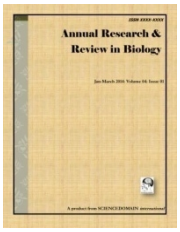

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Effects of Cadmium on Water Content, Soluble Protein, Proline Changes and Some Antioxidant Enzymes in Wheat *(Triticum durum desf.)* Leaves

Amel Alayat[1], Lynda Souiki[2], Nedjoud Grara[2*], Mohamed Reda Djebar[1], Zine Eddine Boumedris[1], Sana Benosmane[1], Rima Amamra[1] and Houria Berrebbah[1]

[1]Cellular Toxicology Laboratory, Department of Biology, University of Badji Mokhtar Annaba, Algeria.
[2]Department of Biology, University of 8 May 1945 Guelma, Algeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author LS managed the analyses of the study. Authors LS, MRD and HB followed and supervised this study. Author ZEB performed the statistical Analysis. Authors NG, SB and RA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The effect of Cadmium stress on plant growth, oxidative stress and antioxidant enzyme of wheat seedlings *(Triticum durum* Desf.*)* was evaluated in this study. Cadmium stress decreased plant growth, lowered the relative water content and caused oxidative damage, as characterised by increased antioxidative enzymes in wheat leaves such as ascorbate peroxidase (APX), guaïacol peroxidase (POX) and catalase (CAT). As a response to increasing Cadmium supply particular increases in antioxidative mechanisms in wheat cultivar Simeto suggest that the high Cadmium sensitivity of Simeto is related to enhanced production and oxidative damage of reactive oxygen species.

**Corresponding author: Email: grara120@yahoo.fr;*

Keywords: Cadmium; wheat; oxidative stress; plant growth; proline; soluble proteins; antioxidant enzymes.

1. INTRODUCTION

Cadmium (Cd) is a non-essential heavy metal, released into the environment by anthropogenic and non-anthropogenic sources. Environmental pollution with cadmium is mainly caused by mining and smelting, dispersal of sewage sludge and the use of cadmium rich phosphate fertilizers [1,2]. At very high concentrations in soils, cadmium can adversely effect plant growth and also human health after introduction to the food chain [3,4].

In plants, the accumulation of cadmium can cause numerous morphological levels, an excessive amount of cadmium causes plant growth retardation; chlorosis, leaf rolls, and necroses [5]. At the physiological level, excess cadmium results in an inhibition of photosynthesis and transpiration [6,7], imbalance of mineral nutrients [8], induction of oxidative stress [9], changes in enzyme activity [10], and modifications to gene expression [11]. Cadmium promotes the accumulation of reactive oxygen spices (ROS), causes severe damage to important cellular components, such as lipids, proteins, DNA, and RNA [12,13] and leads to a decreased growth [14]. ROS include superoxide radical, hydroxyl radical and hydrogen peroxide. Plants have evolved a complex antioxidant system (enzymatic and non enzymatic detoxification mechanisms) for protecting. The antioxidant enzymes, such as peroxidases, superoxide dismutase and catalase scavenge different types of ROS [15].

Both increase and decrease in the activity of many antioxidant enzymes has been observed in cadmium treated plants [4,16,17,18] suggesting that the antioxidant systems, beside being involved in detoxification process, could be sensitive targets of cadmium toxicity. Plants can also tolerate cadmium toxicity by inducing antioxidative defense systems. As mentioned above, cadmium stress can be responsible for production of ROS and peroxidation of critical cell compounds, such as membrane lipids and proteins, chlorophyll and nucleic acids [19,20]. An induced antioxidative defense in response is to Cadmium stress might be, therefore, a relevant mechanism for Cadmium tolerance in plants.

The purpose of this study is to examine the effects of cadmium on the growth, oxidative stress, and antioxidative response of early wheat seedlings *(Triticum durum* Desf.*).* The possible mechanisms of wheat seedlings response to cadmium stress involving free radical metabolism and antioxidative changes are also discussed. In the present study, experiments were conducted to assess the role of antioxidative defense systems in variety wheat Simeto. The objective was to evaluate root and shoot length, dry matter production, development of cadmium toxicity symptoms and to analyze levels of antioxidative defense systems in leaves.

2. MATERIALS AND METHODS

2.1 Plant Material, Growth and Treatments

The experiments are done at the Laboratory of Cellular Toxicology of Annaba, University, Algeria.

