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Vitamin C (Ascorbic Acid) Mediated Structural Integrity: Mitigating the Salinity Stress in Garland Chrysanthemum (*Glebionis coronaria*)

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ABSTRACT

Environmental stress is the primary challenge that severely limiting the global plant growth and productivity, with salinity posing a significant threat. This study was conducted on *Glebionis coronaria* due to its global medicinal and nutritional importance. A pot experiment was conducted to investigate the effects of salt stress. and the growth promoter ascorbic acid (NaCl, NaCl+ASA) on *G. coronaria*. Various concentrations of NaCl (0 mM, 50 mM, 100 mM, and 150 mM) were used, along with combinations of sodium chloride and ascorbic acid (0 mM, 3 mM, 6 mM, and 9 mM). Salinity had a notable diminishing impact on morphological characters such as fresh and dry and weight, shoot length, root length, as well as branch count. However, the growth promoter enhanced root and shoot length, as well as the number of branches. Anatomical parameters such as epidermal thickness, cortical thickness, xylem thickness, or phloem thickness exhibited contrasting outcomes under both salinity and the application of ascorbic acid. Foliar applications of ascorbic acid is an effective strategy to mitigate salinity stress by enhancing the morphological and anatomical integrity of *G. coronoria* stem, root and leaves.

Keywords: Ascorbic acid, salinity, NaCl, *Glebionis coronoria*, Morpho-anatomical adaptations

1. Introduction

Global climatic change is directing this earth toward the un-availability of water bodies for terrain of many countries which is the leading cause of salinity (Ahmed, *et al.*, 2024). On a worldwide scale over 20 % of soils are salt-affected and this number is increasing due to anthropogenic activities (Seleiman *et al.*, 2022). Elevated levels of salt in irrigation water can induce stress in plants with the intensity of this stress influenced by factors such as the type of crop how often and for how long it is exposed to the saline conditions and the concentration of salt present (Yu *et al.*, 2021). Salinity affects plant growth and productivity (Ahmed *et al.*, 2024) development, and seed germination (Parveen *et al.*, 2023). High salinity also has a negative impact on plant photosynthetic rate which can alter protein content and quality and also causes a number of secondary metabolic pathways in plants as well

as antioxidant defenses to be induced (Costan *et al.*, 2020). Increased Na⁺ and Cl⁻ concentrations have a detrimental effect on enzyme activity, plant lipid metabolism and protein synthesis. Additionally, the presence of too many of these ions might encourage the development of ROS which put plants under harmful cellular stress (Hasanuzzaman *et al.*, 2021). Additionally, during salt stress the influx of Na⁺ and the production of organic solutes load cells with energy (Yan *et al.*, 2021).

The garland chrysanthemum scientifically named *Glebionis coronoria* is a member of the Asteraceae family and is also referred to as the annual chrysanthemum (Elsteny *et al.*, 2024). *G. coronoria* now grown as decorative flowers worldwide (Youssef *et al.*, 2020). In addition to its aesthetic appeal, it is also valued as a leafy vegetable. Moreover, this plant offers various health benefits including decreased risk of lung cancer and protection against kidney stones, cardiovascular issues, bloating and bone loss (Satapathy and Mohantay, 2022). Saline soil negatively affects plant growth and productivity (Akram *et al.*, 2022). Plants growing in salty soils face various challenges, including nutrient deficiency, ionic damage and the overall impact decline of productivity (Demo *et al.*, 2025). Salinity diminishes leaf gas exchange, chlorophyll levels, and relative water contents (RWC) (Abbas *et al.*, 2021).

Ascorbic acid (ASA) which serves as the primary source of vitamin C for humans is a compact, water-soluble antioxidant compound. Numerous studies have reported an increase in antioxidant activities when ASA is applied to various crops including maize, sweet pepper, wheat, rice, and barley (Alenazi *et al.*, 2024). Specific oxidative stressors brought on by biotic and abiotic stress can be mitigated significantly by ASA (Azizi *et al.*, 2021). Ascorbic acid could lessen the negative effects of salt by preventing oxidative damage in crops (Naz *et al.*, 2022). Scarcity of literature made it a pressing need for a comprehensive understanding of how economically significant plant (garland chrysanthemum) responds to salinity stress and how ASA can help it mitigate salinity.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Conduction

Research was carried out at the Islamia University of Bahawalpur, Bahdad ul

Jadeed campus Punjab, Pakistan from October 2021 to April 2022 and was repeated in 2023. The soil was prepared for sowing the seeds by mixing sand and clay in 1:3 respectively. Total 32 pots were filled and each pot was filled with 5 kg of soil. To achieve complete soil hydration the pots were watered for two days prior to seeding. A maximum of 8 to 10 seeds were sown at a depth of 2 centimeters in each pot. The experiment followed a completely randomized design (Billah *et al.*, 2024). To assess the impact of salinity stress plants were treated with varying concentrations of salt and a growth promoter.

2.2 Treatment Application

Following the third treatment the plants were harvested to record their morphological characteristics and prepare them for anatomical studies. Plants were delicately extracted from the pots with softened soil to minimize root damage. The stems, roots and leaves were then gently washed with tap water to remove dust particles effectively. (Billah *et al.*, 2017).

Table 1: Layout of the Experiment Showing Different Concentrations of Sodium Chloride (NaCl) and Ascorbic Acid (ASA) used in Treatments

Treatment		NaCl	Ascorbic	Description
Group	Code	(mM)	Acid (mM)	
Control	T0 /S0	0	0	Absolute Control (No Stress, No ASA)
	T1	50	0	Low Salinity
	T2	100	0	Moderate Salinity
Salt Stress Only	T3	150	0	High Salinity
	S1	50	3	Low Salinity + Low ASA
	S2	100	6	Moderate Salinity + Moderate ASA
Salt + ASA	S3	150	9	High Salinity + High ASA

2.3 Morphological Parameters

The following morphological characteristics were measured: fresh weight (g), dried weight (g), root length (cm), shoot length (cm), plant height (cm), and branch count (Shrestha *et al.*, 2021).

2.4 Anatomical Parameters

Specimen of root, stem, and leaf were taken from each treatment of *G. coronaria* for microscopic examination and were fixed in F.A.A. solution (containing 10 ml formalin, 35 ml 95% ethyl alcohol, and 5 ml glacial acetic acid) (Taha *et al.*, 2020). Following free-hand sectioning a standard double-staining technique was applied to the sections. Photomicrographs of the prepared slides were captured using a camera. Various anatomical characteristics of the stem, root and leaf including epidermal and cortical thickness, root area, vascular bundle area, phloem thickness, and xylem cell area were calculated.

2.5 Statistical Analysis

The trial was organized in a completely randomized design. All morphological and anatomical data were analyzed using the analysis of variance technique (ANOVA) with computer software R (version 4.5.1) by using the package agricolae. Tukey's HSD test ($p < 0.05$) was employed for significant mean comparisons. All the data of morphology and anatomical attributes were represented by the bar plots with the ggplot2 package (Ahmed *et al.*, 2024b).

3. RESULTS

3.1 Morphological Parameters

The morphological characteristics of *G. coronaria* indicated a progressive reduction with increasing salt levels. Elevating the salt concentration in the solution provided to *G. coronaria* led to a modest decrease in various growth parameters, including fresh and dry weight (g), shoot length (cm), root length (cm), branch count and plant height (cm), as illustrated in Figure 1. Application of ascorbic acid under salt stress yielded contrasting outcomes. A significant increase was observed in fresh and dry weight (g), root length (cm), and number of branches where there is decline succeeded by growth in plant height (cm) and shoot length (cm) as shown in figure 2.

