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Enhancing Phytoremediation Efficiency in Response to Environmental Pollution Stress

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1. Introduction

Environmental pollution, particularly contamination of soil and water resources, has been accelerated as a result of global industrialization and so is considered as a major risk for human communities throughout the world. Due to the adverse effects of organic and inorganic pollutants on human health and environmental safety, it is necessary to be removed in order to minimize the entry of these potentially toxics into the food chain. There are several methods to remove the soil pollutants which are categorized into 3 main parts including chemical, physical and biological methods. While conventional methods of soil clean-up including solidification, vitrification, electrokinetic, excavation, soil washing and flushing, oxidation and reduction etc. have shown to be effective in small areas, they need special equipments and are labor intensive. However, due to the side effects and highly costs of physical and chemical techniques, the biological methods especially phytoremediation, seem to be promising remedial strategies and so are highlighted as alternative techniques to traditional methodologies. Although phytoremediation as a "green technology" has shown many encouraging results, there have also been numerous inconclusive and unsuccessful attempts, especially in the field conditions, mostly because of biotic and abiotic stresses. "Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment" (http://en.wikipedia.org). Abiotic stressors as the plant stress factors including high concentration of organic and inorganic pollutants, salinity, drought, flooding etc. could be considered as the main general themes adversely affect phytoremediation efficiency. Therefore, decrease of abiotic stresses is considered as a promising approach to introduce phytoremediation technique more applicable even though in the present of environmental stressors which significantly affect plant growth and development (Dimkpa et al., 2009; Gerhardt et al., 2009; Weyens et al., 2009).

In this chapter we have discussed phytoremediation and its various types, as well as the plant response to abiotic stresses and the mechanisms which could be efficient to enhance phytoremediation efficiency regarding to abiotic stresses, especially considering environmental pollutants.

2. Phytoremediation

Phytoremediation is defined as an environmental friendly, cheap and large scale method which uses plants and their associated microorganisms to degrade, stabilize, reduce and/or remove organic and inorganic pollutants from the environment (Pilon-Smits, 2005). It can be achieved in several ways including phytoextraction, phytostabilization, phytodegradation, phytovolatilization, phytorestoration, phytomining, rhizosphere-enhanced degradation and rhizofiltration (Vara Prasad & Freitas 2003; Pilon-Smits, 2005).

2.1 Phytoextraction and phytomining

Phytoextraction is the removal of heavy metals and metalloids by plant roots with subsequent transport to shoots (Vara Prasad & Freitas 2003). Generally, plants which can grow in heavy metal contaminated soils and waters are categorized to "tolerant", "indicators" and "hyperaccumulators" (Bert et al., 2003). A tolerant species can grow in contaminated soils while other plants have not this ability. For indicator species, there is a linear correlation between metal concentration in growth media and plant tissues. While both indicator and hyperaccumulator species are also tolerant, however since tolerant species could prevent entering metals to roots, they are not necessarily hyperaccumulators or indicators (Bert et al., 2003). Hyperaccumulators have a high potential to uptake and accumulate heavy metals or metalloids which could be more than 100 fold in comparison with common plants. A hyperaccumulator species has i) the ability to accumulate more than 100 $\mu g\ g^{-1}$ dry weight Cd, 1000 $\mu g\ g^{-1}$ dry weight Ni, Cu, Co, Pb, Se, As and 10000 $\mu g\ g^{-1}$ dry weight Zn and Mn, and ii) the bioconcentration factor (i.e. the ratio of metal concentration in plant to soil) and translocation factor (i.e. the ratio of metal concentration in shoots to roots) greater than 1.0 (Sun et al, 2008). "The technique of phytomining involves growing a hyperaccumulator plant species, harvesting the biomass and burning it to produce a bio-ore" (Anderson et al., 1999).

2.2 Rhizofiltration

Rhizofiltration is a promising technique for removal of heavy metals from aquatic environments using suitable plants which could accumulate metals in their roots and shoots (Vara Prasad & Freitas, 2003).

2.3 Phytostabilization

Phytostabilization, where plants are used to stabilize rather than clean organic and inorganic pollutants in contaminated soils to prevent their movement to surface and groundwater and/or to prevent translocation of pollutants from plant roots to shoots. The latter would be important for prevention of pollutant's transport to the upper levels of food chain (Pilon-Smits, 2005; Vara Prasad & Freitas, 2003). Additionally, in phytostabilization plants accumulate pollutants in their roots or immobilize, precipitate and reduce soil contaminants. Phytostabilization could also be important for reduction of wind and water erosion (Vara Prasad & Freitas, 2003).

2.4 Phytodegradation and rhizosphere-enhanced degradation

Degradation of organic pollutants which are easily entered into the plant tissues or in the rhizosphere through the plant enzymes called phytodegradation. If this phenomenon occurs in plant rhizosphere by enhancing the activity of degrading microorganisms through the release of root exudates, named rhizosphere-enhanced degradation, which in fact is achieved by microbial enzymes rather than plant enzymes (Vara Prasad & Freitas, 2003; Pilon-Smits, 2005).

