Effect of Exogenically Applied Gibberellic Acid on Morpho-Anatomical Characteristics of *Chenopodium quinoa* under Salt Stress

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***Abstract*:** Environmental changes can cause the stresses including salt stress which may reduce the crop yield. Plants are impacted by salinity through plant height, number of leaves, the length of roots, osmotic impacts, and ion-specific effects. In the present study, a pot experiment was conducted to evaluate the effect of salt stress, growth promoter gibberellic acid, and NaCl+GA on *Chenopodium quinoa*. It is an annual herbaceous plant that is cultivated mainly for its edible seeds, which are higher in protein, dietary fiber, vitamin B, and minerals than many other grains. Salt concentrations of different levels of NaCl (control, 50 mM, 100 mM, 150 mM, 200 mM) moreover, a maximum of 20 mM and a minimum of 10 mM of gibberellic acid were given to plants. The effect of salinity on morphological parameters such as fresh weight, dry weight, shoot length, root length, and number of leaves showed a significant decrease whereas growth promoter enhanced root, shoot length, and number of leaves. Anatomical parameters such as epidermal thickness, cortical thickness, xylem thickness, and phloem thickness showed contrary results with salinity and by the application of gibberellic acid. In conclusion, a decline in plant growth brought on by salt stress may be caused by endogenous hormonal imbalances, suppression of cell division, decrease in water absorption, and the activity of the root metabolic process. These mechanisms have been balanced by the application of growth promoter. The beneficial impact of gibberellic acid on water intake and seed germination of crops under saline conditions. Moreover, the gibberellic acid enhanced the germination regulation mechanism.

***Index-terms*:** Gibberellic acid; Salinity; NaCl; *Chenopodium*; Morphological; Anatomical parameters.

I. INTRODUCTION

Environmental stresses are one of the most important elements that significantly lower crop productivity as a consequence of climate change. Moreover, signal transduction, gene expression, protein expression, and post-translational modifications may fluctuate in response to environmental conditions (Hasanuzzaman *et al.*, 2020). Primary or secondary metabolites can result from biotic and abiotic stress, and their synthesis is strictly regulated by gene and epigenetic mechanisms. Additionally, their transportation is subject to additional restrictions. Plants react differently to biotic and abiotic stress. Abiotic signals are detected by a disruption of the plasma membrane, whereas biotic signals are receptor-mediated (Singh *et al.*, 2020). Plants develop in response to these stress elements since the changing environment will expose the plants to interactive impacts simultaneously (Raza *et al.*, 2020). Plants have evolved a variety of intricate resistance and adaptation mechanisms (Seleiman *et al.*, 2021).

Salinity in the soil has a negative impact on the plant's growth and development. These effects are usually linked to Na^+ toxicity, which can affect K^+ homeostasis and adversely influence a vast number of enzymes when they reach high cytosolic concentrations. Cl is even more poisonous to some plants than Na^+ (Reginato *et al.,* 2021). In salt stress, NaCl induces an increase in plant height with low and medium concentrations and a loss with the highest concentration. Low concentrations had no apparent effects on the number of leaves or leaf area, but two higher concentrations showed a decline. In the presence of high salt concentrations, certain halophyte species may escape ion toxicity and maintain water absorption (Ketehouli *et al.,* 2019).

Quinoa is described as a "pseudocereal" because it produces seeds that can be ground into flour and eaten like a cereal crop while not being a member of the Gramineae family. Quinoa has remarkable tolerance to abiotic stresses and the fact that these plants' enormous genetic variety enables them to adapt to and thrive in even the most unfavorable environmental situations (Pereira *et al.,* 2019). Crop growth and productivity are adversely impacted by saline soil. The main issues that plants on salty soils confront are nutrient shortage, ionic damage, and saline impact. Salinity reduces leaf gas exchange, chlorophyll, and relative water contents (RWC), as a result of the excessive creation of numerous reactive oxygen species, plants also experience oxidative stress ROS (Abbas *et al.,* 2021).

It has been demonstrated that under NaCl concentrations, *C. quinoa* cells vacuoles effectively sequester Na^+ young leaves with little to no vacuolation (Adolf *et al.,* 2021). Plant hormones are involved in the production of plant stress responses and are active promoters of plants (zhu *et al.,* 2019). Gibberellic acid has a beneficial impact on water intake and germination of crops under normal conditions. Moreover, the associated gibberellic acid germination regulation mechanism is currently understood (zhu *et al.,* 2019). Gibberellic acid is a substance that works well at reducing oxidative stress and preventing the oxidation of lipids. Additionally, it reduces the effect of numerous abiotic stresses such as salt stress. Gibberellic acid enhances hydration status and increases photosynthetic capability (Babaei *et al.,* 2022).

II. MATERIAL AND METHODS

2.1 Plant material and experiment conduction

The experiment was conducted at the Baghdad ul Jadeed Campus The Islamia University Of Bahawalpur, Punjab Pakistan. The certified seeds of *C. quinoa* were collected and were grown in pots. The Soil was prepared for the sowing of seeds by mixing sand and clay 1:3 ratio respectively. There were 40 pots of 5-kilogram soil filled in each pot arranged into blocks. Soil in pots was watered for 2 days to keep full moisture. Ten to twelve seeds were sown in each pot at a depth of 1-2 cm in diameter. The experiment was designed as a completely randomized design (CRD). To evaluate the salinity stress the plants were treated with different salt and growth promoter concentrations.

NaCl salt treatment levels
T0 = control
T1 = 50 mM
T2 = 100 mM
T3 = 150 mM
T4 = 200 mM
Treatment levels of salts with growth promoter gibberellic acid
G0 = control
G1=50 mM (salt) + 10 mM (gibberellic acid)
G2=100 mM (salt) + 10 mM (gibberellic acid)
G3=150 mM (salt) + 20 mM (gibberellic acid)
G4=200 mM (salt) + 20 mM (gibberellic acid)

Three-time treatments of salt and growth promoter were given to plants after one-week intervals. Plants were sampled after the completion of treatments to record morphological parameters and to preserve them for anatomical studies. The soil in the pots first softened and the whole plant was removed carefully without any major damage to the roots. The stem root and leaves were washed gently with tap water to eradicate the dust particles properly (Khalil *et al*., 2023).

2.2 Morphological parameters

Fresh weight (g)**,** shoot length(cm)**,** dry weight(g)**,** root length(cm)**,** number of leaves, and leaf area(cm^2) were recorded.

2.3 Anatomical parameters

For microscopic investigation, *C. quinoa* root, stem, and leaf samples were prepared by killing and fixing in F.A.A. (10 ml formalin, 35 ml ethyl alcohol 95%, and 5ml glacial acetic acid). Afterward free-hand sectioning, the standard double-stained technique was used for staining. Photographs of anatomical slides were recorded with a digital camera. Stem, root and leaf anatomy including area (μm^2), epidermal thickness (µm), epidermal cell area (μm^2), cortical cell area (μm^2), cortical thickness (µm), metaxylem vessel area (μm^2), phloem thickness(µm), endodermis thickness (µm) and endodermis cell area (μm^2) were calculated.

2.4 Statistical analysis

The experiment was organized in a completely randomized design. All the morphological and anatomical data were subjected to the analysis of variance technique (ANOVA) by using computer software technique. LSD test was applied for mean comparison and Pearson correlation using SPSS 1997 was used to correlate various attributes with each other.

III. RESULTS

3.1 Morphological characteristics

C. quinoa showed a gradual decrease in all the morphological characteristics due to increasing levels of salt. Increasing concentration of salt solution given to *C. quinoa* showed slight reduction in fresh weight (g)**,** shoot length (cm)**,** dry weight (g)**,** root length (cm)**,** number of leaves**,** leaf area ($cm^{2)}$ (Fig. 1). Moreover, gibberellic acid mitigates the salt stress showed contrary results in all these morphological characteristics. Shoot length (cm)**,** dry weight (g)**,** root length (cm)**,** number of leaves**,** leaf area (cm^2), and fresh weight (g) were increased (Fig. 2).

