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Impact of Cadmium-Induced Stress on Physiological Traits with Induced Osmolyte and Catalase-Mediated Antioxidative Defense in Rice (*Oryza sativa* L.)

J. Yomso and A. Siddique†
Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara-144411, Punjab, India
†Corresponding author: anaytullahsiddique@gmail.com

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ABSTRACT

Cadmium is one of the most carcinogenic and hazardous heavy metals on the earth for causes many serious diseases and disorders in the plant body. The presence of Cd in the soil is equally harmful to the production of rice crops and human beings. A pot experiment was conducted to analyze the consequences of cadmium-induced stress on the antioxidative defense system in rice plants. The assessment of antioxidative defense mechanism based on the cadmium-induced stress in the range of 100 to 300 ppm while the parameters, Chlorophyll Content Index (SPAD), nitrogen (%), relative water content (%), membrane stability index (%), proline content ($\mu g.g^{-1}$), and catalase activity (nm H_2O_2 $mg^{-1}.min^{-1}$) were used. The highest reduction in the Chlorophyll Content Index (CCI), nitrogen (%), RWC (%), and MSI (%) was recorded at the highest concentrations of Cd Cl_2 (300 ppm). However, at the same time, an increase in proline content ($\mu g.g^{-1}$) and catalase activity (nm H_2O_2 $mg^{-1}.min^{-1}$) were also detected at all the intervals of the study. The activity of CCI, amino acid, and enzyme were presented in % increase/decrease over the control of Cd-induced stress in rice plants. The reduction (%) in CCI (SPAD) and RWC (%) was recorded maximum at 75 Days after transplanting (DAT), while nitrogen (%) and MSI (%) were recorded at 50 DAT. However, the increase (%) in proline and Catalase activity was maximum at 75 and 50 DAT.

INTRODUCTION

Heavy metals are serious threats to the health of human beings as well as crop production. The heavy metal cadmium (Cd) is one of them. There are several sources of Cd in the soil in which chemical fertilizers, the use of wastewater as a source of irrigation, sewage sludge, and weathering of rocks are the major sources (Aebi 1984, Ahmad et al. 2015, Ali et al. 2014, Anjum et al. 2015). Being a non-degradable compound, its presence in soil is always considered an alarm situation for soil health and crop production, while its easily absorbing nature makes it more dangerous compared to other heavy metals. Cadmium-infected plant affects several physicochemical processes of the plant during the entire growth period. It reacts directly or indirectly with plant cells, resulting in lipid peroxidation, and consequently produces several kinds of reactive oxygen species (ROS) like O_2^-, OH^* and H_2O_2 (Saidi et al. 2013, Zhang et al. 2014, Howladar 2014).

Moreover, the presence of cadmium in plants shows negative effects in many ways. It can damage the photosystem, especially the thylakoid membrane, affect the uptake and distribution of nutrients throughout the plant body, and alter the activity of antioxidative enzymes (Asgher et al. 2014, Ali et al. 2014, Khan et al. 2015). During heavy stress, anti-oxidative compounds are also present in the plant body, like Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), and Proline. All these compounds help in the safe dissipation of ROS from the plant body while it depends on many other complex processes (Balestri et al. 2014, Siddique et al. 2018).

MATERIALS AND METHODS

Experimental Details

The present piece of work was undertaken to evaluate the consequences of $CdCl_2$-induced stress in the rice plant (*Oryza sativa* L.) over the research farm of Lovely Professional University while the variety of rice (Pusa Basmati 1121) was procured from Punjab Agriculture University, Ludhiana, Punjab. 21 days old seedlings were placed in $CdCl_2$ treated pot (diameter is 46 cm) wherein clay 8 and 2 kg of clay loam soil and FYM was used in each pot while the pots were arranged in a Completely Randomized Design comprises of five replications. To evaluate the effect of externally imposed $CdCl_2$ stress, five different concentrations ranging

from 100 to 300 ppm were used, while a set of pots was used as a control. The recommended Agronomic practices were followed to grow the rice crop. At the same time, the observations of all the physiological and biochemical observations were carried out at regular intervals of 25 days up to 75 DAT.

