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Altered anatomical appraisal of mung bean (*Vigna radiata* (L.) Wilczek) under salinity stress

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Abstract

Mung bean, an important pulse crop, its growth, and development are affected by various abiotic stresses where salinity hampers yield seriously. An experiment was conducted under the hydroponic condition to observe the effect of salinity on the anatomical attributes of mung bean genotypes. The design of the experiment was randomized complete with factorial having five levels of salinities i.e. 0 (control), 6, 8, 10, 12 dSm^{-1}. The results indicated that anatomical features of mung bean plants were affected by the application of salt. The cortex and stele radius and vascular bundle strands were reduced due to salt application. The vascular bundle size especially the length was decreased with the increased levels of salinity. Similarly, the radius of cortex was also decreased with the increased levels of salinity. The results concluded that the application of salts altered the plant's internal structures, especially the vascular bundles and leaf spongy parenchyma tissue.

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Introduction

Mung bean (*Vigna radiata* (L.) Wilczek) is one of the important legume crops having versatile uses and is recognized as an excellent source of plant protein. The demand for these pulses has increased gradually over the decades. The reproductive growth i.e. flower formation and fruit set is an indeterminate type. The morphology, physiology, and yield of this crop are exaggerated by several degrees of abiotic stresses, especially the salinity. The deleterious effect of salinity altered the micromorphology i.e. anatomy of crops in addition to macromorphology. Plant micromorphology especially the internal structure i.e. anatomy of salt-induced plants severely affected thereby changing the tissue structures and tissue system (Broderson and McElrone, 2013). The anatomical changes in roots and leaves of different crops due to salinity stress have been reported by various investigators (Godfrey *et al*., 2019). There are large alterations in the macro- and micro-morphology of plants growing in brackish soils. The consequence of salinity on root and leaf anatomy of a few glycophytes and halophytes has been reported by Hu and Schmidhalter (2001) and Kilic *et al.* (2007). The development of the xylem was declined due to an increase in salinity where diameter and width of the vascular bundle in rice stem were declined under saline conditions (Pimmongkol *et al.,* 2002). Junghans *et al.* (2006) observed that the activity of cambial tissue was reduced due to the presence of higher concentrations of salinity.

The xylem structures especially the vessel size and cambial activity have been decreased due to the imposing of the salinity. In leaf, salinity resulted in the reduction of leaf area but increased the leaf thickness i.e. increase the sizes of mesophyll tissue (Silva *et al*., 2021).

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Anatomical features of non-legumes especially the leaf tissues of rice where the epidermis contained bulliform cells which possess large vacuole that may help as a junkyard for a greater quantity of Na^+/Cl^- ions. The excessive amounts of Na^+ are transported from mesophyll tissue to bulliform cells to keeping normal physiological activities (Tester and Davenport, 2003). The altered anatomical features change the physiological attributes leading to biochemical compounds namely chlorophyll, amino acids, and enzyme synthesis being hampered due to the salinity stress (Rahman *et al.,* 2002). Both anatomical and physiological strategies could foster the growth and development of crop plants in a stressful situation. Like other crops application of salinity reduces the growth, yield, and quality of mung bean have been documented by several researchers. However, the anatomical appraisal of mung beans under saline stress has not comprehensively been explored. Hence, the experiment was undertaken to observe the micromorphological (anatomical) structure of the mung bean plant under salt stress conditions.

Materials and methods

A glass-house and laboratory experiment was carried out at the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. It was conducted in a factorial CRD (completely randomized design) having four replicates. Factor I encompasses five salinity levels namely 0 (control), 6, 8, 10 and 12 dSm^{-1} and factor II two genotypes tolerant and susceptible. The salinity level was maintained by dissolving commercial salt (NaCl) at the rate of 640 mgL^{-1} distilled water for getting 1 dSm^{-1} salinity level. Two mung bean genotypes *viz*. tolerant and susceptible, selected from our earlier experiment were used in the experiment. The seeds of all the genotypes have been kindly supplied by the Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute , Gazipur, Bangladesh.

The mung bean seeds were sterilized with 70% ethanol and thereafter sown in 250 mL size plastic pot covered with clean small gravels. The plastic pots were put on the cork sheet on a plastic tank containing nutrient solution. The NaCl solution of one concentration was applied to the plants at 7 days after germination. Plants were kept in plastic pots which were put in the plastic bigger containers (10 L) with a nutrient solution. Modified Hoagland solution (Hoagland and Arnon, 1950) was used in the experiment. The hydroponic system was aerated by an air pump for keeping it oxygenated and maintaining the homogeneity of the solution.

