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GROWTH AND ENZYME ACTIVITY OF WHEAT UNDER VARYING LEVELS OF CADMIUM AND IRON

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Abstract

Effects of cadmium (Cd) and iron (Fe) on growth of Atir and Negev varieties of wheat in solution culture experiment was investigated. Based on Geochem-PC model, four different combinations of pCd (-log free Cd^{+2} activity) such as (0, 7.9, 8.2 and 8.5) and two levels of pFe (-log free Fe^{+2} activity) such as (17.0 and 17.6) were formulated as experimental treatments. Shoot dry matter yield of wheat cultivars *viz.* Atir and Negev were reduced to 80 and 74% at 7.9 pCd level. Atir was the most sensitive genotype for the rate of phytometallophore (PM) release. Alterations in superoxide dismutase (SOD) and peroxidase (POD) enzyme activities were initiated from lower pCd level for both the varieties.

Introduction

Phytotoxicity of cadmium is well documented and it also badly affects the morphological, physiological and biochemical functions in plant. Besides initial Cd concentrations in soils, the accumulation, transport and supply of Cd within plant systems is reliant on many different parameters, like plant species, also genotypes within the same species (Wang *et al*. 2019), exchange with zinc, calcium and manganese, root structure, peptides adsorption etc. (Hussain 2019). Apart from microbial activity, pH, ionic concentration changes, and phytometallophores secretion by the plant roots may alter the flow of essential nutrients in the rhizosphere. Acquisition of heavy metals by graminaceous species through root exudates currently coined as phytometallophore was recorded by Mehmood *et al.* (2019). Absorption and uptake of heavy metals by plants are principally governed by the free metal ion in soil solution which can be retained at a low level of metal- chelating resins (Hart *et al.* 1998) or by the application of chelator-buffering (Parker *et al.* 1995). Geochem-PC, computerized chemical equilibrium model, was developed recently to determine the required concentrations of chelating agent and metals for plant nutrition research in solution culture experiment (Parker *et al.* 1995). Rizwan *et al.* (2019) reported that interference of heavy metals with metabolic processes of plants caused phytotoxicity (Rizwan 2019). Even at low activity levels Cd badly affect the functions of cellular metabolism without showing any visual symptoms in plant parts. Oxidative stress is the foremost toxic effects of Cd and generally different stress enzymes like POD (Peroxidase), SOD (Superoxide dismutase) etc. are affected by higher concentration of Cd in plant cell (Dhaliwal and Mandal 2019). Against this backdrop, the present investigation was attempted to investigate wheat crop growth at various free Cd^{+2} and Fe^{+2} activities in a chelator buffered nutrient solution culture, to assess the effect of different levels of Cd^{+2} and Fe^{+2} activities on phytometallophore (PM) production in wheat crop, to investigate the effect of Cd on metal uptake by wheat crop and to investigate the effect of different levels of Cd^{+2} and Fe^{+2} activities on SOD and POD enzymatic activity of the wheat crop.

Materials and Methods

Geochem PC Model was used to determine the free metal activity toxic to wheat plant in solution culture experiments. The experiment was carried out under controlled condition in a

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growth chamber. The N-(2-Hydroxyethyl) ethylenediamine-N,N,N'-triacetic acid (HEDTA) concen-trations were used to prepare a 20µM excess of HEDTA above the sum of the Zn, Cu, Fe, Mn, and Cd concentrations. This excess provided to buffer the activities of all micronutrient-cations and to reduce the activity of any contaminant like Cd in those solutions supposed to be low in Cd activity. The 2 (N-morpholino)- ethanesulfonate (MES) was added at 2.0 mM to buffer pH. Cd treatments comprised of four levels of added $Cd(NO_3)_2$, $4H_2O$, varying from 20 to 81 µM for the HEDTA-buffered solution. GEOCHEM-PC- Version 2.0 was used to calculate the chemical activity of Cd^{+2} and other ions in the nutrient solutions. As Cd-HEDTA concentrations were 20, 40 and 81 µm, the calculated Cd^{+2} activity values in solution were 8.5, 8.2 and 7.9 in the HEDTA buffered system (pH 6.0), while the activities of other metals remain unaltered. Analytical-grade reagents and double distilled water were used for preparation of the chelator-buffered nutrient solutions. Four seedlings of each variety of wheat were grown in each 750 ml dark aerated container. The nutrient solutions were aerated continuously and the pH was adjusted to 6.0 (± 0.01) daily by addition of 1(N) NaOH and 1(N) HCl throughout the experiment. Wheat plants were grown under controlled environment conditions with day/night temperature regime 25 /18°C; 14/10 hrs light /dark (photoperiod), photo flux density at leaf level about 400 µ mol cm^{-2}.