Chlorophyll Content Index CCI and Nitrogen

The observation regarding the CCI was recorded through the SPAD meter Model No. 502 in terms of the SPAD unit, while the estimation of total nitrogen was carried out in flag leaf through KEL PLUS.

Relative Water Content

Estimation of RWC% was carried out according to (Weatherly 1962). 0.2 g leaf sample was cleaned properly and recorded fresh weight before being saturated in 100 mL of distilled water for 2 h and recorded turgid weight. Samples were placed in a hot air oven at 80°C for 48 h to record dry weight, while the following formula was used to calculate RWC%.

$$\text{RWC}\ (\%) = \frac{\text{FW of the sample} - \text{DW of the sample}}{\text{TW of the sample} - \text{DW of the sample}} \times 100$$

Membrane Stability Index

MSI% was calculated using the method described by (Sairam et al. 1990), in which 0.5 g of uniformly sized leaf strips were placed in two separate test tubes, each containing 10 mL of distilled water. The test tubes in one set were placed in a water bath at 40°C for 30 min to measure their electrical conductivity (C_1), while the test tubes in the second set were placed in a water bath at 100°C for 15 min. to measure their electrical conductivity [C_2]. The following formula was used to express the amount of MSI %.

$$\text{MSI}\ \% = [-1\ C_1/C_2]$$

Estimation of Proline Content (µg.g^{-1})

The estimation of proline content was carried out according to (Bates et al. 1973), wherein 0.5 g of fresh leaf sample was homogenized in 5 mL of 3% sulphosalicylic acid using a mortar and pestle followed by a centrifugation process at 3000 rpm for 10 min. Two mL of each extracted sample, glacial acetic acid, and ninhydrin reagent were pipette out in a separate test tube and placed in a water bath at 100°C for 30 min. After that, the proline was separated with the help of 6 mL toluene in a separating funnel. The OD of proline present in the samples was measured at 520 nm, and the final calculation was done with the help of the proline standard curve.

Catalase Activity

Catalase activity was determined using (Aebi 1984), in which 0.1 g of leaf sample was pulverized in 5 mL of phosphate buffer (0.1M) and centrifuged at 10000 rpm for 20 min. The enzyme activity was determined by adding 2.6 mL of phosphate buffer, 0.1 mL of enzyme extract, and 0.1 mL of H_2O (1%) to the reaction mixture, while a blank sample was prepared by adding phosphate buffer instead of enzyme extract to the reaction mixture. The OD was measured at 240 nm at 15-second intervals up to 2 min. The unit of CAT activity was calculated as nmol H_2O_2 min^{-1} (extinction coefficient 36 mM.cm^{-1}).

The statistical analysis of results collected from the experimental trial was analyzed through SPSS software (21st Version), whereas the mean comparisons were carried out by using DMRT at 5% probability.

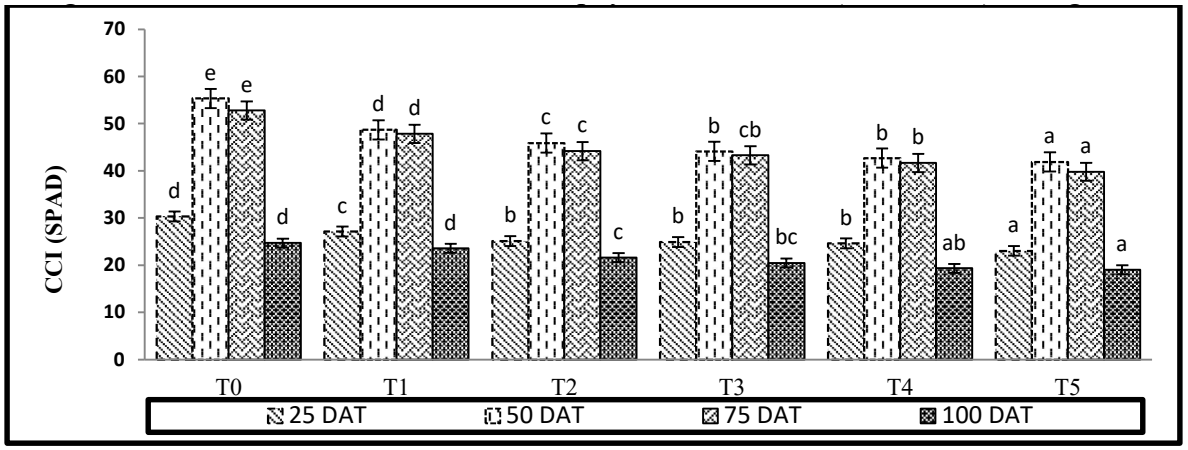

Fig. 1: Effect of $CdCl_2$ treatment on chlorophyll content index (SPAD unit) in flag leaf.