The leaf and stem samples were collected from the different treatments of the experiment at 30 days after sowing. The cross-sections of 2 cm slices from leaf and stem were chosen and softened in a mixture of glycerin 70% (0.5:0.5) for one week. Freehand cross-sections were made by using razor blades. The sections were stained with Safranin thereafter fast green and mounted with glycerin on a glass slide followed by coverslip. The slide was viewed under a digital compound microscope and photographs were taken at 10-40 X magnification. The anatomical parameters were measured by image focus 4.0 software in a computer.

Results and discussion

Vascular bundle strand (Stem xylem and phloem area)

The application of salinity significantly altered the anatomy of the mung bean plant (Plate 1). The vascular bundle strands were affected significantly due to the application of salt stress. Both the xylem and phloem areas were changed remarkably with increasing salinity stress (Fig.1). The highest xylem and phloem areas were observed in control, and the smallest area was found in salt stress condition (12 dSm^{-1}) of both genotypes. A highly negative correlation was found between stem vascular bundle area and salinity. The vascular bundle strands were declining with the increase of salinity stress and the trend was in a straight line as shown in Fig. 1. The decline of vascular bundles indicates that with the increase in salinity level there was a noticeably decrease in the xylem and phloem element's cell growth. However, areas of both xylem and phloem were maximum under the controlled condition which indicates that salinity constrain the immature tissue differentiation process of xylem and phloem. However, cell wall thickness increased with the increased application of salt.

Radius of cortex

The area beneath the epidermis to the endodermis is known as the cortex. The cortical area decreased with the increase of salinity. The radius of the cortex is decreased with the increase of salinity stress and the relationship is a straight line (R^2=0.9387) (Fig. 2 and Fig. 3). The decrease was due to the compactness of the cortical tissue. The deleterious effect of salinity reduced the cell size of the cortical area as those tissues are vulnerable and mainly composed of the parenchyma.

Radius of stele

The results reflected that stele radius was initially increased with the increase of salinity and thereafter declined with further enhancement of salinity levels (Fig.2). The relationship between salinity levels and stele radius is curvilinear (R^2=0.72) (Fig. 4). The initial increase of stele