2.5 Phytovolatization

Plants can also remove toxic substances from soil through phytovolatization. In this process, the soluble contaminants are taken up by the roots, transported to the leaves, and volatized into the atmosphere through the stomata (Vara Prasad & Freitas, 2003; Pilon-Smits, 2005). Some heavy metals such as Hg, As and Se are inactivated when they are translocated from the soil into the atmosphere by bonding to free radicals in the air (Pilon-Smits, 2005).

2.6 Phytorestoration

Phytorestoration involves the complete remediation of contaminated soils to fully functioning soils which is an attempt to return the land to its natural state (Bradshaw, 1997).

3. Plant response to abiotic stresses

Plants react to environmental stresses on various levels including biochemical, cellular and morphological scales depending on type of species or population (Mulder & Breure, 2003). These mechanisms include production of reactive oxygen species by autoxidation and Fenton reaction (for Fe and Cu), blocking of essential functional groups in biomolecules (for Cd and Hg), and displacement of essential metal ions from biomolecules for different kind of heavy metals (Schützendübe & Polle, 2002). Malondialdehyde is a major cytotoxic product of lipid peroxidation and acts as an indicator of free radicals which its production together with chlorophyll, caretonoids, as stress markers, increases in response to metal stress (Ben Ghnaya et al., 2009). Basically, reaction of plants to abiotic stresses depends on type of plant species having fundamental differences in development and anatomy as well as environmental limiting factors (i.e. stressors) (Tester & Bacic, 2005). For example, while a flood may kill most plants in a certain area, but rice would be thrived there.

When plants are faced to metal stress (such as Cd) the abundance of stress-related proteins, like heat shock proteins, proteinases and pathogenesis-related proteins could be changed in leaves and roots. Since roots are not photosynthetical tissues, whereas metal stress could adversely affect CO_2 uptake, electron transport in chloroplasts by damaging photosystem I and II in leaves, proteomic changes in plant tissues upon an abiotic stress exposure would be different (Kieffer et al. 2009). Hence, plant exposed to high level of heavy metals causes reduction in photosynthesis, water and nutrient uptake, growth inhibition and finally death (Yadav, 2010; Soleimani et al., 2010a; Kieffer et al. 2009). The biosynthesis of ethylene as a gaseous plant hormone could be induced in response to environmental stressors which affect germination, growth and development of plant species as well as defence and resistance (Glick, 2004; Kang et al., 2010).

Although, one abiotic stress can usually decrease the ability of plant to resist a second stress (Tester & Bacic, 2005), interaction of various environmental stresses might decrease or increase plant tolerance to the growth limiting factors. For example; though soil salinity usually increases Cd bioavailability in heavy metal polluted soils and subsequently induces their toxicity, chloride salinity increased tolerance of an halophyte species (*Atriplex halimus* L.) to Cd toxicity both by decreasing the absorption of heavy metal and by improving plant tolerance through an increase in the synthesis of osmoprotective compounds in its tissues (Lefevre et al., 2009). The opposite trends were reported in the case of wheat which salinity increased Cd absorption and translocation by plants exposed to the metal in a nutrient solution (Mühling & Läuchli, 2003 In Lefevre et al., 2009; Liu et al., 2007) and for *Elodea canadensis* (Michx.) and *Potamogeton natans* (L.) in the presence of Cd, Cu and Zn (Fritioff et al., 2005). It could also be considered that NaCl might increase the occurance of $CdCl^+$ which may be absorbed by the roots and translocated to the shoots (Lefevre et al., 2009).

Abiotic stresses such as salinity and organic and inorganic pollutants could adversely affect seed germination of plants (Soleimani et al., 2010b; Besalatpour et al., 2008). However, some plants such as *Frankenia* species have been reported to germinate successfully even though in response to abiotic stresses which demonstrate their uses in remediation and revegetation projects in areas affected by salinity (Easton & Kleindorfer, 2009).

Another response of plants upon exposure to heavy metals is oxidative stress which leads to cellular damage. In addition, metal accumulation by plant tissues disturbs cellular ionic homeostasis (Yadav, 2010). Salts and heavy metals could induce oxidative stress in plant which generate active oxygen species and consequently damage plant photosynthetic apparatus resulting in a loss of chlorophyll content and decline in photosynthetic rate and biomass production as well (Qureshi et al., 2005). Total antioxidant activity may increase with increasing environmental pollutants suggesting the capacity of plant to enhance antioxidant defense in response to pollutant stress. Antioxidant enzymes (e.g. dehydroascorbate reductase, glutathione peroxidase, glutathione-S-transferase and superoxide dismutases) may play an important role in plant cell against environmental abiotic stressors (Babar Ali et al., 2005). Reduced forms of phytophenolics act as antioxidant in plant facing to heavy metal stress, while oxidized form (i.e. phenoxyl radicals) can exhibit prooxidant activities under conditions that prolong the radical life time (Dimkpa et al., 2009; Sakihama et al., 2002). Hence, Johnstone et al. (2005) suggested that the test of total antioxidant activity could be mentioned as a new approach to identify putative algal phytoremediator as well as to monitor the effects of water quality on the biological components of polluted aquatic ecosystems.

