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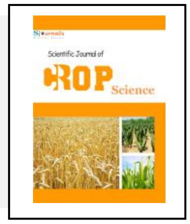

Original article

Investigation physialogical Characteristics of *Ricinus Communis (L.)* and *Brassica juncea (L.)* in cadmium contaminated soil

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ABSTRACT

We have previously reported that Ricinus communis (castor) is more tolerant to soil cadmium (Cd) and more efficient for Cd phytoremediation than Brassica juncea (Indian mustard). This expriment was performed to Investigation physiological and biochemical characteristics of Ricinus Communis (L.) and Brassica juncea (L.) in cadmium contaminated soil. Castor plants showed stronger self- protection ability in form of proline bioaccumulation (r^2= 0.949) than Indian mustard (r^2= 0.932), Proline accumulation increased by 2.6 fold in B. juncea and 17.7 fold in R. communis on 90 DAS applied in cadmium contaminated soil. whereas a lower r2 for malondialdehyde (MDA) and total soluble protein in R. communis (r^2= 0.914 and r^2 = 0.915, respectively) than that of B. juncea (r^2 = 0.947 and r^2= 0.927, respectively) and there was increase of MDA by three fold in B. juncea and 3.36 fold in R. communis over the control that indicated a greater damage to cell membrane in Indian mustard during the stress conditions.

1. Introduction

cadmium (Cd), one of the most hazardous heavy metals, has been released into agriculture soils through anthropogenic activities, such as leather processing, electroplating, appllication of metal containing sewage sludge, and utilization of fertilizers (Santana et al., 2012). can be readily accumulated by agriculturad crops and can inhibit plant growth and cause diseases in animals and human beings (Zhou and Song, 2004). B. juncea have been found to be sensitive to most of the stresses and its green leaves are used as vegetable and fodder in many part of the Indian subcontinent, therefore other alternatives are required to be investigated for removal of the metals from the contaminated agricultural ecosystems. We have recently reported Ricinus communis. (bauddh and Singh, 2012). As better alternative to B. juncea. A considerable portion of the agricultural ecosystem supporting cultivation of B. juncea in Indian subcontinent is affected by cadmium; therefore it will be very significant to investigate comparative physialogical and biochemical characteristics of B. juncea and R. communis from the metal contaminated soil. The present study is planned with this perspective to study the tolerance capability and cadmium removal by these two plants in similar agro climatic conditions from the Cd contaminated soil.

3. Materials and methods

2.1. Plant materials and experimental design

The seeds of Indian mustard (B. juncea L.) and castor (R. communis L.) were obtained from the Hamedan Agricultural Research Institute. Seeds were sown in 30-cm-diameter earthen pots filled with 8 kg soil. Soil was mixed with appropriate amount of CdCl2 to T=0, T=50, T=100 and T=150 mg Cd kg-1 soil. The pots with seeds of B. juncea were kept directly in naturally illuminated net house of research field station in the Department of Agricultur Buali Sina University Lucknow, Hamedana (during October–December months) having the minimum temperature ranged between 6- 20OC, maximum temperature 17- 340C and with relative humidity 62-71 percent. The seeds were germinated in five day and one plant was maintained in each pot for further studies. R. communis needs a comparatively higher temperature for seed germinntion; hence the pots containing its seeds were kept in Green House with controlled conditions for ten day at 300C with 85 percent relative humidity. after germination, the pots of R. communis were shifted in the same net house for comparative study (with B. juncea). All the measurements were performed at 30, 60 and 90 day after sowing (DAS).

2.2. Protein

Protein was estimated by method of (Lowry et al., 1951). Fresh leaves (100 mg)`of the control and treated plants were homogenized separately in 3 ml of 10 percent chilled trichloroacetic acid (TCA) in pestle and mortar and centrifuged at 10,000 rpm for 10 min. After decanting the supernatants, the pellets were washed and heated for 7 min with 3 ml of 1 N NaOH (sodium hydroxide), cooled and centrifuged again at 10,000 rpm for 10 min. The 0.5 ml of extracted sxmpxe was taken in 2.5 ml of 0.5 percent CuSO4 (copper sulfate in 1 percent xotassiumsodium tartarate), 48 ml of 5 percent Na2 Co3 (sodium carbonate) was added. 0.5 ml (1 N) of folin-phenol reagent was added after 10 min. 30-min incubation developed a blue color complex in the mixture. Absorbance was taken at 700 nm against a blank without sample. Protein content was calculated by a standard curve made by bovine serum albumin (BSA).

2.3. Proline

Proline was determined according to the procedure described by bates et al. (1973). Fresh leaves and roots (500 mg) was extracted with 3 percent aqueous 5-sulphosalicylic acid, centrifuged at 5000 r min^{-1}. The sample of the supernatant was used for the proline assay and measured at 520 nm. Proline content was expressed as µg g^{-1} fresh weight (FW).