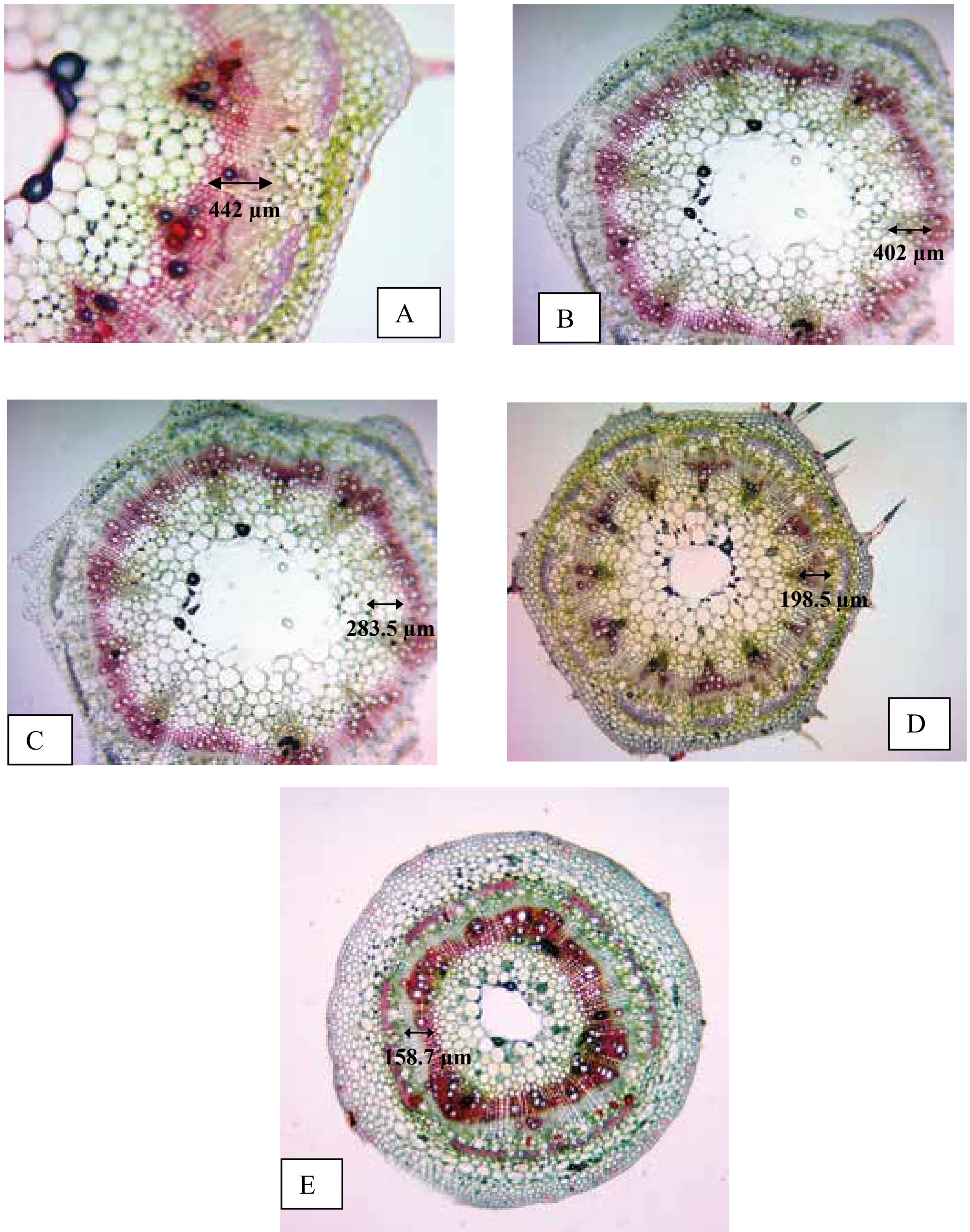

Plate 1. Anatomical features of mung bean stem under salt stress and grown under hydroponic conditions. A-control condition, B-6, C-8, D-10 and E-12 dSm^{-1} levels of salinity
The images have been captured by Digital Compound Microscope (X=40 magnification)

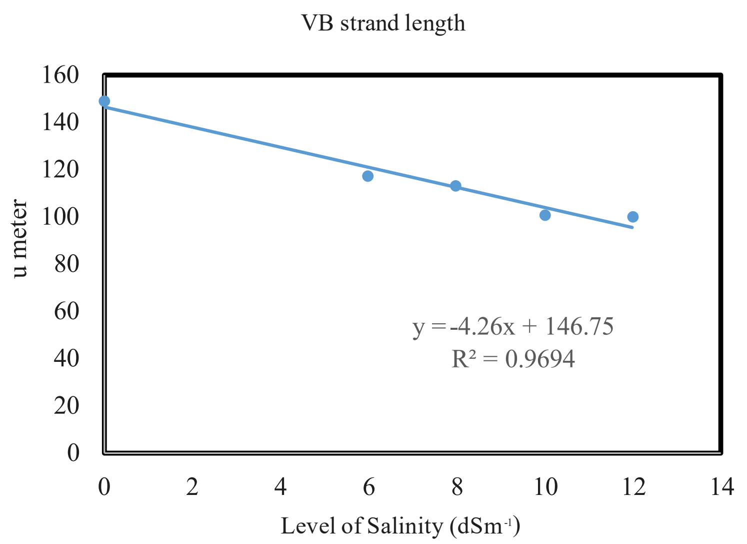

Fig. 1. Correlation between vascular bundle strand length of mung bean and the level of salinity under the hydroponic condition

