Silicon distribution in leaves and roots of rice and maize in response to cadmium and zinc toxicity and the associated histological variations

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At present, the levels of cadmium (Cd) and zinc (Zn) in arable land are high and affect the growth and development of important food crops, including rice and maize. However, the application of silicon (Si) in contaminated areas increases the metal tolerance potential of these plants. This work aimed to study the variations in the distribution pattern of endogenous Si in various tissue regions in roots and leaves of rice and maize exposed to cadmium (Cd) and zinc (Zn) stresses. For these experiments, 45 day-old rice (var. Varsha) and maize (var. CoHM6) seedlings were treated with 1.95 g Zn and 0.45 g Cd kg^{-1} soil. Under Cd stress, the distribution of Si was high in the cortical region of the root, but under Zn stress, the highest Si deposition was found in the endodermis. In leaves, Si deposition was high in both the mesodermis and stelar regions of Cd-treated plants but more Si was deposited in the mesodermis tissue of Zn-treated plants. Heavy metal (Cd and Zn) accumulation and Si deposition showed a strong negative correlation in the roots of rice and maize plants. Complexation with metal ions and redistribution of Si were considered the major mechanisms in Si-mediated mitigation of Cd and Zn stress. Cd- and Zn-induced anatomical changes, such as endodermal thickening, deposits in the xylary elements and aerenchyma formation in the roots of rice and maize, were also associated with the Si distribution.

Introduction

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Rice and maize are the two most important crop plants in the world, and the productivity of these crop plants is adversely affected by heavy metal stress (Fahad et al. 2019). Exceeding the maximum tolerable cadmium (Cd) and zinc (Zn) concentrations in agricultural fields as a consequence of industrialization and various anthropogenic activities is a concern for rice and maize production (Shahid et al. 2015, Fahad et al. 2019). The translocation of toxic metal ions to the grains of these plants increases the possibility of hyperaccumulation of these metals in humans, which leads to bone mineralization and cancer (Ma et al. 2015). In addition, Cd and Zn toxicity negatively affects plant metabolic processes, including photosynthesis, respiration, transpiration, and nitrogen metabolism, ultimately impacting the normal growth and development of plants (Lin et al. 2016). Furthermore, these metals cause an imbalance in the ionic homeostasis of cells, resulting in the accumulation of reactive oxygen species (ROS), which induces oxidative stress in plants (Islam et al. 2014, Anjum et al. 2015). The introduction of novel strategies to mitigate the impact of the intensity of Cd and Zn stress on rice and maize growth can be considered a current need.

Silicon (Si), a metalloid, is the second most abundant element in the Earth's crust and has the ability to enhance the metal tolerance of plants. Most Gramineae members accumulate Si to an extent of 10% of their dry weight, although Si is not considered an essential element (Song et al. 2014, Bhat et al. 2019). Under different biotic and abiotic stresses, plants increase their absorption of Si from soil, highlighting the role of Si in stress tolerance (Song et al. 2011). External application of Si increases Cd and Zn tolerance in rice plants by decreasing metal absorption and translocation (Song et al. 2011). The reduction in metal transport to the shoot is attributed to the accumulation of Si in the endodermis (Gu et al. 2012). The mechanisms of Si-mediated Cd and Zn stress mitigation include the elicitation of antioxidant defense, maintenance of membrane integrity, and complexation and compartmentalization of metal ions in plant tissues (da Cunha and do Nascimento 2009, Song et al. 2011). Si-induced manganese (Mn) and aluminum (Al) stress alleviation has also been demonstrated via increasing the apoplastic binding or complexation of these metals with the cell wall (Iwasaki et al. 2002, Wang et al. 2004). A reduction in bioactive Zn ions and colocalization of Zn and Si in the cell wall have been reported in rice plants exposed to Zn toxicity (Gu et al. 2012).

Cd- and Zn-induced histological modifications have been reported in different plants, such as maize (Vaculík et al. 2012), rice (Huang et al. 2019), *Sorghum bicolor* (Kasim 2006), *Arundo donax* (Guo and Miao, 2010), and *Pistia stratiotes* (e Silva et al. 2013). When *Cajanus cajan* was exposed to Cd stress, a significant reduction in vessel width and density was observed. Cell wall thickening, cell degradation, and crystal formation have also been observed as responses of different species to metal stress (Sruthi and Puthur 2019).

We hypothesized that the distribution of Si plays an important role in the metal tolerance and associated anatomical modifications of rice and maize plants. Detailed studies on the influence of external Si application on Cd and Zn stress alleviation in various plants have been conducted by many researchers, but no studies have been carried out to determine the role of the endogenous Si content in imparting heavy metal tolerance to important crop plants. Therefore, the primary objective of the present study was to evaluate the impact of the internal Si distribution on heavy metal stress mitigation in rice and maize. The anatomical modifications induced by Cd and Zn toxicity were also examined in the context of the Si distribution pattern in rice and maize plants.

Materials and methods

Experimental design

Maize and rice seeds were surface sterilized with a 0.1% $HgC1_2$ (w/v) solution for 5 min and placed at a depth of 8 cm in sterilized soil (soil:sand in 1:1) in polythene bags (18 × 13 cm). Soil sterilization was performed according to the method of Raj and Sharma (2009). The polythene bags were kept in a polyhouse maintained at 60 ± 2% relative humidity, with a light intensity of 250 ± 75 μmol m^{-2} s^{-1} and 25 ± 2°C temperature. After 45 d of growth, plants were treated with 40 ml (the field capacity of the soil) of solutions containing 1.95 g Zn kg^{-1} soil, supplied as $ZnSO_4$, and 0.45 g Cd kg^{-1} soil, supplied as $CdCl_2$. These concentrations were selected as stress-imparting concentrations that caused ~50% growth reduction in both plants after 12 d of exposure (data not shown). The second lower healthy leaves and root systems of the maize and rice plants were collected for various analyses on day 8 of the treatment.

SEM analysis

The anatomical modifications in the leaves and roots of rice and maize plants were evaluated using scanning electron microscopy (SEM). Leaves collected from plants subjected to different treatments were fixed in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer (pH 7.2) for 5 min. The fixed specimens were rinsed twice with double-distilled water and dehydrated by passing through an ascending acetone series. The dried specimens were mounted on grooves cut on aluminum stubs using double-sided adhesive conducting carbon tape to expose the sections. After gold-palladium coating, photomicrographs of the specimens were captured using the photographic attachment of a field emission scanning electron microscope (Carl-Zeiss Gemini 300 FESEM).