The tested wheat seeds (*Triticum durum* Desf.*)* were provided by the Algerian Office Inter Cereals (AOIC) El Hadjar Annaba, Algeria. Wheat (*Triticum durum,* cv. Simeto) was used in

the experiments. Seeds were surface sterilized in 5% Sodium hypochlorite (NaCiO) solution for 10 min and rinsed with distilled water. Germination was performed in the dark on Whatman filter papers in dishes Petri. Cadmium chloride ($CdCl_2$, Fluka) was used as Cadmium salt and prepared freshly for the treatments. Different amounts of $CdCl_2$ were added to the culture solution to form the following six treatments: 0 (control), 2, 5µM, 25µM, 50µM, 75µM, 100µM. The leaves were collected after 14 days for analysis of various parameters.

2.2 The Relative Water Contents (RWC)

The relative water content (RWC) was determined in fresh leaf discs of 2 cm^2 diameter; discs were weighed quickly and immediately floated on distilled water in Petri dishes to saturate them with water for the next 24h, in dark. The adhering water of discs was blotted and tugor mass was noted. Dry mass of the discs was recorded after dehydrating them at 70°C for 48h [21]. RWC was calculated by using the following formula [22]:

$$\text{RWC}= \frac{fresh\ mass - dry\ mass}{tugor\ mass - dry\ mass} \text{ x } 100$$

2.3 Determination of proline content

The method of Troll and Lindsley [23] was used to determine the concentration of proline in wheat leaves. Absorbance was measured at 528 nm by spectrophotometer Jenway 3600. The proline concentration in the sample was determined from a standard curve using analytical grade proline and calculated on fresh weight basis (mg/g FW).

2.4 Determination of Soluble Proteins

The method of Bradford [24] was used to determine the concentration of soluble proteins in wheat leaves with BSA as standard. Absorbance was recorded at 595 nm. Soluble proteins were expressed as mg/g FW.

2.5 Antioxidant Enzyme Activity

Extraction of POX, APX and CAT was as described by Loggini et al. [25]. Leaves of wheat (1g fresh weight) were homogenized in ice cold 50mM phosphate buffer (pH 7,5). The homogenate were centrifuged at 12000g for 20 min, and the supernatants were used for enzyme activity assays.

Guaiacol peroxidase (POX) activity was measured according to the method of Fielding et al. [26]. The reaction mixture (3ml) consisted of 100µl enzyme extract, 8mM Guaiacol, 50mM phosphate buffer (pH=7,2), and 50 µl H_2O_2 (300mM). An increase in the absorbance due to oxidation of guaiacol was measured spectrophotomtrically at 470 nm (ε= 24, $7mM^{-1}$ cm^{-1}).

Ascorbate peroxidase (APX) activity was assayed according to the method of Nakano and Asada [27]. The reaction mixture consisted of 100µl enzyme extract, 0,5mM ascorbate, 50mM phosphate buffer (pH=7,2) and 50µl H_2O_2 (300mM). The oxidation of ascorbate was determined by the change in absorbance at 290 nm (ε= 2, $8mM^{-1}$ cm^{-1}).

Catalase (CAT) activity was determinated according to Cakmak and Horst [28]. The assay mixture (3,0ml) consisted of 100µl enzyme extract, 50µl H_2O_2 (300mM) and 2, 85ml 50mM phosphate buffer (pH=7,2). CAT activity was assayed by monitoring the decrease in the absorbance at 240 nm as a consequence of H_2O_2 disappearance (ε=39, $4mM^{-1}$ cm^{-1}).

2.6 Statistical Analysis

The experiment was arranged in a completely randomized design, with three independent replicates. Data was analyzed by ANOVA and means were compared by the Tukey test at the 95% level of confidence. The standard deviation was plotted in all graphs.

3. Results

3.1 Dry Weight and Relative Growth Rate

The dry weight (%), shoot and root growth of the wheat genotype Simeto, is shown in Table 1.