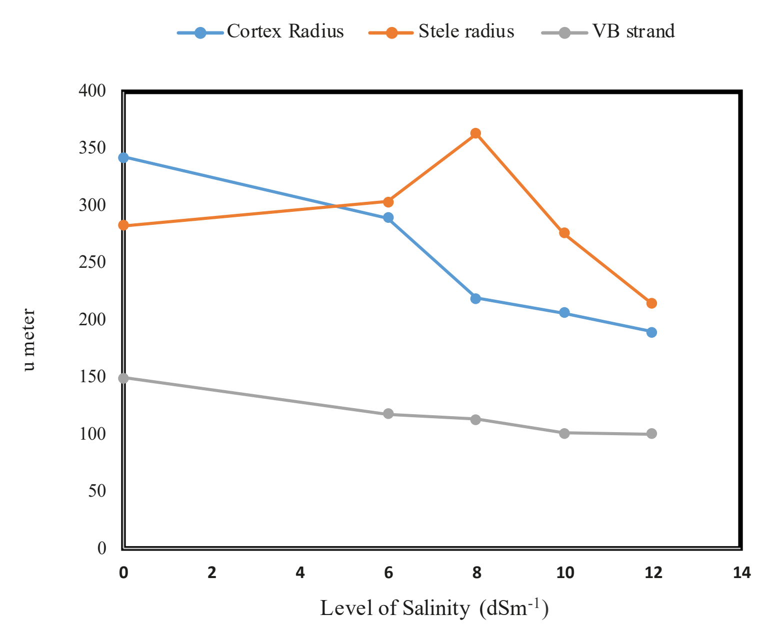

Fig. 2. The effect of salt stress on the cortex and stele radius and vascular bundle strand length of mung bean grown under the hydroponic condition

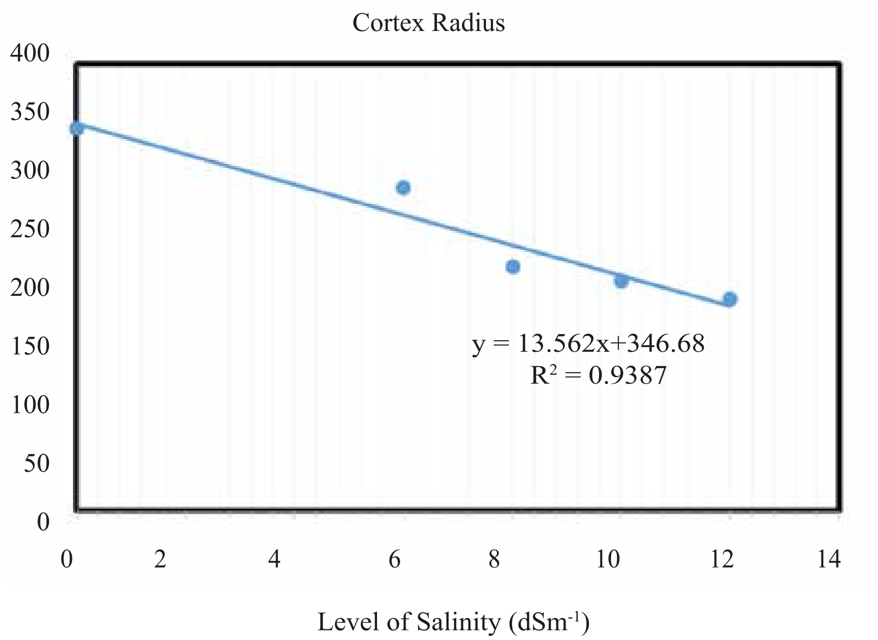

Fig. 3. Effect of salinity stress on the trends of cortex radius of mung bean grown under the hydroponic condition

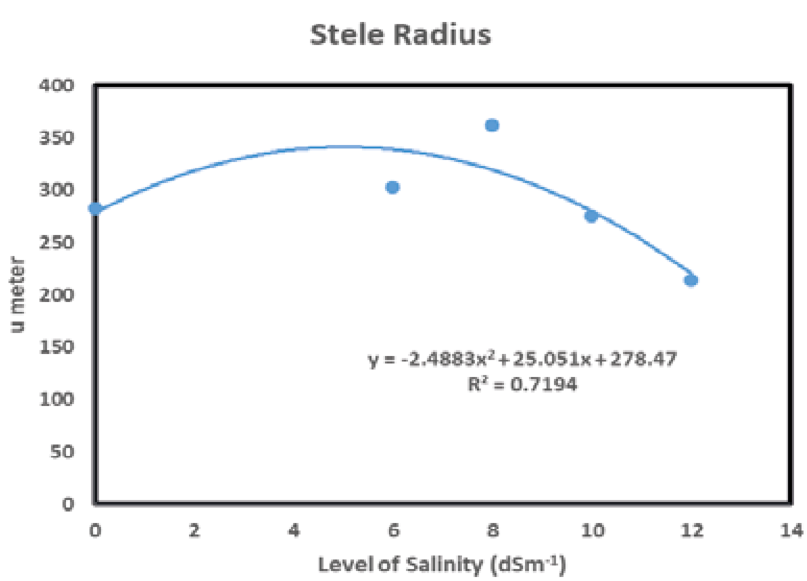

Fig. 4. Effect of salinity stress on the trends of stele radius of mung bean grown under the hydroponic condition

area might be due to enhancement of cell size due to accumulation of Na^+ and Cl^- ions resulting in accumulation of more water vis-à-vis increase the cell size.