Analysis of elemental distribution patterns

To study the elemental distribution, dehydrated root and leaf samples of *O. sativa* and *Z. mays* were analyzed using an advanced, high resolution, field emission scanning electron microscope (Carl-Zeiss Gemini 300 FESEM). A quantitative compositional analysis of Si, Cd and Zn was carried out on an Inca analyzer EDX (energy-dispersive X-ray) spectrophotometer at 20 kV according to the protocol of Cocozza et al. (2008). Three separate root microspots and two separate leaf microspots from the control and metal-treated plants were analyzed by EDXMA (energy-dispersive X-ray microanalysis). The microspots are represented as Spectrum 1, Spectrum 2 and Spectrum 3.

Estimation of bioaccumulated Zn and Cd

The rice and maize plants were harvested and dried in an oven at 100°C for 1 h and then at 60°C until a constant weight was achieved. The material was used for Zn and Cd bioaccumulation studies according to the method described by Allan (1969). From each sample, 1 g was digested in Kjeldahl flasks heated at 60°C using sulfuric:perchloric acid (5:2) until the solution became colorless. Subsequently, the digest was filtered and transferred to a standard flask, and the volume was brought to 100 ml. An atomic absorption spectrophotometer (Shimadzu AA-7000) was used to estimate the Zn and Cd levels present in the digested samples. The bioconcentration factor (BCF) was calculated using the following equation (Chaudhuri et al. 2014):

$$\mathrm{BCF} = \frac{\text{Metal concentration in plant tissue at harvest (mg/kg)}}{\text{Initial metal concentration in external medium (mg/kg)}}$$

Results

Si distribution relative to Cd and Zn

The distribution of Si in the root tissues of rice and maize has a crucial role in Cd and Zn stress tolerance. Maize roots subjected to Cd stress showed a slight increase in Si deposition in the stelar region; conversely, under Zn toxicity, there was a 49% decrease in Si deposition in the stelar region (Table 1). However, Si deposition was increased in the endodermis of maize roots by 12% and 44% in Cd- and Zn-treated maize, respectively. Similarly, maize roots also showed a slight increase in Si contents in the epidermis and cortex when subjected to Cd and Zn stress (Fig. S1). In the control, the maximal Si deposition was observed in the stele, but in the roots of Cd-treated maize, more Si was distributed to the epidermis and cortical regions. In Zn-treated maize roots, the maximal Si deposition was observed in the endodermis.

In rice roots, the Si content in the stelar region was reduced by 30% and 50% in Cd- and Zn-treated plants, respectively, compared with the control (Table 1). The Si content was also slightly reduced in endodermal cells of Cd-treated rice roots, but the level of Si was enhanced by 20% in the epidermal and cortical regions. In the control, the maximal Si deposition was observed in the stele, but in the roots of Cd-treated rice, more Si was distributed to the epidermis and cortical regions (Fig. S2). In Zn-treated rice roots, the maximal Si deposition was observed in the endodermis. Therefore, the Cd- and Zn-induced modifications in the pattern of the maximal Si deposition in rice roots were consistent with those observed in maize roots.

The patterns of the Cd and Zn distributions showed significant modifications relative to the Si deposition. In maize roots, the maximal Cd accumulation was observed in the epidermis and cortical region, but Zn was mainly deposited in the endodermal region (Fig. S1). In rice roots, higher Cd accumulation was observed in the stele than in the endodermis, cortex and epidermis of Cd-treated plants (Fig. S2). Higher Zn accumulation was observed in the epidermis and cortex than in the stele and endodermis of Zn-treated rice plants. The accumulation of metal ions and Si showed a negative correlation in rice roots (R^2 = -0.635 and $P \leq 0.05$) and in maize roots (R^2 = -0.088 and $P \geq 0.05$), as presented in supplementary table 1.

The distribution of Si in maize leaf tissues was dependent on the heavy metal stress to which the plant was exposed. While Cd induced a slight increase in the Si content in the stelar region, a decrease was observed in the stelar region of Zn-treated maize leaves (Fig. S3). The Si content was slightly reduced in the mesophyll of Cd-treated maize leaves, whereas it increased slightly in Zn-treated maize leaves. Si was distributed mainly to the mesophyll regions of both control and Zn-treated maize leaves. However, in leaves of Cd-treated maize, Si was largely redistributed into the stelar regions (Table 1).

Cd induced modifications in the distribution of Si in rice leaves. Increases of 30% and 27% in the Si content were observed in the stele and mesophyll tissue, respectively, compared with the control (Fig. S4). However, in rice leaves exposed to Zn stress, the Si content was reduced by 16% in the stele, and a 24% increase was observed in mesophyll tissue. The maximal Si deposition was observed in mesophyll tissues of control and heavy metal (Cd and Zn)-treated rice compared with the other tissue regions (Table 1).

In maize leaves, Cd and Zn accumulation was higher in the stele and mesophyll tissues, while the Si content was reduced in the mesophyll cells of Cd-treated plants compared with the control. In rice leaves, both Cd and Zn were highly accumulated in the stelar region compared with mesophyll tissue. The complexation of Cd and Zn metal ions was visualized in SEM images of roots from maize and rice plants exposed to Cd and Zn stress (Figs 1 and 2).

Anatomical modifications in roots

The anatomical modifications induced in the roots of rice and maize by elevated exposure to Cd and Zn included parenchymal degradation, cell wall thickening and blockage of xylary elements; these modifications were more severe in maize plants than in rice plants. The cortical region of the maize root consists of 6-7 layers of parenchymal cells, and degradation of these cells was the prominent response observed in maize roots upon exposure to Cd and Zn toxicity (Fig. 3).

The parenchymal tissues in the stelar region were completely damaged, and the cortical parenchyma was shrunken in roots of Cd-treated maize plants (Fig. 3C). In the roots of Zn-treated plants, the inner cortical parenchyma was significantly damaged, the outer cortical parenchyma cells were larger in size, and there was an increase in the intercellular space (Fig. 3E). In rice roots, within the epidermal layer, a single sclerenchymatous layer of the exodermis was observed, followed by a large aerenchymatous cortex (Fig. 4). This sclerenchymatous exodermis was not observed in maize plants, and this exodermal layer in rice acts as a physical barrier against the entry of metal ions.

Under Cd and Zn stress, the cortical regions of rice did not exhibit any significant anatomical modifications (Fig. 4C, E). This result indicates that rice roots are more tolerant to Cd and Zn toxicity than maize roots.