2.4. Malondialdehyde (MDA)

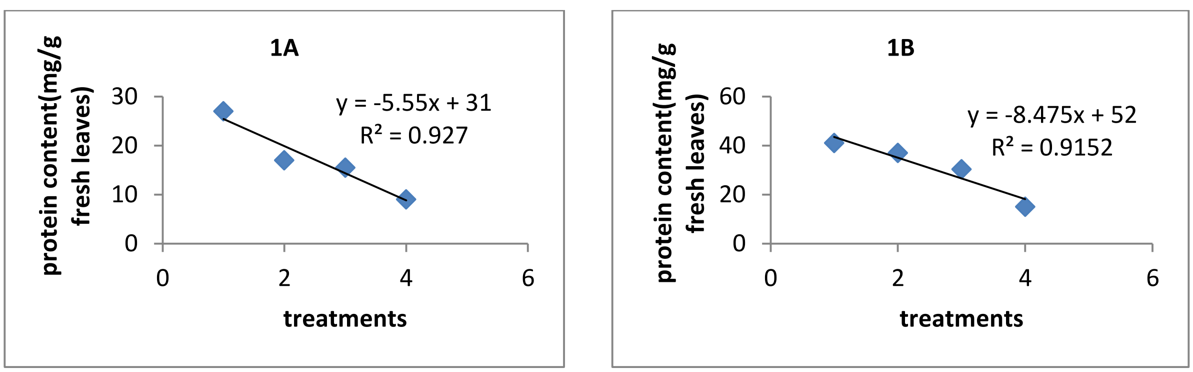
The level of lipid peroxidation products in leaf samples was expressed as MDA content and was determined by Heath and Packer (1968). About 200 mg fresh leaves were ground in 0.25 percent 2-thiobarbituric acid (TBA) in 10 percent trichloroacetic acid (TCA) using a mortar and pestle. After heating at 950C for 30 min, the mixture was quickly cooled in an ice bath and centrifuged at10,000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same at 600 nm. The blank was 0.25 percent TBA in 10 percent TCA. The concentration of lipid peroxides together with oxidatively modified proteins of plants were thus quantized in terms of MDA level using an extinction coefficient of 155mM^{-1} cm^{-1} and expressed as n mol g-1 fresh weight (FW).

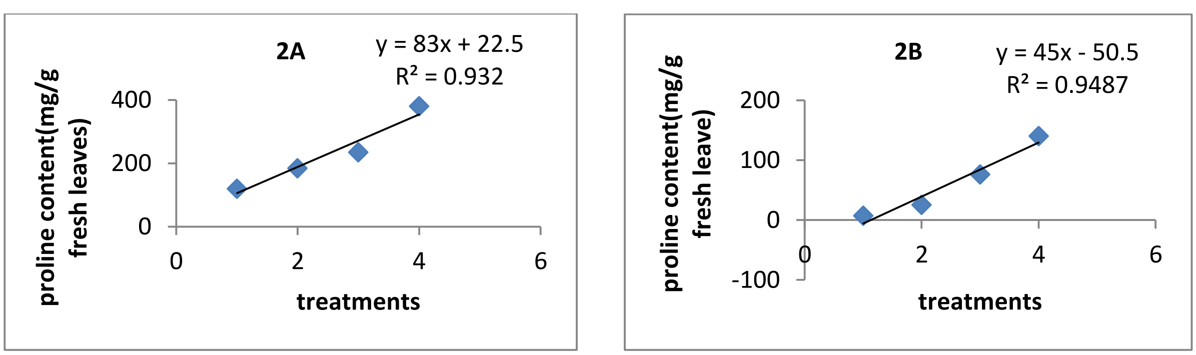
3- Results and discussion

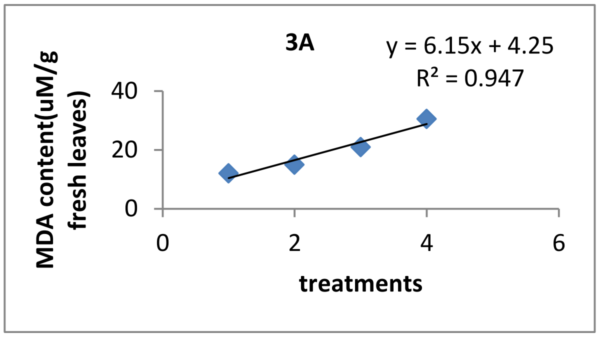
3.1. Levels of protein, proline and MDA in the plants in response to cadmium

The effect of Cd on leaf total soluble proteins of B. juncea and R. communis was shown in Fig. 1(A–B). The protein content of leaves on 30, 60 and 90 DAS significantly ($p<0.01$) decreased in both the species with application Cd stress. The decrease in total soluble protein was higher in B. juncea (61.72 percent) on 90 DAS under 25µM cadmium compared to R. communis (48.43 percent). The magnitude of decrease in leaf protein was higher in B. juncea (r^2=0.927) than that of R. communis (r^2 =0.915) under 25µM cadmium (Fig. 1A–1B). 25µM Cadmium was more detrimental in decreasing leaf protein.