3.2 Anatomical characteristics

In anatomical characteristics *C. quinoa* stem, root, and leaf showed different modifications. In the stem, most of the anatomical characters showed a slight decrease in epidermal thickness, cortex thickness, endodermis thickness, and endodermal cell area (Fig. 3,4, and plate 1). Stem phloem thickness and pith area also reduced under salt stress. Whereas, gibberellic acid increased stem area, epidermis thickness, cortex thickness, endodermis thickness, and endodermal cell area. Stem phloem thickness and pith area also increased (Fig. 4,5 and plate 2). Root area, epidermal thickness, cortex thickness, endodermis thickness, and endodermal cell area showed reduction (Fig. 6,7, and plate 3). Root phloem thickness also decreased. Moreover, the growth regulator increased root area, epidermis thickness, cortex thickness, endodermis thickness, and endodermal cell area. Root phloem thickness was also enhanced by the application of gibberellic acid (Fig. 8,9, and plate 4). In the leaf, the following anatomical parameters decreased at a moderate level of salt solution (Fig. 10,11, and plate 5). However, growth promoters caused an increase in the leaf area, epidermis thickness, cortex, midrib cell area, and metaxylem vessel thickness. Leaf phloem thickness also increased (Fig. 12,13, and plate 6).

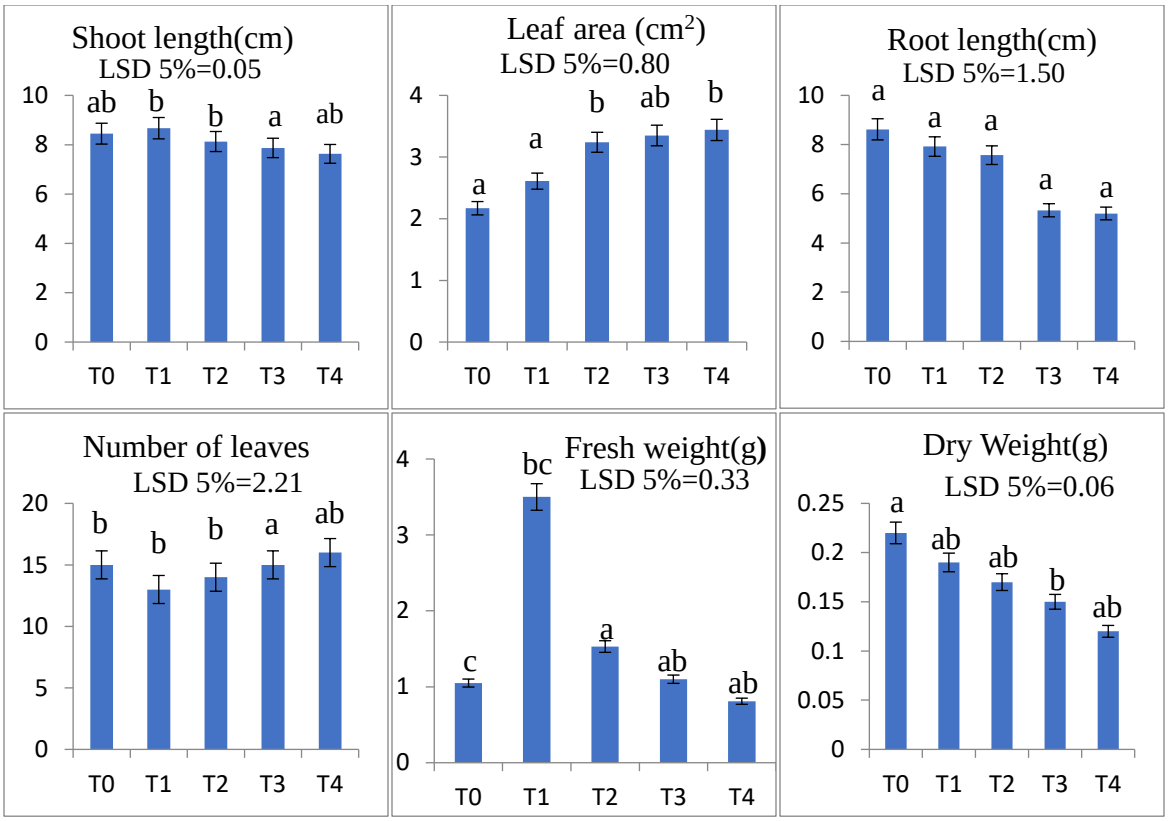
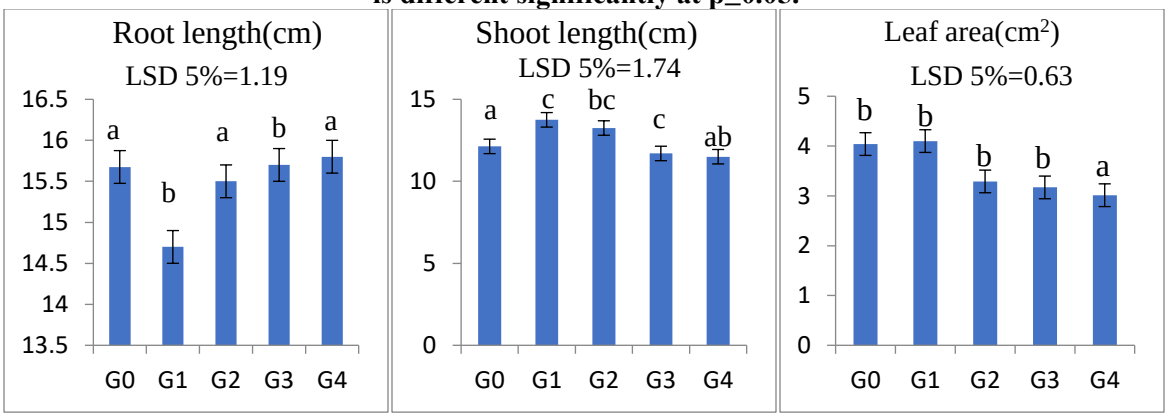


Figure 1: Morphological parameters root length, shoot length, Number of leaves, and Leaf area fresh and dry weight of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150 mM, T4=200 mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.



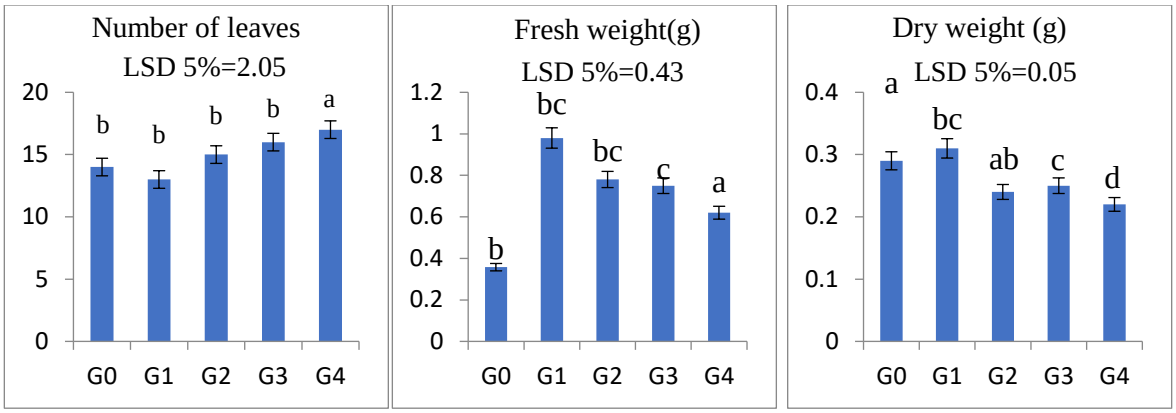


Figure 2: Morphological parameters Root length, shoot length, number of leaves, and Leaf area fresh and dry weight of *C. quinoa* are shown in graphs at different levels of salts with growth promoter gibberellic acid (control, G1=50 mM+10 mM, G2=100 mM+10 mM, G3=150 mM+20 mM, G4=200 mM+20 mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