The most remarkable modification observed in response to Cd and Zn treatment in rice and maize was wall thickening in the root xylem elements along with the inner transverse wall of the endodermal cells (Table 2). Upon exposure to $CdCl_2$, the inner transverse wall of the endodermal cells in the root of maize plants showed a 68% enhancement in thickening compared with the control. This thickening of the walls of the xylem elements was even more pronounced in the roots of plants exposed to $ZnSO_4$ toxicity, which were 105% thicker than the control (Fig. 3D, F). However, in rice roots, Cd and Zn toxicity induced thickening of epidermal cells (by 270% and 93%, respectively), and the walls of xylem elements were thickened by only 50-56% compared with the control. The cell walls of the endodermis in roots did not show any significant modifications under Zn toxicity in rice.

The depositions in the xylem vessels were similar to a network and were observed in both Cd- and Zn-treated rice (Fig. 4) and maize (Fig. 3). These thickenings in the xylem and endodermis showed a strong negative correlation with the Si content of the xylem and endodermis in rice (R^2 = -0.769 and $P \geq 0.05$) and maize roots (R^2 = -0.715 and $P \geq 0.05$), as presented in Table S2.

Anatomical modifications in leaves

The major anatomical modifications observed within the leaves of rice and maize under Cd and Zn toxicity were increases in the number and thickness of the sclerenchymatous bundle sheath cells, increases in the wall thickness of xylem elements and the blockage of xylem vessels. Cd-treated maize plants (Fig. 5) showed more significant modifications in leaf anatomy than rice plants (Fig. 6). The loss of turgidity of mesophyll cells and the collapse of bulliform cells were clearly visualized in the SEM images of maize leaves (Fig. 5C).

A reduction in the phloem area and depositions and blockages in xylem vessels were also observed in Cd- and Zn-treated maize plants. In rice leaves, thickening of the walls of the bundle sheath and xylem elements were observed (Fig. 6C), but xylem vessels were not blocked as observed in Cd-treated maize plants. Interestingly, the thickening of the cell walls induced by the presence of Cd and Zn was different in tissues of both maize and rice. Cd-treated maize roots showed an increase in wall thickening of endodermal cells, whereas in Zn-treated maize roots, the xylem elements showed the most thickening. These thickenings showed a strong negative correlation with the Si content and a positive correlation with the metal content.

Bioaccumulation

Cd and Zn accumulation was increased in plants with higher exposure to external metal concentrations. The uptake and transport of Cd and Zn ions depend on the distribution of endogenous Si, which reduces metal uptake. The bioaccumulation patterns of Cd and Zn were different in these crop plants (Table 3). Cd accumulation was higher in maize plants (5.65 mg g^{-1} DW) than in rice plants (5.04 mg g^{-1} DW). When plants were exposed to Zn toxicity, Zn accumulation was increased to 1.28 and 0.86 mg g^{-1} DW in rice and maize, respectively.

The bioconcentration factor (BCF) value represents the ratio between the metal content within the plant and that present in the growth medium. The BCFs of Cd and Zn were also modified as metal accumulation increased. The BCFs of Cd were 12 and 11 for Cd-treated maize and rice plants, respectively. However, the BCF of Zn was lower in plants subjected to Zn toxicity than in the control plants; the BCFs of Zn were 5.78 and 0.66 in the control and stressed rice plants, respectively. In maize, the BCFs of Zn were 2.47 and 0.44 in the control and stressed plants, respectively.

Discussion

Silicon distribution relative to Cd and Zn

Silicon-induced Cd and Zn stress alleviation has been reported in rice, maize and *Solanum nigrum*, and the metal chelation property of Si has been studied by previous researchers (Wang et al. 2000, Zhang et al. 2008, Liu et al. 2013). Moreover, Si accumulation renders the cell wall more negative and heterogeneous, and the interaction between the Si-hemicellulose matrix and Cd leads to complexation, which aids in decreasing the cytoplasmic Cd content (Ma et al. 2015, Głazowska et al. 2018). In the present study, different regions of the roots showed variations in endogenous Si accumulation patterns under Cd and Zn stress. The results observed in maize and rice roots indicate that a redistribution of Si from the stelar region to other regions of root tissue occurs under Cd and Zn stress, which may help to protect plants from metal stress. Although a portion of Si underwent complexation with Cd or Zn and precipitated in the cell walls or assembled as cellular inclusions, the remaining portion was redistributed to other regions of the root and to the leaves, possibly due to the replacement of Si by Cd and Zn ions.

In this study, it was evident that tissues with high Cd and Zn accumulation exhibited a reduction in Si content in maize and rice, which may be due to the redistribution of Si from tissue regions where other metal ions (Cd and Zn) are concentrated at high levels. Similar to our results, when rice roots under Zn stress were treated with exogenous Si, minimum Zn accumulation and maximum Si deposition were observed in the stelar region of the root (Gu et al. 2012). In contrast, in a study conducted in the roots of rice plants, maximal Cd accumulation was observed in the epidermis and endodermis and Si accumulation was also higher in the epidermis and endodermis, indicating complexation of Si with Cd and its precipitation in these two regions (Shi et al. 2005).

The endodermis plays a crucial role in reducing metal transport towards the central cylinder, and in the present study, Si deposition was increased in the endodermis during Cd and Zn stress in rice and maize, which potentially increases the physical defense exhibited by the endodermis. Si is a normal component of the endodermis of cereals, and the mode of Si deposition may be impregnation or Si-aggregate formation (Lux et al. 2020). Similar to our results, when maize plants were grown on Cd- and Zn-enriched soil, the deposition of silica bodies was prominently observed in the endodermis of roots (da Cunha and do Nascimento 2009). In maize, the precipitation of Si in the endodermis and pericycle of roots is considered a mechanism of Cd and Zn stress tolerance (da Cunha and do Nascimento 2009, Tripathi et al. 2012).

In maize and rice roots, the epidermal and cortical regions showed an increase in Si deposition during Cd stress, which may result in the coprecipitation of metal ions with Si in the outer region, efficiently preventing the uptake of excess metal ions. Earlier studies also support our results; for example, when *Minuartia verna was* exposed to excess Zn concentrations, Zn-Si coprecipitation was prominent in epidermal tissues (Bhat et al. 2019). At the same time, reports have shown that exogenous application of Si reduces Zn uptake, simultaneously increasing the inactivation of Zn ions (Kaya et al. 2019). Wang et al. (2000) investigated the metal detoxification mechanisms of Si in strawberry and found that precipitation of Si and Cd complexes in the cell walls and intercellular spaces is a means of reducing metal ion interference in metabolism. The differential distribution pattern of Si within root tissues is largely due to the differential affinities of Si, Cd and Zn for the cell walls of different root tissues from maize and rice under Cd and Zn stress.

