction is associated with restricted water flow, decreased cellular turgor, metabolic stress, and hormonal changes (Khan et al., 2018; Betzen et al., 2019), resulting in a constriction of conducting vessels that affects water and nutrient transport.

The diameter of the vascular bundle in plants decreases linearly with increasing concentrations of applied proline, with a reduction of 15.12% at the highest concentration (20 mM) compared to plants

without the use of the mitigator (Figures 5b and 6). High concentrations of proline, acting as an osmotically active solute, lead to water loss from vascular tissues. When combined with the metabolic stress induced by proline, it negatively affects the regulation of cellular growth (Yong et al., 2017), resulting in a decrease in the diameter of vascular bundles and, consequently, in the area of xylem vessels, which are essential components for the transport of nutrients and water (Ortega et al., 2006).

The longest vascular bundle length observed in *C. chinense* plants was 910.14 μM , cultivated with ECw of 5.99 dS m^{-1} and a concentration of 0.05 mM proline (Figures 5c and 6). This observation suggests a potential adaptation of plants to salt stress, where a longer vascular bundle may facilitate more efficient transport of water and nutrients, helping to mitigate the adverse effects of stress and ensuring plant survival (Abdelaal et al., 2021; Pandey et al., 2022).

The greatest leaf thickness (404.39 μM) was recorded in plants cultivated under an ECw of 5.99 dS m^{-1} without application of proline (0.00 mM), as shown in Figures 7a and 8. This pattern was also observed in the thickness of the spongy parenchyma, with higher values (251.76 μM) under the same salinity conditions and absence of proline (Figures 7b and 8). This result suggests that the increased leaf thickness, particularly in the spongy parenchyma, may be an adaptation of *C. chinense* plants to reduce water loss under saline stress conditions. The spongy parenchyma with fewer intercellular spaces indicates an adaptive strategy to maximize photosynthetic efficiency, allowing for faster and more efficient gas exchange when water is available (Elias et al., 2003; Wall et al., 2022).

Regarding the thickness of the palisade parenchyma, a reduction was observed with increasing concentrations of proline, being more significant (140.62 μM) at a concentration of 11.42 mM (Figures 7c and

8), followed by a trend of growth with higher concentrations of the mitigator. On the other hand, the ratio between palisade and spongy parenchyma was higher (0.95 μM) in plants cultivated under an ECw of 3.61 dS m^{-1} and with proline application at a concentration of 19.99 mM (Figures 7d and 8). Proline plays a crucial role in plant adaptation to saline stress (Mansour and Ali, 2017; Shafi et al., 2019). At higher concentrations, it can influence both cellular morphology and physiology, affecting the organization of the palisade parenchyma (Mbarki et al., 2018; Patriarca et al., 2021).

For the stomatal density of the abaxial epidermis (Figures 9 and 10), the highest value (53.29 μm^2) was obtained in plants subjected to an ECw of 0.50 dS m^{-1} and a proline concentration of 19.97 mM. Proline can influence the levels of plant hormones, such as abscisic acid, stimulating cellular development and promoting the formation of stomatal cells. Additionally, in response to stress, proline can trigger plant adaptation, including an increase in stomatal density to improve gas exchange and reduce water loss (Freitas et al., 2018; Ghosh et al., 2022).

In saline environments, the reduction in stomatal density can be important to minimize water loss, suggesting that under high salinity, plants decrease the abaxial leaf surface, resulting in a lower transpiration rate, considered a survival strategy under water scarcity conditions (Wasim and Naz, 2020). Similar results have been observed in halophytes, such as *Sporobolus ioclados* (Naz et al., 2016).

Indeed, the application of proline in pepper plants under salt stress promoted significant adaptations, such as increased leaf thickness and stomatal density, facilitating photosynthesis and gas exchange. Proline also regulated antioxidants, helping to maintain cellular integrity and cuticle thickness. Reductions in vascular bundle diameter and changes in epidermis and parenchyma thickness showed adaptive responses to salt stress,

highlighting proline as an important mitigator for plant resistance and survival under adverse conditions.