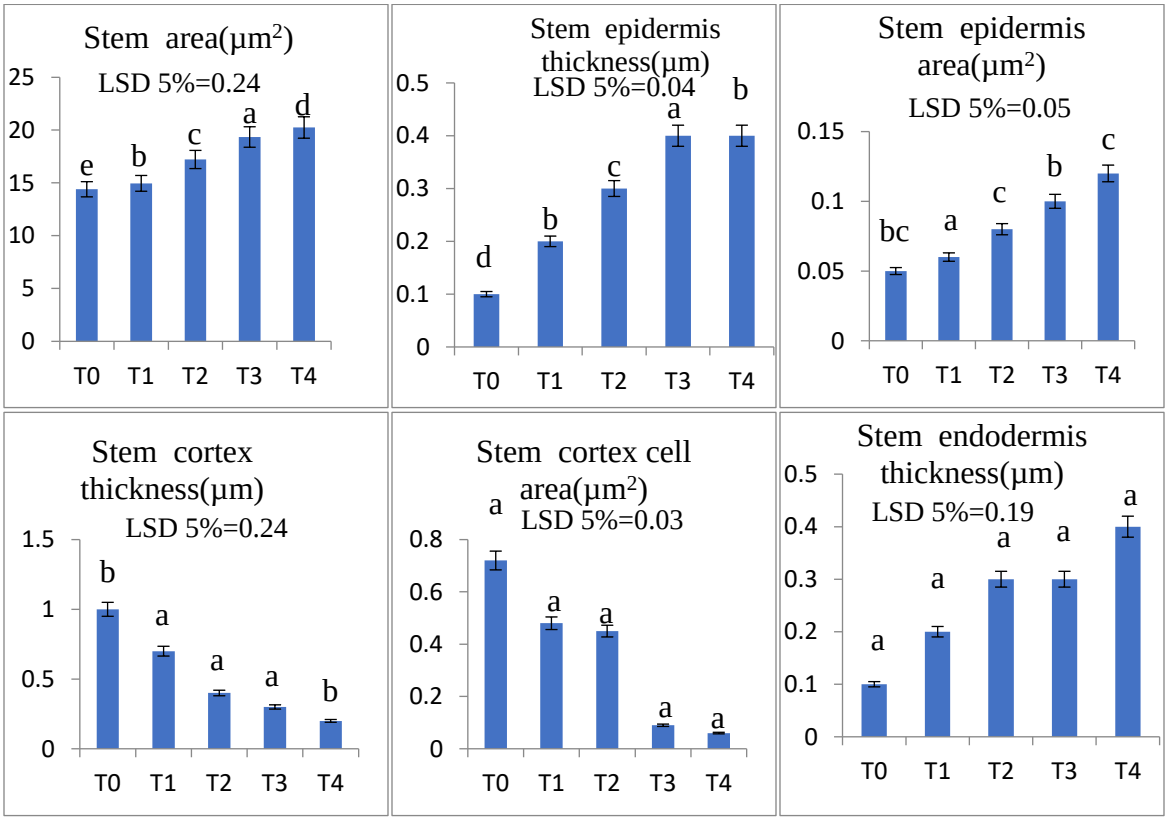


Figure 3: Anatomical parameters of stem area, cortex, and endodermis of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150 mM, T4=200 mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

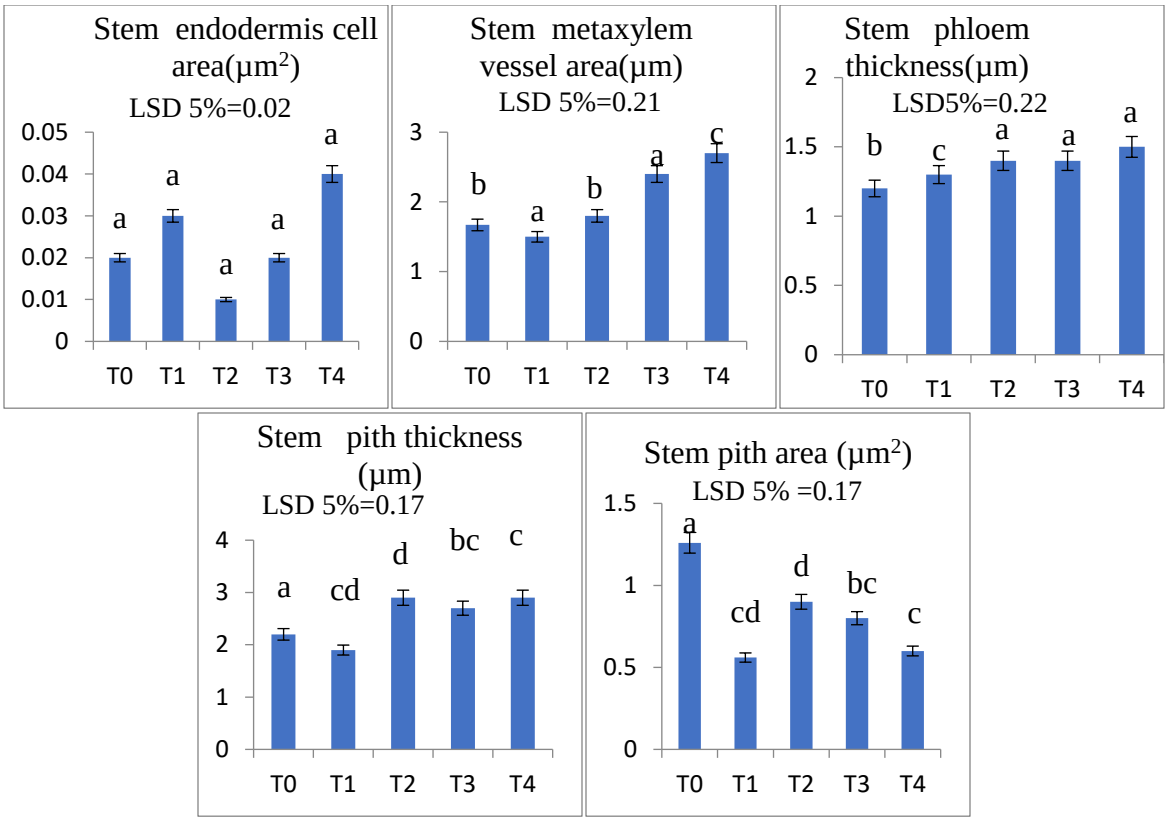
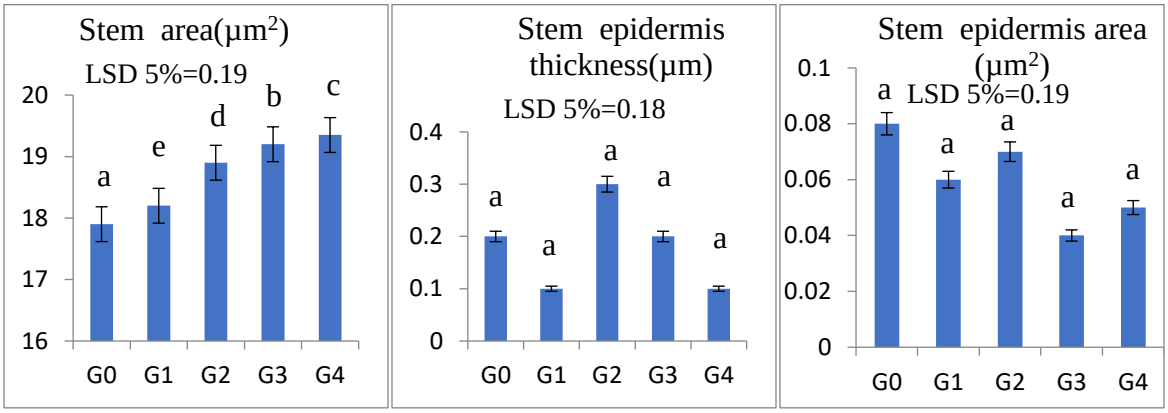


Figure 4: Anatomical parameters of stem endodermis and vascular region of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150mM, T4=200mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.



