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Effect of Cadmium on Growth and Antioxidant Enzyme Activities in Broccoli

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Abstract

Vegetable crops like cauliflower and broccoli are occasionally grown in heavy metal contaminated soils on the outskirts of industrial cities. Among the various toxic metals, cadmium (Cd) is of particular concern because it is readily absorbed and accumulated in plants, thus increasing the potential for contamination of food chain. The present investigation was thus designed to study the partitioning of Cd among various parts of broccoli plants grown in Cd treated soil (10 and 100 mg Cd/kg soil). The effect of Cd on photosynthesis and antioxidant enzymes was also studied. Plants accumulated larger portion of Cd in the shoots followed by roots and heads. Photosynthesis was adversely affected in Cd-treated plants due to reduced leaf area accompanied by decline in chlorophyll content and lowered Hill reaction activity in leaves. However, activity of antioxidant enzymes such as catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) was increased in Cd-treated plants as compared to the control possibly to combat the oxidative stress caused by Cd.
Key words : Broccoli, Cadmium, Catalase, Chlorophyll, Peroxidase.

Cadmium (Cd) is a pollutant element and potential toxin that has no known function in any biological organism. Agricultural soils are mainly contaminated with Cd from phosphate fertilizer use, sewage sludge dispersal and atmospheric deposition (Wagner 1993). High concentrations of Cd in the soil may inhibit seed germination and exert a wide range of adverse effects on the growth and metabolism of plants (Hegedus et al. 2001). Cd directly or indirectly inhibits physiological processes such as respiration, photosynthesis, water relations and gas exchange (Van Assche and Clijsters 1990). One of the main toxic effects of Cd is oxidative stress, linked with lipid peroxidation of cellular membranes. Oxidative stress results in the production of active oxygen species such as superoxide radicals (O_2), singlet oxygen ($^1O_2$), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2), which affect various cellular processes concerned with the functioning of membrane systems. While plant growth may be severely restricted by heavy metals such as Cd, some plant species possess a unique ability to adapt rapidly and develop tolerance to toxic or lethal levels of heavy metals. The ecological adaptation of plant communities to chronically high heavy metal exposure stands as a classical example of rapid evolution under extreme selection pressures (Woolhouse 1983). The aim of present work was to study the effect of Cd on growth and physiology of broccoli and to explore the role of antioxidant enzymes for metal tolerance in plants.

Methods

The seeds of broccoli (*Brassica oleracea* L. var *italica* Plenck) cultivar Punjab Broccoli 1 were obtained from Department of Vegetables, Punjab Agricultural University, Ludhiana. A field experiment was designed to investigate the effect of Cd on growth and physiology of broccoli. The seeds of broccoli were grown in nursery and then 40 day old seedlings were transplanted in field. Cd (as cadmium acetate) was added into the soil at two rates (10 and 100 mg Cd/kg soil) before transplanting.

Estimation of Cd. Samples of root and shoot from control and Cd-treated broccoli plants were collected at 70 days after transplanting (DAT) stage. However, head samples were collected at final harvest stage. Plant samples were allowed to dry in an oven at 65 ± 2 C for 72 hours. About 500 mg of dry

Table 1. Effect of Cd application in soil on Cd accumulation in various plant parts of broccoli cultivar Punjab Broccoli-1.

Cd treatments (mg/kg) soil	Cd accumulation (μg/g) Root	Shoot	Head
0	1.54	2.15	0.38
10	10.47	24.18	5.37
100	73.15	74.99	29.51

Table 2. Effect of Cd on total leaf area per plant and shoot dry matter of broccoli cultivar Punjab Broccoli-1.

Cd treatments (mg/kg soil)	Total leaf area per plant (cm^2/plant)	Shoot dry matter (g)
0	683.50	26.10
10	489.50	20.33
100	413.00	18.50
CD at P = 0.05	110.48	4.22

plant material was digested in an acid mixture of HNO_3 : $HClO_4$ (3 : 1 vol/vol). Acid mixture was allowed to evaporate and the metal residues were dissolved in 10 ml of deionized water. Cd estimation was then carried out by ICAP-AES (Inductively Coupled Argon Plasma-Atomic Emission Spectrophotometer).

Leaf Area. Leaf area of control and treated plants was measured at 70 DAT stage using graphic method.

Dry Matter Accumulation. Dry weight of shoots was also recorded at 70 DAT after drying the plant samples in an oven at 65 ± 2°C for 72 hours.

Biochemical Analysis. Total chlorophyll content (Arnon 1949) and Hill reaction activity (Lerer and Bar-Akiva 1979) were determined in fresh leaf tissue at 35 and 70 DAT stages. Activities of catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) were assayed in fresh leaf tissue extracts as described by Palmiano and Juliano (1973) and Mitra et al. (1970), respectively.

