Journal of Plant Biochemistry & Physiology
ISSN: 2329-9029

Gowthami and Vasantha, J Plant Biochem Physiol 2015, 3:3
DOI: 10.4172/2329-9029.1000151

Research Article Open Access

Cadmium Induced Changes in the Growth and Oxidative Metabolism of Green Gram-*Vigna radiata* Linn

Gowthami N*[1] and Vasantha S[2]

[1]Research Scholar, Department of Botany, PSGR Krishnammal College for Women, Coimbatore, India
[2]Dean for student affairs, Department of Botany, PSGR Krishnammal College for Women, Coimbatore, India

Abstract

The effect of cadmium chloride on green gram (*Vigna radiata Linn.*) was studied using the parameters like carbohydrates, protein, chlorophyll and *oxidative enzymes* like superoxide dismutase, catalase and peroxidase of leaves in order to know the possible involvement of cadmium metal in the generation of oxidative stress. Results obtained in this work showed that the application of cadmium decreased the content of carbohydrate, protein, chlorophyll and superoxide dismutase, catalase and peroxidase, compared to the control plants.

Keywords: *Vigna radiata linn;* Cadmium phytotoxicity; Oxidative enzymes

Introduction

Phytotoxicity is a term used to describe the degree of toxic effect by a compound on the growth of plants. It is caused by a wide variety of compounds, including heavy metals, salinity, pesticides, phytotoxins or allelopathy. The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as uptake and accumulation of metals from cytoplasm to extra cellular exudates and cell wall constituents; efflux of heavy metals from cytoplasm to extra nuclear compartments including vacuoles; complexation of heavy metal ions inside the cell by various substances, for example, organic acids, phytochelatins, amino acids and metallothioneins; accumulation of osmolytes and induction of antioxidative enzymes; activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures [1]. Cadmium (Cd), being highly toxic metal pollutants of soils, which inhibits the root and shoot growth (yield), affects nutrient uptake. The application of sewage sludge, waste and cadmium containing fertilizers causes the increase of cadmium content in soils. As green gram is a stress sensitive legume, the present investigation was undertaken to analyze whether cadmium produces oxidative stress during early stages of seedling development.

Materials and Methods

Sample collection and preparation

The healthy seeds of Green gram (*Vigna radiata* Linn) were collected from Tamil Nadu Agricultural University (TNAU), Coimbatore. The seeds (3 sets of 1 gram each) were soaked in 10, 50 µM solution of Cadmium chloride overnight. Then the seeds were sown in the earthern pots and allowed to grow naturally for a week. Every alternate day they were watered with distilled water in the green house. At the 7th day seedlings were removed, used for the content of carbohydrate, protein, chlorophyll and superoxide dismutase, catalase and peroxidase.

Estimation of total carbohydrate: The total carbohydrate was estimated by anthrone method [2].

Reagents

1. 2.5N- HCl

2. Anthrone reagent

500 mg of the leaves was weighed and ground well with a pestle and mortar in 5-10 ml of 80% ethanol. The supernatant was collected and 0.5, 1 ml aliquots were taken for analysis. Standard solution was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard (glucose) as blank. The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added and was heated in a boiling water bath for 10 minutes and made to cool. Green to dark green colour was recorded at 630 nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of carbohydrate present in the tube was calculated from the graph.

Estimation of protein: Protein content was estimated by Lowry's method [3].

Reagents

A. 2% sodium carbonate in 0.1N NaOH

B. 1% sodium potassium tartarate solution

C. 0.5% copper sulphate solution

D. 48 ml of A, 1 ml of B, 1 ml of C

E. 1 part Folin-Phenol (2N): 1 part water

500 mg of the leaves was weighed and ground well with a pestle and mortar in 5-10 ml of the phosphate buffer. This solution was centrifuged and the supernatant was used for protein estimation. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standards (Bovine Serum Albumin) were pipetted into a series of test tubes 0.1 ml and 0.2 ml of the sample extract was pipetted in two other test tubes. The volume was made to 1 ml in all the test tubes. The tube with 1 ml of water served as the blank. 5 ml of reagent D was added to each tube including the blank. This was mixed well and allowed to stand for 10 min. Then 0.5 ml of reagent E was added, mixed and incubated at room temperature in the dark for 30 min. Blue colour was developed. The readings were recorded at 660 nm. Standard graph was drawn and the amount of protein in the

***Corresponding author:** Gowthami N, Department of Botany, PSGR Krishnammal College for Women, Coimbatore, India, Tel: +919524855522; E-mail: gowthami488@gmail.com

Received June 10, 2015; **Accepted** June 29, 2015; **Published** July 08, 2015

Citation: Gowthami N, Vasantha S (2015) Cadmium Induced Changes in the Growth and Oxidative Metabolism of Green Gram-*Vigna radiata* Linn. J Plant Biochem Physiol 3: 151. doi:10.4172/2329-9029.1000151

sample was calculated.