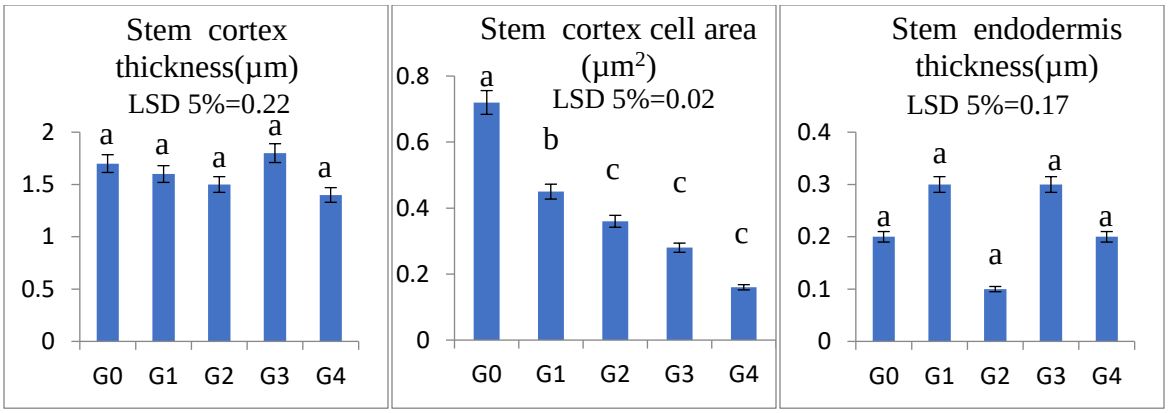


Figure 5: Anatomical parameters *C. quinoa* are shown in graphs at different levels of salts (control, G1=50+10 mM, G2=100+10 mM, G3=150+20 mM, G4=200+20 mM) with gibberellic acid. Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

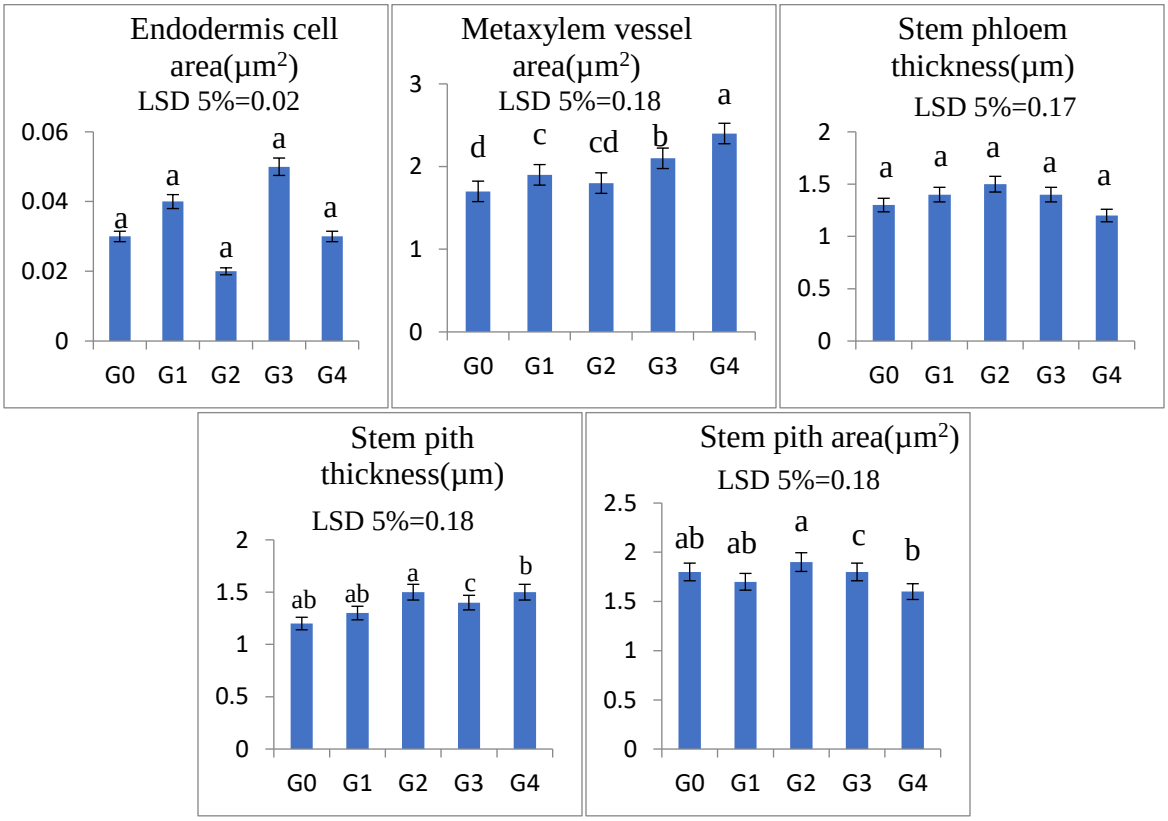


Figure 6: Anatomical parameters of endodermis, pith, and vascular region of *C. quinoa* are shown in graphs at different levels of salts (control, G1=50+10 mM, G2=100+10 mM, G3=150+20mM, G4=200+20mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

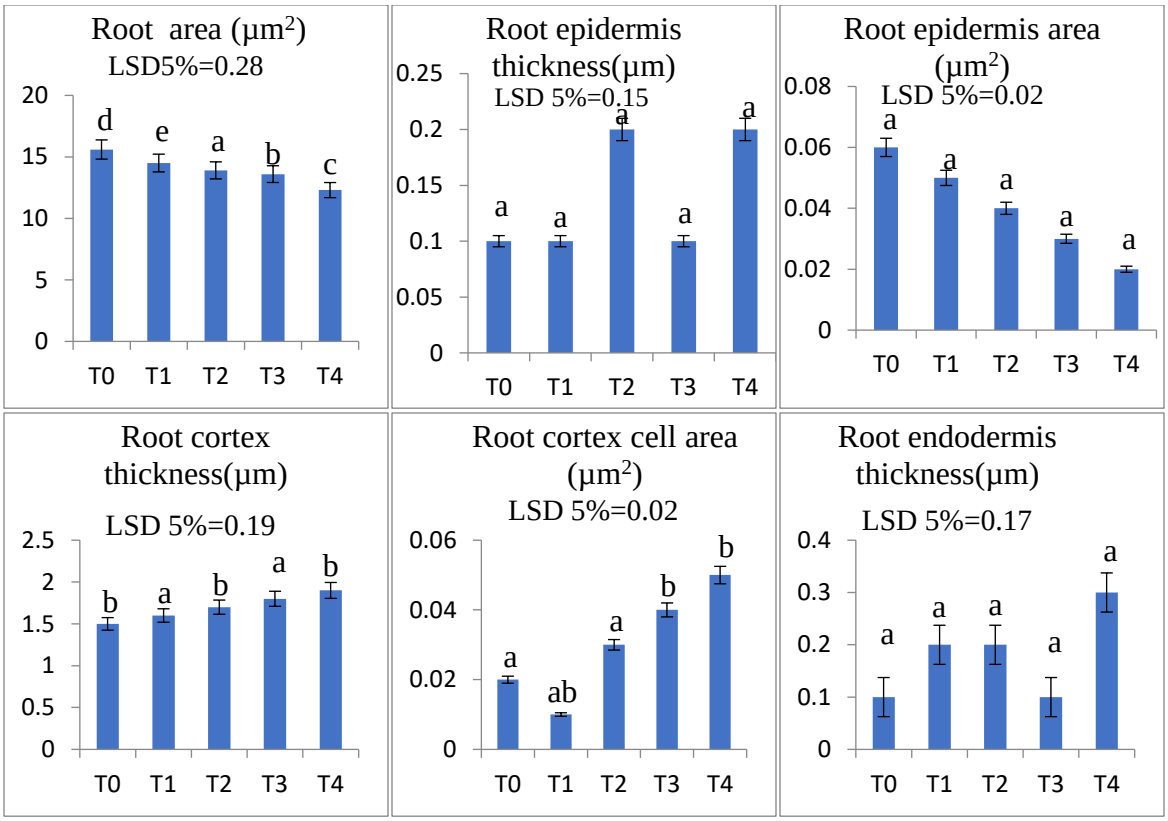


Figure 7: Anatomical parameters of root area, cortex, and endodermis of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150 mM, T4=200 mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