In leaves, the Si content in mesophyll cells was reduced in Cd-treated maize plants compared with the respective control, which could be due to the displacement of Si from mesophyll cells by excess Cd ions. The accumulation of Zn and Cd in mesophyll tissue makes maize very sensitive to Cd and Zn toxicity, as the accumulation of these metals in metabolically active sites hinders very important metabolic functions (Gu et al. 2012). Compared with the mesophyll tissue, xylem elements, such as the xylem sclerenchyma, are metabolically inactive; therefore, the accumulation of more metal ions does not cause harm, which may be the reason for the higher metal tolerance exhibited by rice leaves. Moreover, the metals are precipitated as complexes in xylem and mesophyll tissue. These complexes attach to the cell wall as inactive forms.

Anatomical modifications

The greater degradation of parenchymal cells observed in maize roots could be the result of oxidative stress induced by the elevated metal concentration in the tissue. Similar results were found in *Brachiaria decumbens* under Cd and lead toxicity, which induced cell wall degradation in roots (Gomes et al. 2011). Vitória et al. (2004) reported that Cd-induced reduction in the cellular hydraulic potential was the reason for the disintegration of the cortical parenchyma of radish roots. This extensive cell degradation significantly reduced the rates of root growth and maturation under heavy metal stress (Sandalio et al. 2001, Shackira and Puthur 2019). Rice roots did not show any significant parenchymal cell degradation, in contrast to maize roots, which indicates lower oxidative stress elicited in rice roots than in maize.

Cell wall thickening in the endodermis and vascular tissues is considered an important anatomical modification in rice and maize under Cd and Zn stress. Shackira and Puthur (2019) reported wall thickening in the root cells of *Acanthus ilicifolius* under Cd stress, which supports the results of the present study. This cell wall thickening is largely due to lignification that occurs in response to the presence of high metal concentrations. Cd- and Zn-induced lignification in the cell wall has been reported in the roots of different plants, such as *Glycine max* (Finger-Teixeira et al. 2010), tea (Zagoskina et al. 2007), wheat (Li et al. 2012) and maize (da Cunha and do

Nascimento 2009). Heavy metals have the capacity to bind to the cell walls, and the increased cell wall content facilitates this process. The capacity to bind heavy metals in the cell wall is a mechanism to protect cytoplasmic metabolism from the deleterious effects of heavy metals (da Cunha and do Nascimento 2009). Moreover, the thickening of exodermal and endodermal cells in maize and rice roots indicates the adaptation and tolerance of maize to elevated concentrations of heavy metals. This thickening acts as an apoplastic barrier for the uptake and transport of metal ions (Lux et al. 2004). ROS molecules accumulated in the roots act as signaling molecules for lignin deposition in the cell wall, leading to cell wall thickening (Rahoui et al. 2017). Such an arrangement prevents the transportation of Cd and Zn to the shoot, which could be achieved by Casparian band thickening of the endodermis, as observed in the roots of maize plants. In addition, when maize plants were exposed to Cd stress, the development of Casparian bands and suberin lamellae aided in the reduction of the symplasmic Cd content (Vaculík et al. 2012).

Xylem plays a crucial role in water and mineral transport, and metal toxicity induces the deposition of electron-dense compounds in xylary elements, which further reduces the longitudinal transport of water and minerals. In this study, roots of rice and maize showed metal deposition in the xylem element and reduced apoplast water transport through xylem vessels. This unequal thickening due to depositions in the xylary elements in the roots of rice and maize hinders water transport to shoots, subsequently hindering plant growth and development. *Phaseolus vulgaris* exposed to Zn stress exhibited less water transport due to Zn deposition in xylem vessels, supporting the results of our study. At the same time, the walls of the xylem sclerenchyma are metabolically inactive, and the cell wall compartmentalization of metals in this area helps the plant tolerate metal stress.

Interestingly, the cell wall thickening induced by Cd and Zn was different in different tissues of maize and rice. Cd-treated maize roots showed an increase in wall thickening in endodermal cells, whereas in the roots of Zn-treated maize, xylem elements showed maximum thickening. These thickenings showed a strong negative correlation with the Si content and a positive correlation with the metal content. Si deficiency induced the thickening of cell walls in rice leaves and increased the contents of cell wall components, such as hemicellulose, cellulose and lignin (Yamamoto et al. 2012). Upregulation of the genes involved in secondary wall synthesis was also observed under Si-deficient conditions, and secondary wall formation was specifically observed in the sclerenchyma and xylem tissues. The antagonistic action of Si towards heavy metals was made clear with the application of Si in heavy metal-contaminated areas, resulting in reduced uptake of heavy metals and the prevention of metal-induced lignification in the plant cell wall (Schwanz et al. 2001).

Bioaccumulation

Exogenous application of Si has been found to alleviate Cd toxicity by reducing Cd uptake by rice roots and shoots. Likewise, the present study suggested that the endogenous Si distribution under Cd and Zn stress also plays an important role in metal tolerance. The reduction in Cd uptake in rice was due to the accumulation of more Si towards the periphery of the root, which plays a greater role in the prevention of Cd ion uptake by roots (Bhat et al. 2019). Maize roots also distributed the Si content to the root periphery but at a lower level than the Si content in the epicortical regions of rice during Cd stress, which also protects the root from the overaccumulation of Cd in roots. Therefore, the redistribution of Si to the root periphery can be considered a prominent metal tolerance mechanism in the roots of these two crop plants. Rice and maize plants distributed more Si to the endodermis and thus accumulated the least Zn in the endodermis, which helped minimize the translocation of Zn ions to xylem vessels. The increase in Zn accumulation, higher metal tolerance of rice and reduced translocation to roots indicates the potential of rice roots to stabilize more Zn ions in the roots.