3.2 Anatomical Parameters

In the anatomical features of *G. coronaria* the stem, root, and leaf, various alterations were observed. Specifically, in the stem, several anatomical parameters exhibited a minor reduction, including number of vascular bundles, and phloem thickness while there was an initial increase followed by a decline in stem area. There is an increase

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in endodermis with an increased level of salt while there is a dip followed by growth in the cortical cell area, cortical cell thickness, xylem vessel length, epidermal thickness, vascular bundle thickness, and pith area (Figure 3 and plate 1). Whereas ascorbic acid increased the number of vascular bundles, epidermal thickness, endodermis, and pith area while stem area decreased. The phloem thickness initially decreased before increasing, whereas the cortex thickness exhibited opposite trends (Figure 4 and plate 2). Root area, vascular bundle area, and phloem thickness were decreased while cortex thickness, epidermal and endodermis thickness, xylem cell area, and pith thickness were increased with increased salt concentration (Figure 5 and plate 3). Moreover, growth promoter ascorbic acid increased root area, phloem thickness, xylem cell area, cortex thickness, and pith thickness while epidermal, endodermis thickness, and vascular bundle area decreased (Figure 6 and plate 4). In the leaf, several anatomical parameters decreased with increased salt concentration i.e. midrib thickness, leaf's lamina thickness, phloem thickness, vascular bundle region, and cortical cell size while abaxial and adaxial epidermis, xylem cell area, and cortex thickness increased (Figure 7 and plate 5). However, the growth promoter increased all these parameters except the adaxial epidermis and cortical cell area (Figure 8 and plate 6).

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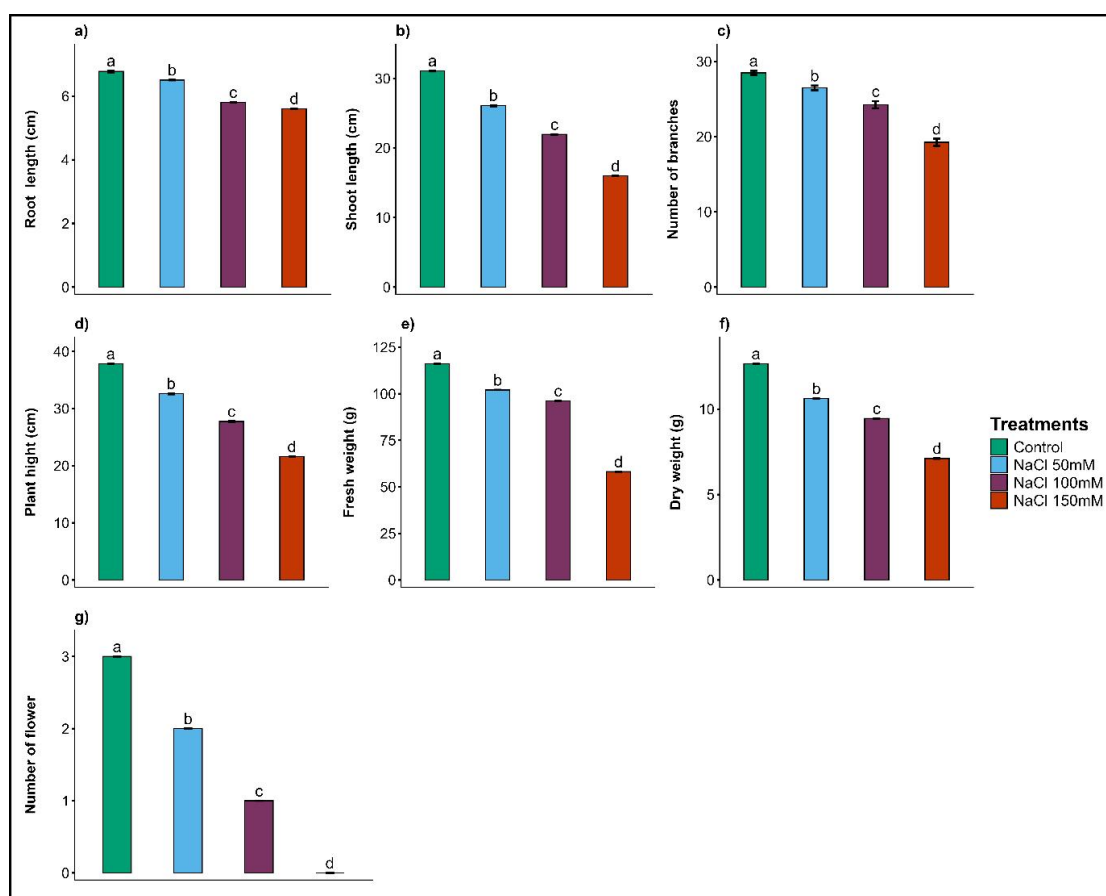


Figure 1: Morphological parameters of *Glebionis coronaria* at various concentrations of NaCl indicating the means + SE (n=4), different alphabets above the bar represented as significant, and the same letters indicate no significant difference.

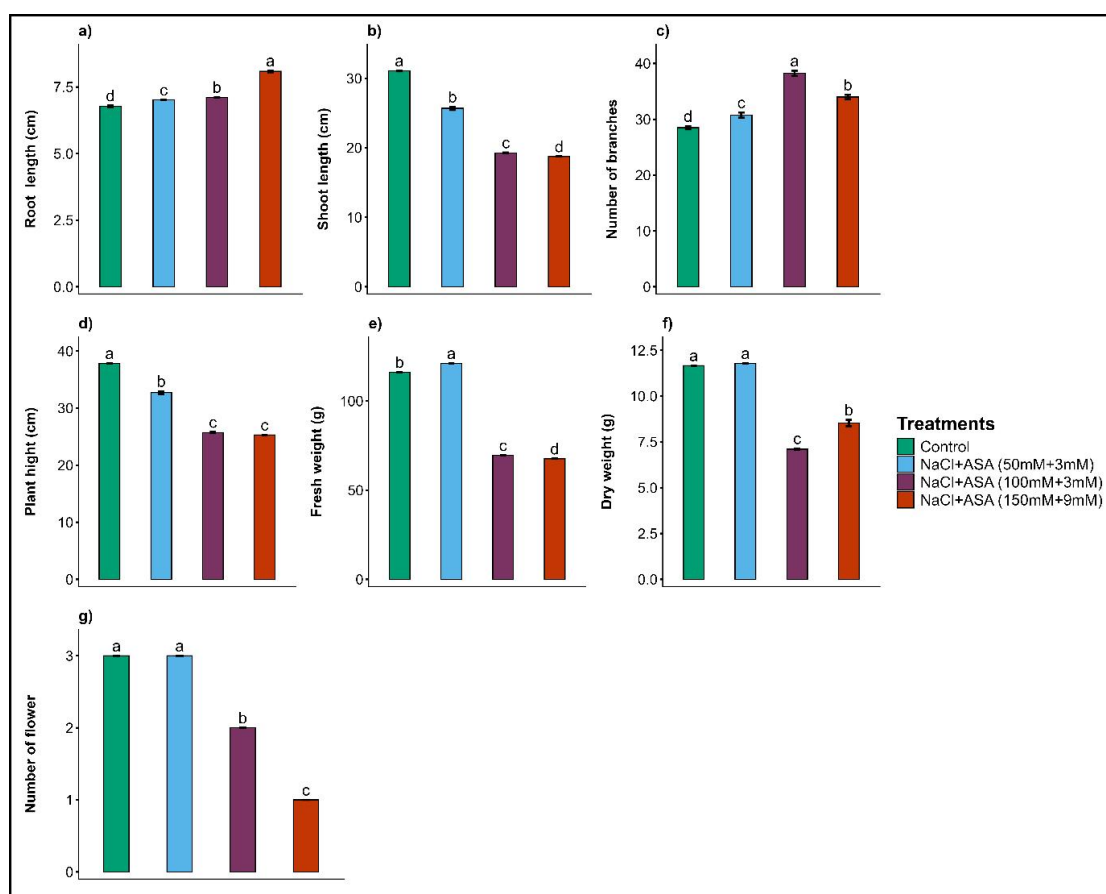


Figure 2: Morphological parameters of *Glebionis coronaria* at various concentrations of NaCl+ASA indicating the means + SE (n=4), different alphabets above the bar represented as significant, and the same letters indicate no significant difference.

