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Plant Genetic Resource Utilization: An Appraisal

Plant Genetic Resources are crucial for the sustainable utilization and development of all ecosystems. The vast diversity in our country, an invaluable treasure, has to be wisely managed and utilized. Exploration of genetic diversity for crop improvement, food and agriculture, development of pharmaceuticals and identification of bioactive molecules with unique properties are of great significance in the current scenario of emerging diseases, climate change, and food scarcity. Most of our genetic resources are not thoroughly studied, and some are threatened /or at the verge of extinction due to destructive activities and invasive species. Awareness about the PGR among the younger generation is needed to conserve the resources for the future generation. There is a need to understand diversity to conserve and utilize resources in a sustainable way. Department of Botany is presenting here a collection of papers concerning plant genetic resources and sustainable utilization.

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Contents

Physiological and Anatomical Changes in Roots and Leaves of Maize Seedlings Subjected to Cadmium Stress

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ABSTRACT

The aim of the study was to resolve the impact of $CdCl_2$ toxicity on the physiology and anatomy of maize plants. *Zea mays* plants were treated with $CdCl_2$ (100 mM) solution on 45 d of growth. The results showed leaf chlorosis along with the reduction in leaf moisture content under $CdCl_2$ exposure. These physiological changes could be correlated with the anatomical modifications induced by $CdCl_2$ in the leaves of maize plants. Cell degeneration, thickening of cell walls, and blockages in the tracheary elements of vascular cylinder were the major modifications observed due to the accumulation of cadmium. Light microscope studies showed that exposure to $CdCl_2$ caused dark deposits in the vascular cylinder, wall thickening in endodermis as well as rupturing of parenchyma tissues in roots of maize. Moreover, cadmium toxicity caused a reduction in the root volume and loss of cell viability, which is associated with the internal structural changes of roots. The result indicated that maize plants have peculiar anatomical and physiological adaptations to impart tolerance towards $CdCl_2$.

KEYWORDS

Abiotic stress, Anatomy, Cadmium, Maize

INTRODUCTION

Cadmium (Cd), a divalent cation, is one of the most toxic heavy metals and has no described biological function in the plant system. But in the present scenario, Cd level in the environment exceeds its limit due to various anthropogenic activities like mining, smelting, unmanaged agricultural practices, and industrial waste deposition. High water solubility, relative mobility, the potential of biomagnification, and long biological half-life make Cd more toxic to the living world (Gill and Tuteja, 2011; Marques and do Nascimento 2013). Cd affects several basic events of plant growth, development, and physiology, including mineral nutrition, water balance, and gas - exchange, membrane

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function, cell metabolism, photosynthesis, and the defense against oxidative stress (Bi et al., 2009; Liu et al., 2010).

Plants exhibit different mechanisms to tolerate higher concentrations of Cd taken up into the plant cells. The important mechanisms are metal binding to cell walls, reduced transport across the cell membrane, active efflux of metal, compartmentalization, and chelation, which leads to modifications in the anatomical characters (Cobbet and Goldsbrough, 2002).

Maize, the queen of cereals, is one of the most versatile crops in India. In the Indian agricultural economy, *Zea mays* L. production has an important place because it is the third most important food crop after rice and wheat. India's estimated total maize production in 2017-2018 was 26.26 million tons (DAC and FW, 2018). Moreover, maize belongs to the grass family, and it was reported as a Cd tolerant crop (Malekzadeh et al., 2007).

The objective of the present study was to identify the internal structural changes and associated physiological alterations in maize due to Cd stress.

MATERIALS AND METHODS

Collection of Biological material

Maize (CoHM 6) seeds were collected from the Centre for Plant Breeding and Genetics, Department of Millets, Tamil Nadu Agriculture University (TNAU), Coimbatore, India.

Experimental design

Maize seeds were surface sterilized with 0.1% $HgC1_2$ solution for 5 min and placed at 8 cm below the sterilized soil filled in polythene bags (18×13 cm). The soil was sterilized by solarization according to the method of Raj and Sharma (2009). After 45 d of growth, plants were treated with 100 mM $CdCl_2$ solution (selected as the stress imparting concentration after preliminary analysis). The second lower leaves of the maize plants were taken for various analyses on 0, 4, 8 and 12 d of treatment.

