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- Title of the thesis: Proteomic and Biochemical Investigations to study the response of *Vigna radiata* to Fe-deficiency and Cd stress.

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Abstract

Pulses are the major staple food not only for India but also the entire world. *Vigna radiata* L. is one of the most important pulses among them. The selection of *V. radiata* for the present had two major reasons i) its importance as a major protein source in food and ii) being efficient in N_2-fixation due to presence of nodules in the roots. Thus, leaf and root-nodule were the major organs to study. Among toxic metals that plant encounter through roots, cadmium (Cd) is one of much concern as it is capable not only to alter normal metabolic and growth processes but also because it induces Fe-deficiency like symptoms clearly seen on the shoots. Iron (Fe) is very important trace metal essential for normal growth of plants such as by mediating a number of catalystic/enzymatic reactions, association with the proteins which are structural in nature and may catalyze electron transport reactions. Iron may be further associated with sulphur metabolism. Thus, the major objectives of this thesis was to study the role of iron (Fe) under Cadmium (Cd) stress on concerned biochemical parameters (chlorophyll content, total soluble protein content, root-shoot ratio, nodule biomass, leghemoglobin content and ferritin content), levels of cellular antioxidants either non-proteincious (ascorbate and glutathione) or enzymatic (APX, SOD, CAT, and GR), structural analysis (electron microscopy) and last but not the least changes in the proteomic profile. Considering the above objective, study was started by designing two different sets containing iron (Fe) and iron-deficient (-Fe). Each set was latter subjected to cadmium (Cd) stress (50µM) keeping their controls as such (+Fe) and (-Fe) to make (+Fe+Cd) and (-Fe+Cd). Ultimately, there were four set of treatments T0 (+Fe-Cd), T1 (-Fe-Cd), T2 (-Fe+Fe) and T3 (+Fe+Cd). The motive of these combinations was to compare impact of cadmium in presence and absence of iron and of iron-deficiency. Thirty days old plants were exposed to $CdCl_2$ (50 µM) for a period of 24 hours and 72 hours. Leaf samples and root-nodule samples were collected at said 24 hours after treatment (24 HAT) and 72 hours after treatment (72 HAT).

The major objective of this thesis was to investigate the interactive and protective role of Fe under Cd stress in *Vigna radiata* L. (Green gram). 30 days old control and Fe-deficient plants, grown in sandy-loams, were subjected to $CdCl_2$ (50 µM/1ppm) stress for a period of 24 and 72 hours as per WHC. Fe-deficiency lead to an elevation in TBARS (oxidative stress indicator) which further increased by the addition of Cd. However, a significant reduction was observed in Cd treated plants when Fe was present. Activity of several enzymatic cellular

antioxidants viz., superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) and contents of non-enzymatic cellular antioxidants viz., ascorbate and glutathione decreased under Fe-deficiency and Cd stress during Fe-deficiency. However, the activity of antioxidant enzymes was not much influenced when Fe was supplied during Cd exposure. Consequently, the total soluble protein and chlorophyll content, length of shoot and root under Fe deficiency was found to be decreased but increased slightly in the presence of Cd, however, increase was prominent in presence of iron. N_2-fixing organs i.e., root nodules were analyzed for the number of root nodules per plant, and contents of leghemoglobin and ferritin which were decreased under Fe-deficiency and Cd, but found to be less affected under Cd stress. Ultrastructral investigations showed absence of symbiosomes (effective area for N_2-fixation) under Fe-deficiency and Cd stress, but was less affected in presence of Fe under Cd stress. Proteomic study of leaves revealed that proteins were differentially expressed in response to Fe-deficiency and Cd stress. About 238 spots were reproducibly detected and analyzed in leaves in which some proteins were up regulated and some were down regulated. Eight differentially expressed proteins like oxygen evolving enhancer protein 1, RuBisCo, oxygen evolving enhancer protein 2, HSP70, RuBP/oxygenase activase, RuBP carboxylase, rubber elongation protein and PR10 protein were identified by LC/MS-MS. In root-nodules around 159 proteins were detected among which 32 protein spots were over expressed and under expressed analyzed by PD quest software (Bio-Rad USA). These differentially expressed proteins were tryptic digested and analyzed by LC/Q-TOF followed by the identification by matching with protein data bank with the help of MASCOT. The present study shows that both Fe-deficiency and Cd exposure induces oxidative stress in *V. radiata* which was severe with Cd-stress under Fe-deficiency. High sensitivity of cellular antioxidant system, photosynthetic pigments, leghemoglobin, ferritin and symbiosome were also sensitive to Fe-deficiency and Cd-stress under Fe-deficiency. However, iron tends to minimize stress effects of Cd, even in nodules. Proteome of leaf and root-nodule were also more affected under Fe-deficiency and Cd exposure. Since, Fe declines the toxic effects of Cd and was found to up-regulate some important proteins. The role of the said identified proteins was found associated in protection against Cd-stress and if not, as per literature, their identification by expected role has been discussed.