The BCF value represents the ratio between the metal content within the plant and that present in the growth medium. The BCF of Zn in the control stands was high because the low Zn content in the medium resulted in the uptake of a higher proportion of Zn given it is an essential element. As the Zn content of the growth medium was high under the treatment conditions and the uptake of this element was restricted from exceeding the limit, the calculated BCF was lower in treated plants than in the control. A similar result was obtained when *Bruguiera cylindrica* was exposed to another essential element, *i.e.*, copper, where BCF was higher in the control than in the metal-treated plants (Sruthi and Puthur 2019). However, in the case of Cd, the increase in the BCF value in treated plants indicates an increased concentration of this toxic metal in the growth medium, while the plant could regulate the uptake and transport of Cd. Similar results were found in *Acanthus ilicifolius* upon exposure to different concentrations of $CdCl_2$ (Shackira and Puthur 2016).

Conclusion

Endogenous Si deposition and heavy metal (Cd and Zn) accumulation showed a negative correlation in the roots of rice and maize, indicating that the partitioning of Si in the roots of these two grasses under metal stress, either by complexation with metal ions and/or by redistribution to specific tissue regions, can further prevent metal uptake. The anatomical modifications induced by the higher concentrations of Zn and Cd showed the hypersensitivity of maize versus rice towards Cd. Moreover, these anatomical modifications, specifically cell wall thickening, showed a correlation with Si distribution in rice and maize.

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Author contributions

E. J. performed the analysis, processed the experimental data and interpreted the results.
J.T.P. provided critical feedback and helped draft the manuscript. P.A. revised the manuscript to the present form.

Data availability Statement

The data pertaining to this manuscript are not shared in any other source but will be readily made available upon request.

Figure legends

Fig. 1. SEM image and EDX spectra of electron-dense depositions in the root vessels of maize and rice exposed to $CdCl_2$ stress.

Fig. 2. SEM image and EDX spectra of electron-dense depositions in the root vessels of maize and rice exposed to $ZnSO_4$ stress.

Fig. 3. Scanning electron micrograph of root C.S. of maize exposed to $ZnSO_4$ and $CdCl_2$. Control (A and B); $CdCl_2$ (C and D) and $ZnSO_4$ (E and F). E-epidermis; C-cortex; St-stele and X-xylem.

Fig. 4. Scanning electron micrograph of root C.S. of rice exposed to $ZnSO_4$ and $CdCl_2$. Control (A and B); $CdCl_2$ (C and D) and $ZnSO_4$ (E and F), E-epidermis; C-cortex; St-stele; En-endodermis and X-xylem.

Fig. 5. Scanning electron micrograph of leaf C.S. of maize exposed to $ZnSO_4$ and $CdCl_2$. Control (A and B); $CdCl_2$ (C and D) and $ZnSO_4$ (E and F), E-epidermis; C-cortex; St-stele; En-endodermis and X-xylem.

Fig. 6. Scanning electron micrograph of leaf C.S. of rice exposed to $ZnSO_4$ and $CdCl_2$. Control (A and B); $CdCl_2$ (C and D) and $ZnSO_4$ (E and F), UE-upper epidermis; LE-lower epidermis and X-xylem.

Treatments		Si (% weight)		Treated metal (% weight)	
		Rice root	Maize root	Rice root	Maize root
Control	**stele**	97.5 ± 4.9[a]	65.24 ± 3.2[a]		
	endodermis	92.59 ± 0.45[a]	46.66 ± 3.5[b]		
	Epidermis+cortex	54.51 ± 1.5[b]	60.58 ± 5.1[a]		
$CdCl_2$	**stele**	53.57 ± 2.5[b]	77.19 ± 1.9[a]	6.43 ± 0.001[a] (Cd)	22.80 ± 3.2[b] (Cd)
	endodermis	50.54 ± 0 .5[b]	58.13 ± 2.4[b]	3.76 ± 0.02[b] (Cd)	34.88 ± 8.1[a] (Cd)
	Epidermis+cortex	91.49 ± 3.1[a]	79.87 ± 5.4[a]	0.02 ± 0.001[c] (Cd)	20.12 ± 3.5[b] (Cd)
$ZnSo_4$	**stele**	46.31 ± 1.6[b]	16.66 ± 2.1[c]	11.44 ± 2.4[b] (Zn)	22.22 ± 3.1[b] (Zn)
	endodermis	87.02 ± 2[a]	90.43 ± 4.6[a]	3.10 ± 3.4[c] (Zn)	0.04 ± 0.002[c] (Zn)
	Epidermis+cortex	49.93 ± 5.1[b]	69.90 ± 0.4[b]	26.28 ± 2.6[a] (Zn)	37.68 ± 1.8[a] (Zn)
Treatments		**Si**		**Treated metal**	
		Rice root	**Maize root**	**Rice root**	**Maize root**
Control	**stele**	57.39 ± 4.5[b]	76.92 ± 3.7[a]		
	mesophyll	72.67 ± 5.3[a]	80 ± 5.4[a]		
$CdCl_2$	**stele**	87.99 ± 3.6[a]	92.98 ± 3.4[a]	0.08 ± 0.001[a] (Cd)	31.36 ± 3.6[a] (Cd)
	mesophyll	99.94 ± 4.6[a]	73.43 ± 2.8[b]	0.05 ± 0.003[b] (Cd)	3.62 ± 0.05[b] (Cd)
$ZnSo_4$	**stele**	41.38 ± 5.2[b]	33.52 ± 3.5[b]	6.14 ± 0.04[a] (Zn)	1.13 ± 0.06[b] (Zn)
	mesophyll	96.37 ± 6.3[a]	90.75 ± 4.6[a]	7.81 ± 0.02[a] (Zn)	19.32 ± 0.3[a] (Zn)

Table 1: SEM-EDX microanalysis data in the root and leaves of maize and rice plants subjected to Cd and Zn toxicity. Different letters following values indicate a significant difference between treatments (Duncan's test, $P \leq 0.05$). The data is an average of recordings from three independent experiments each with three replicates (i.e. n = 9). The data represent mean ± standard error