Proline accumulation increased by 2.6 fold in B. juncea and 17.7 fold in R. communis on 90 DAS when was applied in 25µM cadmium. (Fig. 2A- 2B). Cumulative capacity of free proline is a manifest action of self- protection ability of plants grown in stressed environment. The proline bioaccumulation in the plants subjected Cd stress was well correlated with the decrease in protein content in leaves. Castor plants showed stronger self-protection ability in form of proline bioaccumulation (r^2 =0.961) than Indian mustard (r^2 = 0.918) (Fig. 2A–2 B). The level of malondialdehyde (MDA), one of the major thiobarbituric acid (TBA) reactive metabolites, increased significantly in both the plants. On 90 DAS there was increase on MDA by three fold in B. juncea and 3.36 fold in R. communis over the control (Fig. 3A–3B). a higher correlation values for MDA which indicates more Lipid peroxidation in leaves was calculated for B. juncea (r^2 =0.947) with multiple Cd stress as compared to R. communis r^2 =0.914) (Fig. 3A–3B).







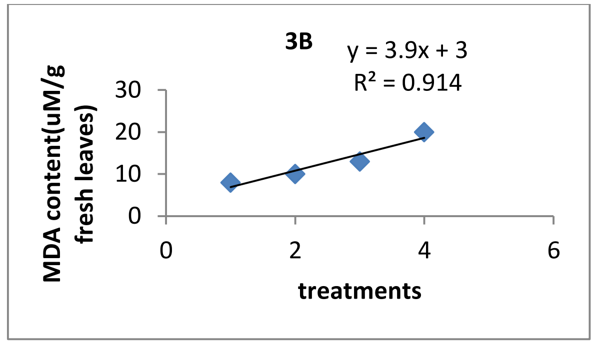


Fig. 1. Correlation analysis between different treatments and protein, proline and MDA content in fresh leaves of B. juncea [A] and R. communis [B]after 90 day of sowing. Where: T0=control, T1 =Cd 50 (mg),T2 =Cd 100(mg) and T3 =Cd 150(mg).

4-Discussion

The study revealed that Cd in the soil simultaneously influenced not only growth, protein, proline and Malondialdehyde (MDA) content but also Cd bioaccumulation in roots and its translocation to the shoots in R. communis and B. juncea. R. communis appeared relatively less sensitive towards the deteriorating effects of Cd, as compared to B. juncea. The results are in accordance to our earlier report on superiority of R. communis in relation to growth, enhanced levels of stress metabolites and phyto-accumulation of the metal over B. juncea, when Cd stress was applied 25µM (Bauddh and Singh, 2012).

The effect of varying doses of Cd on leaf soluble proteins of B. juncea and R. communis was shown in Fig. 1. Cadmium contamination has decreased soluble protein in leaves of both the plants. The decrease in protein contents in plant parts as a result of increasing Cd as stress have been reported in many plants (Mohammadkhani and Heidari, 2008; Best et al., 2011; Farouk et al., 2011; Mafakheri et al., 2011; Bauddh and Singh, 2012). It appears that similar to growth and biomass production, leaf protein levels also decrease more effectively when multiple abiotic stress occur simultaneously. Abiotic stresses heavy metal are known to cause oxidative damage to plants through the formation of reactive oxygen species, which cause damage to membrane lipids and proteins etc., however, to resist this damage plants have an antioxidative defense system by producing antioxidant compounds as stress metabolites (Bauddh and Singh, 2012; Chutipaijit et al., 2012).

Proline is one of these metabolites, which has been shown to accumulate high under the exposure of heavy metal including cadmium (Muneer xt al., 2011). It plays an important role in osmo regulation and osmo- tolerance. R. communis appeared to have relatively stronger self- protection ability in terms of proline bioaccumulation ($r2$ =0.949) than that of B. juncea ($r\ 2$ =0.0.932) grown in Cd contaminated soil (Fig1. 2A–2B). It has been suggested that protein degradation might contribute to proline accumulation in the metal treated plants (Chen et al., 2001). The level of lipid peroidation that was determined by malondialdehyde content showed to be increased in a time and stress amount dependent manner for both the species, though the MDA content was slightly higher in B. juncea than that in R. communis (Fig1. 3A–3B). An increased MDA levels have been reported in many plants under Cd (Zhang et al., 2010). When the correlation was plotted between stresses applied (Cd) and MDA content in the leaves, lower value was found in R. communis($r2$ =0.914) than that of B. juncea ($r2$ =0.947) whith indicate the greater damage to cell membrane in the B. juncea than that in R. communis (Fig1. 3A–3B).

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