Based on the results of a preliminary experiment conducted in the growth chamber with two varieties of wheat viz. Negev and Atir in the chelator-buffered nutrient solution culture, treatments like pCd levels (0, 7.9, 8.2 and 8.5) (Cd1, Cd2, Cd3) and pFe levels (17.0 and 17.6) (Fe1, Fe2) were selected to conduct the present experiment. The main experiment was carried out with pCd levels: 0, 7.9, 8.2 and 8.5 and pFe levels: 17.0, and 17.6. Phytometallophore release by the wheat varieties was estimated by following the standard protocol (Shenker *et al.* 1995). To assay the enzyme activity like peroxidase (POD) and superoxide dismutase (SOD), gel electrophoresis and determination method was followed (Bradford 1976). Samples were electrophoresed through 14% polyacrylamide native gels using the gel system (Laemmli 1970).

Factorial randomized block design was used to analyze the experimental data (Gomez and Gomez 1984). Least significant differences (LSD) were determined at the 5 % probability level, and Duncan's Multiple Range Tests was also used.

Results and Discussion

Highest biomass production was recorded in Negev variety of wheat. Primarily the yield of wheat declined progressively with continuous increase in pCd level (from 7.9 to 8.5). It was noted that at all levels of pCd and pFe in solution, dry shoot weight differed significantly for both the wheat varieties. The response generally decreased with concomitant rise in Cd status in the solution and it was showed by the response of DM production to Cd application. The results illustrated in the Fig. 1 revealed that in case of Atir, at CdlFe1 and Cd1Fe2 levels, the dry shoot weights were 5.04 and 14.33 times, respectively less than control. It was recorded at lower activity level of Fe (pFe 17.6), dry matter production was severely affected due to acute stress of iron deficiency.

In case of Negev, the dry shoot weight (Fig. 1) decreased 1.29 times at pFe 17.6 level, without Cd and with Cd level dry shoot weight declined 1.79 times. Furthermore, in comparison to control the dry shoot weights of Negev variety at CdlFel and CdIFe2 levels were 3.73 and 5.18 times, respectively declined. Results showed that Cd stress was prominent at high Fe status and reduced under Fe stress condition. It was also observed that Fe stress was more acute in wheat plants at pFe 17.6 level, thus Cd effect was lesser. Bouziani *et al.* (2019) also reported that cadmium repression results in continuation of growth activity. At CdlFel and CdIFe2 levels, the dry shoot weights (DRW) of Atir, were 5.04 and 14.33 times less as compared to control respectively. DRW

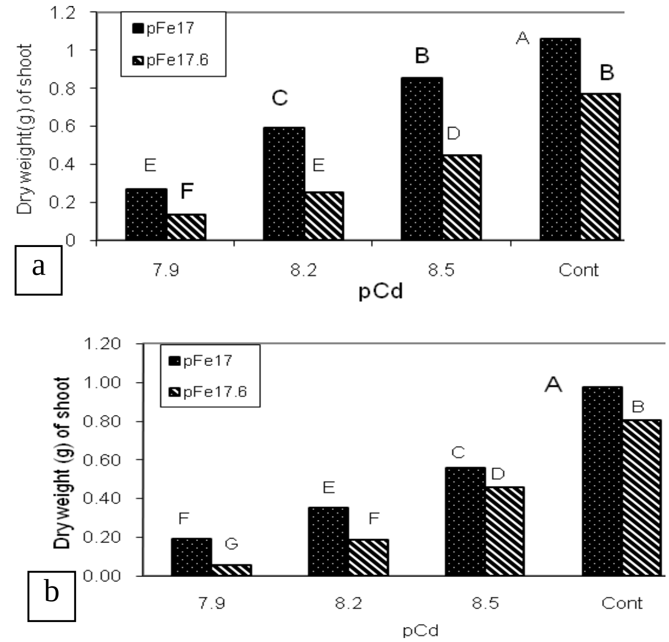

Fig. 1. Dry shoot weight (g) of Negev (a) and Atir (b) variety of wheat. [A]Mean values followed by different letter within columns differ significantly at P < 0.05 according to Duncan Multiple Range Test (DMRT).