Conclusions

Salt stress negatively affected the anatomy of 'Biquinho' pepper leaves. The application of proline at a concentration of 5.35 mM increased the thickness of the adaxial cuticle. On the other hand, 20 mM of proline stimulated the thickness of the abaxial epidermis, palisade parenchyma to spongy parenchyma ratio, and stomatal density. The results obtained reinforce the idea that proline enhances the anatomy of pepper leaves, potentially serving as an alternative to increase tolerance to the effects of salt stress. However, further studies are needed to better understand the mechanism of action of proline in plant defense system signaling.

Thus, salt stress caused a reduction in abaxial cuticle thickness and an increase in adaxial epidermis thickness. The vascular bundle diameter and leaf thickness were also affected. Proline application mitigated some effects, improving cuticle thickness and stomatal density, as well as influencing the palisade parenchyma.

Acknowledgments

We would like to thank the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES - financial code 001) and the National Council for Scientific and Technological Development (CNPq).

References

Abdelaal, K.; Alkahtani, M.; Attia, K.; Hafez, Y.; Király, L.; Künstler, A. The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. *Biology*, v. 10, n. 6, 520, 2021. <https://doi.org/10.3390/biology10060520>

Aboryia, M. S.; El-Dengawy, E. R. F.; El-Banna, M. F.; El-Gobba, M. H.; Kasem, M. M.; Hegazy, A. A.; Hassan, H. M.; El-Yazied, A. A.; El-Gawad, H. G. A.; Al-Qahtani, S. M.; Al-Harbi, N. A.; Dessoky, E. S.; Ismail, I. A.; El-Mogy, M. M.; El-Boraie, E. S. A. Anatomical

and physiological performance of Jojoba treated with proline under salinity stress condition. *Horticulturae*, v. 8, n. 8, 716, 2022. <https://doi.org/10.3390/horticulturae8080716>

Acosta-Motos, J. R.; Ortuño, M. F.; Bernal-Vicente, A.; Diaz-Vivancos, P.; Sanchez-Blanco, M. J.; Hernandez, J. A. Plant responses to salt stress: Adaptive mechanisms. *Agronomy*, v. 7, n. 1, 18, 2017. <https://doi.org/10.3390/agronomy7010018>

Al-Elwany, O. A.; Hemida, K. A.; El-Mageed, T. A. A.; El-Saadony, M. T.; Abuqamar, S. F.; El-Tarabily, K. A.; Taha, R. S. Impact of folic acid in modulating antioxidant activity, osmoprotectants, anatomical responses, and photosynthetic efficiency of *Plectranthus amboinicus* under salinity conditions. *Frontiers in Plant Science*, v. 13, 887091, 2022. <https://doi.org/10.3389/fpls.2022.843572>

Alnajjar, M. A. H.; Ubaid, A. R.; Al-Presem, W. F. F. Effect of proline treatment on seed germination and growth seedlings of date palm *Phoenix dactylifera* L. irrigated by Shatt-Alarab water. *Basrah Journal for Date Palm Research*, v. 10, n. 2, p. 1-19, 2011.

Alnajjar, M. A.; Alpresem, W. F.; Ibrahim, M. A. Effect of amino acid proline treatment on anatomical characteristics of leaves and roots of date palm seedlings *Phoenix dactylifera* L. developed under saline stress conditions. *Plant Archives*, v. 20, n. Supplement 1, p. 755-760, 2020.