Table 1. Effect of cadmium stress on growth traits (root and shoot growth) and relative water content of wheat plants

Treatments	Root length (cm)	Shoot length (cm)	Relative water content (%)
Control	11.116±1.245a	12.53±2.46a	95.436±3.054a
2,5µM Cd	6.813±1.424b	10.363±1.880ab	87.123±2.999ab
25µM Cd	4.65±0.435bc	9.38±0.844ab	84.002±3.287b
50µM Cd	4.03±0.678c	8.343±0.333b	78.616±3.506bc
75µM Cd	3.693±0.346c	7.933±0.404b	69.933±3.506cd
100µM Cd	3.413±0.306c	7.58±0.166b	61.135±4.893d

Mean pairs followed by different letters are significantly different (p=0.05); n=3. The same letters after the data within a column indicates there was no significant difference at a 95% probability level

Shoot and root growth of wheat seedlings were significantly affected by cadmium, and showed a continuous decrease with the increase of cadmium concentration. According to the statistic analysis (ANOVA), the decrease of shoot and root length was high significant (P<0,001).

Exposure of the plants to different concentration of $CdCl_2$ inhibited plant growth, which led to a significant decrease in dry weight and relative growth rate.

These results indicate that the wheat seedlings were sensitive to cadmium. According to the results at the concentration of 100µM there was significant reduce in root and shoot growth and dry weight content.

3.2 Proline Content

The effect of cadmium stress on the proline content of wheat leaves is presented in Fig. 1.

Exposure of wheat plants to cadmium significantly increased proline content. According to the Fig. 1, Proline content was highly affected with the CdCl2 concentrations equal of higher than 50uM, what was proved by statistical analysis (p< 0,001).

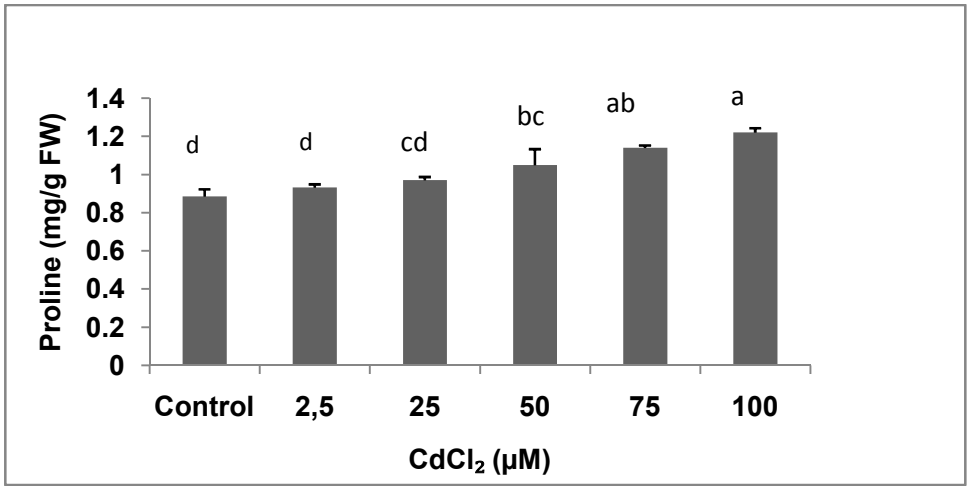

Fig. 1. Effect of cadmium stress on the proline content of wheat leaves

Data are the mean ± S.E. of three replicates. Mean pairs followed by different letters are significantly different (p=0.05). The same letters after the data indicates that there was no significant difference at a 95% probability level

3.3 Soluble Proteins

Fig. 2 shows the effect of cadmium on the soluble proteins content of wheat leaves.