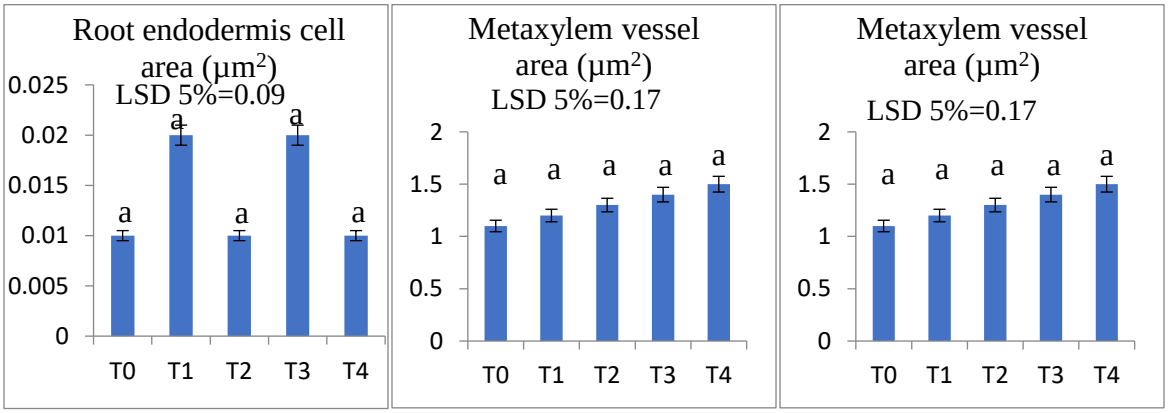


Figure 8: Anatomical parameters of root endodermis area, xylem, and phloem thickness of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150 mM, T4=200 mM Mean sharing the same letter do not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

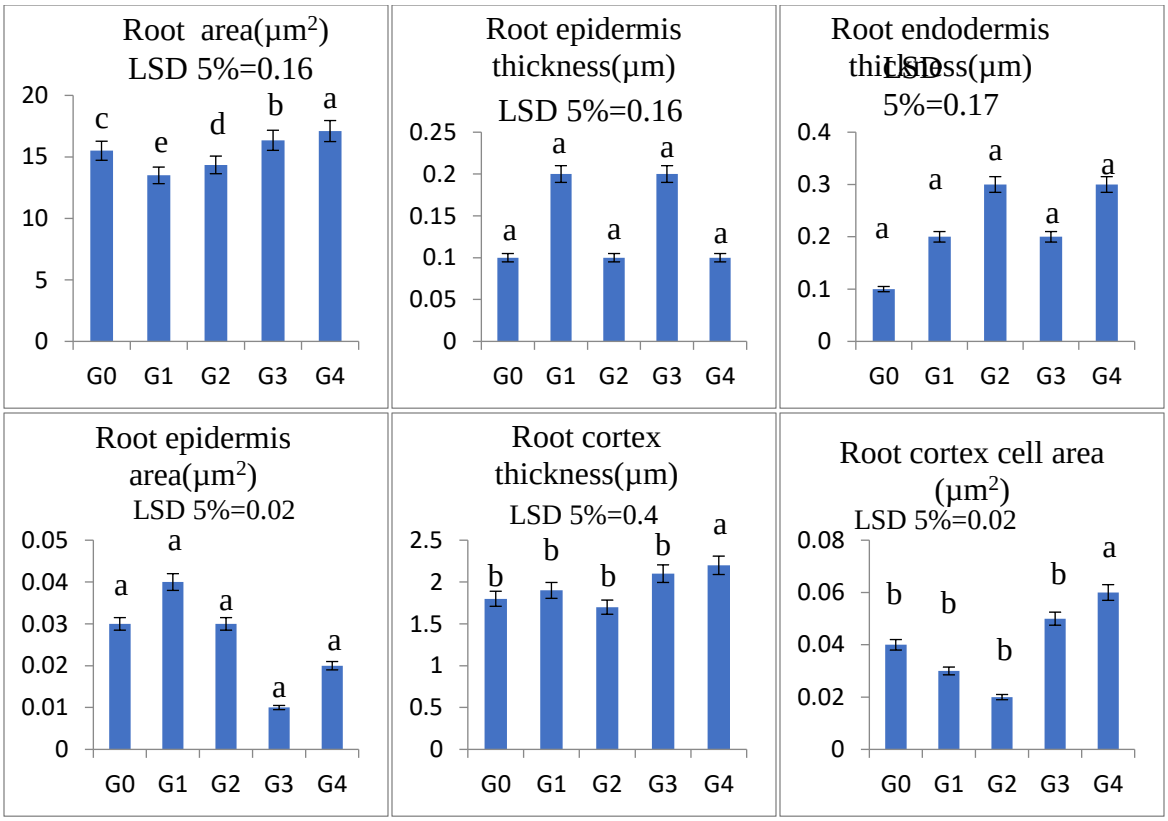


Figure 9: Anatomical parameters *C. quinoa* is shown in graphs at different levels of salts with gibberellic acid. Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

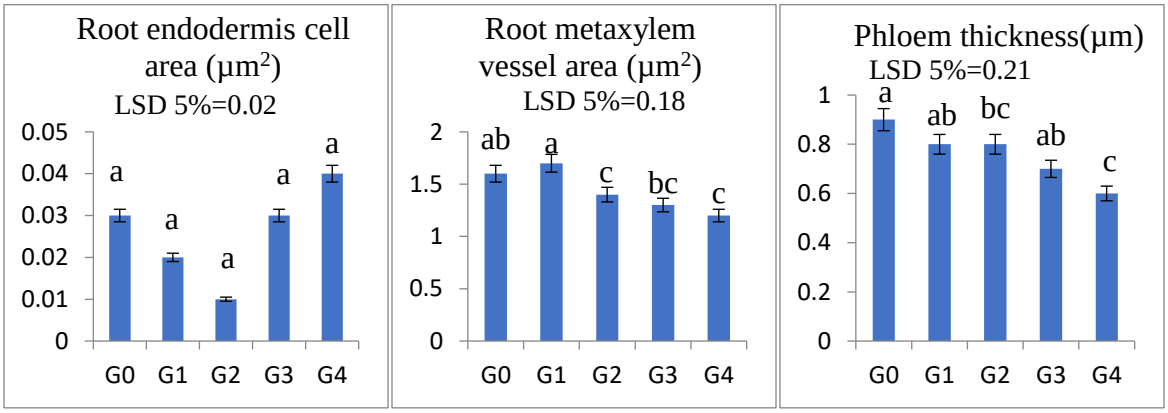


Figure 10: Anatomical parameters of root endodermis area, xylem, and phloem thickness of *C. quinoa* are shown in graphs at different levels of salts (control, G1=50+10 mM, G2=100+10 mM, G3=150+20 mM, G4=200+20 mM) with gibberellic acid Mean sharing the same letter do not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

