PHYTOCHELATINS
(\( \gamma \)-GluCys)\(_n\) Gly

PC-Cd

Cd

Cys

Cys

Cys

Cys

INTRODUCTION
CHAPTER 1

INTRODUCTION

All the extreme environmental conditions that lead to alterations in plant metabolism resulting in decreased rate of plant growth processes, loss in productivity or inducing damaging effect in any of plant’s organ/part, alteration in anatomical, biochemical or molecular regulation are termed ‘Environmental Stress’.

Over the last century human activities have resulted in an unprecedented increased level of environmental pollutants such as heavy metals, salinity, UV-light and ozone levels, and caused increased stressful challenges to the plants. Pollutants like heavy metals, salt and some biocides (soil contaminants) are mainly introduced in the plant through the root system, whereas much extent of oxidative stress is caused through the way of foliage. The stress may cause osmotic imbalance in the plant system causing decrease in water availability essential for vital plant metabolic processes, oxidation of plant pigments (resulting in decreased photosynthesis), proteolysis (lyses of anabolic enzymes), etc. that ultimately result in retarded growth. Some of the plants develop a strategy to combat stress conditions and are termed as tolerant plants (Fig. 1).

It is not just so much the quantity of pollution that is in evidences but also the chemical nature of the pollution (Connell et al., 1999). In recent years many chemical substances have entered the commercial world without a full understanding of their toxicity, or their environmental fate (Baarschers, 1996). Some of these substances have subsequently been blamed directly for various environmental consequences, while others like heavy metals, have since been shown to be involved in synergistic reactions, or even to bioaccumulate (Baarschers, 1996).

Cadmium, the most well known heavy metal, poisoning was first recognized in the Fechu area of Japan in postmenopausal women during the 1940s. The number of cases peaked in the years 1955-68. Japan's
CHAPTER 1

INTRODUCTION

Environmental stress

\[ \rightarrow \]

Physiological alterations

\[ \rightarrow \]

Signaling molecules generation

\[ \rightarrow \]

Signaling molecules transportation

\[ \rightarrow \]

Triggering of expression for signaling molecule responding genes

\[ \rightarrow \]

Type I pathway

\[ \rightarrow \]

Expression for stress protein

\[ \rightarrow \]

Mediation of anti-stress reactions

\[ \rightarrow \]

Stress tolerance to plants

Type II pathway

\[ \rightarrow \]

Expression for stress protein absent

\[ \rightarrow \]

Stressful conditions in cell

\[ \rightarrow \]

Damage

Fig. I: Different events in plant cell during environmental stresses. If pathway I is dominant the plant is stress tolerant and if pathway II dominates plant is susceptible.

Ministry of Health and Welfare declared that exposure to cadmium must participate in causing the syndrome called “Itai-Itai (Ouch-Ouch)” that results in pain upon walking, particularly in joints. Exposure to cadmium resulted from consumption of rice from paddies that were contaminated with mining wastes.
Because of the adverse effects of increased metal concentrations on most living organisms, techniques have been developed to remediate contaminated soils. Current remediation methods applicable to soils contaminated with heavy metals are expensive, environmentally invasive, and labor intensive. A remediation technique that is of low cost, but protective to human health and the environment would be a valuable addition to current remediation methods. Phytoremediation is such a technique, (the use of plants to remove, or render environmental contaminants harmless) that has gained an increasing interest during the past few years. A plant cover effectively prevents contaminant spread by minimizing wind erosion and surface run-off, as well as by reducing percolation to the ground water. Plants may be used to remove contaminants from soil by phytoextraction and then harvested for processing (Cunningham et al., 1995; Chaney et al., 1997). There are some promising results suggesting that these techniques might become viable alternatives to mechanical and chemical approaches in remediation of metal contaminated soils through biotechnological assets.