Sample	Treatments	Endodermis	Xylem	Epidermis
Maize root	Control	2.033 ± 0.09 [b]	2 ± 0.294 [c]	1.103 ± 0.062 [c]
	$CdCl_2$ treated	3.433 ± 0.237 [a]	2.933 ± 0.301 [b]	1.167 ± 0.09 [b]
	$ZnSO_4$ treated	1.317 ± 0.162 [c]	4.1 ± 0.212 [a]	1.073 ± 0.038 [a]
Rice root	Control	2.2 ± 0.155 [a]	2.133 ± 0.206 [b]	0.897 ± 0.067 [b]
	$CdCl_2$ treated	2.013 ± 0.009 [a]	3.2 ± 0.155 [a]	3.333 ± 0.897 [a]
	$ZnSO_4$ treated	2.3 ± 0.256 [a]	3.333 ± 0.448 [a]	1.733 ± 0.179 [ab]
		Xylem	**Bundle cap**	
Maize leaf	Control	3 ± 0.059 [a]	2.967 ± 0.09 [b]	
	$CdCl_2$ treated	2.767 ± 0.148 [a]	3.567 ± 0.237 [ab]	
	$ZnSO_4$ treated	1.967 ± 0.09 [b]	3.867 ± 0.189 [a]	
Rice leaf	Control	1.837 ± 0.171 [b]	3.183 ± 0.348 [a]	
	$CdCl_2$ treated	2.267 ± 0.148 [b]	3.167 ± 0.448 [a]	
	$ZnSO_4$ treated	2.867 ± 0.189 [a]	3.367 ± 0.323 [a]	

Table 2: Cell wall thickening in the root and leaves of maize and rice plants subjected to Cd and Zn toxicity. Different letters following values indicate a significant difference between treatments (Duncan's test, $P \leq 0.05$). The data is an average of recordings from three independent experiments each with three replicates (i.e. n = 9). The data represent mean ± standard error

	Treatments	Metal concentration	BCF
Rice	Control	BDL	
	$CdCl_2$ treated	5.040 ± 0.711 (Cd mg g^{-1} DW)	11.20158
	Control	0.143 ± 0.006 (Zn mg g^{-1} DW)	5.783902
	$ZnSO_4$ treated	1.28 ± 0.043 (Zn mg g^{-1} DW)	0.658621
Maize	Control	BDL	
	$CdCl_2$ treated	5.658 ± 0.177 (Cd mg g^{-1} DW)	12.57373
	Control	0.061 ± 0.0010 (Zn mg g^{-1} DW)	2.469716
	$ZnSO_4$ treated	0.863 ± 0.073 (Zn mg g^{-1} DW)	0.4426

Table 3: Zn and Cd accumulated in the rice and maize plants (mg g^{-1} DW) with BCF value under the exposure of Cd and Zn toxicity; BDL-below detectable level.

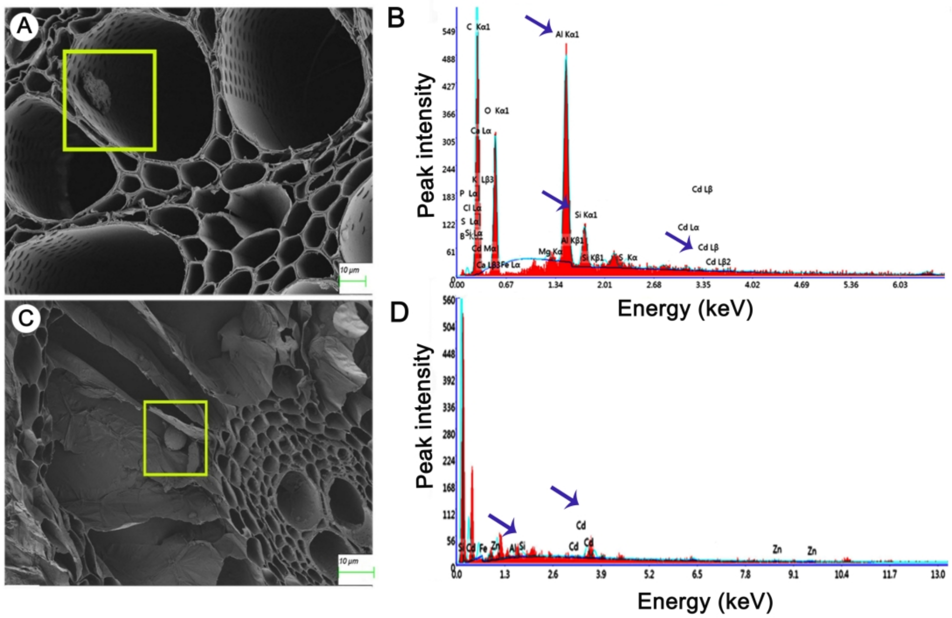

Fig.1. SEM image and EDX spectrums of the electron dense depositions in the root vessel of maize and rice exposed to CdCl2 stress.

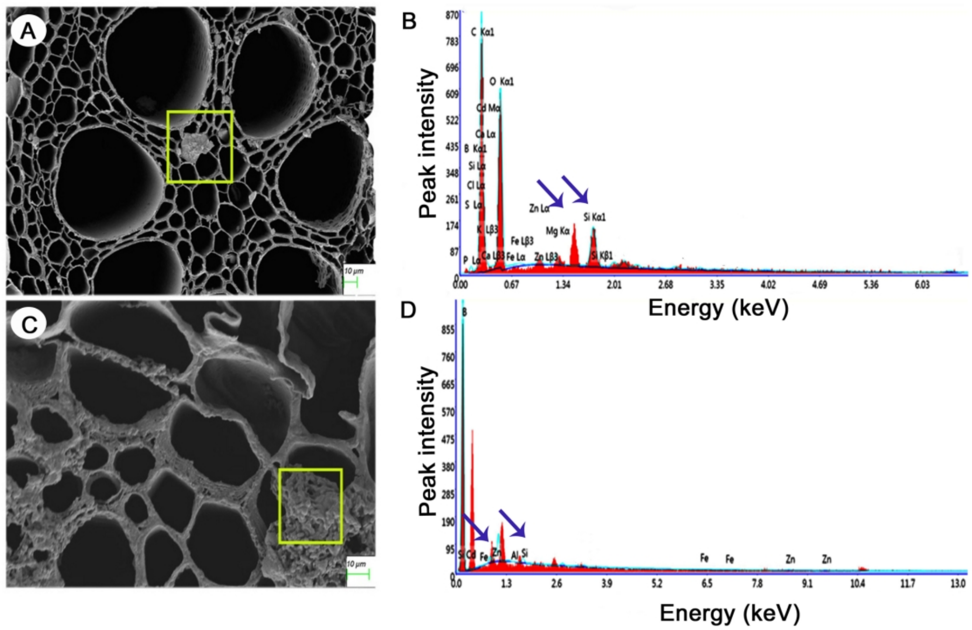

Fig. 2. SEM image and EDX spectrums of the electron dense depositions in the root vessel of maize and rice exposed to ZnSO4 stress.