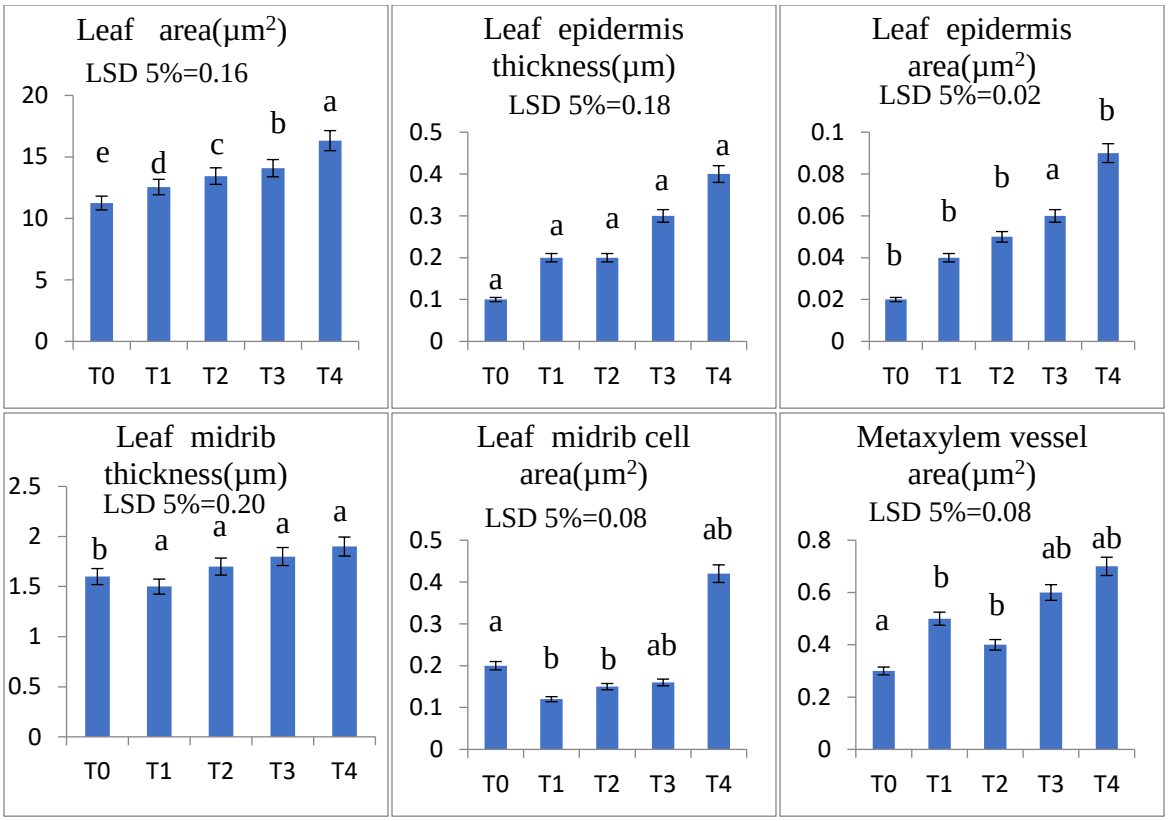


Figure 11: Anatomical parameters of leaf area, cortex, midrib, and metaxylem of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150 mM, T4=200 mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

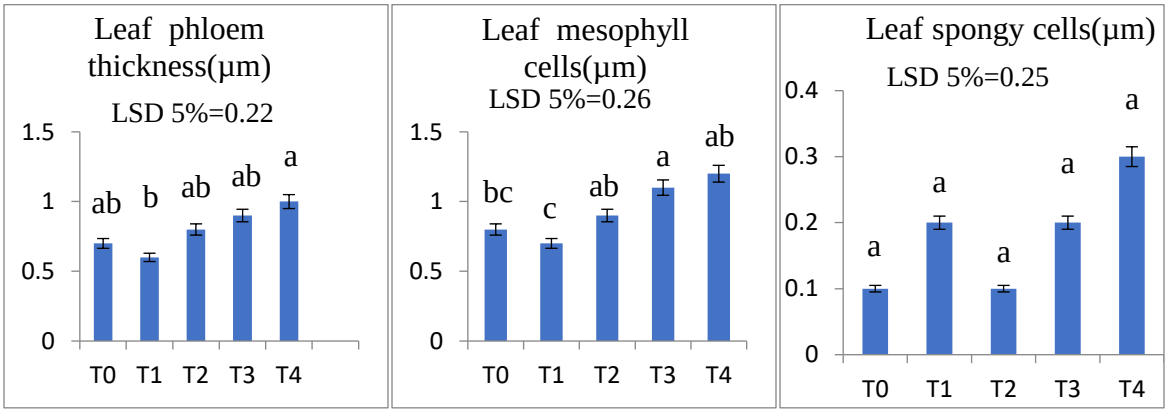


Figure 12: Anatomical parameters of leaf phloem, mesophyll, and spongy cells of *C. quinoa* are shown in graphs at different levels of salts (control, T1=50 mM, T2=100 mM, T3=150mM, T4=200mM). Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

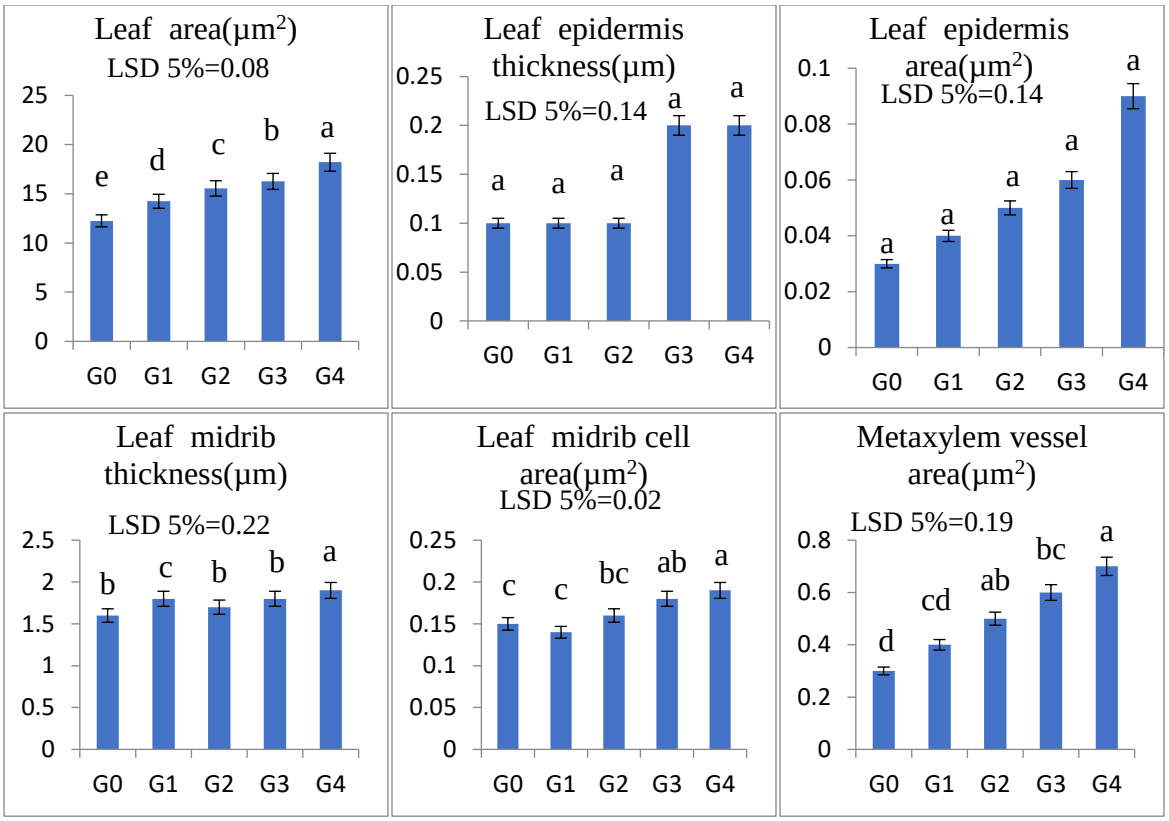


Figure 13: Anatomical parameters of leaf area, epidermis, and midrib of *C. quinoa* are shown in graphs at different levels of salts (control, G1=50+10 mM, G2=100+10 mM, G3=150+20 mM, G4=200+20 mM) with gibberellic acid. Mean sharing the same letter does not differ significantly at p≤0.0.5; Mean sharing different letters is different significantly at p≥0.05.

