BIOCHEMICAL CHANGES IN DUCK WEED AFTER CADMIUM TREATMENT. ENHANCEMENT IN SENESCENCE

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Abstract. The effect of Cd on biochemical changes in the free floating duck weed (*Spirodela polyrrhiza* L. SP20) was studied. Cadmium enhanced senescence; enzymes like peroxidase, protease and phosphatases, which are known as a marker of senescence, were increased after Cd treatment. Nitrate reductase activity was also increased. With increasing Cd levels, Ca, Mg, and Zn concentrations in the plant were decreased while the Fe concentration was increased.

1. Introduction

Cadmium is an environmental pollutant which is highly toxic to plants and animals. Exposure to Cd will decrease growth of plants although the mechanism of Cd toxicity has not been elucidated. In whole plants, Cd is readily absorbed and translocated, and the concentration of Cd in various tissue is directly related to levels of exposure to Cd (Jastrow and Koeppe, 1980). One reason for the phytotoxicity of Cd results from competition of Cd ions with essential microelements (Borges and Wollum, 1981; Khan and Khan, 1983). Other biological effects induced by Cd are inactivation of the mitochondrial membrane (Bittell *et al.*, 1974), distruction of photosynthetic membranes which affects both the photosynthesis (Weigel, 1985a, b) and transpiration (Jastrow and Koeppe, 1980). These results demonstrate that Cd affects energy producing systems in plants. To obtain further evidence on the nature of Cd toxicity in plants, we investigated the effect of Cd on the biochemical changes in duck weed. Owing to their minute size and easy manipulation under aseptic conditions, *Spirodela* provides excellent material for studying various aspects of Cd toxicity.

2. Materials and Methods

Stock culture and experimental cultures of duck weed (*Spirodela polyrrhiza* L. Sp20) were maintained on Bonner and Deverian medium (1939) supplemented with 1% sucrose. The medium was adjusted to pH 5.5 before autoclaving at 0.1 to 0.2 MPa for 10 min. Cultures were grown under continuous light of 54 μmol m⁻² s⁻¹ at 25 °C.

From 10 to 12 days old stock culture, a three frond colony was inoculated into a 50 mL aliquot of media supplemented with various concentrations of Cd.

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Subsequently after 14 days, fronds were washed with distilled water, and their fresh weight determined. Fresh frond were used for measuring enzyme activities and other biochemical changes.

Acid and alkaline phosphatase enzymes were analyzed at pH 4.0 and 9.0, respectively, as described elsewhere (Yamaya and Matsumoto, 1981). Peroxidase was extracted by grinding 500 mg frond in 5 mL of 0.2 M potassium phosphate buffer (pH 7) at 4 °C, and the homogenate was centrifuged at 11 000 g for 30 min, the supernatant was used. A cuvette was filled with 4 mL phosphate buffer (pH 6.1), 0.5 mL 0.02 M \( \text{H}_2\text{O}_2 \) and 0.5 mL 0.09 M guiacol solution and 0.1 mL of enzyme extract. The increase in optical density (at intervals of 15 up to 180 s) was recorded at 420 nm and enzyme activity expressed as units g\(^{-1}\) fresh weight min\(^{-1}\).

For protease measurement extraction was the same as peroxidase but the supernatant was dialyzed against the grinding buffer for 12 hr at 2 °C using cellophane tubing (cut off molecular weight 1000) to remove free amino acids. The activity was measured by adding 0.2 mL of enzyme extract into 2 mL of bovine hemoglobin prepared in 0.1 M potassium malate buffer (pH 3.5). The mixture was incubated for 30 min at 37 °C. The reaction was stopped by precipitating the undigested protein with 1 mL of 10% TCA. After removing the precipitate by centrifugation, the supernatants were analyzed for free amino acids by the method of Rosen (Rosen, 1957). The protease activity was expressed as \( \mu \text{g} \) of released glycine hr\(^{-1}\) at 37 °C. Nitrate reductase activity was measured by the method developed by Hageman and Flesher (1960).

Nitrogen was estimated by modified microKjeldhal process (Humphries, 1956). Phosphorus was determined colorimetrically by metavanadate molybdate method (Betramson, 1942). Potassium content was estimated by using flamephotometer (Jackson, 1967). Total Ca and Mg contents of the plant were estimated by using Alternate Versenate method as described by Kanwar and Chopra (1976). Zinc, Fe and Cd were determined by Perkin-Elmer model 373 atomic absorption spectrophotometer. Five replicates were used for each treatment and all the experiments were repeated at least 3 times. Results are expressed with standard error.

3. Results and Discussion

Cadmium treated plants showed a significant reduction in number of fronds and fresh weight (Table I). At 0.1, 1.0, and 10 mg L\(^{-1}\) of Cd about 17, 23, and 69% inhibition, respectively in the number of fronds was observed. At higher concentrations of Cd the size of fronds was also decreased which resulted in decreased fresh weight.

After Cd treatment, stimulation in acid and alkaline phosphatase activities were recorded (Figure 1). Enhancement in the activity of both enzymes were insignificant at 0.1 mg L\(^{-1}\) of Cd, which increased about 2-fold in presence of 10.0 mg L\(^{-1}\) of Cd. Increased acid phosphatase activity in soyabean after Pb treatment has been reported by Lee et al. (1976). They related increased activity of phosphatase to