Biotechnology is transforming the world of agriculture, adding new traits to crop plants at a greatly accelerated rate. Plants are becoming more efficient producers of food, fiber, medicines, and construction materials. In addition to these conventional uses, biotechnology opens doors to unique uses of plants that are gaining greater acceptance in public attention as well as in the scientific community. Improvement of plants by genetic engineering opens up new possibilities for phytoremediation of metal polluted soils. However, this approach can be fully exploited only when the mechanisms of metal tolerance, accumulation and translocation in plants are better understood (Karenlampi et al., 2000).

The experimental material selected for the present study consists of different Brassica juncea L. genotypes. Brassica juncea belongs to family Brassicaceae. The family includes 375 genera and 3,200 species widely distributed throughout the world. There are six species of Brassica that
merit attention for their economic importance. Among the six species, three are diploid: *B. campestris*, *B. nigra*, and *B. oleracea*, and the other three are amphidiploids: *B. juncea*, *B. napus*, and *B. carinata*. The botanical or genomic relationship between these six species was established by U. (1935) and is represented in the form of a triangle usually known as U’s triangle (Fig. 2).

![Genomic relationship between the six Brassica species](image)

**Fig. 2: Genomic relationship between the six Brassica species**

*B. juncea* L. is generally thought to have originated in the Middle East, where the *B. rapa* and *B. nigra* species overlapped in the wild, but Central Asia and China being suggested as sites of primary origin (Prakash, 1980). Hemingway (1976), however, considers that *B. juncea* L. may also have arisen by independent hybridization at secondary centers in India, China and Caucasus, as *B. nigra* was widely used as the commercial spice from early times.

So far the largest numbers of hyperaccumulating species in the Temperate Zone belong to Brassicaceae with *Brassica juncea*, *Alyssum* and *Thlaspi* species as most significant (Kärenlampi et al., 2000; Singh et al.,
2001). *Brassica juncea* has been found to accumulate more than 400μg g⁻¹ dw Cd in the shoot (Haag-Kerwer *et al.*, 1999) and has following advantages to be used as a potential phytoremediator:

- Fast growing and produces high biomass (10t/ha biomass shoot yield), during a short period.
- Naturally equipped for the heavy metal tolerance due to better genetic make up synthesis of "S" and "N-"compounds that provide basis for biotic stress tolerance in general and toxic metal tolerance specifically.
- Cultivated extensively in varied agro climatic conditions throughout the world.
- Oil yielding crops, so that the oil can be obtained as an additional return

So far the plant has been used at a number of polluted sites for the removal of various metals in its natural as well as in transgenic form (Table I & II). The procedure involved in cleaning of the metal contaminated sites through phytoremediation is given in fig. 3.

Table I: Natural population of *B. juncea* L. used for phytoremediation of heavy metal from contaminated soil

<table>
<thead>
<tr>
<th>Plant genotype</th>
<th>Metal target</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. juncea</em> L.cv.17387</td>
<td>Se</td>
<td>de Souza <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>-do-</td>
<td>Se</td>
<td>de Souza <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>-do-</td>
<td>Se</td>
<td>de Souza <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><em>B. juncea</em> L.cv.426308</td>
<td>Cd</td>
<td>Salt <em>et al.</em>,1995</td>
</tr>
<tr>
<td>-do-</td>
<td>Pb</td>
<td>Vassil <em>et al.</em>,1998</td>
</tr>
<tr>
<td><em>B. juncea</em> L.</td>
<td>¹³⁷Cs, ⁹⁰Sr</td>
<td>Adler, 1996</td>
</tr>
<tr>
<td><em>B. juncea</em> L.</td>
<td>Pb</td>
<td>Raskin <em>et al.</em>,1994</td>
</tr>
</tbody>
</table>
Table II: Transgenic *B. juncea* L. used for phytoremediation of heavy metal contaminated soil