Estimation of photosynthetic pigment content

The estimation of chlorophyll was done according to Arnon (1949) and it was calculated using the following equation,

$$\text{Total chlorophyll} = \frac{20.12\ (A_{646} - A_{750}) + 8.02\ (A_{663} - A_{750}) \times \text{volume}}{\text{Fresh weight of the sample}}$$

Leaf moisture content

The tissue moisture content was determined by measuring the fresh and dry weights of the leaves. Leaf moisture content percentage (MC %) was calculated using the following equation (Lokhande et al., 2011).

$$\text{Moisture content \%} = [(FW - DW)/FW] \times 100$$

Root volume

The root volume of maize plants was measured according to the protocol of Rahul et al. (2019).

Determination of cell viability

The loss of cell viability in the roots of metal-treated plants was evaluated by the modified method of Šimonovičová et al. (2004), using Evans blue stain. Maize roots were stained in 0.25% (v/v) aqueous solution of Evans blue for 15 min at room temperature. The stained roots were washed three times with distilled water for 10 min each. Root tips (5 mm) were excised and soaked in 50% (v/v) methanol along with 1% (w/v) sodium dodecyl sulfate (SDS) at 60°C for one h. Optical density was measured spectrophotometrically at 600 nm.

Light microscopic studies

Leaves and roots of the $CdCl_2$ treated and control maize plants were fixed in FAA. Freehand sections were stained with toluidine blue O according to the procedure of Khasim (2002). Further, the sections were examined under the microscope (Zeiss, 888-014536, Germany), and photomicrographs were captured.

Field emission scanning electron microscopic (FESEM) studies

Root and leaf sections of 100 mM $CdCl_2$ treated and control plants were fixed in 2.5% glutaraldehyde, prepared in 0.2 M sodium cacodylate buffer (pH 7.4) for 5 min. After fixing, sections were dehydrated by passing through acetone series with five minutes incubation in each. Then the specimens were gold-palladium coated, and further micrographs were taken with the help of photographic attachment of the FESEM (Carl-Zeiss, 300, Germini).

RESULTS AND DISCUSSION

Anatomical studies of leaves and roots of maize plants treated with $CdCl_2$showed distinct changes in internal structure compared to control (Figure 2, 3 and 4), which was highly associated with some of the physiological characteristics of plants. The maize root cross-section (C.S.) consists of the epidermis, cortex, and stelar region. The cortex was parenchymatous and thin walled. The vascular tissues consist of pericycle, phloem, and xylem vessels consisting of protoxylem, early metaxylem and late metaxylem. The maize leaf C.S. consists of epidermis, mesophyll tissues, and stelar region and characterized by the presence of bulliform cells in the epidermis (Janeeshma et al., 2020). $CdCl_2$ induced anatomical modifications in leaves was significantly observed in bulliform cells, stomata and vascular bundles (Figure 2G and 4C), whereas in roots the parenchyma cells were found to be collapsed in Cd treated plants (Figure 2D) but not in control plants (Figure 2A).

The collapse of bulliform cells, depressed stomata, chlorophyll degradation, were the major modifications observed in maize leaves under the influence of a higher concentration of $CdCl_2$. Potential of heavy metals to disrupt ionic and hormonal balance results in the degeneration of cells (Novais et al., 2011). Structural loss of bulliform cells was developed as a result of water loss, and it could be correlated with the reduced MC% under **a** higher concentration of $CdCl_2$. MC% of leaves was decreased by 35% in plants subjected to 100 mM $CdCl_2$ treatment on 12 d of treatment as compared with the control (Figure 1A); further, this decrease in MC% was observed to be dependent on the severity and duration of exposure of plants to the stress. Similar findings were also reported in lettuce (Costa et al., 1994) and radish seedlings (Costa and Morel, 1994), wherein it was shown that exposure to Cd stress reduced the water content of the plant tissues. The

possible reason for the reduction in water content is the heavy metal induced blockage of the xylem vessels (Shackira et al., 2017).