Statistical Analysis. The data on various parameters were subjected to statistical analysis. Critical difference values were calculated doing analysis of variance (ANOVA) (Singh et al. 1991).

Results and Discussion

Root, shoot and head of broccoli showed a progressive accumulation of Cd as a function of the external Cd concentration. However, for both the Cd concentrations studied, Cd concentration was more pronounced in the shoot than in the root followed by the head (Table 1). Effective translocation of heavy metals from root to leaves is a distinguish-ing feature of hyper accumulators (Brown et al. 1995). Hyper accumulation of metals also depends on the genetic capability of plant and metal bioavailability. Five-day old *Brassica juncea* seedlings exposed to 10 μmol Cd concentration in liquid media accumulated 8 μmol Cd/g dry weight after 48h (Salt et al. 1997). *Colocasia esculentum* plants accumulated larger portion of Cd in the roots followed by stem and leaf (Patel et al. 2005). However, broccoli in our experiment accumulated higher concentration of Cd in shoots than roots and head indicates the capability of this plant to hyper accumulate Cd in shoots. In contrast, heads accumulated Cd at much lower levels suggesting that only a limited translocation of Cd occurs through shoots to heads of broccoli. Exposure of broccoli to different concentrations of Cd showed pronounced effect on leaf area and dry weight of shoots (Table 2). There was a gradual decrease in total leaf area per plant with the increase in soil Cd concentration. This reduction in leaf area consequently resulted in lowered shoot dry matter. The decrease in shoot dry weight was 22 and 29% with 10

Table 3. Effect of Cd on total chlorophyll content and Hill reaction activity in leaves of broccoli cultivar Punjab Broccoli-1.

Cd treatments (mg/kg soil)	35 DAT	70 DAT
Total Chlorophyll Content (mg/g FW)		
0	1.13	1.46
10	0.92	1.02
100	0.88	1.01
CD at P = 0.05	0.05	0.07
Hill Reaction Activity (Decrease in Absorbance per g FW/h)		
0	2.35	2.61
10	1.01	1.42
100	0.70	0.81
CD at P = 0.05	0.43	0.41

Table 4. Effect of Cd on activity of catalase and peroxidase in leaves of broccoli cultivar Punjab Broccoli-1.

Cd treatments (mg/kg soil)	35 DAT	70 DAT
Catalase (Change in Absorbance per Min/g FW)		
0	27.48	31.64
10	31.64	33.30
100	34.96	47.45
CD at P = 0.05	0.15	2.84
Peroxidase (ml H_2O_2 Decomposed per Min/g FW)		
0	37.46	39.14
10	39.21	46.38
100	42.63	60.42
CD at P = 0.05	2.91	3.14

and 100 mg Cd/kg soil, respectively. A reduction in dry matter accumulation has also been observed in cabbage, rye grass, maize and white clover with increased Cd level (Yang et al. 1998).

Plants also exhibited a gradual reduction in total chlorophyll content and Hill reaction activity in leaves with the increase in soil Cd concentration at 35 and 70 DAT stages. However, total chlorophyll content and Hill reaction activity in leaves was higher at 70 DAT stage than 35 DAT stage in controls and in Cd-treated plants (Table 3). It has been reported that Cd primarily affected the photosynthetic pigments, before photosynthetic function. Cd inhibits several processes of chlorophyll synthesis like synthesis of δ-aminolevulinic acid (Stobart et al. 1995) and also the integration of chlorophyll molecules into pigment protein complexes (Horvath et al. 1996). Cd assimilation in the plant was accompanied by an increase in the activities of antioxidant enzymes such as catalase and peroxidase with respect to control plants (Table 4). The activity of both catalase and peroxidase showed a linear increase with increasing Cd concentrations. Further, a higher activity of these enzymes was observed at 70 DAT stage than 35 DAT stage in control and Cd-treated plants. Previous studies have demonstrated that Cd toxicity induces the production of reactive oxygen species (ROS) resulting in significant damage to cellular constituents (chaoui et al. 1997, Metwally et al. 2003). This oxidative stress caused by Cd leads to increased activities of antioxidant enzymes which enable the cell to quench ROS, and help the plant to adapt rapidly and evolve tolerance to lethal levels of heavy metals.

Thus, increased Cd availability led to accumulation of Cd in root, stem and head of broccoli plants with concomitant decrease in biomass due to lowered leaf area, total chlorophyll content and Hill reaction activity in leaves. Activity of antioxidant enzymes significantly increased with increasing Cd concentrations that possibly lowered oxidative stress caused by metal toxicity.

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