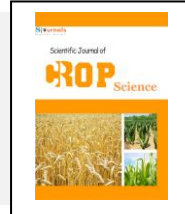


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ROP ScienceJournal homepage: www.Sjournals.com**Original article****Investigation physiological Characteristics of *Ricinus Communis* (L.) and *Brassica juncea* (L.) in cadmium contaminated soil****N. Gharehbaghli***Member's of Scientific Boards of Department of Agriculture, Payame Noor university, P.Obox 19395- 3687 Tehran, Iran.***Corresponding author; Member's of Scientific Boards of Department of Agriculture, Payame Noor university, P.Obox 19395-3687 Tehran, Iran.*

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ABSTRACT

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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 experiment was performed to investigate 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 r^2 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.

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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, application of metal containing sewage sludge, and utilization of fertilizers (Santana et al., 2012). Cd can be readily accumulated by agricultural crops and can inhibit plant growth and cause diseases in animals and human beings (Zhou and Song, 2004). *B. juncea* has been found to be sensitive to most of the stresses and its green leaves are used as vegetable and fodder in many parts 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*. (Baudh and Singh, 2012). As a 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 physiological 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 Agriculture Buali Sina University Lucknow, Hamedana (during October–December months) having the minimum temperature ranged between 6–20°C, maximum temperature 17–34°C and with relative humidity 62–71 percent. The seeds were germinated in five days and one plant was maintained in each pot for further studies. *R. communis* needs a comparatively higher temperature for seed germination; hence the pots containing its seeds were kept in Green House with controlled conditions for ten days at 30°C 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 days 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 supernatant was taken in 2.5 ml of 0.5 percent CuSO₄ (copper sulfate in 1 percent potassium sodium tartarate), 48 ml of 5 percent Na₂CO₃ (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) were 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 95°C for 30 min, the mixture was quickly cooled in an ice bath and centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant was

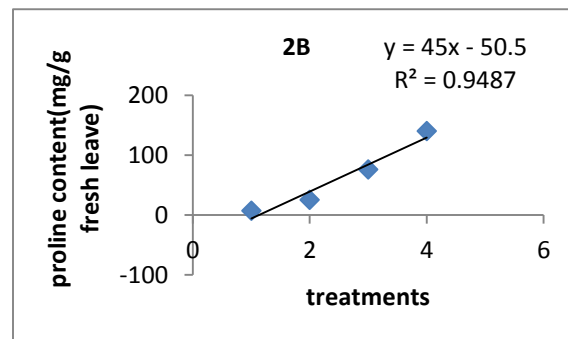
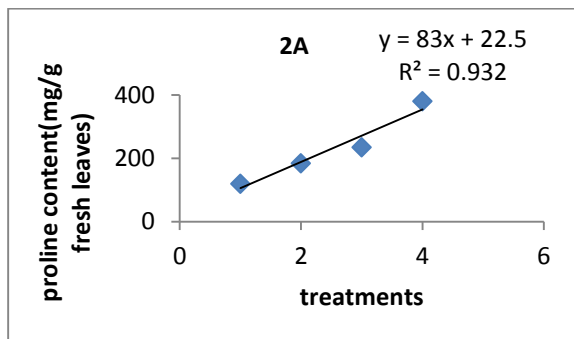
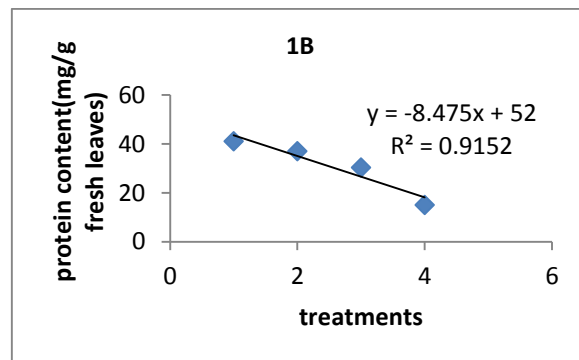
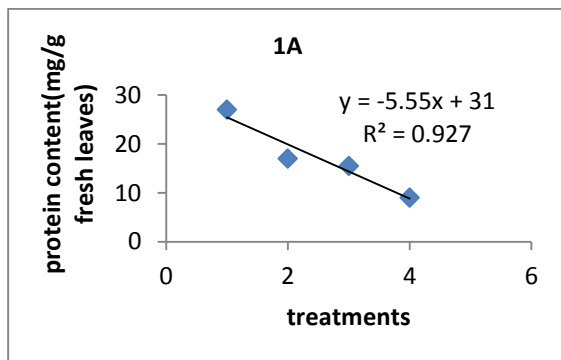
read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same at 600 nm. The blank was 0.25 percent TBA in 10 percent TCA. The concentration of lipid peroxides together with oxidatively modified proteins of plants were thus quantized in terms of MDA level using an extinction coefficient of $155\text{mM}^{-1}\text{cm}^{-1}$ 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 $25\mu\text{M}$ 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 $25\mu\text{M}$ cadmium (Fig. 1A–1B). $25\mu\text{M}$ 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 $25\mu\text{M}$ 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).