Betzen, B. M.; Smart, C. M.; Maricle, K. L.; Maricle, B. R. Effects of increasing salinity on photosynthesis and plant water potential in Kansas salt marsh species. *Transactions of the Kansas Academy of Science*, v. 122, n. 1-2, p. 49-58, 2019. <https://doi.org/10.1660/062.122.0105>

Butt, M.; Ayyub, C. M.; Amjad, M.; Ahmad, R. Proline application enhances growth of chilli by improving physiological and biochemical attributes under salt stress. *Pakistan Journal of Agricultural Sciences*, v. 53, n. 1, p. 43-49, 2016. <https://doi.org/10.21162/PAKJAS/16.4623>

Butt, M.; Sattar, A.; Abbas, T.; Sher, A.; Ijaz, M.; Ul-Allah, S.; Shaheen, M. R.; Kaleem, F. Foliage applied proline induces salt tolerance in chili genotypes by regulating photosynthetic

- attributes, ionic homeostasis, and antioxidant defense mechanisms. *Horticulture, Environment, and Biotechnology*, v. 61, n. 4, p. 693-702, 2020. <https://doi.org/10.1007/s13580-020-00236-8>
- Cavalcanti, F. J. A. Recomendações de adubação para o estado de Pernambuco: 2a. aproximação. 3 ed. rev. Recife: Instituto Agronômico de Pernambuco, 2008. 212p.
- Chourasia, K. N.; More, S. J.; Kumar, A.; Kumar, D.; Singh, B.; Bhardwaj, V.; Kumar, A.; Das, S. K.; Singh, R. K.; Zinta, G.; Tiwari, R. K.; Lal, M. K. Salinity responses and tolerance mechanisms in underground vegetable crops: an integrative review. *Planta*, v. 255, n. 3, 68, 2022. <https://doi.org/10.1007/s00425-022-03845-y>
- Elias, S. R. M.; Assis, R. M.; Stacciarini-Seraphin, E.; Rezende, M. H. Anatomia foliar em plantas jovens de *Solanum lycocarpum* A. St.-Hil. (Solanaceae). *Brazilian Journal of Botany*, v. 26, n. 2, p. 169-174, 2003. <https://doi.org/10.1590/S0100-84042003000200004>
- Feng, S.; Ren, L.; Sun, H.; Qiao, K.; Liu, S.; Zhou, A. Morphological and physiological responses of two willow species from different habitats to salt stress. *Scientific Reports*, v. 10, n. 1, 18228, 2020. <https://doi.org/10.1038/s41598-020-75349-2>
- Ferreira, E. B.; Cavalcanti, P. P.; Nogueira, D. A.; Ferreira, M. E. B. Package 'ExpDes. pt'. R package – version 1, 2018. 68p.
- Freitas, P. A. F.; Carvalho, H. H.; Costa, J. H.; Miranda, R. S.; Saraiva, K. D. C.; Oliveira, F. D. B.; Coelho, D. G.; Prisco, J. T.; Gomes-Filho, E. Salt acclimation in sorghum plants by exogenous proline: physiological and biochemical changes and regulation of proline metabolism. *Plant Cell Reports*, v. 38, p. 403-416, 2019. <https://doi.org/10.1007/s00299-019-02382-5>
- Freitas, P. A. F.; Miranda, R. S.; Marques, E. C.; Prisco, J. T.; Gomes-Filho, E. Salt tolerance induced by exogenous proline in maize is related to low oxidative damage and favorable ionic homeostasis. *Journal of Plant Growth Regulation*, v. 37, n. 3, p. 911-924, 2018. <https://doi.org/10.1007/s00344-018-9787-x>
- Fu, Y.; Ma, H.; Chen, S.; Gu, T.; Gong, J. Control of proline accumulation under drought via a novel pathway comprising the histone methylase CAU1 and the transcription factor ANAC055. *Journal of Experimental Botany*, v. 69, n. 3, p. 579-588, 2018. <https://doi.org/10.1093/jxb/erx419>
- Ghosh, U.; Islam, M.; Siddiqui, M.; Cao, X.; Khan, M. Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. *Plant Biology*, v. 24, n. 2, p. 227-237, 2022. <https://doi.org/10.1111/plb.13363>
- Huang, H.; Ullah, F.; Zhou, D. X.; Yi, M.; Zhao, Y. Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science*, v. 10, 800, 2019. <https://doi.org/10.3389/fpls.2019.00800>
- Johansen, D. A. *Plant microtechnique*. New York: McGraw-Hill Book Company, Inc., 1940. 523p.
- Kaya, C.; Tuna, L.; Higgs, D. Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. *Journal of Plant Nutrition*, v. 29, n. 8, p. 1469-1480, 2006. <https://doi.org/10.1590/S0100-84042003000200004>
- Khan, M. N.; Alsolami, M. A.; Siddiqui, Z. H.; Alomrani, M. A. M.; Kalaji, H. M. Regulation of Na⁺/H⁺ antiport system and nitrogen metabolism by melatonin and endogenous hydrogen sulfide confers resilience to drought and salt stress. *South African Journal of Botany*, v. 164, p. 152-166, 2024. <https://doi.org/10.1016/j.sajb.2023.11.033>
- Khan, W. U. D.; Aziz, T.; Maqsood, M. A.; Farooq, M.; Abdullah, Y.; Ramzani, P. M. A.; Bilal, H. M. Silicon nutrition mitigates salinity stress in maize by modulating ion accumulation, photosynthesis, and antioxidants. *Photosynthetica*, v. 56, n. 4, p. 1047-1057, 2018. <https://doi.org/10.1007/s11099-018-0812-x>
- Kong, X.; Tian, H.; Yu, Q.; Zhang, F.; Wang, R.; Gao, S.; Xu, W.; Liu, J.; Shani, E.; Fu, C.; Zhou, G.; Zhang, L.; Zhang, X.; Ding, Z. PHB3 maintains root stem cell niche identity through ROS-responsive AP2/ERF transcription factors in *Arabidopsis*. *Cell Reports*, v. 22, n. 5, p. 1350-1363, 2018. <https://doi.org/10.1016/j.celrep.2017.12.105>