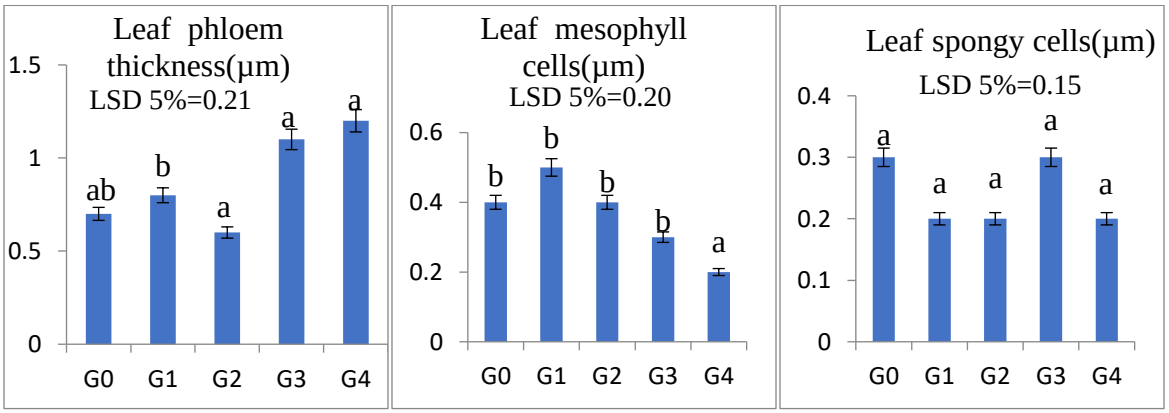


Figure 14: Anatomical parameters, leaf phloem, mesophyll, and spongy cells of *C. quinoa* are shown in graphs at different levels of salts (control, G1=50+10 mM, G2=100+10 mM, G3=150+20 mM, G4=200+20 mM) with gibberellic acid. Mean sharing the same letter does not differ significantly at p≤0.05; Mean sharing different letters is different significantly at p≥0.05.

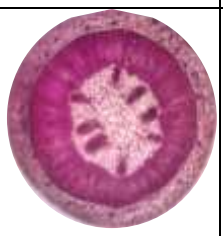
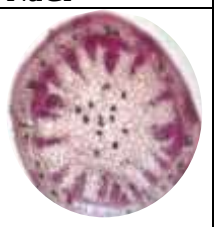
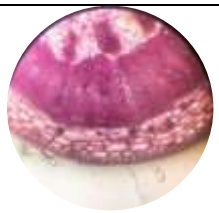
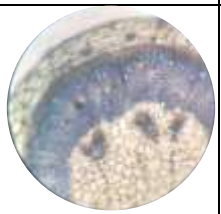
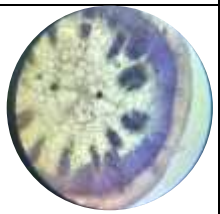
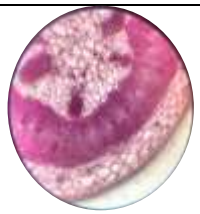
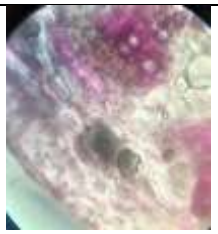
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
4X Ground tissue					
10X Dermal region					
40X Vascular region					

Plate 1: Stem anatomy of *C. quinoa* under different levels of salt stress (control, T1=50 mM, T2=100 mM, T3=150 mM, T4=200 mM).

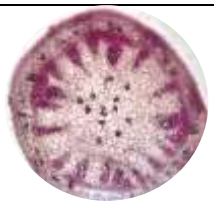
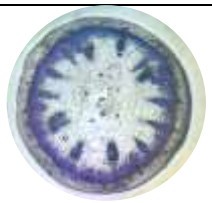
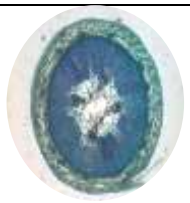
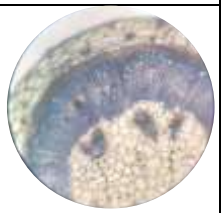
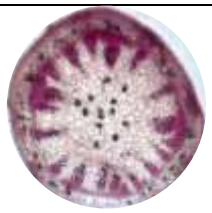
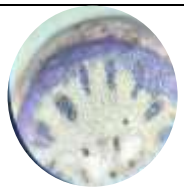
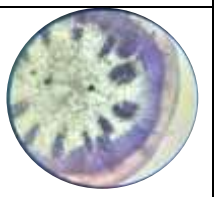
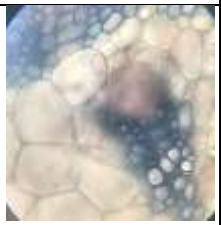
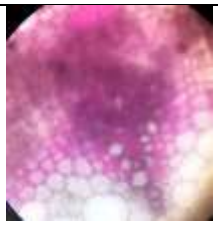
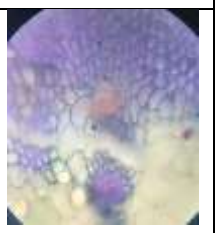
	Control	50 mM NaCl+GA	100 mM NaCl+GA	150 mM NaCl+GA	200 mM NaCl+GA
4X Ground tissue					
10X Dermal region					
40X Vascular region					

Plate 2: Stem anatomy of *C. quinoa* under different levels of salt stress along with gibberellic acid (control, T1=50 mM+10 mM GA, T2=100 mM+10 mM GA, T3=150 mM+20 mM GA, T4=200 mM+20 mM GA).

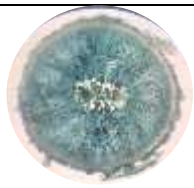
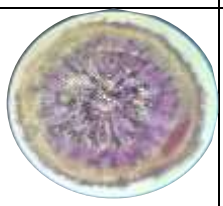
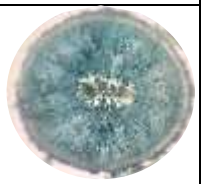
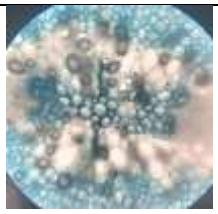
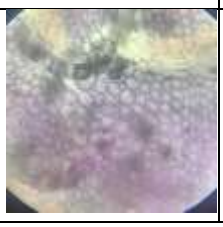
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
4X Ground tissue					
10X Dermal region					
40X Vascular region					

Plate 3: Anatomy of root under different levels of salt (control, 50 mM, 100 mM, 150 mM, 200 mM).

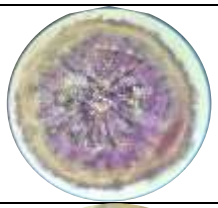
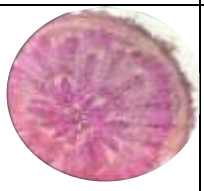
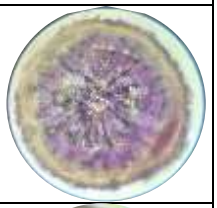
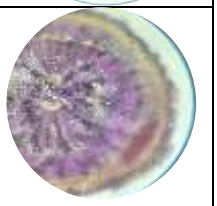
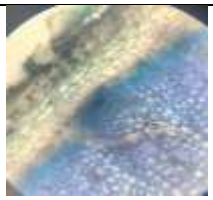
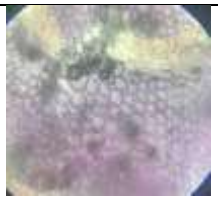
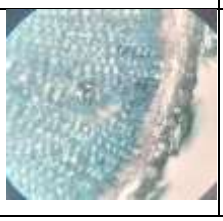
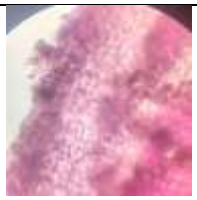
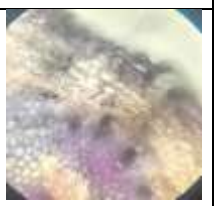
	Control	50 mM NaCl+GA	100 mM NaCl+GA	150 mM NaCl+GA	200 mM NaCl+GA
4X Ground tissue					
10X Dermal region					
40X Vascular region					

Plate 4: Root anatomy under different levels of salt with gibberellic acid at 10 mM and 20 mM.