Table 1: Effect of cadmium chloride-induced stress on % increase/decrease in rice plant.

Days After Transplanting Percentage Increase/decrease between Control to HLCT	25 DAT	50 DAT	75 DAT
CCI (SPAD)	-24.06	-24.30	-24.60
Nitrogen (%)	-31.09	-35.67	-24.44
RWC (%)	-28.04	-29.07	-38.28
MSI (%)	-13.19	-37.77	-35.81
Proline content (μg g^{-1})	+46.23	+38.10	+38.34
Catalase activity (nm H_2O_2 mg^{-1} min^{-1})	NA	+77.91	+77.76

*HLCT= Highest level of cadmium toxicity, NA= not analyzed

RESULTS AND DISCUSSION

The present study was executed to evaluate the consequences of $CdCl_2$-induced stress in rice plants. The data depicted in Fig. 1 indicated that the maximum CCI reading was recorded at 50 DAT in all the sets of treatments compared to the remaining DATs, *i.e.*, 25, 75 and 100 DAT, while the significant detrimental effect of $CdCl_2$-induced stress among the treatments was recorded at maximum in T_5 at all the time of observations (23.01, 41.91, 39.81 and 19.06) contains the highest concentration of $CdCl_2$ (300 ppm) compared to a control set T_0 (30.38, 55.36, 52.80 and 24.71). The data regarding the increase/decrease (%) over the control Table 1 also showed the highest reduction of CCI (SPAD) reading in T_5 (24.06, 24.30 and 24.60%).

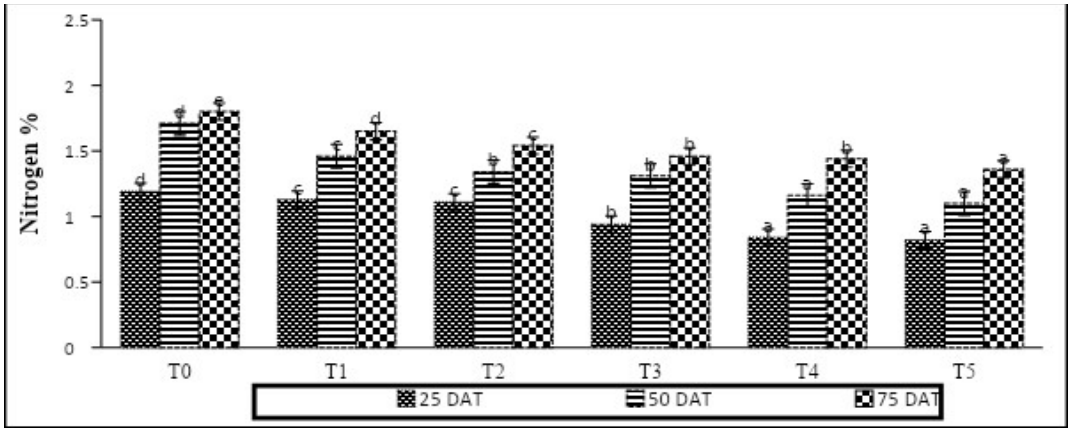

Fig. 2: Effect of $CdCl_2$ treatment on Nitrogen content (%) in dry flag leaf.