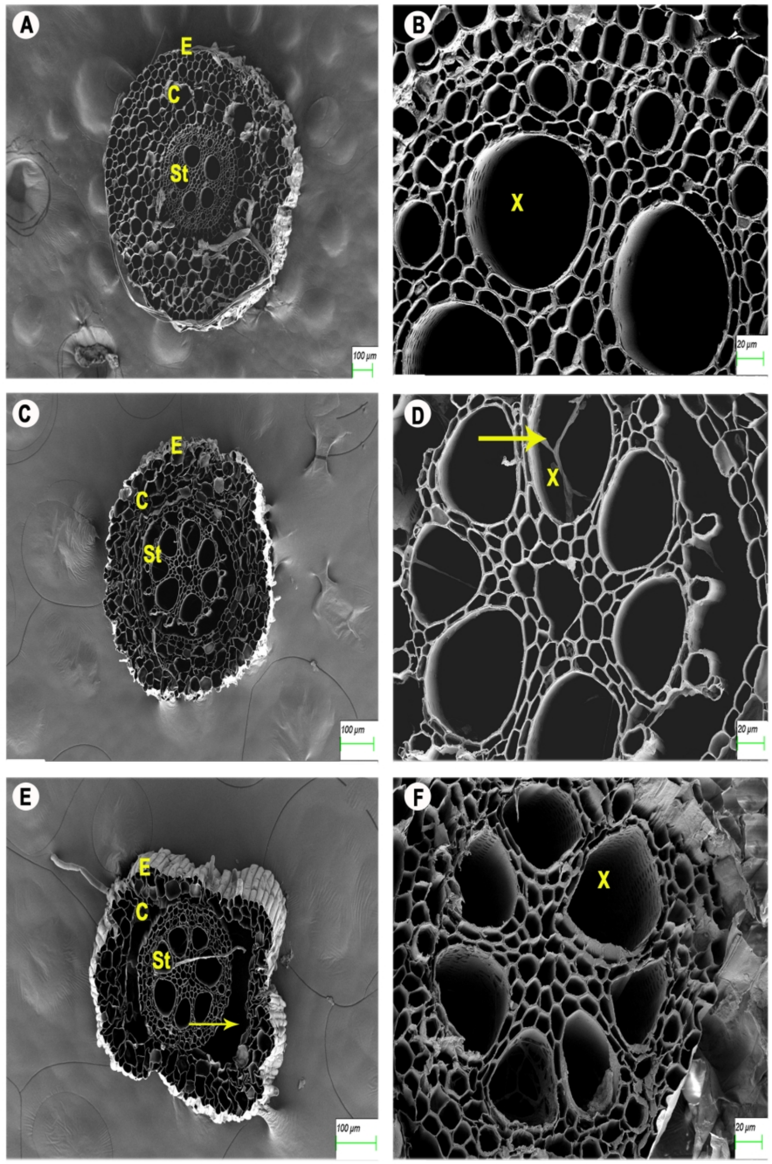

Fig. 3. Scanning electron micrograph of root C.S. of maize exposed to ZnSO4 and CdCl2. control (A and B); CdCl2 (C and D) and ZnSO4 (E and F). E-epidermis; C- cortex; St-stele and X-xylem.

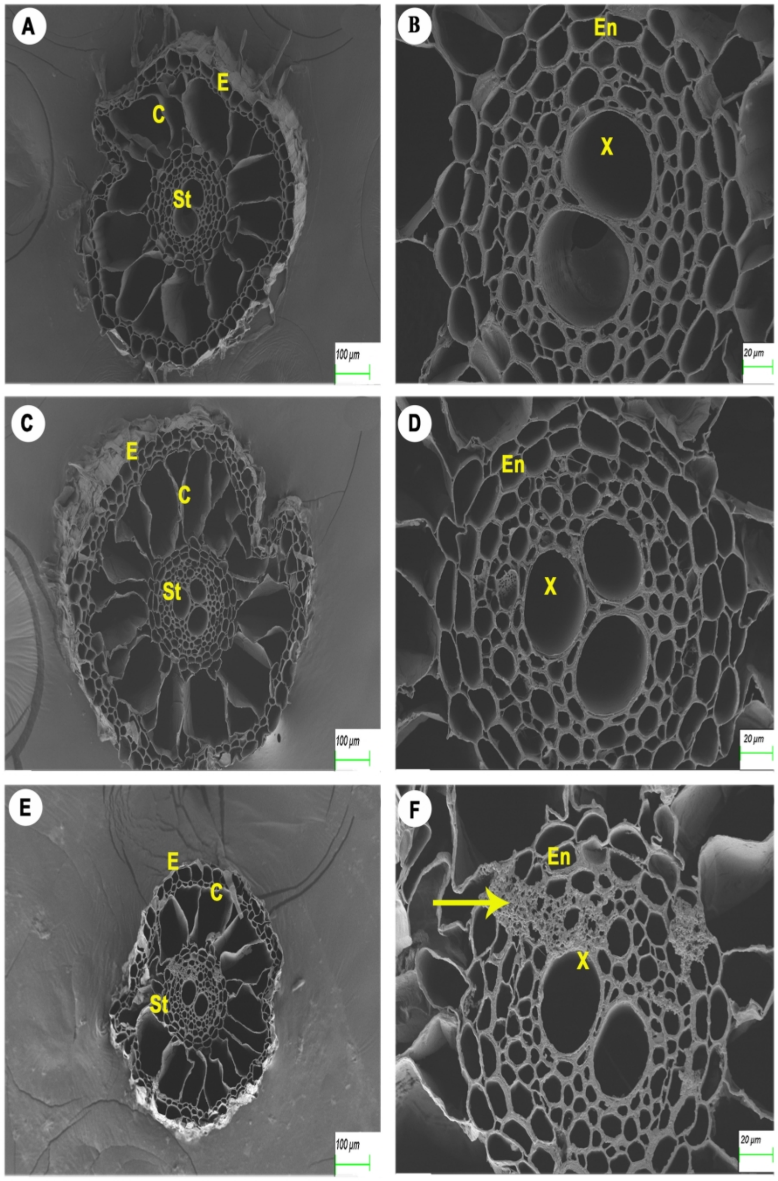

Fig. 4. Scanning electron micrograph of root C.S. of rice exposed to ZnSO4 and CdCl2. control (A and B); CdCl2 (C and D) and ZnSO4 (E and F), E-epidermis; C- cortex; St-stele; En-endodermis and X-xylem.