<table>
<thead>
<tr>
<th>Foreign gene introduced</th>
<th>Promoter</th>
<th>Vector</th>
<th>Response obtained</th>
<th>Phytoremediation efficiency of transformed plants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis</em> APSI encoding ATP-Sulphurylase</td>
<td>CMV 35S</td>
<td><em>A. tumefaciens</em></td>
<td>Overexpression of plastidic ATP-Sulphurylase</td>
<td>2-3 fold higher Se accumulation in shoots and 1.5 fold higher Se in roots as compared to wild type</td>
<td>Pilon-Smits et al., 1999</td>
</tr>
<tr>
<td><em>E. coli</em> gshII encoding glutathione synthetase (-do-)</td>
<td>-do-</td>
<td>-do-</td>
<td>Overexpression of cytosolic glutathione synthetase</td>
<td>3 fold higher Cd accumulation in transformed plants</td>
<td>Zhu et al., 1999a</td>
</tr>
<tr>
<td><em>E. coli</em> gshI encoding γ-glutamyl cysteine synthetase (GS)</td>
<td>-do-</td>
<td>-do-</td>
<td>Overexpression of γ-glutamyl cysteine synthetase targeted to the plastid</td>
<td>Increased tolerance to Cd, due to the higher accumulation of phytochelatins, glutathione and total non-protein thiols. Transgenic plants accumulated more Cd (40-90% higher) in shoots than wild plants</td>
<td>Zhu et al., 1999b</td>
</tr>
<tr>
<td><em>E. coli</em> gor gene encoding glutathione reductase (GR)</td>
<td>-do-</td>
<td>-do-</td>
<td>Overexpression of the glutathione reductase in the plastids (cpGR) as well as in cytosol (cyst GR)</td>
<td>Reduced Cd uptake and/or translocation; Cd levels in shoots of transgenic plants were half compared to wild plants. 2 times more root GSH levels in transformed plants</td>
<td>Pilon-Smits et al., 2000</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Identification and selection of contaminated sites

\[ \downarrow \]

Cultivation of selected cultivars of *B. juncea* L. at contaminated sites

\[ \downarrow \]

Harvest of plants and removal from that area

\[ \downarrow \]

Separation of pods and seeds

\[ \downarrow \]

Extraction of oil and separation of oil cake

Estimation of toxic metal levels and forms in oil and cake

\[ \downarrow \]

If, present below permissible limit available for market

\[ \downarrow \]

Plant biomass excluding seeds

Burn the cake

\[ \downarrow \]

Smelting the bio ore

\[ \downarrow \]

Recovery of metals

If, present above permissible limits, filter with metal chelating filter

\[ \downarrow \]

Fig. 3: The phytoremediation of contaminated sites employing *Brassica juncea* L.

Centre for Biotechnology
Hamdard University

7
The present study was aimed to find out the difference in the degree of tolerance/resistance among *Brassica juncea* L. genotypes to Cd stress. Emphasis was laid on the characterization of the mechanisms used by these genotypes to hyperaccumulate, translocate and tolerate cadmium. It is expected that an understanding of these processes will make it possible to apply the tools of molecular biology to genetically manipulate the crop plants to improve their metal accumulating efficiency. The findings of this study will also help to genetically engineer other plants for high Cd accumulation efficiency. The following investigations were carried out in this study:

- The effect of varied concentrations of cadmium on growth characteristics of *Brassica juncea* L. genotypes.
- The effect of varied concentrations of cadmium on the distribution and accumulation of Cd in *Brassica juncea* L. genotypes.
- The effect of varied concentrations of cadmium on pigment concentrations of *Brassica juncea* L. genotypes.
- The effect of varied concentrations of cadmium on physiochemical characteristics of *Brassica juncea* L. genotypes.
- The effect of varied concentrations of cadmium on enzymatic and non-enzymatic antioxidant systems of *Brassica juncea* L. genotypes.
- The effect of varied concentrations of cadmium on protein and fatty acid profiles of selected *Brassica juncea* L. genotypes.
- The effect of varied concentrations of cadmium on ultrastructure of chloroplasts and mitochondria of selected *Brassica juncea* L. genotypes.