RESEARCH ARTICLE | International Journal of Plant Sciences, Vol. 4 Issue 1 : 71-74 (January to June, 2009)

Heavy metal mediated enzymic antioxidants profiling in *Brassica juncea* Linn. plantlets cultured *in vitro*

PRIYANKA MISHRA AND JITENDRA SINGH BALIYAN

Accepted : September, 2008

SUMMARY

As a part of systematic *in vitro* selection and characterization of heavy metal tolerance in higher plants for biotechnological breeding program heavy metal (Zn/Cd) tolerant plantlets of *Brassica juncea* PCR-7 were developed on half strength MS basal salts supplemented with 1 per cent sucrose but devoid of growth regulator with varying regimes of Zn and Cd. Plantlets were capable of growing *in vitro* in the presence of 250mg/L Zn and 30mg/L Cd. Enzymic antioxidants (superoxide dismutase, catalase, peroxidase) profiling were used as indicators of heavy metal tolerance.

Key words : *Brassica juncea*, Heavy metal, Enzymic antioxidants

Phytotoxicity of heavy metals such as Cd, Cu and Zn is long known and well documented. However, the mechanisms or metal toxicity induction are not fully understood. Metal ions may directly interfere with the metabolic activities by altering the confirmation of proteins, for example enzymes, transporters or regulator proteins, owing to their strong affinities as ligands to sulfhydryl and carboxylic group (Van Asche and Clijesters,1990). This is taken to be a major cause for metal imposed toxic effects. Besides, some ions with strong redox properties e.g. Cu, but also those lacking them. e.g, Zn and Cd are known to initiate membrane lipid peroxidation and stimulate the peroxidation of reactive oxygen species (ROS). Thus, plants exposed to HM stress frequently face oxidative stress.

One of the biochemical changes occurring when plants are subjected to environmental stresses is the production of ROS (Dat *et al.*,2000).Impairment of the electron transport in chloroplast and mitochondria causes the generation of superoxide (O^{-2}) and hydrogen peroxide (H_2O_2) which are generally not harmful at optimum physiological conditions. However, their metal (Fe^{+3} or Cu^{+2}) catalysed conversion into one of the most reactive molecules, known in nature hydroxyl radical (OH^-), lead to cellular damage via membrane peroxidation and DNA damage. In order to avoid the production of these reactive molecules plants have to evolve an effective scavenging system involving antioxidant molecules like carotenoids, ascorbate, glutathione and tocopherols as well as antioxidant enzymes such as superoxide dismutase (SOD: EC1.15.1.1), catalase (CAT:EC 1.11.1.6), ascorbate peroxidase (APX: EC 1.11.1.11) and glutathione reductase (GR: EC 1.8.5.1).

Removal of heavy metal contamination on soil is also difficult. Remedial measures could be taken using some hyperaccumulator species like Indian mustard (*Brassica juncea*) , generally used for phytoremediation of polluted soil. So far, there are very few reports on *in vitro* selection of metal tolerant plants. In the present study, selected metal tolerant plants on the basis of germination frequency and enzyme activity of *Brassica juncea*, an important crop plant cultivated for edible oil, seed, vegetable and fodder. Experiments were conducted with a major objective to examine whether the sequence of enzyme activities controlling oxidative stress plays a role in the tolerance mechanism towards Zn and Cd using aseptically *in vitro* grown *Brassica juncea* PCR-7 seedlings as model experimental plant system.

MATERIALS AND METHODS

Certified seeds of Indian mustard *Brassica juncea* PCR-7 were obtained from National Research Center on Rapseed-mustard (NRCRM), Sewar, Bharatpur (Rajasthan) India. Seeds were sterilized by rinsing one minute in 95% ethanol then treated with 20 per cent bleaching powder for 30 minutes and finally washed five times with sterile water for 10 minutes. Sterilized seeds were germinated aseptically (Zhu *et al.*, 1999) on half strength MS basal salts supplemented with 1% sucrose and 0.8% agar but devoid of growth regulator with varying regimes of Zn (2.5, 10, 250 mg/L) and Cd (30 mg/L). Erlenneyer flasks containing seeds were placed in cold (4°C) for two days and then transferred to a incubator at

Correspondence to:
JITENDRA SINGH, Department of Biotechnology, S.B.S. (P.G.) Institute of Biomedical Sciences and Research, Balawala, DEHRADUN (UTTARAKHAND) INDIA
Authors' affiliations:
PRIYANKA MISHRA, Department of Biotechnology, S.B.S. (P.G.) Institute of Biomedical Sciences and Research, Balawala, DEHRADUN (UTTARAKHAND) INDIA