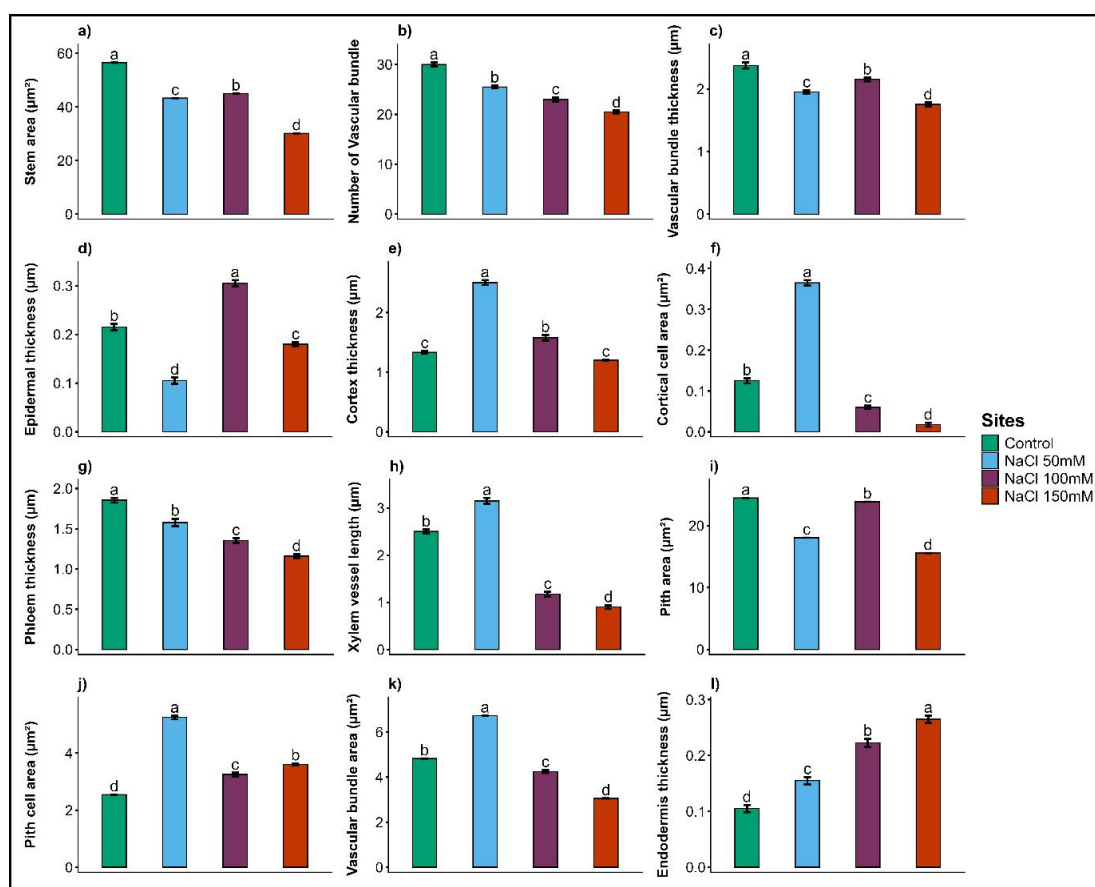


Figure 3: Comparison of anatomical parameters of stem at various concentrations of NaCl indicating the means + SE (n=4), different alphabets above the bar represented as significant, and the same letters indicate no significant difference.

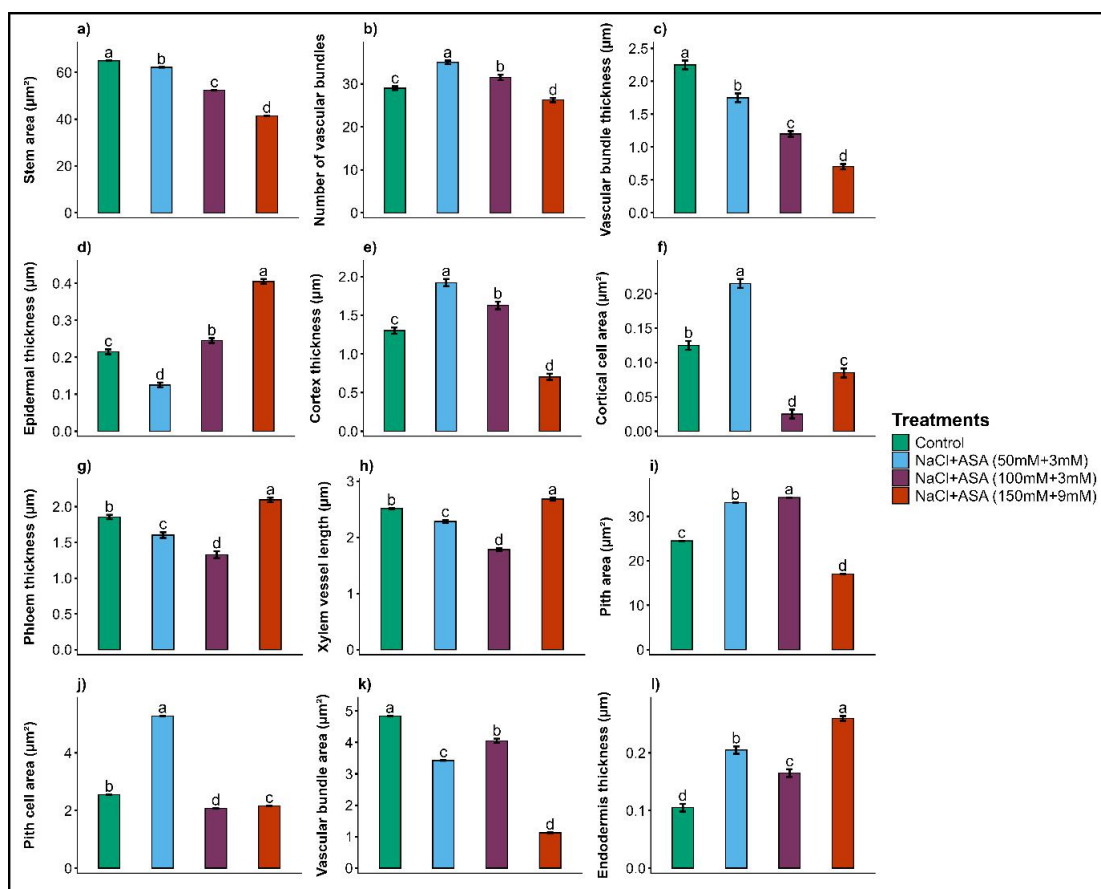


Figure 4: Comparison of anatomical parameters of stem at various concentrations of NaCl+ASA indicating the means + SE (n=4), different alphabets above the bar represented as significant, and the same letters indicate no significant difference.