Estimation of chlorophyll: The chlorophyll content in leaves was estimated by the method of Arnon [4].

Reagent is 80% acetone.

Procedure: 1 g of the leaf tissue was weighed and ground with 20 ml of 80% acetone. This solution was centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred to 100 ml volumetric flask. This procedure was repeated until the residue becomes colorless. This was made to 100 ml with 80% acetone. This absorbance was recorded at 645, 663 and 652 nm against the solvent (80% acetone) blank. The amount of chlorophyll present in the extract mg chlorophyll per g tissue was calculated using the following equations.

$$Mg\ chlorophyll\ a/\text{g}\ tissue = 12.7(A663) - 2.69(A645) \times \frac{V}{1000 \times W}$$

$$Mg\ chlorophyll\ b/g\ tissue = 22.9(A645) - 4.68(A663) \times \frac{V}{1000 \times W}$$

$$Mg\ total\ chlorophyll/g\ tissue = 20.2(A645) + 8.02(A663) \times \frac{V}{1000 \times W}$$

where

A=Absorbance at specific wavelength

V=Final volume of chlorophyll extract 80% acetone

W=Fresh weight of tissue extracted

Assay of superoxide dismutase (SOD): SOD was assayed according to the method of [5].

Reagents

1. Sodium pyrophosphate buffer (0.025M, pH 8.3)

2. Phenazine methosulphate (PMS) (186 µM)

3. Nitroblue tetrazolium (NBT) (300 µM)

4. NADH (780 µM)

5. Glacial acetic acid

6. n-butanol

7. Potassium phosphate buffer (50 mM, pH 6.4)

Procedure:

Preparation of enzyme extract: The leaves (0.5 g), were ground with 3.0 ml of potassium phosphate buffer, centrifuged at 2000 g for 10 minutes and the supernatants were used for the assay.

Assay:

The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.2 ml of the enzyme preparation and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2 ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was then shaken with 4.0 ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

Assay of catalase (CAT): Catalase activity was assayed following the method [6].

Reagents

1. Phosphate buffer: 0.067M (pH 7.0)

2. Hydrogen peroxide (H_2O_2) (2 mM) in phosphate buffer

Procedure:

Preparation of enzyme extract: 20% homogenate of the leaves was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay.

Assay: H_2O_2-phosphate buffer (3.0 ml) was taken in an experimental cuvette, followed by the rapid addition of 40 µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

Assay of peroxidase (POD): The method proposed by [7] was adopted for assaying the activity of peroxidase.

Reagents

1. Pyrogallol: 0.05M in 0.1M phosphate buffer (pH 6.5)

2. H_2O_2: 1% in 0.1M phosphate buffer, pH 6.5

Procedure:

Preparation of enzyme extract: 20% homogenate of the leaves was prepared in 0.1M phosphate buffer (pH 6.5) centrifuged at 3000 rpm and the supernatant was used for the assay.

Assay: To 3.0 ml of pyrogallol solution, 0.1 ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 ml of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

Results and Discussion

The results revealed a drastic decrease in the levels of carbohydrate and protein in cadmium treated plants in both the concentrations compared to control plants, coincides with the massive decrease in barley plant and [8] who found decrease in soluble protein content in plant when grown on various amendments of tannery waste containing heavy metals (Table 1).

The level of chlorophyll a, b was found to be 1.011 ± 0.24, 0.26 ± 0.04 in control plants which has been reduced in 10 and 50 µM cadmium treated plants (Table 2). The decline in chlorophyll content in plants exposed to Cd^{2+} stress is believed to be due to (a) Inhibition of important enzymes, such as protochlorophyllide reductase [9] and δ-aminolevulinic acid dehydratase (ALA- dehydratase) associated with chlorophyll biosynthesis [10] (b) Impairment in the supply of Mg^{2+}, Fe^{2+}, Zn^{2+}, and Mg^{2+} [11]. The decrease in chlorophyll content was also reported in sunflower and in almond [12] (Table 3).

Units

SOD - Amount that causes 50% reduction in the extent of NBT oxidation.

CAT - Amount of enzyme required decreasing the optical density by 0.05 units.

PEROXIDASE - 1 Micro Molar pyrogallol oxidized per min.