- Lenth, R. V. Response-surface methods in R, using rsm. *Journal of Statistical Software*, v. 32, n. 7, p. 1-17, 2009. <https://doi.org/10.18637/jss.v032.i07>
- Liang, X.; Zhang, L.; Natarajan, S. K.; Becker, D. F. Proline mechanisms of stress survival. *Antioxidants & Redox Signaling*, v. 19, n. 9, p. 998-1011, 2013. <https://doi.org/10.1089/ars.2012.5074>
- Mansour, M. M. F.; Ali, E. F. Evaluation of proline functions in saline conditions. *Phytochemistry*, v. 140, p. 52-68, 2017. <https://doi.org/10.1016/j.phytochem.2017.04.016>
- Mansour, M. M. F.; Hassan, F. A. How salt stress-responsive proteins regulate plant adaptation to saline conditions? *Plant Molecular Biology*, v. 108, n. 3, p. 175-224, 2022. <https://doi.org/10.1007/s11103-021-01232-x>
- Martinez, M.; Santos, C. P.; Verruma-Bernardi, M. R.; Carrilho, E. N. V. M.; Silva, P. P. M.; Spoto, M. H. F.; Ciarrocchi, I. R.; Sala, F. C. Agronomic, physical-chemical and sensory evaluation of pepper hybrids (*Capsicum chinense* Jacquin). *Scientia Horticulturae*, v. 277, 109819, 2021. <https://doi.org/10.1016/j.scienta.2020.109819>
- Mateus, N. B.; Barbin, D.; Conagin, A. Viabilidade de uso do delineamento composto central. *Acta Scientiarum. Agronomy*, v. 23, n. 6, p. 1537-1546, 2001. <https://doi.org/10.4025/actascitechnol.v23i0.2795>
- Mbarki, S.; Sytar, O.; Cerda, A.; Zivcak, M.; Rastogi, A.; He, X.; Brestic, M. Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. In: Kumar, V.; Wani, S. H.; Suprasanna, P.; Tran, L.-S. P. (ed.). *Salinity responses and tolerance in plants, Volume 1: Targeting sensory, transport and signaling mechanisms*. Cham: Springer, 2018. p. 85-136. https://doi.org/10.1007/978-3-319-75671-4_4
- Mittler, R. ROS are good. *Trends in Plant Science*, v. 22, n. 1, p. 1-19, 2017. <https://doi.org/10.1016/j.tplants.2016.08.002>
- Naz, N.; Fatima, S.; Hameed, M.; Naseer, M.; Batool, R.; Ashraf, M.; Ahmad, K. S. Adaptations for salinity tolerance in *Sporobolus ioclados* (Nees ex Trin.) Nees from saline desert. *Flora*, v. 223, p. 46-55, 2016. <https://doi.org/10.1016/j.flora.2016.04.013>
- Ortega, L.; Fry, S. C.; Taleisnik, E. Why are *Chloris gayana* leaves shorter in salt-affected plants? Analyses in the elongation zone. *Journal of Experimental Botany*, v. 57, n. 14, p. 3945-3952, 2006. <https://doi.org/10.1093/jxb/erl168>
- Pandey, A. K.; Zorić, L.; Sun, T.; Karanović, D.; Fang, P.; Borišev, M.; Xu, P. The anatomical basis of heavy metal responses in legumes and their impact on plant-rhizosphere interactions. *Plants*, v. 11, n. 19, 2554, 2022. <https://doi.org/10.3390/plants11192554>
- Patriarca, E. J.; Cermola, F.; D'Aniello, C.; Fico, A.; Guardiola, O.; Cesare, D.; Minchiotti, G. The multifaceted roles of proline in cell behavior. *Frontiers in Cell and Developmental Biology*, v. 9, 728576, 2021. <https://doi.org/10.3389/fcell.2021.728576>
- Rai, G. K.; Khanday, D. M.; Choudhary, S. M.; Kumar, P.; Kumari, S.; Martínez-Andújar, C.; Pérez-Alfocea, F. Unlocking nature's stress buster: Abscisic acid's crucial role in defending plants against abiotic stress. *Plant Stress*, v. 11, 100359, 2024. <https://doi.org/10.1016/j.stress.2024.100359>
- Ribeiro, C. S. C.; Carvalho, S. I. C.; Heinrich, A. G.; Reifschneider, F. J. B. BRS Tui: a new Biquinho-type pepper cultivar released by Embrapa. *Horticultura Brasileira*, v. 36, n. 4, p. 526-528, 2018. <https://doi.org/10.1590/S0102-053620180417>
- Sá, F. V. S.; Souto, L. S.; Paiva, E. P.; Torres, S. B.; Oliveira, F. A. Initial development and tolerance of pepper species to salinity stress. *Revista Caatinga*, v. 32, n. 3, p. 826-833, 2019. <https://doi.org/10.1590/1983-21252019v32n327rc>
- Sachdev, S.; Ansari, S. A.; Ansari, M. I.; Fujita, M.; Hasanuzzaman, M. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, v. 10, n. 2, 277, 2021. <https://doi.org/10.3390/antiox10020277>
- Sanatombi, K.; Rajkumari, S. Effect of processing on quality of pepper: A review. *Food*