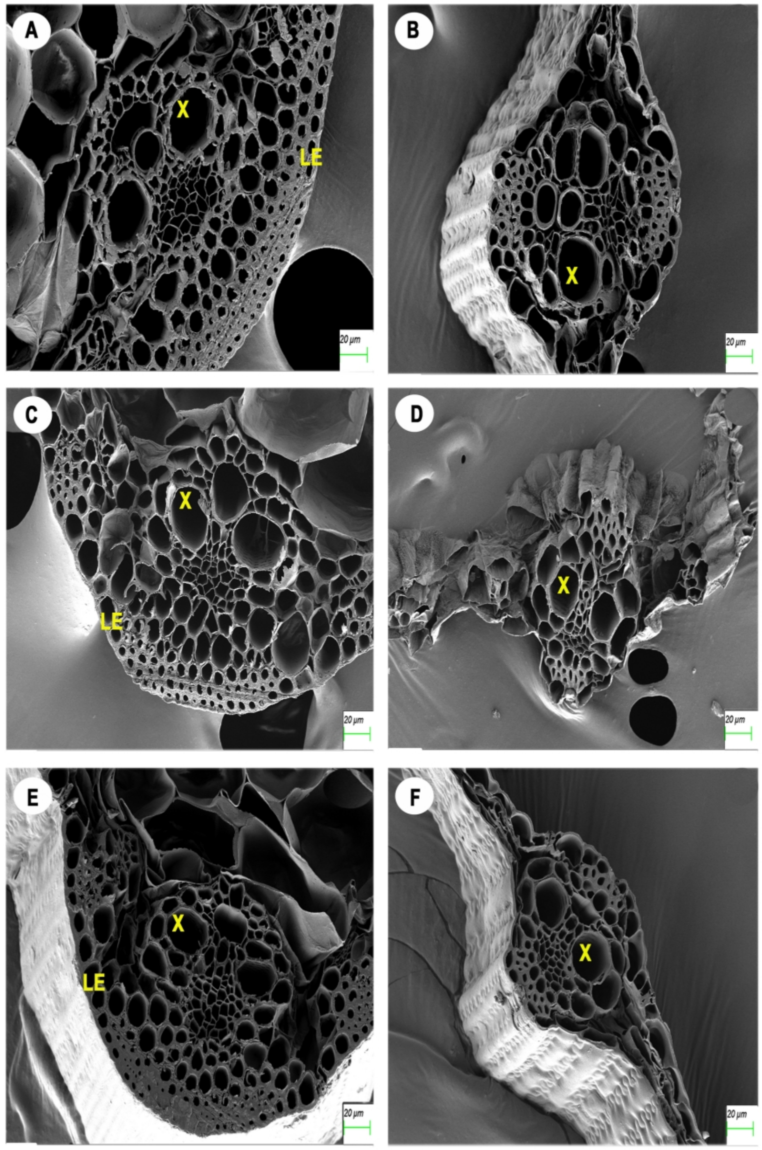

Fig. 5. Scanning electron micrograph of leaf C.S. of maize exposed to $ZnSO_4$ and $CdCl_2$. control (A and B); $CdCl_2$ (C and D) and $ZnSO_4$ (E and F), E-epidermis; C- cortex; St-stele; En-endodermis and X-xylem.

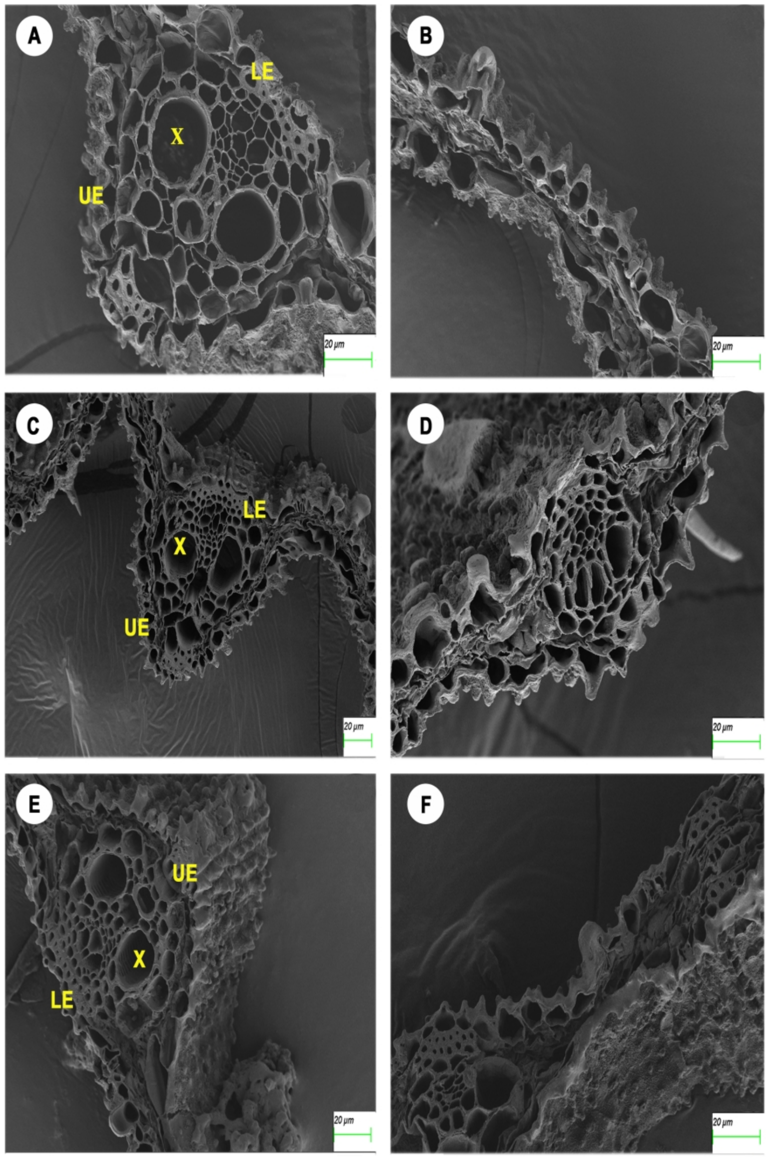

Fig. 6. Scanning electron micrograph of leaf C.S. of rice exposed to $ZnSO_4$ and $CdCl_2$. control (A and B); $CdCl_2$ (C and D) and $ZnSO_4$ (E and F), UE- upper epidermis; LE-lower epidermis and X-xylem.