The level of SOD was found to be 18.83 ± 0.24 in control plants, which has been reduced in 10 and 50 µM cadmium treated plants. SOD is a key enzyme in the plant antioxidant defences and is encharged of

S.No	Treatments	Carbohydrate (mg/g)	Protein (mg/g)
1	Control	29.8 ± 0.26	110.0 ± 1.48
2	10 µM	23.3 ± 0.24	87.8 ± 1.29
3	50 µM	17.6 ± 0.19	72.8 ± 0.99

Table 1: Effect of cadmium chloride on carbohydrate and protein content on the leaves of *Vigna radiate*.

S.No	Treatments	Chlorophyll a (mg\g)	Chlorophyll b (mg/g)
1	Control	1.011 ± 0.24	0.26 ± 0.04
2	10 µM	0.77 ± 0.10	0.23 ± 0.02
3	50 µM	0.66 ± 0.09	0.21 ± 0.02

Table 2: Effect of cadmium chloride on chlorophyll content on the leaves of *Vigna radiate*.

S.No	Treatments	Superoxide dismutase (SOD)	Catalase (CAT)	Peroxidase
1	Control	18.83 ± 0.24	283.33 ± 1.99	24.69 ± 0.31
2	10 µM	13.83 ± 0.17	204.12 ± 1,68	14.69 ± 0.22
3	50 µM	10.71 ± 0.11	168.53 ± 1.29	9.10 ± 0.13

Table 3: Effect of cadmium chloride on superoxide dismutase, catalase, peroxidase activity on the leaves of *Vigna radiate*.

the dismutation of O_2^- radicals to H_2O_2 and O_2 [13] Reduction of SOD activity induced by Cd was reported in wheat [14] and bean plant [15]. The level of Catalase was found to be 283.33 ± 1.99 in control plants, which has been reduced in 10 and 50 µM cadmium treated plants. The decrease of Catalase activity in the presence of Cd ions was also described in pea plant [16]. The level of Peroxidase was found to be 24.69 ± 0.31 in control plants, which has been reduced 10 and 50 µM cadmium treated plants. Peroxidase was down regulated probably by enzyme inhibition by the Cd metal, reported in wheat roots [17]. Hence the present investigation also supports the fact that $CdCl_2$ can induce a concentration-dependent oxidative stress situation in leaves, characterized by the inhibition of the antioxidant systems.

References

1. Cho M, Chardonnens AN, Dietz KJ (2003) Differential heavy metal tolerance of Arabidopsis halleri and Arabidopsis thaliana: A leaf slice test. New Phytol 158: 287-293.
2. Hedge JE, Hofreiter BT (1962) In carbohydrate chemistry 17, Academic press, New York.
3. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein Measurement with the Folin Phenol Reagent. J Biol Chem 193: 265-275.
4. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol 24: 1-15.
5. Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 21: 130-132.
6. Luck H (1965) Catalase. In: Methods of Enzymatic Assays, New York, Academic Press, pp. 885-894.
7. Reddy KP, Subhani SM, Khan PA, Kumar KB (1995) Effect of light and benzyl adenine and dark-treated graving rice (Oryza sativa) leaves - changes in peroxidases activity. Plant Cell Physiology 26: 987- 994.
8. Singh S, Sinh S (2005) Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv.Rohini) grown on various amendents of tannery waste. Ecotox Environ safe 62: 118-127.
9. Assche FV, Clijisters H (1990) Effects of metals on enzyme activity in plants. Plant cell & Environment 13: 195-206.
10. Padmaja K, Prasad DDK, Prasad ARK (1990) Inhibition of chlorophyll synthesis in Phaseolus vulgaris seedlings by cadmium acetate. Photosynthetica 24: 399-405.
11. Arduini I, Bold DLG, Onnis A (1996) Cadmium and copper uptake and distribution in Mediterranean tree seedlings. Physiol Plantarum 97: 111-117.
12. Breckle CW (1991) Growth under heavy metals. In: Waisel YA, Eshel U, Kafkafi U (eds) Plant roots: the hidden half, New York, Marcel Dekker, pp. 351-373.
13. Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53: 1331-1341.
14. Milone TM, Cristina S, Herman C (2003) Antioxidative responses of wheat treated with realistic concentration of cadmium. J Environ Sci Exp Bot 50: 265-276.
15. Cardinaels C, Put C, Van Asche F, Lijsters H (1984) The superoxide dismutase as a biochemical indicator, discriminating between zinc and cadmium toxicity. Biol Plantarum 40: 131-135.
16. Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (Pisum sativum L. cv. Azad). J Exp Bot 52: 1101-1109.
17. Converso DA, Fernández ME, Tomaro ML (2000) Cadmium inhibition of a structural wheat peroxidase. J Enzyme Inhib 15: 171-183.