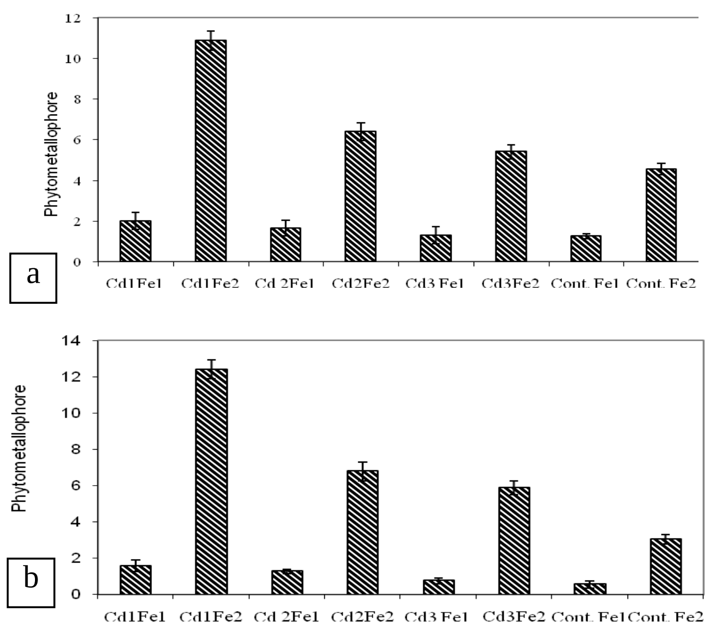

Fig. 2. Effect of Cd and Fe on phytometallophore (μmol g^{-1} root DW) release by Negev (a) Atir (b) variety of wheat. [A]Mean values followed by different letter within columns differ significantly at P < 0.05 according to Duncan Multiple Range Test (DMRT).

production was 1.21 times more at higher activity level of Fe (pFe 17.0). At pCd 7.9 level, DRW production was 3.44 times more at pFe 17.0 level as compared pFe 17.6. In case of Atir (Fig. 2) at the same level of pCd, the severe stress situation occurred at pFe 17.6 level and caused higher amount of PM production which was 5.46 times more than the pFe 17.0 level. The solubility of Cd in soil was governed by root exudates partially (Kamal *et al.* 2019). Root exudates could have controlled the solubility of Cd in the soil. Furthermore, it was obvious that under control condition, at pFe 17.6, PM production was 3.64 times more than at pFe 17.0 level. It was also noted that with concomitant rise in pCd level (from 8.5 to 7.9), production of PM was enhanced at any pFe level. But at CdlFe2 level, the maximum amount of PM production in case of both the varieties was observed. Under Fe stressed condition, PM release rate for both the wheat varieties was increased by a factor 25 to 30. Negev (Fig. 2) is more effective in producing PM as compared to Atir of wheat. The highest production of phytometallophore was noted at Cd1Fe2 1evel. Experimental results confirmed that Cd may influence the release rate of PM synthesis in wheat plant.

Heavy metals contents in wheat crop were illustrated in Fig. 3. Significant increase in Cd content in wheat plant with concomitant rise in levels of pCd was recorded. The cadmium concentration in shoot of Negev varied from 252.28 to 451.68 mg kg^{-1} at normal iron level. At pFe