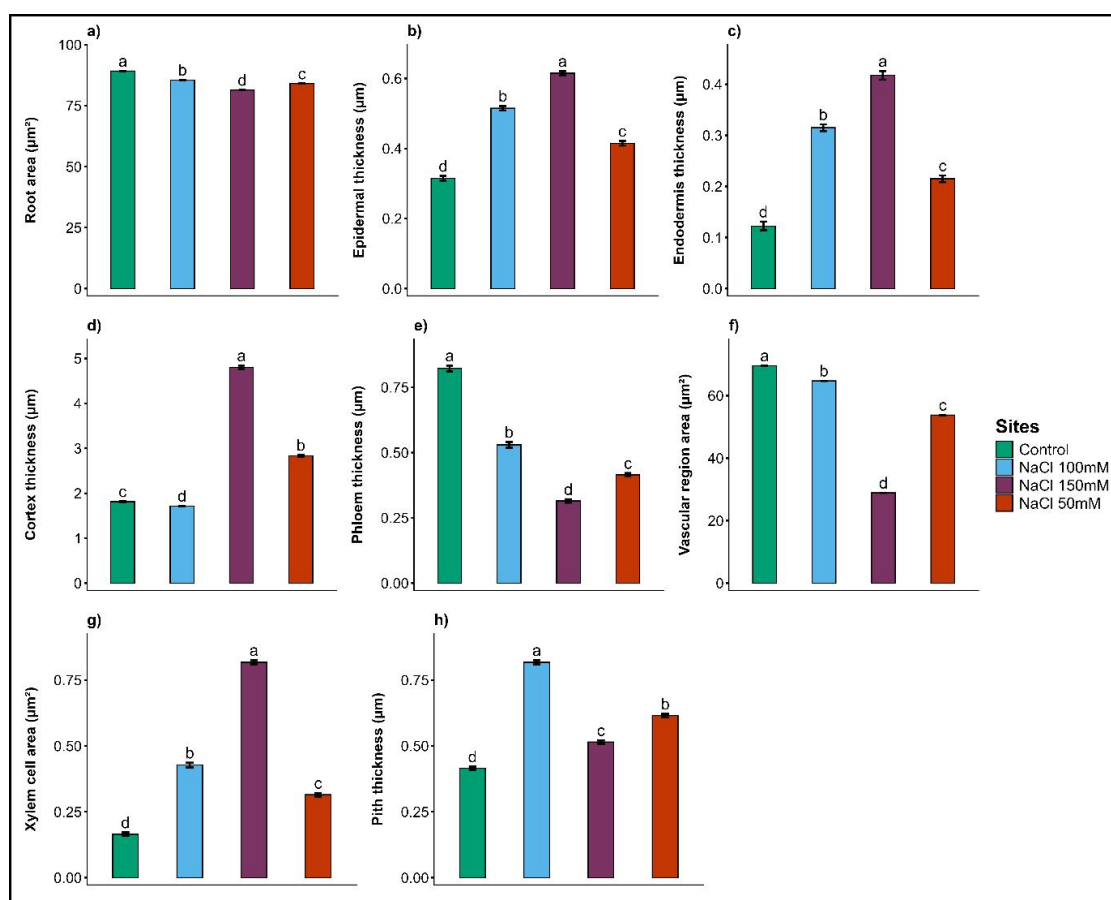


Figure 5: Comparison of anatomical parameters of Root at various concentrations of NaCl, indicating the means + SE (n=4), different alphabets above the bar represented as significant, and the same letters indicate no significant difference.

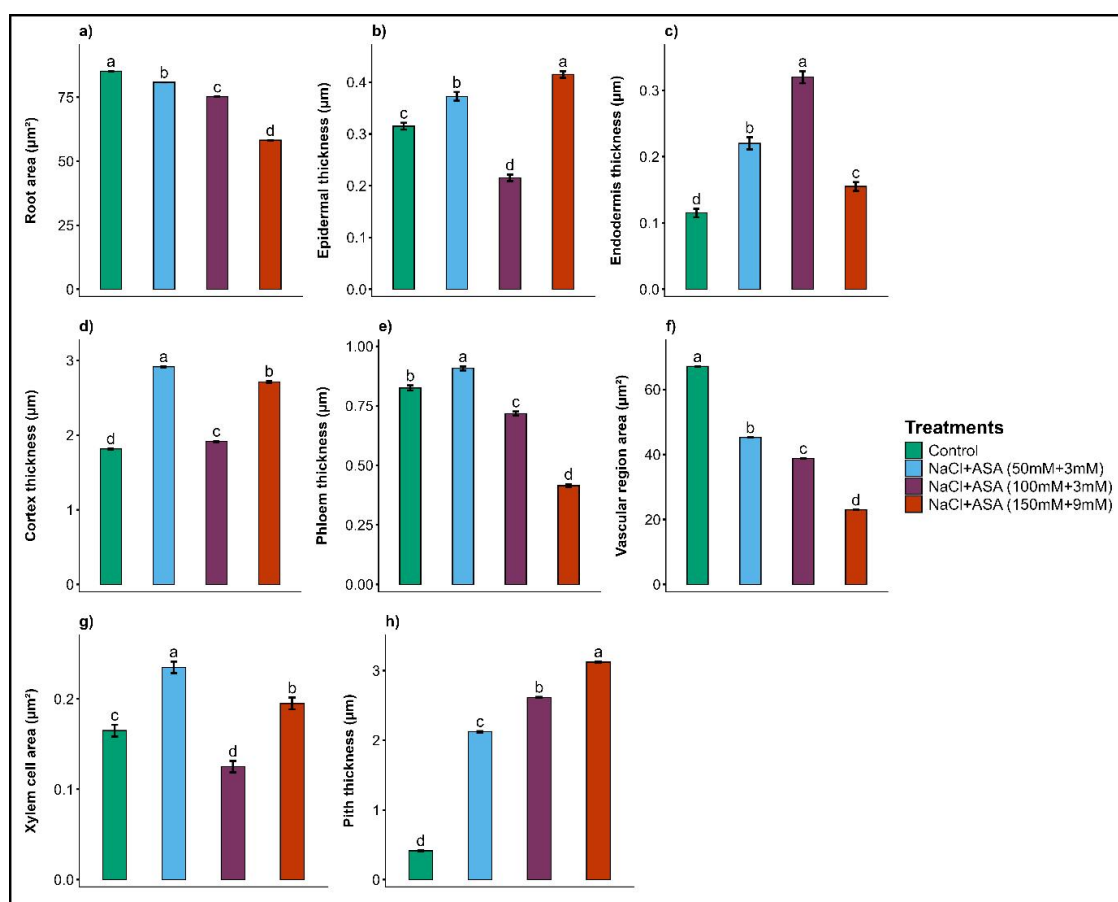


Figure 6: Comparison of anatomical parameters of Root at various concentrations of NaCl+ASA indicating the means + SE (n=4), different alphabets above the bar represented as significant and same letters indicates no significant difference.