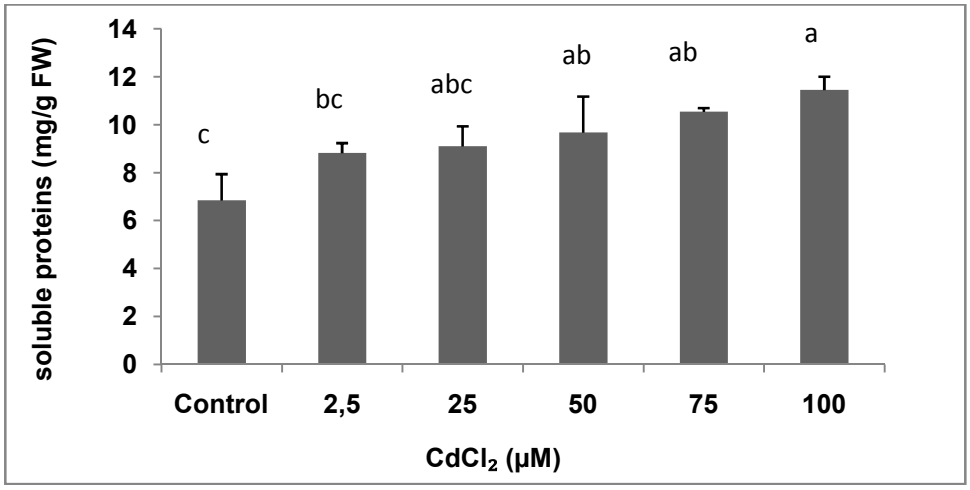

Fig. 2. Effect of cadmium stress on the soluble proteins of wheat leaves

Data are the mean ± S.E. of three replicates. Mean pairs followed by different letters are significantly different (p=0.05). The same letters after the data indicates that there was no significant difference at a 95% probability level

Increase of protein content in wheat leaves with the increased exposure to the cadmium concentration has been observed (Fig. 2). Total protein content was 6,841mg/g FW in control leaves, 9,671 mg/g FW at 50µM and 11,445mg/g FW at 100µM of Cadmium

respectively. The statistical analysis (ANOVA) indicated that the increase of soluble proteins was highly significant (P<0,01).

3.4 Antioxidant Enzyme Activity

The changes in antioxidant enzyme activities in wheat leaves, including POX, APX and CAT induced by cadmium at different concentrations are shown in Figs. (3,4 and 5).

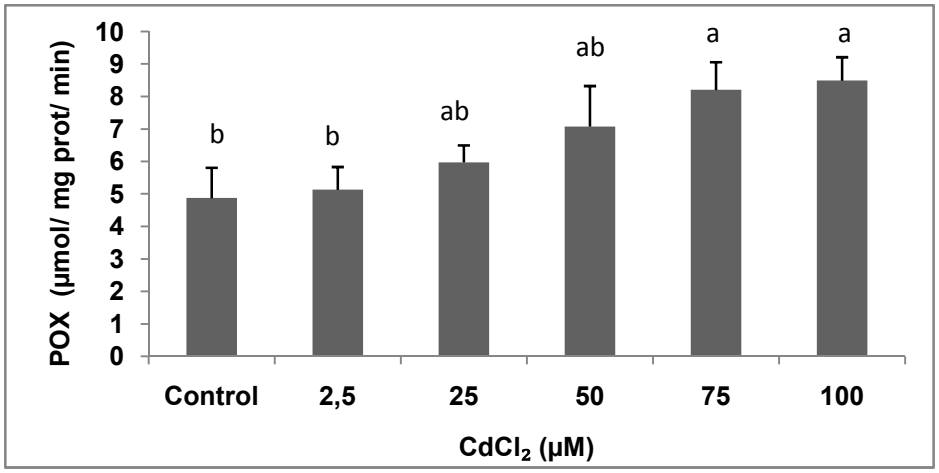

Fig. 3. Effect of cadmium stress on the activity of POX content of wheat leaves
Data are the mean ± S.E. of three replicates. Mean pairs followed by different letters are significantly different (p=0.05). The same letters after the data indicates that there was no significant difference at a 95% probability level

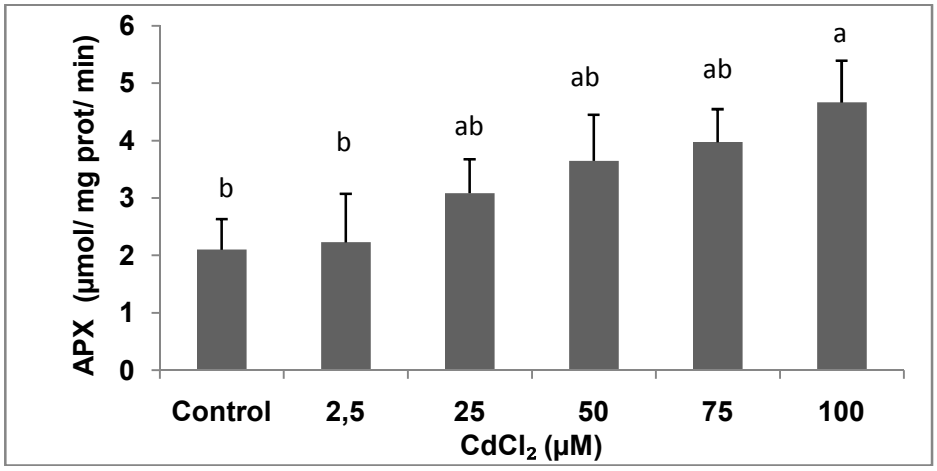

Fig. 4. Effect of cadmium stress on the activity of APX content of wheat leaves
Data are the mean ± S.E. of three replicates. Mean pairs followed by different letters are significantly different (p=0.05). The same letters after the data indicates that there was no significant difference at a 95% probability level