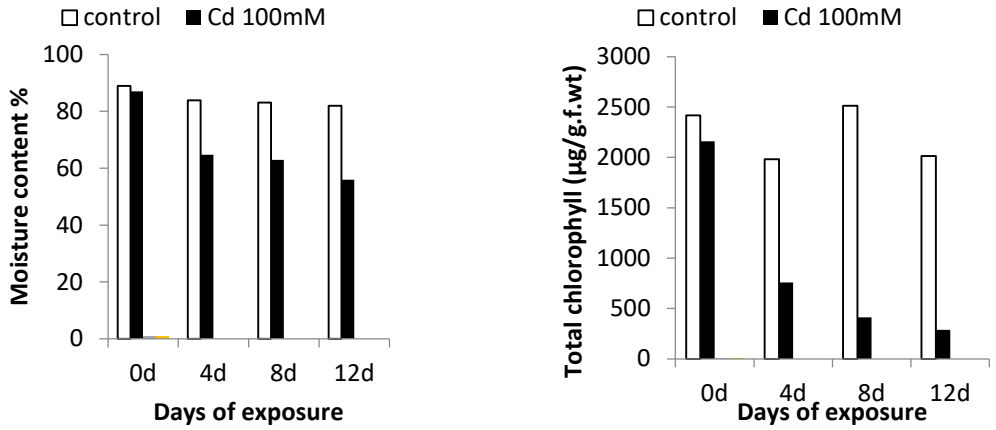

Figure 1: Moisture content % (A) and total chlorophyll content (B) of *Zea mays* subjected to 100 mM $CdCl_2$ solution. Values are the mean ± SE of three independent experiments.

The total chlorophyll content of maize plants was analyzed to evaluate the extremity of chlorophyll degradation in leaves subjected to $CdCl_2$ stress. The total chlorophyll content was decreased on exposure to 100 mM $CdCl_2$, i.e., 86% of reduction on 12 d of exposure (Figure 1B). Generally, metal treated plants show a reduction of chlorophyll content and photosynthesis rate due to degradation of chlorophyll occurring as a result of the replacement of Mg^{2+} by Cd ion. Cd toxicity leads to a decrease in the synthesis of chlorophyll and even causes inhibition of the activity of some enzymes in the Calvin cycle (Baryla et al., 2001). According to Baryla et al. (2001) leaf chlorosis observed in oilseed and rape grown in Cd contaminated soil could be related to reduced chloroplasts and the resulting reduction in chlorophyll content. In barley and maize seedlings, Cd has been shown to inhibit the chlorophyll synthesis by affecting the activity of photochlorophyllide reductase, which further leads to the chlorosis (Stobart et al., 1985; Rascio et al., 1993) and these observation are comparable with the results observed in the present study.

Thickened cell walls, dark deposits in cell walls of vascular tissue, and breakdown of parenchyma tissue were observed in the roots of maize plants exposed to Cd (Figure 2D). Both xylem elements and pith of roots showed dark deposition in its cells, a structural abnormality observed as a result of Cd stress (Alfaraas et al., 2016). Wall thickening in the root metaxylem and protoxylem along with the inner transverse wall of endodermis was the most significant anatomical modification observed due to $CdCl_2$ treatment (Janeeshma et al., 2020). The inner transverse wall of the endodermis showed an increase in the wall thickening compared to the control. In the stelar region, xylary elements also got thickened due to Cd toxicity compared to the control. The ability of Cd ions to adhere to the cell wall prevented the entry of Cd ions into the cytosol. Moreover, wall thickening can help to maintain the hydraulic capacity of roots and can also act as a barrier to water loss. The thickening of exodermal and endodermal cells indicates the adaptation and tolerance of maize to high concentrations of heavy metals (Lux et al., 2004).

Scanning electron micrographs also showed that the tracheary elements of xylem in root and leaf had an accumulation of some electron dense particles causing blockage of vessels, which may help the plant to reduce the uptake of Cd ions (Figure 3F). Energy dispersive X-Ray analysis of the electron dense depositions confirmed it as aluminum

oxalate crystals, which could be accumulating in response to the higher concentration of Cd in the cytosol (Barcelo et al., 1988; Janeeshma et al., 2020).