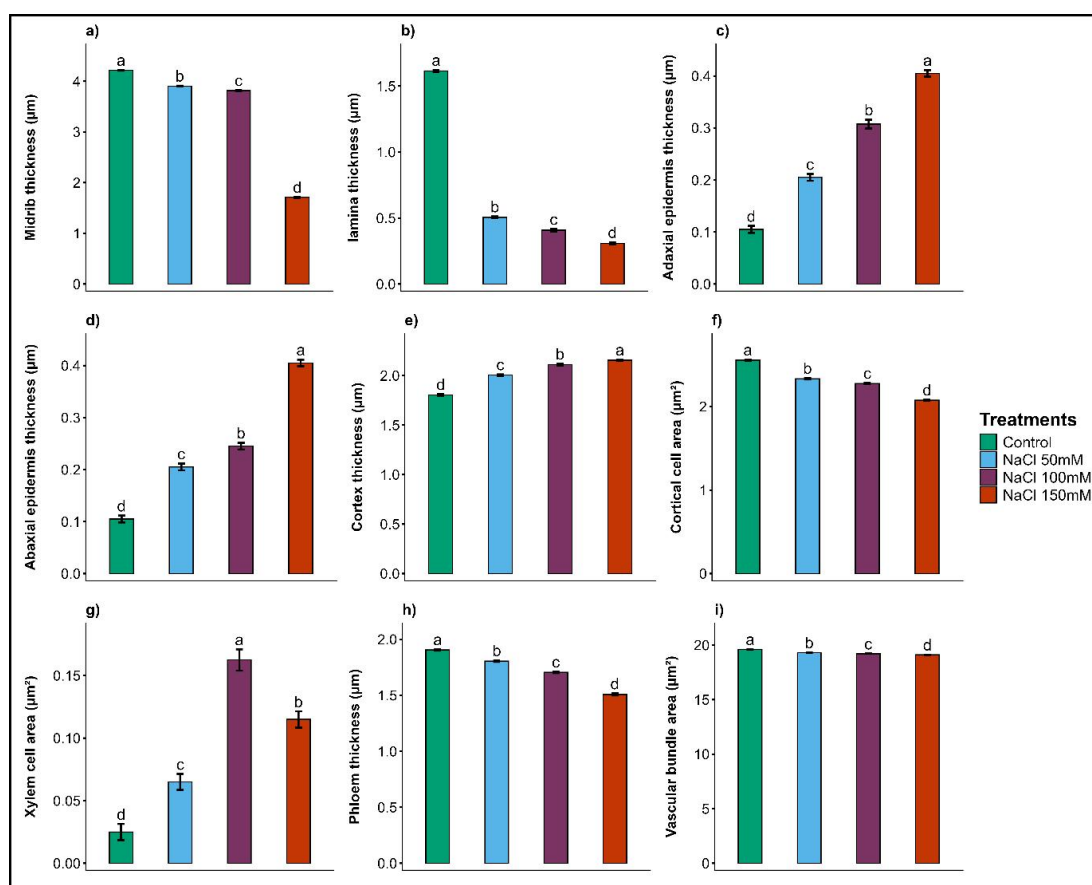


Figure 7: Comparison of anatomical parameters of the leaf at represent concentrations of NaCl indicating the means + SE (n=4), different alphabets above the bar represented as significant and same letters indicates no significant difference.

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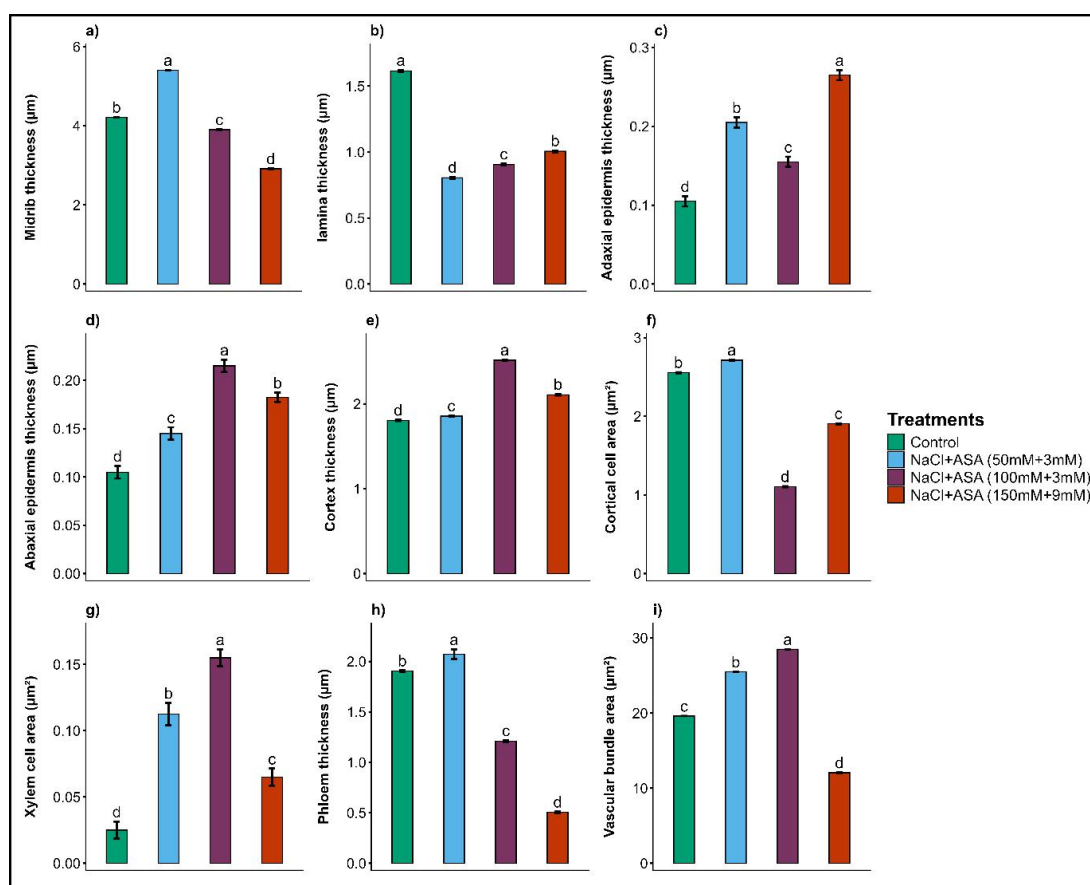
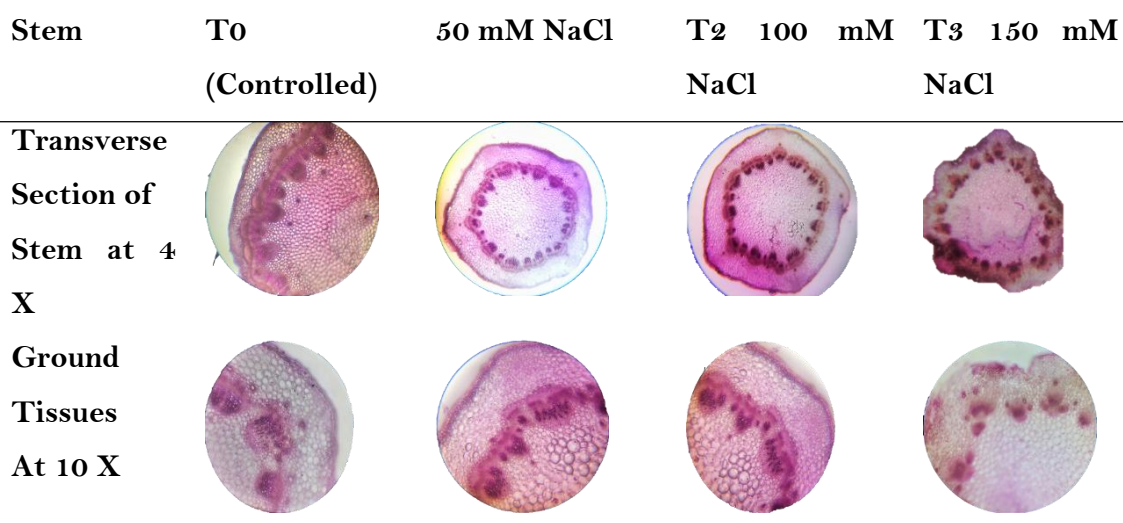
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Figure 8: Comparison of anatomical parameters of the leaf at various concentrations of NaCl + ASA indicates the means + SE (n=4), different alphabets above the bar represent a significant and same letters indicate no significant difference.