Reviews International, v. 36, n. 6, p. 626-643.
<https://doi.org/10.1080/87559129.2019.1669161>

Scrucca, L. GA: A package for genetic algorithms in R. Journal of Statistical Software, v. 53, n. 4, p. 1-37, 2013.
<https://doi.org/10.18637/jss.v053.i04>

Shaaban, A.; Al-Elwany, O. A. A. I.; Abdou, N. M.; Hemida, K. A.; El-Sherif, A. M. A.; Abdel-Razek, M. A.; Semida, W. M.; Mohamed, G. F.; El-Mageed, T. A. A. Filter mud enhanced yield and soil properties of water-stressed *Lupinus termis* L. in saline calcareous soil. Journal of Soil Science and Plant Nutrition, v. 22, n. 2, p. 1572-1588, 2022.
<https://doi.org/10.1007/s42729-021-00755-y>

Shafi, A.; Zahoor, I.; Mushtaq, U. Proline accumulation and oxidative stress: Diverse roles and mechanism of tolerance and adaptation under salinity stress. In: Akhtar, M. S. (ed.). Salt stress, microbes, and plant interactions: Mechanisms and molecular approaches – volume 2. Singapore: Springer, 2019. p. 269-300. https://doi.org/10.1007/978-981-13-8805-7_13