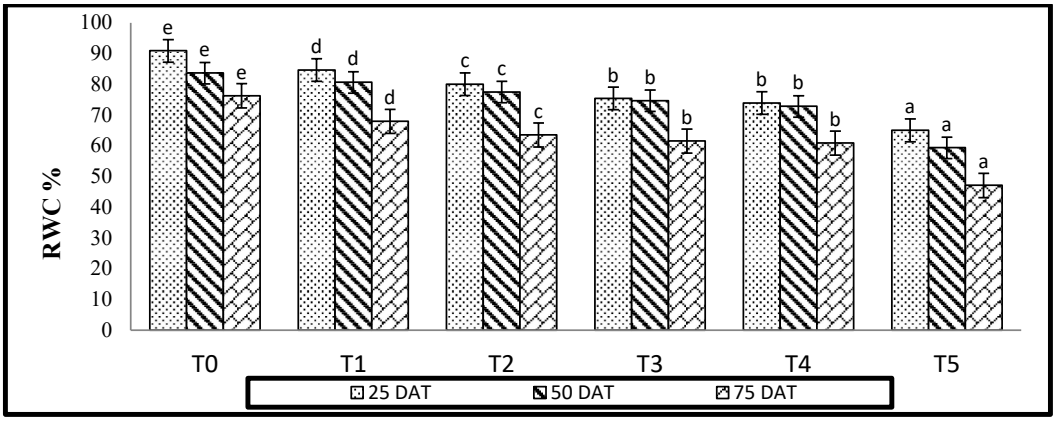

Fig. 3: Effect of $CdCl_2$ treatment on RWC (%) in flag leaf.

Data presented in Fig. 2 indicate that the nitrogen content present in the leaf gradually increased along with the age of the plant up to 75 DAT in all the sets of treatments, including control, while it decreased simultaneously with the elevation of $CdCl_2$ concentration from 100 to 300 ppm. The maximum reduction in nitrogen content was found in T_5 (0.82, 1.10, and 1.36%) compared to control one (1.19, 1.71, and 1.80%), which was followed by T_4> T_3> T_2> and T_1. Data presented in Table 1 also showed that the % reduction of nitrogen content was recorded maximum in T_5 at 50 DAT (35.67%) followed by 25 and 75 DAT (31.09 and 24.44 %). Data pertaining in Fig. 3 and 4 represented the effect of externally imposed $CdCl_2$ stress on RWC (%) and proline content (μg.g^{-1}) was recorded at regular intervals of 25, 50, and 75 DAT in rice and found that the amount of RWC % was decreased while the amount of proline increased gradually along with the advancement of growth phases of rice plant from 25 to 75 DAT. A similar trend was also observed concerning $CdCl_2$ treatment from 100 to 300ppm concentrations whereas the close analysis of both the data indicated that RWC% and proline content have a negative relationship among them Fig. 5. The data of increase/ decrease (%) over the control for the proline content and RWC % was found in T_5 (46.23) at 25 DAT and (38.28) at 75 DAT (Table 1). while a reduction (%) for RWC% was recorded at 25 DAT and 75 DAT (46.23 and 38.28) over the range of externally induced $CdCl_2$ stress Table 1.

The data regarding the membrane stability index (MSI%) presented in Fig. 6 reveals that the minimum stability of the membrane due to the elevated levels of $CdCl_2$ was found in T_5 (13.9, 21.32, and 25.50%) at all the DAT compared to the rest of the treatments including control (18.19, 34.41 and 39.65%). In comparison, the correlation studies among the MSI % and Proline content (μg.g^{-1}) show a negative