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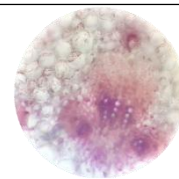
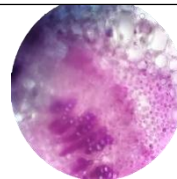
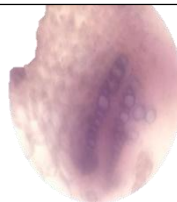
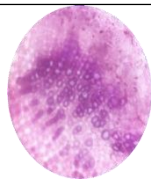
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Vascular
bundles at
40 X



Pith region
at 40 X

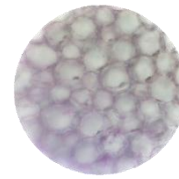
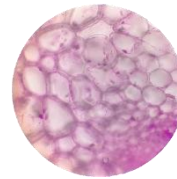
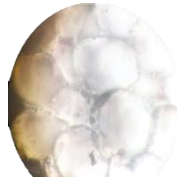
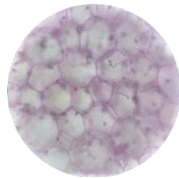
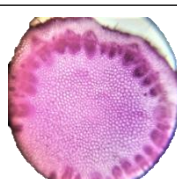
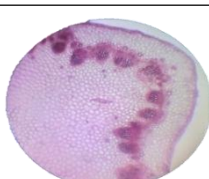


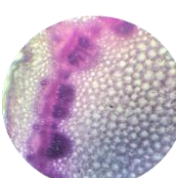
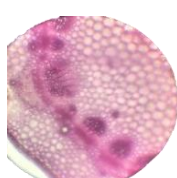
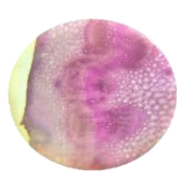
Plate 1: Stem anatomy of *Glebionis coronaria* under different levels of NaCl (control, 50 mM, 100 mM and 150 mM).

Stem	S0 (Controlled)	S1 3mM ASA+ 50 mM NaCl	S2 6mM ASA+ 100 mM NaCl	S3 9mM ASA+ 150 mM NaCl
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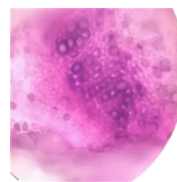
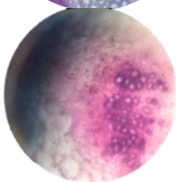
Transverse
Section of
Stem at 4
X



Ground
Tissues
At 10 X



Vascular
bundles at
40 X



Pith
region at
40 X

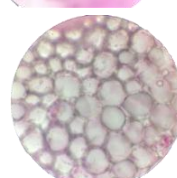
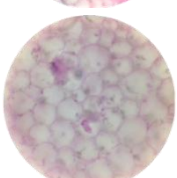
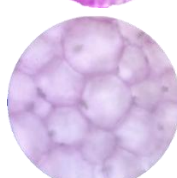
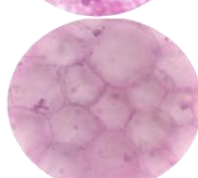


Plate 2: Stem anatomy of *Glebionis coronaria* under different levels of NaCl + ASA (control, 50 mM + 3 mM, 100 mM + 6 mM and 150 mM + 9 mM respectively).

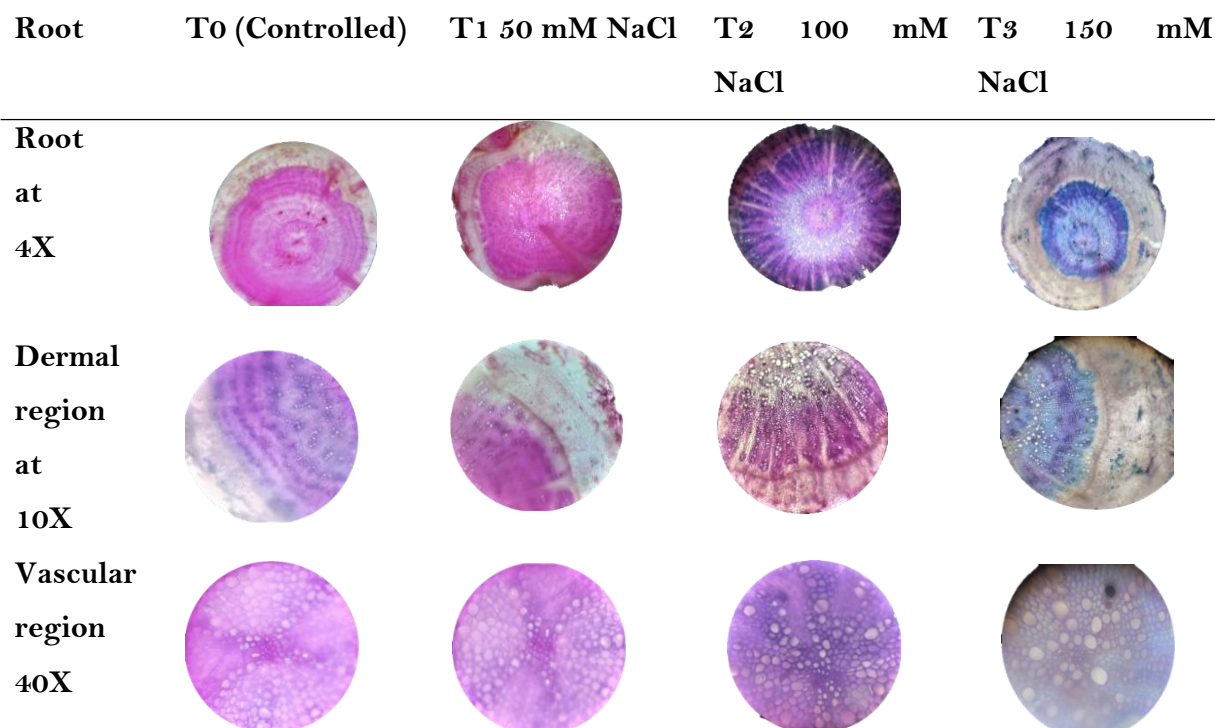


Plate 3: Root anatomy of *Glebionis coronaria* under different levels of NaCl (control, 50 mM, 100 mM, and 150 mM)