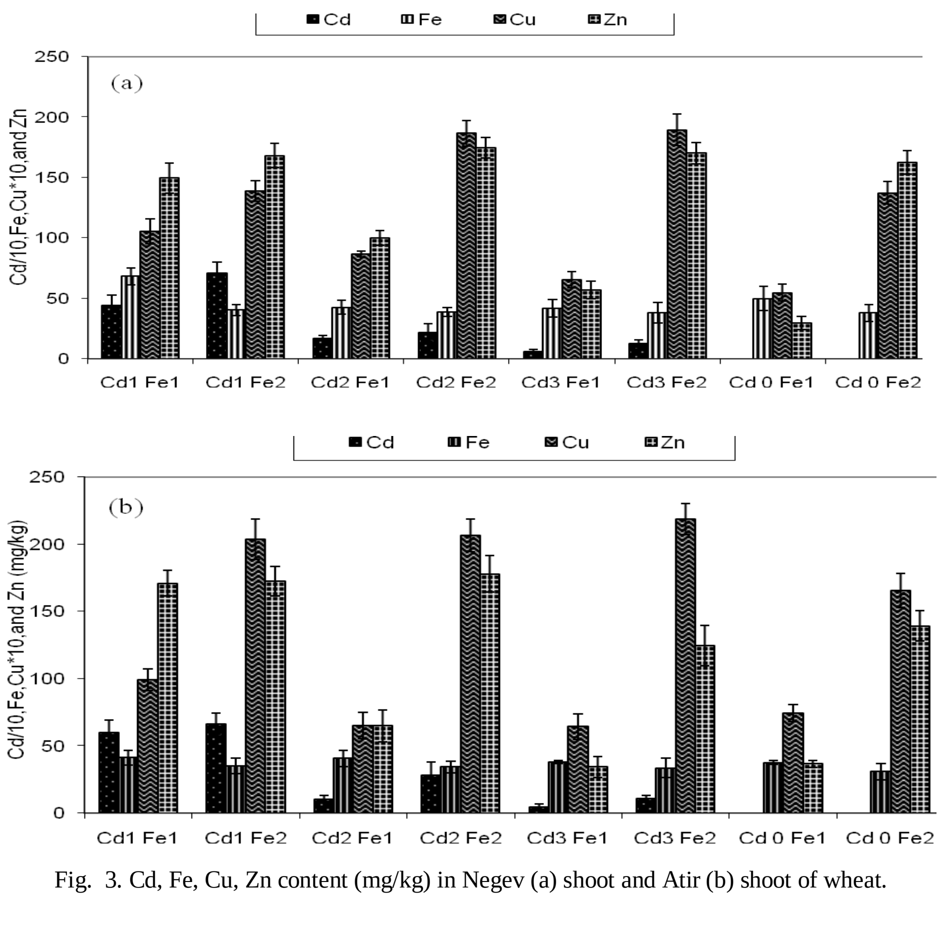

Fig. 3. Cd, Fe, Cu, Zn content (mg/kg) in Negev (a) shoot and Atir (b) shoot of wheat.

17.6 level, Cd concentration in shoot was more than at pFe 17.0 level. Maximum mean Cd content in shoots was recorded in the Negev, whereas the same was minimum in the Atir. Such variability is attributed to the inherent genetic differences in ability of the cultivars to mine Cd from the solution. Higher Cd content was recorded in case of Negev indicated that the variety can extract more Cd from solution Cd. Plants have developed an intricate antioxidant system like production of superoxide dismutase and peroxidase enzymes to restrict oxidative and radical damage (Foy *et al.* 1978). Enhanced peroxidase activity was observed in the 3rd and 4th leaf of wheat varieties. In case of Atir variety of wheat (Fig. 4) peroxidase activity increased from control to higher activity of pCd. The maximum activity was observed at Cd1Fe1 and Cd1Fe2 level. It indicated that under stress condition due to Cd, the enzyme activity may increase. The SOD activity also enhanced with pCd 7.9 level irrespective of pFe level. At Cd1Fel and Cd1Fe2 levels, the highest enzyme activity was recorded. The POD enzymatic activity similar results were recorded for Negev variety of wheat (Fig. 5). The enzyme activity enhanced with concomitant rise in the activity level of pCd. At both the levels of pFe enzyme activity increased with pCd 7.9 levels. The similar trend was observed for SOD enzyme activity.