Shahid, M. A.; Sarkhosh, A.; Khan, N.; Balal, R. M.; Ali, S.; Rossi, L.; Garcia-Sanchez, F. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. Agronomy, v. 10, n. 7, 938, 2020.
<https://doi.org/10.3390/agronomia10070938>

Shahid, S. A.; Zaman, M.; Heng, L. Soil salinity: Historical perspectives and a world overview of the problem. In: Zaman, M.; Shahid, S. A.; Heng, L. (ed.). Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques. Cham: Springer, 2018. p. 43-53.
<https://doi.org/10.1007/978-3-319-96190-3>

Sousa, H. C.; Sousa, G. G.; Cambissa, P. B. C.; Lessa, C. I. N.; Goes, G. F.; Silva, F. D. B.; Abreu, F. S.; Viana, T. V. A. Gas exchange and growth of zucchini crop subjected to salt and water stress. Revista Brasileira de Engenharia Agrícola e Ambiental, v. 26, n. 11, p. 815-822, 2022.
<https://doi.org/10.1590/1807-1929/agriambi.v26n11p815-822>

Spiekerman, J. J.; Devos, K. M. The halophyte seashore paspalum uses adaxial leaf papillae for

sodium sequestration. Plant Physiology, v. 184, n. 4, p. 2107-2119, 2020.
<https://doi.org/10.1104/pp.20.00796>

Srivastava, S. Morpho-anatomical adaptation against salinity. In: Kimatu, J. N. (ed.). Plant defense mechanisms. London: IntechOpen, 2022. p. 1-13.
<http://dx.doi.org/10.5772/intechopen.101681>

Taha, H. S.; Bekheet, S. A.; Saker, M. M. Factors affecting in vitro multiplication of date palm. Biologia Plantarum, v. 44, n. 3, p. 431-433, 2001. <https://doi.org/10.1023/A:1012423601467>

Tekindal, M. A.; Bayrak, H.; Özkaya, B.; Yavuz, Y. Second-order response surface method: factorial experiments an alternative method in the field of agronomy. Turkish Journal of Field Crops, v. 19, n. 1, p. 35-45, 2014. <https://doi.org/10.17557/tjfc.78787>

Tognetti, V. B.; Bielach, A.; Hrtyan, M. Redox regulation at the site of primary growth: auxin, cytokinin and ROS crosstalk. Plant, Cell & Environment, v. 40, n. 11, p. 2586-2605, 2017.
<https://doi.org/10.1111/pce.13021>

Wall, S.; Vialet-Chabrand, S.; Davey, P.; Van Rie, J.; Galle, A.; Cockram, J.; Lawson, T. Stomata on the abaxial and adaxial leaf surfaces contribute differently to leaf gas exchange and photosynthesis in wheat. New Phytologist, v. 235, n. 5, p. 1743-1756, 2022.
<https://doi.org/10.1111/nph.18257>

Wasim, M. A.; Naz, N. Anatomical adaptations of tolerance to salt stress in *Cenchrus ciliaris* L., a saline desert grass. Journal of Animal & Plant Sciences, v. 30, n. 6, p. 1548-1566, 2020.
<https://doi.org/10.36899/JAPS.2020.6.0176>

Yong, B.; Xie, H.; Li, Z.; Li, Y. P.; Zhang, Y.; Nie, G.; Zhang, X. Q.; Ma, X.; Huang, L. K.; Yan, Y. H.; Peng, Y. Exogenous application of GABA improves PEG-induced drought tolerance positively associated with GABA-shunt, polyamines, and proline metabolism in white clover. Frontiers in Physiology, v. 8, 1107, 2017. <https://doi.org/10.3389/fphys.2017.01107>

Zeng, J.; Dong, Z.; Wu, H.; Tian, Z.; Zhao, Z. Redox regulation of plant stem cell fate. The EMBO Journal, v. 36, n. 19, p. 2844-2855, 2017. <https://doi.org/10.15252/embj.201695955>