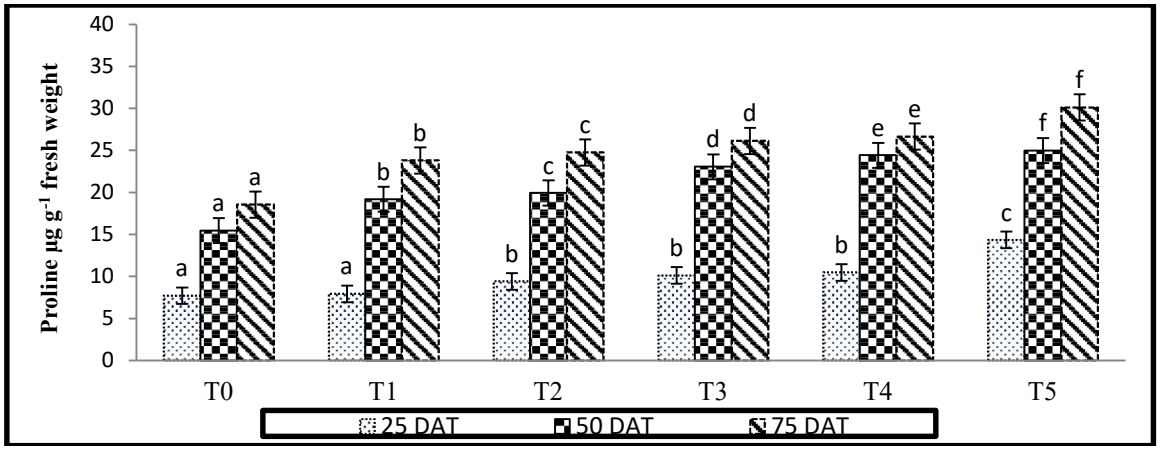

Fig. 4: Effect of $CdCl_2$ treatment on Proline content (μg g^{-1} Fresh weight) in flag leaf.

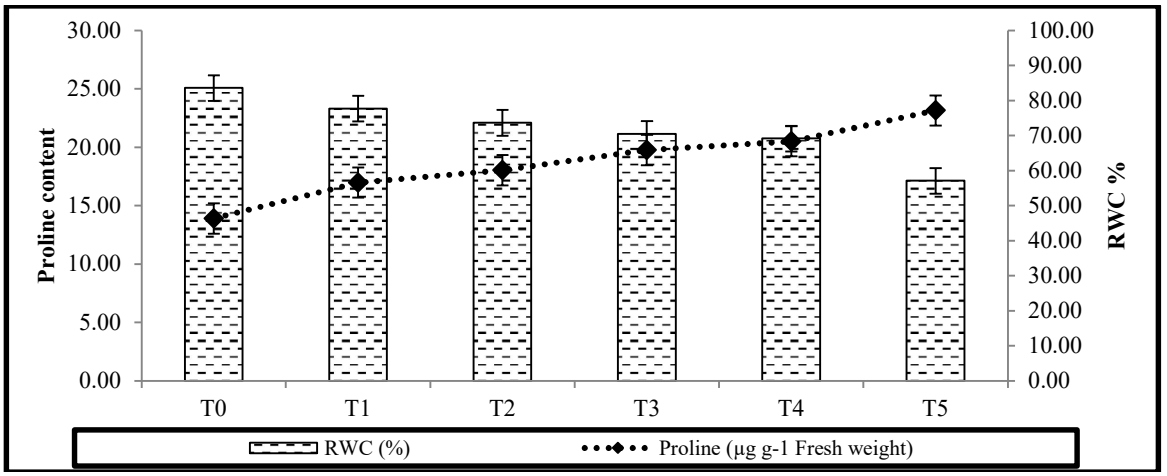

Fig. 5: Effect of different concentrations of cadmium chloride ($CdCl_2$) treatment on Proline content and RWC.

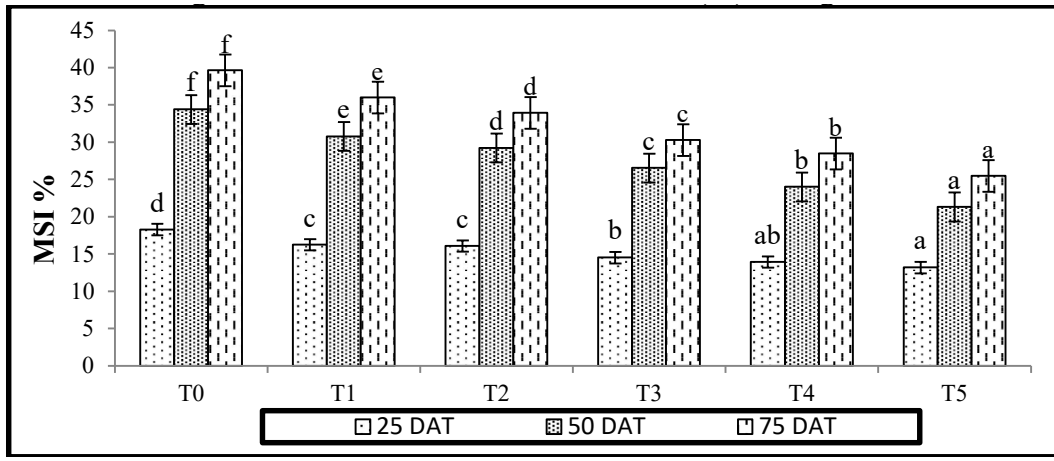

Fig. 6: Effect of $CdCl_2$ treatment on MSI (%) in flag leaf.