Ratio of cortex and stele

The results reflected that the ratio between cortex and stele radius is decreased with the increase in salinity levels (Fig. 5). These findings reveal that the cortex areas were decreased more than the stele area i.e. the cortical tissues are more vulnerable than the stele tissue. The cortical tissues are mainly composed of parenchyma whereas the stele is composed of both parenchyma and sclerenchyma the later tissues are more tolerant to salinity rather than parenchyma tissue.

tissue, columnar in shape which contains numerous chloroplasts and are considered a principal site for photosynthesis. The palisade tissue thickness in the leaf of mungbean genotypes remains unchanged in control and lower salinity (6 dSm^{-1}) but it decreased at the higher salinity (8, 10 and 12 dSm^{-1} NaCl) level compare to the control.

Xylem area and phloem area were maximum under normal (control) condition. This indicates that salinity induces structural changes in xylem and phloem of stems. The increasing salinity reduces the stems vascular area while cell thickness increased with salinity level compared to control treatment (Khan *et al*., 2019). Salinity caused the gradual changes in plant internal structure which reduces the xylem and the phloem area.

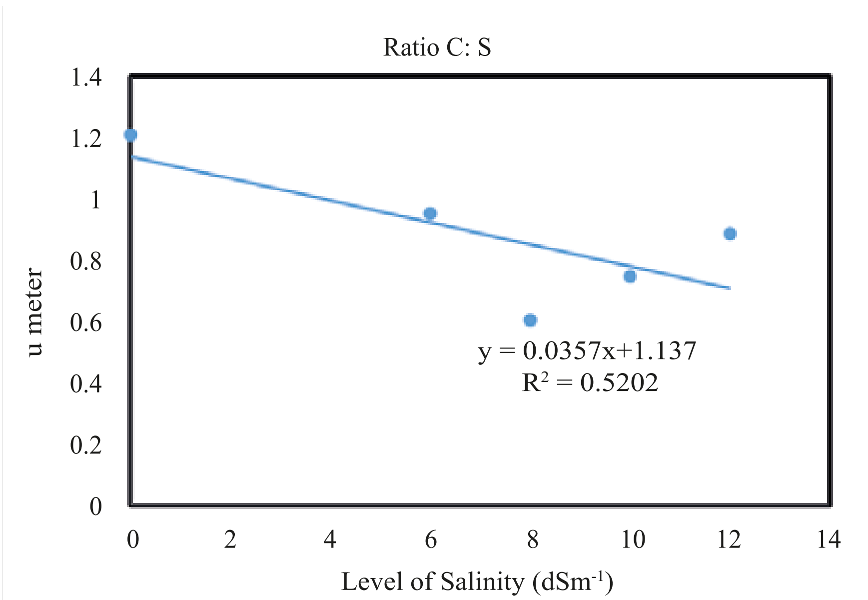

Fig. 5. Effect of salinity stress on the trends of cortex and stele radius ratio; vascular bundle strand length of mung bean grown under the hydroponic condition

Leaf anatomy

At higher concentrations (8, 10 and 12 dSm^{-1}) of salinity stress, the thickness of the upper epidermis of leaves of almost all the genotypes showed a tendency to increase. The anatomical image showed that palisade tissue became less affected than spongy parenchyma tissue. Although both are resulted in compactness due to the imposition of salinity. Anyway, the breakdown of chloroplast resulting in chlorosis was clearly visible in the image at higher levels of salinity. The palisade tissues are the parenchymatous mesophyll

Salt stress affected the structure of the vascular bundle where both the xylem and phloem area were altered significantly. A negative correlation between the areas of the vascular bundle (xylem and phloem area) and salinity was observed in the stem. This indicates that the accumulation of excessive Na^+ and Cl^- ions affected the growth and differentiation of the elements of the xylem and phloem resulting in the shrinkage of the vessel and sieve tube. Application of diluted seawater resulted in a considerable decrease of stem dimension, the xylem, and phloem tissue thickness (Eisa *et al.,* 2017).