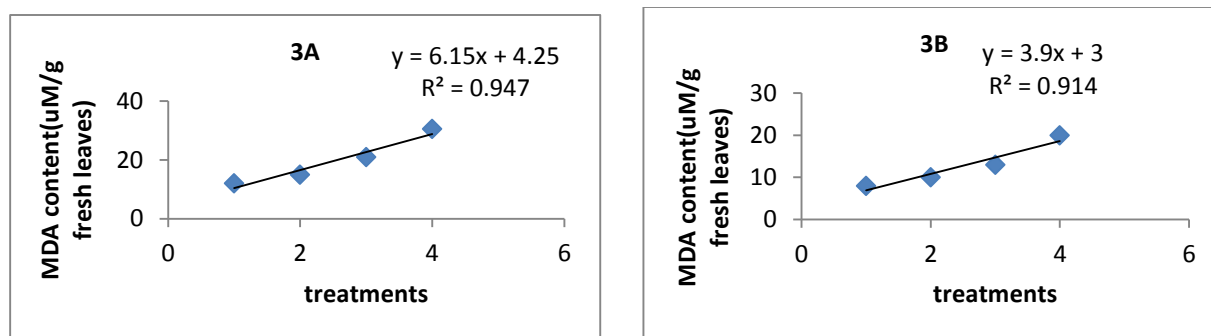


Fig. 1. Correlation analysis between different treatments and protein, proline and MDA content in fresh leaves of *B. juncea* [A] and *R. communis* [B] after 90 day of sowing. Where: T0=control, T1 =Cd 50 (mg),T2 =Cd 100(mg) and T3 =Cd 150(mg).

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 (Baudhd 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; Baudhd 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 (Baudhd 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 et 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 ($r^2 = 0.949$) than that of *B. juncea* ($r^2 = 0.932$) 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 peroxidation 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* ($r^2 = 0.914$) than that of *B. juncea* ($r^2 = 0.947$) which indicate the greater damage to cell membrane in the *B. juncea* than that in *R. communis* (Fig1. 3A–3B).

References

- Baudhd, K., Singh, R.P., 2012. Cadmium tolerance and its phytoremediation by two oil yielding plants *Ricinus communis* (L.) and *Brassica juncea* (L.) from the contaminated soil. *Int. J. Phytorem.* 14, 772–785.
- Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of proline for water stress studies. *Plant Soil* 39, 205–207.

- Best, M., Koenig, K., McDonald, K., Schuelder, M., Rogers, A., Ferrieri, R.A., 2011. Inhibition of trehalose breakdown increases new carbon partitioning in to cellulosic biomass in *Nicotiana tabacum*. *Carbohydr. Res.* 346, 595–601.
- Chen, C.T., Chen, L.M., Lin, C.C., Kao, C.H., 2001. Regulation on proline accumulation in detached rice leaves exposed to copper stress. *Plant Sci.* 160, 283–290.
- Chutipaijit, S., Cha-um, S., Sompornpailin, K., 2012. An evaluation of water deficit tolerance screening in pigmented indica rice genotypes. *Pak. J. Bot.* 44 (1), 65–72.
- Farouk, S., Mosa, A.A., Ibrahim, H.M., EL-Gahmery, A.M., 2011. Protective effect of humic acid and chitosan or radish (*Raphanus sativus*, L. var. *sativus*) plants subjected to cadmium stress. *J. Stress Physiol. Biochem.* 7 (2), 99–116.
- Heath, R.L., Packer, L., 1968. Phytoperoxidation in isolated chloroplasts. I. Kinetic and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198.
- Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y., 2011. Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. *Aust. J. Crop Sci.* 5 (10), 1255–1260.
- Mohammadkhani, N., Heidari, R., 2008. Effects of drought stress on protective enzyme activities and lipid peroxidation in two maize cultivars. *Pak. J. Biol. Sci.* 10 (21), 3835–3840.
- Lowry, O.H., Sosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Zhao, Y. (2011). Cadmium accumulation and antioxidative defenses in leaves *Triticum aestivum* L. and *Zea mays*. *Journal Biotechnol.* (15):2936- 2943
- Santana, K.B., de Almeida, A.F., Souza, V.L., Mangabeira, P.A.O., Silva, D., da, C., Gomes, F.P., Dutruich, L., Loguercio, L.L., 2012. Physiological analyses on *Genipa americana* L. reveals a tree with ability asphytostabilizer and rhizofilterer of chromium ions for phytoremediation of polluted watersheds. *Environ. Exp. Bot.* 80, 35–42.