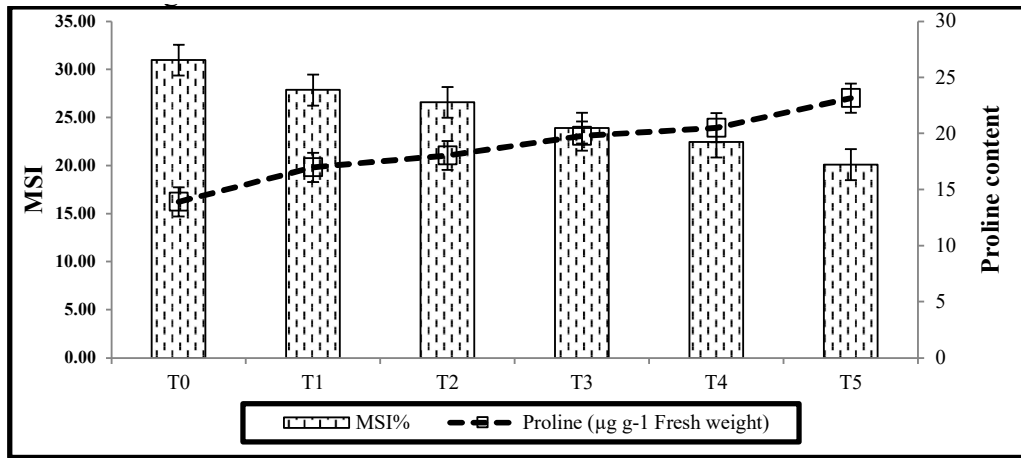

Fig. 7: Effect of $CdCl_2$ treatment on MSI and Proline content.

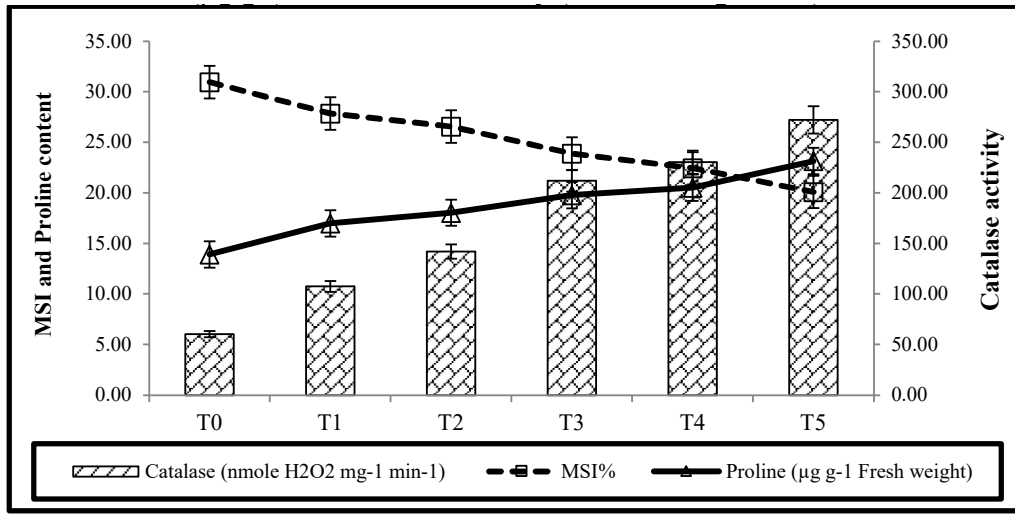

Fig. 8: Effect of $CdCl_2$ treatment on relationship between MSI %, Proline content (µg g^{-1}) and Catalase activity (nm H_2O_2 $mg^{-1}min^{-1}$).

relationship. In contrast, the MSI % was gradually decreased while the proline was increased Fig. 7. The maximum % reduction of MSI was recorded in T_5 at 50 DAT (37.77%) over the range of externally induced $CdCl_2$ stress Table 1.