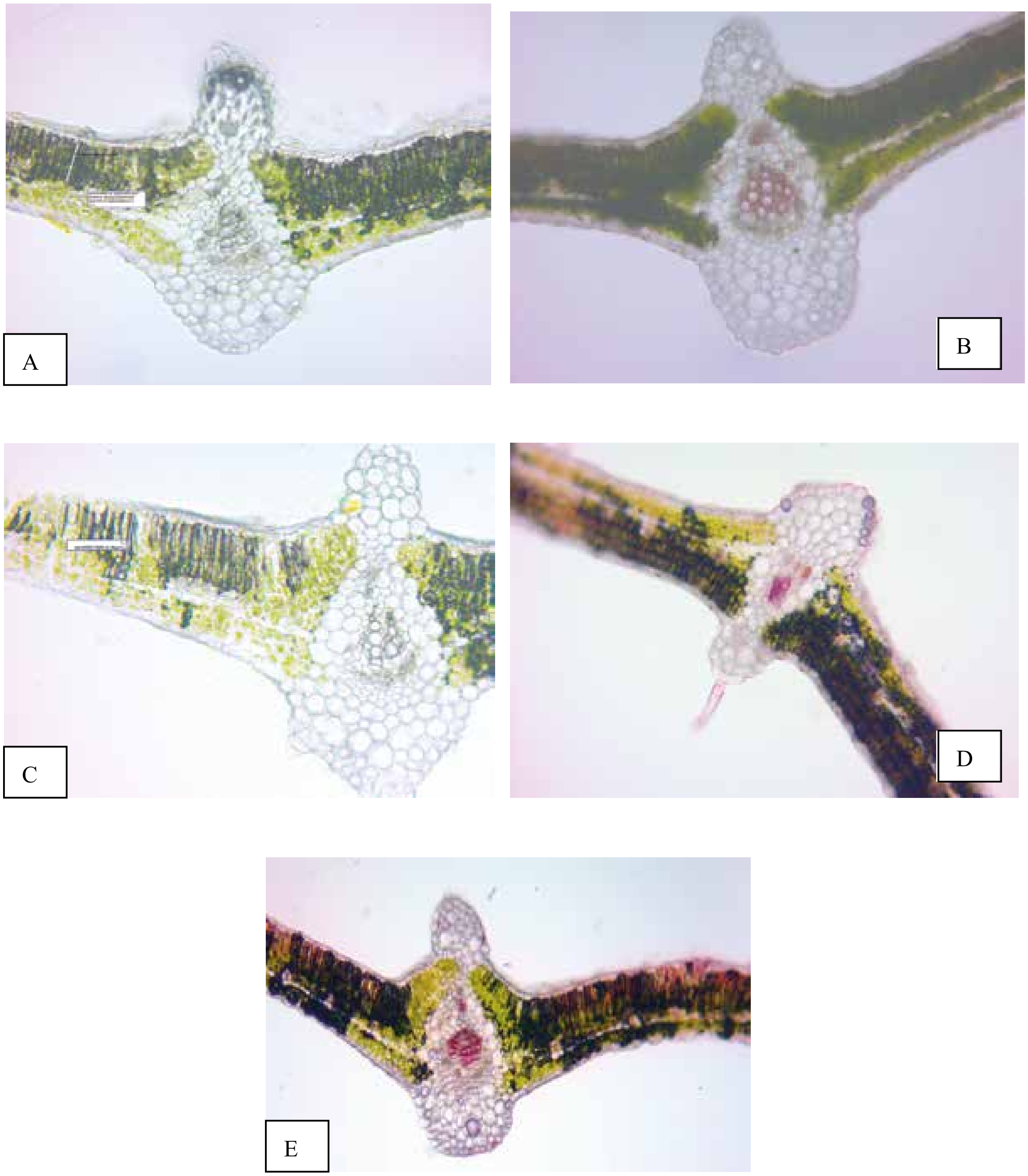

Plate 2. Anatomical features of mung bean leaf under salt stress and grown under hydroponic conditions. A-control condition, B-6, C-8, D-10 and E-12 dSm^{-1} levels of salinity
The images have been captured by Digital Compound Microscope (X=40 magnification)

Salinity showed a subtractive effect on shoot anatomy of pulse crops. It is indicated that, with the increase in salinity level, there was a significant decrease in stem xylem and phloem area (Khan *et al*., 2019). Relative stem xylem area (%) was reduced due to salinity stress on pulse crops. Stem area of *Gazania harlequin* was decreased remarkably by the increment of NaCl level as reported by Younis *et al.* (2014). In our study, the decreased vascular bundle area of the stem was due to slower cell growth. On the contrary, salinity induces the cell wall thickening process due to higher level of lignin synthesis. Nikpour-Rashidabad *et al*. (2019) reporeted the decrease of vascular cylinder (VC) and cortical parenchyma (CP) areas, affecting the VC/CP ratio in mung bean with the salinity inposition. Younis *et al*. (2014) reported that xylem and phloem area of stem showed a decreasing trend under salt stress condition which indicates that with the increase in salinity level there was noticeably decrease in the xylem and phloem cell growth.