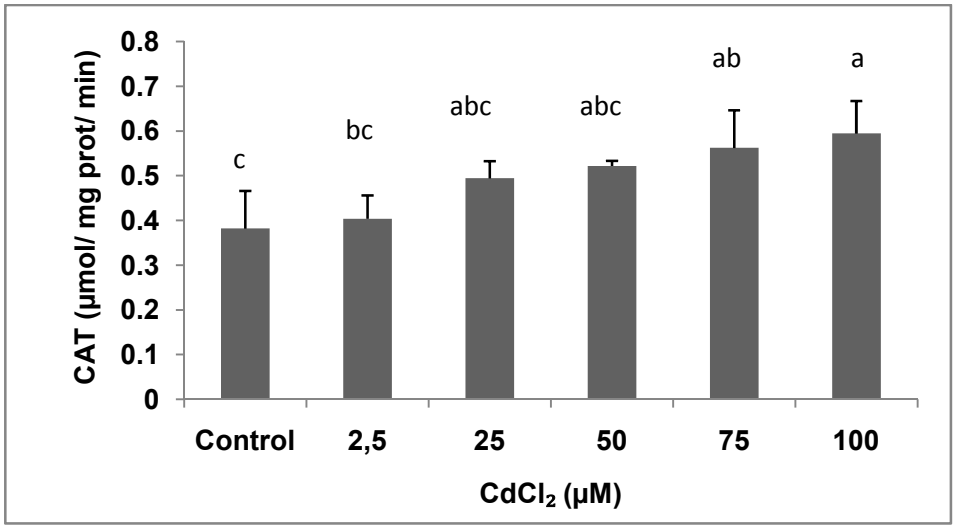

Fig. 5. Effect of cadmium stress on the activity of CAT content of wheat leaves
Data are the mean ± S.E. of three replicates. Mean pairs followed by different letters are significantly different (p=0.05). The same letters after the data indicates that there was no significant difference at a 95% probability level

The treatment with different concentrations of cadmium resulted high significant increase in APX, POX and CAT activities (Figs. 3, 4 and 5). The expressed toxicity symptoms at higher cadmium concentrations corresponded to increased enzymes activities in comparison to controls (P<0,01).

4. DISCUSSION

Exposure of plants to toxic metals can lead to numerous physiological and biochemical disorders. The inhibition of plant seedling growth can be regarded as general responses associated with heavy metal toxicity [29,30].

The present study shows that cadmium markedly reduced root elongation and shoot length. Occurrence of these symptoms was associated with reductions in dry matter production. Cadmium affected root growth more than shoot growth, especially at elevated Cadmium levels, confirming the results found in wheat [31], radish [32] and barley [33]. Greater sensitivity of roots to cadmium than shoots might be related to the fact that roots are the first organs to be in contact with cadmium, accumulating it at much higher amounts than shoots [19,34].

The water content in wheat plants decreased gradually and significantly (P<0,001) with the increase of Cd concentration (Table 1). To examine the osmotic effect of abiotic stress treated plant tissues, the water content was frequently measured [35] and it was observed that plant water status was highly affected by heavy metal stress [36-38]. These results indicate that an excess level of cadmium has a toxic and an osmotic effect on wheat plants.

In higher plants, proline is accumulated under stress, both due to an increase in production by reducing its degradation [39]. The accumulation of proline occurs after the development of resistance is a consequence rather than a cause of hardening [40]. In the present study,

proline increased significantly in the cadmium treated wheat plants. Enhanced proline accumulation in reponse to Cd toxicity has been earlier demonstrated in *Triticum aestivum, Vigna radiate, Helianthus annus and Phaseolus vulgaris* [41,42,43]. Thus, proline accumulation is a potential indicator of stress tolerance [44]. Proline also acts directly as an antioxidant to protect the cell from free radical damage and maintains a more reducing environment that is favorable for phytochelation synthesis and cadmium sequestration [45].