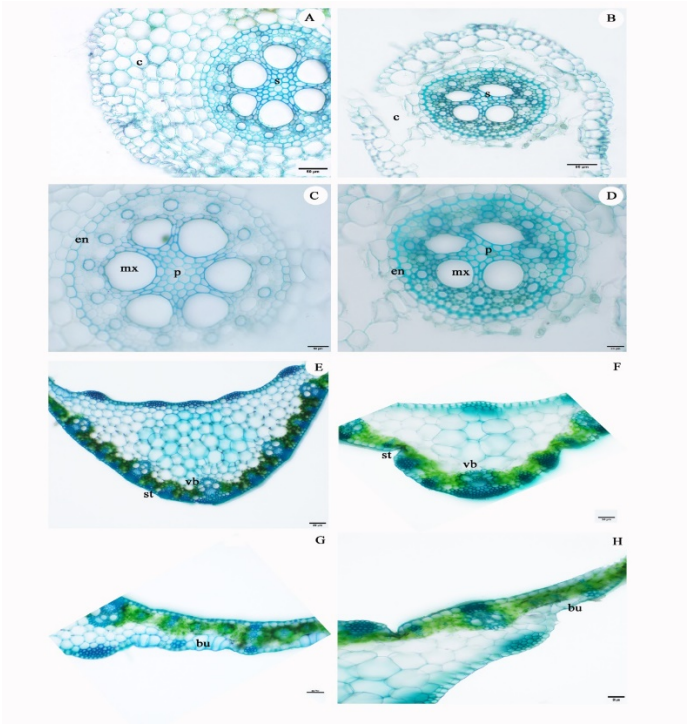

Figure 2: Micrographs of the anatomical changes in the roots and leaves of of *Zea mays* subjected to 100 mM $CdCl_2$ solution as compared to control. A&C) control root, C&D) Cd treated root, E&F) control leaf and G&H) Cd treated leaf (c-cortex; s-stele; p-pith; mx-metaxylem; en-endodermis; st-stomata; vb-vascular bundle and bu-bulliform cells).

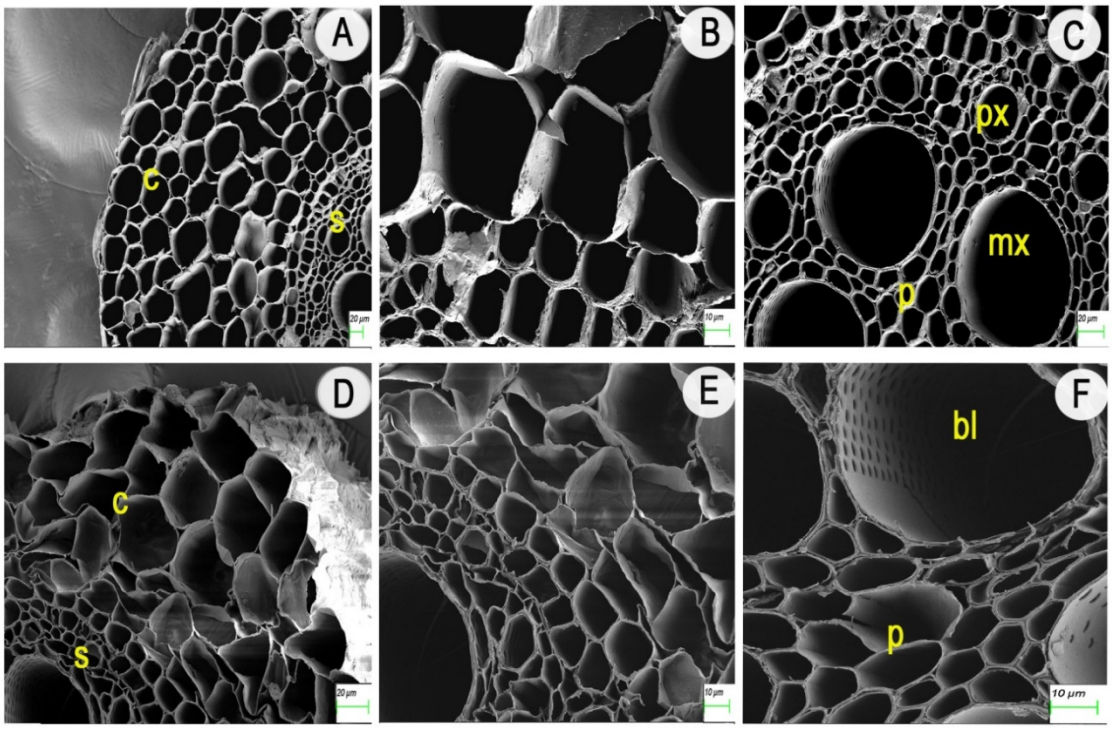

Figure 3: Scanning electron micrographs of the anatomical changes in the roots of *Zea mays* subjected to 100 mM $CdCl_2$ solution as compared to control. A, B&C) control D, E&F) Cd treated root (c-cortex; s-stele; p-pith; mx-metaxylem; px-protoxylem; and bl-blockage in vessels).