Hanumantha Rao *et al.* (2016) reported that mung bean seed germination is quick under saline conditions and reduced the time of germination. They also mentioned decline in root and shoot lengths and fresh mass. The stem wall thickness of faba bean as a result of salinity stress could be attributed to the decrements induced in all included tissues(Dawood *et al*., 2014). The thicknesses of the epidermis, cortex, fibre tissue, phloem tissue, xylem tissue and parenchymatous area of the pith were decreased in saline treated plant.

Hameed *et al.* (2010) reported that salinity resulted in the gradual alteration of micromorphology of *Cynodon dactylon* consequently reducing the xylem and the phloem area. Induction of NaCl increases the lignification of vessel and sieve tube has been documented by Nja *et al.* (2018) in *Medicago sativa* cv. Gabès. At lower concentration of NaCl (50 mM), the thickness of the epidermis of barley variety Odesskaya-100 resulted in an increase over the control by 40% as reported by Atabayeva *et al.* (2013).

Enhancement of salinity level increased cutin layer thickness of leaf compared to control treatment. The salinity tolerant plants reduce their transpiration resulting the reduced dehydration loss like under drought conditions developing a thick layer of cutin (Samuels *et al.,* 2008). Our results show a thicker cuticle in the tolerance genotype which corroborated the results of Dolatabadian *et al.* (2011). Generally, under saline conditions leaves of the most plants become thicker, succulent, and lower specific leaf areas (Kozlowski, 1997). Additionally, the thicker epidermis has been observed by several investigators which are mainly salt-sensitive dicot species namely *Kandelia candel* and kidney bean (Hwang and Chen, 1995; El-Araby and Hegazi, 1999). It might be the most effective tolerant appraisal in halophyte or salt-tolerant crop species where water loss could be minimized significantly (Zhu *et al*., 2001).

In contrast, Dawood *et al*. (2014) reported the reduction in midvein and lamina thickness due to salinity than that of control plants. The thinner leaflets made by salt could be ascribed to the lessening in the thickness of both the palisade and spongy tissues, as well as in the proportions of midvein bundles. Abdelaal (2015) revealed that the thickness of leaf lamina and vascular tissue reduced with the drought stress. Upsurge in epidermal cutin along with decline in lamina thickness is also reported by Boghdady (2009) in mung bean and Petrov *et al*. (2012) in barley plants.

In our results, the thicknesses of both upper and lower epidermis were found to be increased in the leaf of salt-treated plants compared to control. Sánchez-Aguayo *et al.* (2004) showed that salt stress enhanced xylem development by increasing lignification in vascular bundle with a gradually less evident effect. Both collenchyma and parenchyma lost their regular contours with increasing salt concentrations. Under control conditions, the spongy parenchyma was characterized by scattered cells separated by air spaces but it became denser under stress conditions, since the intercellular spaces decreased (Hoffmann *et al*., 2021).

The thicker epidermis not only improves the water use efficiency of plants but also gives additional space for the sequestration of Na^+ in the leaf epidermis (Shabala *et al*., 2012). In halophyte *Salvadora persica*, the upper epidermal layer was thickened by 40, 54, and 41%, and the thickness of the lower epidermal layer was increased by 35, 55, and 34% respectively in 250, 500, and 750 mM NaCl-treated plants as compared to control (Parida *et al.,* 2016). Parida *et al*. (2016) also mentioned severe damage and complete destruction of palisade layer at higher saline concentration in the leaf of *S. persica.*

Conclusion

Salt stress altered the internal structures i.e. micromorphology of the plant especially the vascular bundle and ground tissue of the leaf. The anatomical features of the stem were affected by the increased application of salinity. The cortex and stele radius and vascular bundle strands were reduced due to salt application. The spongy parenchyma of mesophyll tissue was more vulnerable than palisade parenchyma tissue.

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