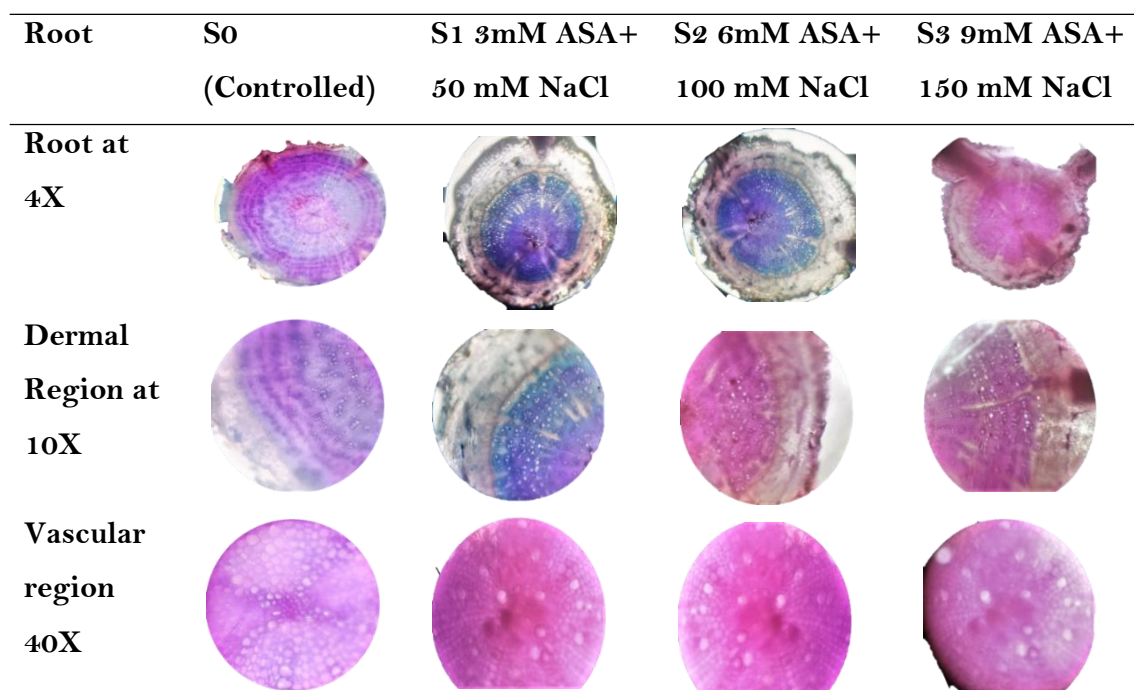


Plate 4: Root anatomy of *Glebionis coronaria* under different levels of NaCl + ASA (control, 50 mM + 3 mM, 100 mM+6 mM, and 150 mM+9 mM)

respectively)

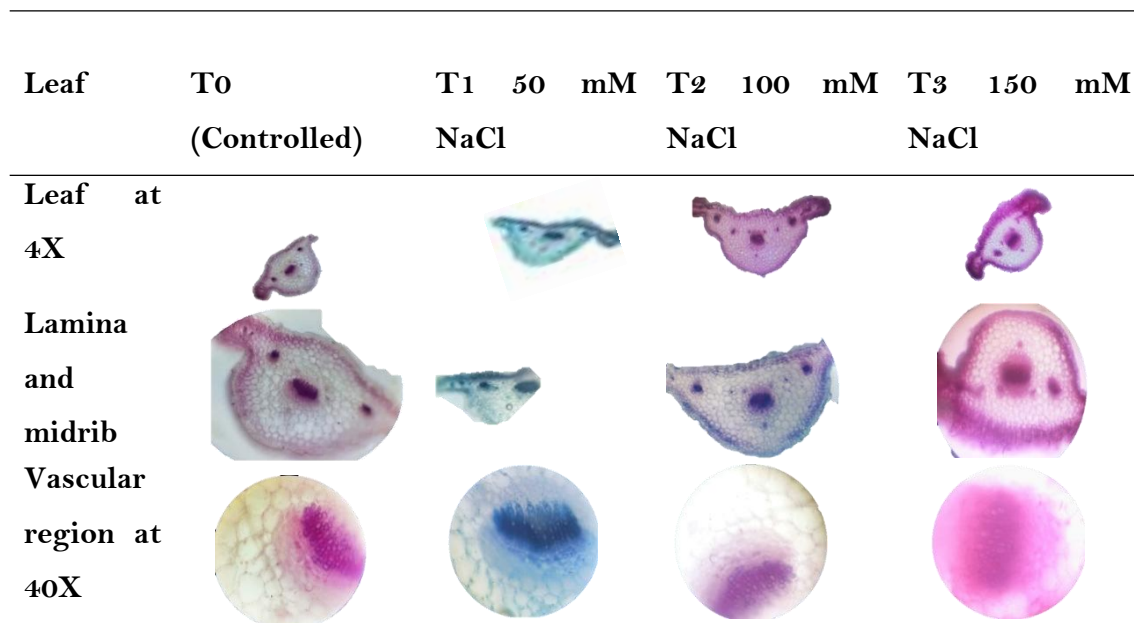


Plate 5: Leaf anatomy of *Glebionis coronaria* under different levels of NaCl stress (control, 50 mM, 100 mM, and 150 mM).

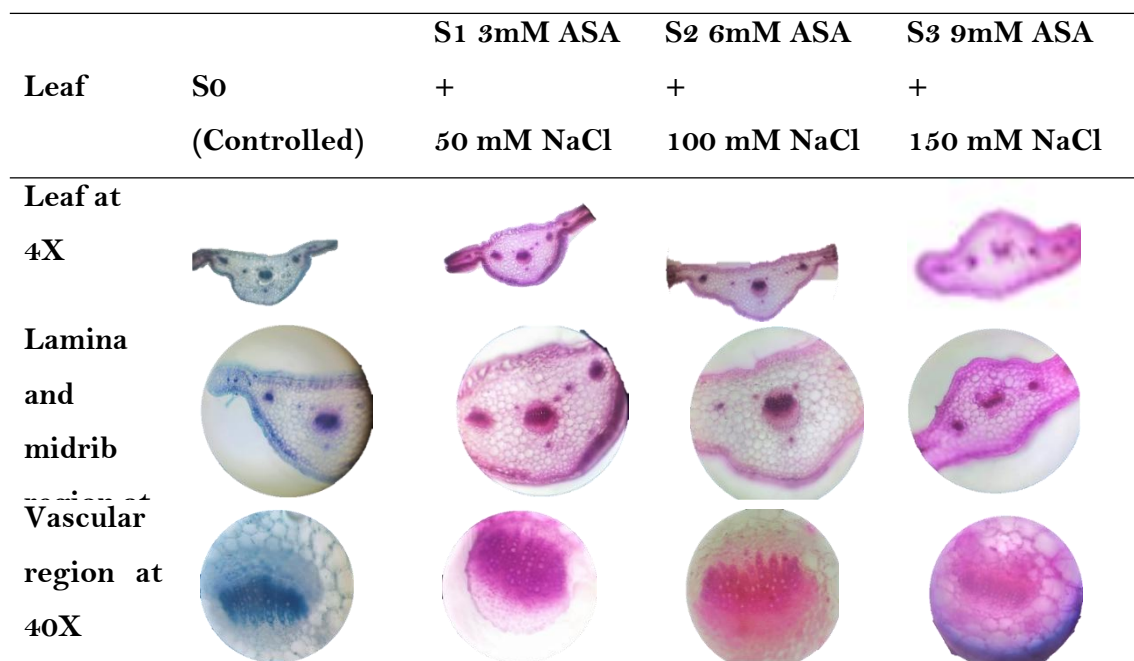


Plate 6: Leaf anatomy of *Glebionis coronaria* under different levels of NaCl + ASA (control, 50 mM + 3 mM, 100 mM + 6 mM and 150 mM + 9 mM respectively).

DISCUSSION

Salt stress not only affect plant growth and development, but it also has a significant influence on crop yield. Under salt conditions, tolerant metabolic processes are critical for plant survival and productivity (Javeed *et al.*, 2023; Kumari *et al.*, 2021). Salt stress has a direct impact on plant development, with leaf growth limitations being one of the first noticeable results. Higher salt concentrations cause considerable increases in leaf thickness, breadth, and length. Lower salt levels enhance leaf length and width, while greater salt concentrations accelerate leaf thickness (Yao *et al.*, 2023)

The lengths of roots and shoots reduced as salt stress increased. According to **Ren et al., (2023)** lengths of roots and shoots decreased with the increase in salinity because of lowers the osmotic potential of the soil solution that reduced the cell turgor pressure which required for the cell elongation and expansion. The plant height of *Glebionis coronaria* decreased with increased levels of the salinity. The similar results were observed on tomato plants and sweet paper (El-Beltagi *et al.*, 2022).