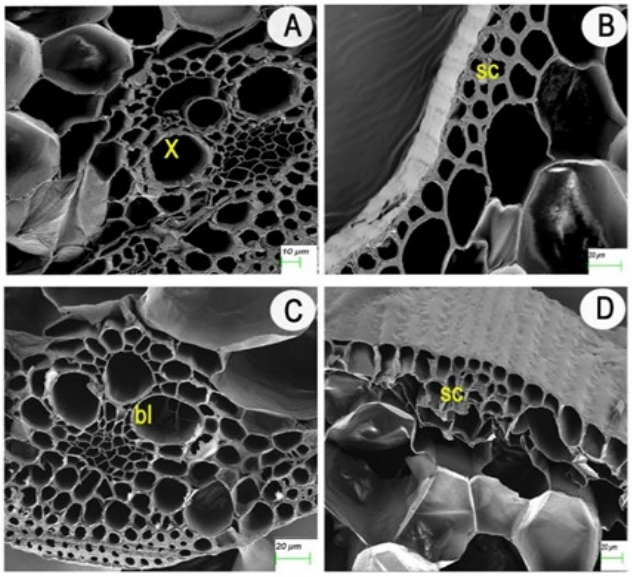

Figure 4: Scanning electron micrographs of the anatomical changes in the leaves of *Zea mays* subjected to 100 mM $CdCl_2$ solution as compared to control. A&B) control C&D) Cd treated leaf (x-xylem; bl-blockage in vessels; and sc-sclerenchyma).

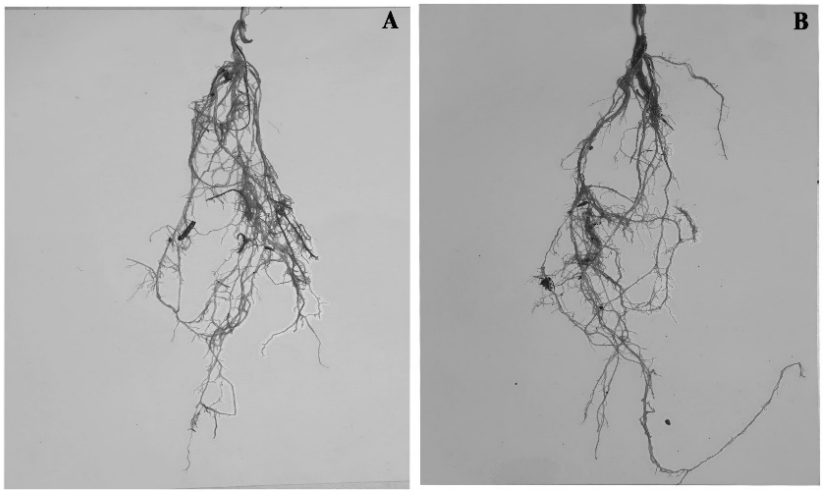

Figure 5: Reduction in the root volume of *Zea mays* subjected to 100 mM $CdCl_2$ solution as compared to control. A) control B) roots of $CdCl_2$ treated plants.

Sample	Root volume (ml)	Absorbance of Evans blue stain
Control	5.93 ± 0.600	0.380 ± 0.050
$CdCl_2$ treated	3.83 ± 0.726	0.509 ± 0.081

Table 1: Cadmium induced reduction in root volume and cell viability in *Zea mays*.

These anatomical changes in the roots of maize plants, such as the degradation of parenchyma cells in the cortex and stelar region of roots exposed to Cd stress, could finally result in the reduction of root volume (Table 1). Root volume was significantly decreased under Cd stressor and the decrease was to the extent of 44 % as compared to control (Figure 5). The most significant amount of Evans blue uptake denoting the death of the cells was detected in the roots of Cd treated plants as compared to the control

plants, and the increase was 33 %. The increase in cell death is related to the apoptosis in the cortex due to the Cd stress. Similar to our observation, rice seedlings when exposed to Cu toxicity, showed enhanced cell death in roots (Chen et al. 2004).

The above results indicate that maize plants exhibit gradual changes in the root and leaf internal structure under exposure to higher concentrations of Cd, so as to encounter the metal stress. Understanding these changes would help to analyze metal tolerance mechanisms operational in maize and also to find ways and means to improve the same.

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