In the present work, Cadmium treatment increased soluble protein content; these results suggest that this increase is due to the increase of antioxidant enzymes and reactive oxygen species. Although, some ROS contact as signaling molecules by altering the expression of certain genes and modulating the activity of specific defense proteins, in high concentrations can be extremely harmful to organisms [46]. They can induce oxidation of proteins, lipids and nucleic acids, leading to alterations in cell structures and mutagenesis [47]. Increase of soluble proteins could results from the activation of genes for synthesis of specific proteins associated with stress such as proteins “LEA” that protect the vital set of cellular proteins [48], and the heat shock proteins which permit maintains membrane protein and the plant cell structures [49]. The acquisition of resistance to stress process is accompanied by an important synthesis of soluble protein; this is the result of a slower development and storage of molecules in the hyaloplasm or in some organelles (chloroplasts, mitochondria). It seems that the synthesis of specific proteins is necessary for the hardening [40].

Reactive oxygen species (ROS) are an unenviable part of aerobic life. Their steady state concentration is a balance between production and elimination providing certain ROS level [50]. This equilibrium can be disturbed by metal stress, leading to enhanced ROS level and damage to cellular constituents, which is called oxidative stress [34,51,52]. In plants, toxic metals induce oxidative stress by generating ROS via hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), hydroxyl radicals (OH^-) and singlet oxygen ($O_2^{\cdot}$) [53]. In response to the increased ROS, the antioxidant defense system comprising POD, APX and CAT plays important roles in scavenging ROS [54]. Both increase and decrease in the activity of many antioxidant enzymes have been observed in cadmium treated plants [55,56,18,17].

Our results showed that, Cd treatment significantly increased POD, APX activities in leaves of wheat plants (Figs. 3 and 4). Similarly, Milone et al. [57] showed that cadmium could increase POD and APX activities in wheat seedling leaves. Increase in ascorbate peroxidase and guaïacol peroxidase activities could represent an appropriate protection against overproduction of peroxides when heavy metals accumulate in wheat [58].

The role of POD is to eliminate the excess of H_2O_2 [59]. POD catalyzes H_2O_2 dependent oxidation of substrate, while CAT and APX eliminate H_2O_2 by breaking it down directly to form water and oxygen [60]. APX reduces H_2O_2 to H_2O, subsequently producing monodehydroascorbate radicals (MDHA) from ascorbate (Asc) [61].

Catalase (CAT) is an important enzyme in the protection against oxidative stress in all aerobic organisms. It catalyzes, rapid decompisition of hydrogen peroxide into oxygen and water, thereby protecting cells from oxidizing effects caused of excessive H_2O_2 [62]. Earlier data in the literature concerning the catalase response in plants leaves exposed to cadmium stress are contradictory since both enzyme activation [63,64] and inhibition [65,16,66] have been described. In our investigations exposure of wheat plants to cadmium markedly induced an increase of CAT activity in leaves. In response to the in ROS accumulation, the antioxidant defense system comprising SOD and CAT plays important roles in their scavenging [67]. SOD could eliminate superoxide, a harmful substance to cell membranes,

produced in the aero-metabolism process. H_2O_2 is also toxic to plant cells, could be eliminated by CAT [68].

Comparing the activity of H_2O_2 eliminating enzymes, many authors assume that APX plays a central role in H_2O_2 detoxification at the chloroplast level where as at the cytosol level POD is the most important H_2O_2 scavenger [69,70]. Furthermore, POD participating barrier against poisoning heavy metals. In contrast to APX and POD, CAT activity is primarily regulated by the amount of H_2O_2 produced by photorespiration due to its peroxisome location [19].

5. CONCLUSION

The present results allow us to conclude that the wheat plants showed a negative response to cadmium toxicity. The physiological and biochemical process in plants was significantly affected by stress of $CdCl_2$. To deal with the cadmium induced oxidative stress, wheat plants activated antioxidant enzymes such as CAT, APX and POD to diminish the reactive oxygen species. These biochemical responses can be interpreted as an internal tolerant mechanism and may allow us to develop strategies for reducing the risks of the cadmium contamination to crop production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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