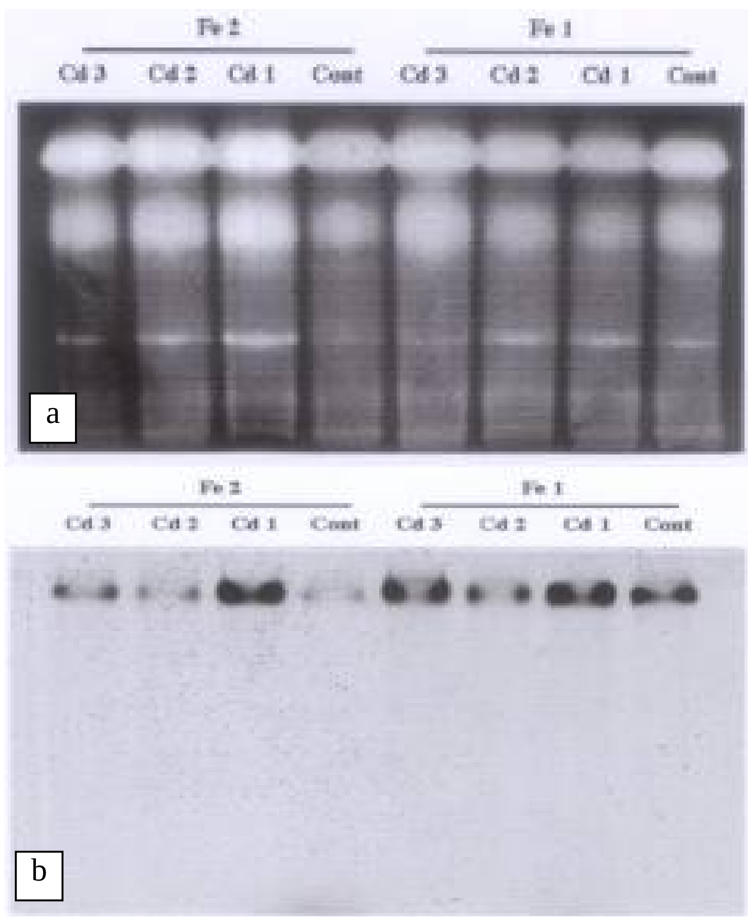

Fig. 4. Effect of Cd and Fe on SOD (a) and POD (b) activity of Atir variety of wheat.

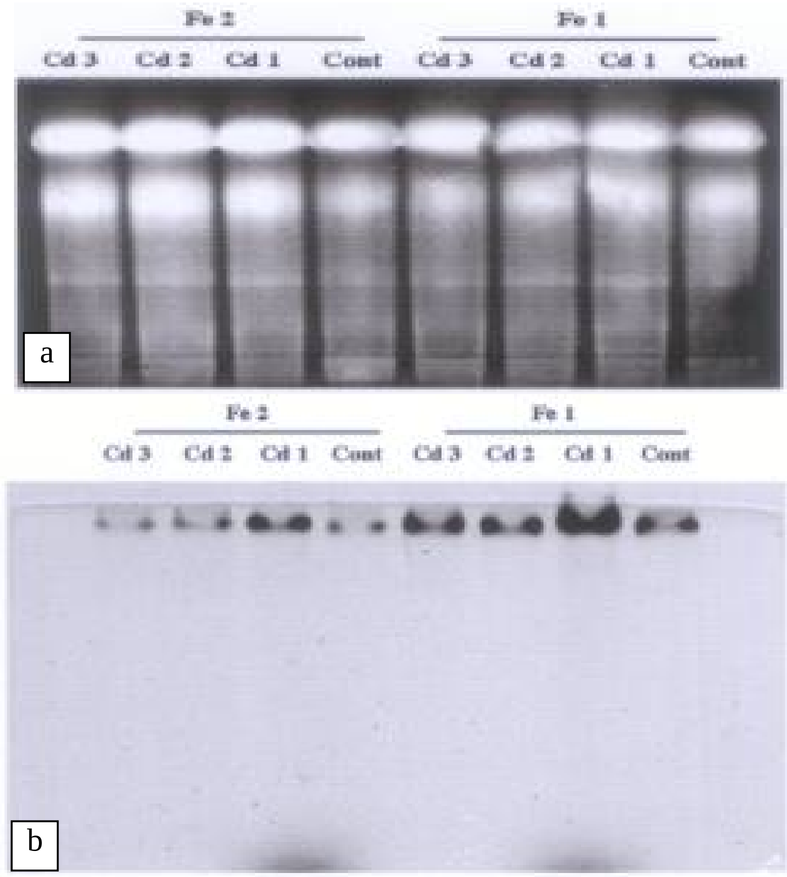

Fig. 5. Effect of Cd and Fe on (a) SOD and (b) POD activity of Negev variety of wheat.

Overall experimental results depicted that higher levels of Cd concentration drastically reduced the shoot dry matter yields irrespective of iron sufficient and iron stressed plants. At initial growth stage of wheat crop, phyto-toxicity of Cd can be confirmed by estimating the stress enzyme activities which may be considered as a diagnostic criterion. Experimental findings may be useful to mitigate Cd pollution in plant-human-animal chain by proper selection of suitable varieties of crops for cultivation.

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