Data depicted in Fig. 8 shows a relationship among MSI%, proline content ($\mu g.g^{-1}$), and catalase activity (nm H_2O_2 $mg^{-1}.min^{-1}$), where it was observed that proline content and catalase activity were increased significantly along with the elevation of $CdCl_2$ treatments while a negative relationship of MSI % was observed compared to proline and catalase activity.

The present study was executed to know the consequences of an antioxidative defense system in rice under the elevated conditions of $CdCl_2$ stress, whereas the entire sets of $CdCl_2$-treated plants showed a gradual change in the leaf color from green to yellow and a reduction in nitrogen content Fig. 1 and 2. The greenness of leaves was measured through a SPAD meter, and it found that T_5 which was represented the highest concentration of $CdCl_2$. Enough evidence is available to understand the reason behind chlorosis in such types of treated plants, consequently reducing the rate of photosynthesis. The absorption of Cd by the roots and its translocation towards the leaf has a detrimental effect on the biosynthesis of chlorophyll because it inhibits the synthesis of the most important enzymes, amino levulinic acid (ALA) and δ-amino levulinic dehydrogenase (ALDA) in which ALA is substantial than ALDA (Zhao et al. 2021, Morsch et al. 2002) The results of the present work was positively related with the finding of (Pireh et al. 2017) who reported that elevated levels of cadmium toxicity not only inhibit the biosynthesis of chlorophyll but also affect the key enzymes of nitrogen assimilation in the plant that impaired rate of photosynthesis followed by growth and development (Djebali et al. 2005, Zhang et al. 2020, Gratao et al. 2019). It seems from the data depicted in Fig. 3 that the relative water content (RWC %) decreased when the concentrations of externally imposed $CdCl_2$ were increased, while the parallel increase in proline content Fig. 4 and MSI% Fig. 6 was also detected in rice plants.

Moreover, the relation between the RWC% versus proline content Fig. 5 and MSI % versus proline endorse a negative relationship among them. The results are according to the findings of (Rahman et al. 2016), who reported that the presence of Cd in the soil alters the relationship of soil plant water relation that impairs the cell structure and its membrane; hence, RWC % decreased in the plant (Ahmad et al. 2015), a remarkable reduction in membrane stability % (Janmohammadi 2010, Anjum et al. 2015). It was observed from the data that the synthesis of proline and catalase activity increased due to the externally imposed $CdCl_2$ Fig. 4 and 6. The findings are consistent with those of (Siddique et al. 2018), who found that higher concentrations of Cd in the plant cell caused oxidative stress, which increased the production of ROS (O_2^-, OH*, and H_2O_2). ROS overproduction interferes with ETC and mineral uptake (Hasanuzzaman et al. 2012, Ajum et al. 2015, Siddique et al. 2018). However, a natural defense mechanism already exists in the plant body that aids in detoxification because proline is an osmoregulatory compound that maintains water balance and osmotic homeostasis. The enzyme catalase facilitates the scavenging process of overproduced ROS (Qadir et al. 2004, Hasanuzzaman et al. 2012).

CONCLUSION

The present study was conducted to know the consequences of externally induced $CdCl_2$ stress on the antioxidative defense mechanism. Externally induced $CdCl_2$ stress negatively affected the biosynthesis of chlorophyll, nitrogen assimilation, RWC%, and MSI% during the crop growth period. However, at the same time, an upregulation of proline biosynthesis synthesis and activity of catalase enzyme (CAT) was recorded. The defensive role of the compound proline and catalase activity is well known for the antioxidative process that coordinates in balancing soil plant water relationship and scavenging overproduced ROS in rice plants. To overcome the damage that occurs due to the high concentration of $CdCl_2$, further studies are required to find out the substantial treatments that protect the plant in prevailing situations rather than the naturally available antioxidative defense system.

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ORCID DETAILS OF THE AUTHORS

A. Siddique: https://orcid.org/0000-0001-6349-4472