The fresh and dry weights of the plants decreased as the salt levels increased. Similar findings were reported by (Ors and Suarez, 2017) in spinach. Findings of Atta et al., 2023 analyzed that high concentration of soluble salts lowers the osmotic potential of soil. All of this prevented the water uptake, with the accumulation of the toxic ions disturbed the metabolic processes of the photosynthesis.

According to our findings, root length of *Glebionis coronaria* enhance when the plant is treated with ascorbic acid, counteracting the negative effects of salt. Similar results were reported by (Wang *et al.*, 2019), who found that ascorbic acid increased root length in Okra plants and mitigated the effects of the salinity. Study of Kanwal et al., (2024) that ascorbic acid under salinity levels minimizes oxidative stress caused by reactive oxygen species. This also improve the cell division in root apical meristem due to ionic homeostasis. Based on our results, lower concentrations of ASA had no significant impact on plant height compared to the control, resulting in reduced growth relative to the control and failing to mitigate the negative effects of salt. However, higher concentrations of ASA increase plant height compared to

plants treated with salt alone, demonstrating that ASA alleviates the negative effects of salt and promotes increased plant height contrary results have also been documented. For instance, (Naeem *et al.*, 2020) observed on basil plants, and (Kandil *et al.*, 2020) on soybean plants that the application of ascorbic acid accelerated cell division and growth, significantly increasing plant height. They found that compared to saline-treated plants, both fresh and dry weights increased when treated with ASA, except at 6 mM ASA.

In *Glebionis coronaria* the stem area initially increased at 100 mM NaCl but decreased as salt levels go higher. Findings reported by Hameed *et al.*, (2010) stem area of *Cynodon dactylon* consistently increased as the salt levels increased. Despite the stems being generally thinner, they observed an increase in stem area with higher NaCl concentrations. The vascular bundles of the stem undergo structural adaptations due to salinity, as due to a high level of salt stress vascular bundle number and phloem thickness are significantly decreased in plants under salinity stress. The same results were found by (Rashid and Ahmed, 2011) that salinity alters the phloem and vascular bundle of the stem structurally. Cortical cell area and cortical cell thickness first declined and then continued to expand as salt levels increased, reaching up to 100 mM. The same results were found by (Silva *et al.*, 2021) with anatomical reactions intended to reduce oxidative stress. The cortical cell area grew at 50 mM NaCl and then declined as the outer salt level rose, although cortical thickness improved consistently with the amount of salt used. Increased vascular bundle space in comparably less tolerant plants (*Zizyphus mauritiana*) (Liu *et al.*, 2022), and the same results were found here when the vascular bundle area increased at 50 mM of NaCl.

Root area normally decreased with increasing salt stress levels modest increase in root area was seen at a salt stress concentration of 100 mM, however. Sarkar and Rashid *et al.*, 2015 and (Hameed *et al.*, 2010) published similar findings, suggesting that root area decreased under salt stress conditions. The cortex thickness, epidermal thickness, xylem cell area, pith thickness, and endodermis thickness increased with the salt stress mostly at 150 mM of NaCl Alike consequences were stated by (Hameed *et al.*, 2010). The plasmalemma of cortical

cells of the young root is actively involved in the absorption and establishment of a surface in taking the water and salt from the apoplast. So, the reduction of cortical cells at 100m M of NaCl is the reduction of this absorption. There was also a drop in vascular bundle area and phloem thickness. This decrease is due to high salinity. High salinity levels caused a reduction in the translocation of water (Céccoli *et al.*, 2011).

The root area of plants subjected to NaCl+ASA increased, demonstrating that foliar ascorbic acid treatment mitigates the effects of salt stress. These findings are consistent with (Stevens *et al.*, 2006) research, which highlighted salicylic acid's part in improving salt tolerance mechanisms in plants, a role that has been shown across a variety of crops. However, when ASA was given as a foliar spray under salt chloride stress, root area decreased. In contrast to plants under sodium chloride stress, ASA foliar spray application lowered epidermal thickness, endodermis thickness, and vascular bundle area. The opposite results were seen by (El-Taher *et al.*, 2021) in the current investigation, cowpea plants subjected to 6000 ppm of salt stress received a foliar treatment of 100 ppm of SA, which improved the anatomical characteristics of leaves. These outcomes line up with what (Nour *et al.*, 2012) have noticed. However, an increase was observed in phloem thickness, cortex thickness, xylem cell area, and pith thickness when the plant was treated with ASA. Similarly, in tough saline locations increasing xylem cell size and phloem thickness is critical for aiding water and photosynthate movement. Similar results were reported by (El-Taher *et al.*, 2021), who found that salicylic acid increased these parameters as well.

Results showed a significant decrease in the anatomical structure with increasing salinity levels. When compared to the control, the midrib thickness reduced as saline stress increased. The midrib was shortest at the greatest salinity level. Alike results were reported by (Ola *et al.*, 2012) where there is a decline in midrib thickness [Salt Range Ecotype]. There was also a reduction in the leaf's lamina width, phloem thickness, vascular bundle region, and cortical cell size. The leaf margin, in particular, showed a considerable drop in lamina width. The same outcomes were stated by (Awasthi *et al.*, 1999) who described that there is a decrease in the lamina thickness of the leaf of barley seedlings. Also, the findings of (Hameed

et al., 2013) showed that there is a decrease in lamina thickness. This decrease results from the physiological functions of the leaf being affected by salt. The vascular bundle area also decreased. The findings concur with those of (Hameed *et al.*, 2009) who examined the anatomical adaptation of a cylindrical *Imperata* leaf [Faisalabad ecotype] and the vascular bundle region and cortical cell size decreased as the salt content in the growth media rose, whereas the phloem area rose. The abaxial and adaxial epidermis, xylem cell area, and cortex thickness increased with increased levels of salt stress. The results are related to (Hameed *et al.*, 2009) where the abaxial and adaxial epidermis increased.

When a foliar application of ASA was sprayed on the plant, the midrib width, lamina width, and vascular bundle area all increased. Alike outcomes were reported by (Nassar *et al.*, 2019) who observed a rise in lamina and midrib thickness of basil leaf with foliar application of ASA. (Khafagy *et al.*, 2009) stated that ascorbic acid and glycine betaine increased these parameters of sweet pepper. The lower or abaxial epidermis when sprayed with ASA under NaCl increased as compared to saline conditions but the adaxial epidermis decreased as compared to saline conditions. The cortical cell area first increases when the plant is applied with 3m M ASA then decreases. The cortex thickness, cortex thickness, xylem cell area, and phloem thickness first decreased with sprayed with 6 mM ASA then there was an increase in it. Results are similar to those (Nassar *et al.*, 2019) stated that xylem rows increased when sprayed with ascorbic acid. The ascorbic acid lessens the effect of salt on leaf anatomical parameters (Desoky and Merwad, 2015).

CONCLUSION

Reduced plant growth due to salt stress may result from internal hormonal imbalances, inhibited cell division, and reduced water uptake. The study revealed that applying ascorbic acid during salt stress improves morphological and anatomical characteristics especially leaf anatomy, thereby enhancing plant growth mechanisms.

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