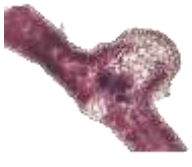
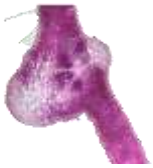
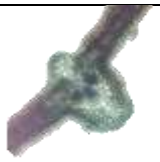
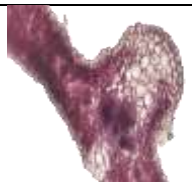
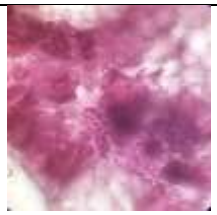
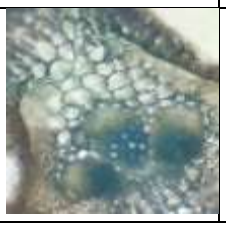
	Control	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
4X Ground tissue					
10X Dermal region					
40X Vascular region					

Plate 5: Anatomy of leaf under different concentrations of salt (control, 50 mM, 100 mM, 150 mM, 200 mM).

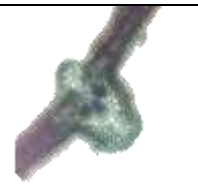
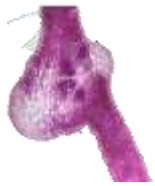
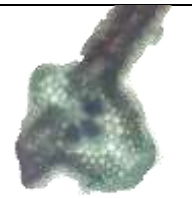
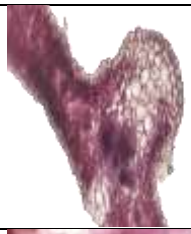
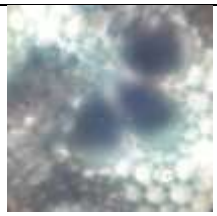
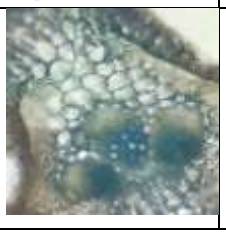
	Control	50 mM NaCl+GA	100 mM NaCl+GA	150 mM NaCl+GA	200 mM NaCl+GA
4X Ground tissue					
10X Dermal region					
40X Vascular region					

Plate 6: Leaf anatomy under different levels of salt with gibberellic acid at 10 mM and 20 mM

IV. DISCUSSION

Morphological parameters such as the fresh and dry weight of *C. quinoa* showed a decrease with an increase in the salt concentration. Our results were supported by Souri *et al.* (2019) that the fresh and dry weight of the plant is less at

higher salt concentrations as compared to the control condition. The growth of plants was inhibited by NaCl. Similarly, NaCl effect on modifications to growth and ion accumulation high intraspecies morphological diversity fresh and dry mass of shoots and roots (Jekabsone *et al.*, 2022). Our study revealed that Sodium (Na) and chloride (Cl) concentrations cause a decrease in the shoot and root length. Shoot and root length showed a greater decrease at the level of treatment having greater salt concentration. There were analogous results reported by (Zhu *et al.*, 2019) that shoot length increased at low salinity but decreased significantly at high salinity. Root length was reduced. Sodium chloride reduced shoot length, root length, fresh weight, dry weight, and the amount of nutrients while increasing levels of loss of electrolytes, peroxidation of lipids, and protective enzyme activities (Wang *et al.*, 2019).

A considerable increase in plant morphological characters was noticed by the application of growth promoters. In terms of plant dry weight, plants demonstrated the greatest level of growth enhancer increased dry and fresh weight. Plants receiving GA demonstrated an increased number of leaves relative to control in a dose-dependent manner (Ali *et al.*, 2021). Gibberellins (GAs), influence plant development by regulating several physiological functions. There were some results were investigated by Miceli *et al.* (2019) that GAs can promote seed germination, leaf expansion, stem and root extension, and dormancy. Our results are analogous to the work of Pethybridge *et al.* (2023) who illustrated that the effects of gibberellic acid (GA) on hormone transcriptional regulatory systems resulted in root growth suppression. GA has also been reported to stimulate the formation of secondary xylem, reduce secondary phloem production, and improve lignification in roots. GA foliar treatments resulted in increased plant height, leaf area, number of leaves, root length, and tuber growth (Cornea-Cipcigan *et al*., 2020). An increase in root weight/plant, root length, was found by Othman *et al*. (2021) at significantly high gibberellic acid concentrations.

In our results leaf area increased at greater salt concentration at 200mM, contrary results were found by (Yu Shi *et al.*, 2019), who concluded that the leaf is an essential part of vegetation to maintain regular development. Plant health status may be indicated by the state of the leaves. When exposed to salt stress, the foliage will show similar reactions. Salt stress prevents plants from growing. Under saline-sodic soil conditions in the current study, all of the evaluated growth metrics were drastically reduced (Niamat *et al*., 2019). Chauhan *et al.* (2019) concluded that a growth promoter solution reduces the toxic effects of salinity by boosting germination rates and shoot as well as length of roots, and total dry and fresh weight. According to our study in comparison with the control condition the stem area, epidermis thickness, xylem, phloem, and cortex gradually increase. With increased salt concentration stem area increased. Kheloufi *et al*. (2019) confirmed that stem thickness could boost their capacity for storing water, helping them to retain more of it and combat the unfavorable moisture conditions brought on by salt stress. The length and thickness of the stem layer and the elongated pith in plants increased after foliar treatment. Epidermis, cortex, phloem, and xylem tissues, as well as the thickness of the parenchymatous portion of the pith and vessel diameter, may all have increased in thickness, contributing to the rise in stem wall thickness (El-Taher *et al.*, 2021).

Our results showed that the salt stress cause an increase in the epidermal leaf area. It was observed that with an increase in the concentration of NaCl leaf epidermis area also increased. There were contrary results proposed by Nassar *et al.* (2020) that salt stress reduced leaves lamina thickness to less than the control. This decrease could be attributed to a decrease in mesophyll tissue thickness as well as an increase in the length and diameter of the major vascular bundle. The diameter of the metaxylem vessel and the overall thickness of the tissue inside of it both fell below the value used as a control. To reduce the effects of salt in cells, high salinity causes a rise in leaf succulence (Naskar *et al.*, 2021). The salinity of the soil, and these anatomical structural alterations restrict transpiration. Over the complete mesophyll thickness, an increase in the lamina parenchyma's intercellular gaps was seen. Plant tolerance to salt is tightly correlated with the anatomical characteristics of the roots. Anatomical attributes coordinate to prevent salt toxicity in plants grown in very saline zones, a comparative examination of the morphological traits of roots under diverse salinity is necessary (Feng *et al.*, 2021). (Salsinha *et al.*, 2021) revealed that the variations in the concentrations of salt were adversely connected with the characteristics of the leaf, including epidermal thickness, stomatal density and size, bundle-sheath size, vascular-bundle size, and length.

V. CONCLUSION

In conclusion, a decline in plant growth brought on by salt stress may be caused by endogenous hormonal imbalances growth of the *C. quinoa* by suppression of cell division, a decrease in water absorption, and the activity of

root metabolic processes. The results showed that the morphological and anatomical parameters are enhanced by the application of gibberellic acid under salt stress. Gibberellic acid causes an increase in the plant's growth mechanism.

Conflict of interest

The authors declare no conflict of interest.

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