Generally, the main mechanisms of higher plants in the presence of a metal stress include: stimulation of antioxidant systems in plants, complexation or co-precipitation, immobilization of toxic metal ions in growth media, uptake processes and compartmentation of metal ions within plants (Pilon-Smits, 2005; Liang et al., 2007; Jahangir et al., 2008). To minimize the detrimental effects of heavy metal stress, plants use detoxification mechanisms which are mainly based on chelation and subcellular compartmentalization (Mejáre & Bülow, 2001; Yadav, 2010). A principal class of heavy metal chelator known in plants is phytochelatins (PCs), a family of Cys-rich peptides. PCs are synthesized non-translationally from reduced glutathione in a transpeptidation reaction catalyzed by the enzyme phytochelatin synthase. Therefore, availability of glutathione is very essential for PCs synthesis in plants at least during their exposure to heavy metals (Yadav, 2010). One strategy of plants against xenobiotic stress such as phytotoxic chlorophenols is increasing of extracellular peroxidases enzymes capable of catalyzing their oxidative dechlorination which could be a protection approach of some aquatic plants (e.g. *Spirodela punctata*) against pollution stress (Jansen et al., 2004).

In the case of hyperaccumulators which are extensively used to remediate soil contaminated with heavy metals, the major involved processes in response to excess amounts of metals are i) bioactivation of metals in the rhizosphere through root–microbe interaction, ii) enhanced uptake by metal transporters in the plasma membranes, iii) detoxification of metals by chelation with phytochelatins, metallothioneins, metal-binding proteins in the cytoplasm and/or cell wall, and iv) sequestration of metals into the vacuole by tonoplast-located transporter proteins (Yang et al., 2005).

Understanding the plant response to abiotic stresses, mainly due to excess environmental pollutants, can be important in selecting a suitable approach to prevent decreasing phytoremediation efficiency.

4. Enhancing phytoremediation efficiency

Due to limitations of phytoremediation such as low biomass of hyperaccumulator species, plant sensitivity to high concentrations of environmental pollutants as well as other abiotic stresses and less efficiency of ions and compounds which have low bioavailability to uptake by plants, several approaches have been mentioned in recent decays to boost the efficiency of this technology. Although there are some chemicals (e.g. surfactants and ligands) which may increase phytoextraction, phytodegradation or phytostimulation of pollutants through the enhancement of bioavailability of organic and in-organic compounds in media, nature-based methods like using plant-microorganisms symbiosis not only seem to be more acceptable due to having less side-effects by protection of food chain but could also be efficient in remediation process by increasing plant biomass (Weyens et al., 2009). In the following, we mainly discuss several approaches including plant symbiosis with fungi and bacteria as well as plant genetic engineering which have revealed improvement of phytoremediation efficiency of various environmental pollutants consequently.

4.1 Plant-bacteria symbiosis

Generally there are several bacterial species in the rhizosphere called rhizobacteria. Root zone bacteria which have shown beneficial effects on various plants are named plant growth-promoting rhizobacteria (PGPR) and categorized into 2 main parts; extracellular and intracellular PGPR (Dimkpa et al., 2009). The latter group includes bacteria which are capable of entering the plant as endophytic bacteria and are able to create nodules, whereas extracellular PGPR are found in the rhizosphere, rhizoplane or within the apoplast of the root cortex, but not inside the cells (Dimkpa et al., 2009; Rajkumar et al., 2009). Since endophytic bacteria live within the plant, they could be better protected from biotic and abiotic stresses in comparison to rhizospheric bacteria (Rajkumar et al., 2009).

Plant-associated bacteria can promote plant growth as well as reduce and/or control of environmental stresses which together affect phytoremediation efficiency through several approaches directly and indirectly, within the plant and/or in the rhizosphere (Dimkpa et al., 2009; Glick, 2004, 2010; Kang et al., 2010; Rajkumar et al., 2009; Weyens et al., 2009; Yang et al., 2009). Furthermore, in the case of organic pollutants, there are a number of soil microorganisms that are capable of degrading xenobiotic compounds and consequently reduce their related stress to plants in contaminated soils (Glick, 2010).

Regarding to plant-bacteria symbiosis, there are several mechanisms which induce abiotic stress tolerance within the plant or in the rhizosphere which are mentioned in the following.

4.1.1 Mechanisms underlying abiotic stress tolerance within the plant

They are as follows:

1. Production of phytohormones (e.g. auxins, cytokinins, gibberellins) which can change root morphology is an adaptation mechanism of plant species exposed to environmental stresses (Dimkpa et al., 2009; Weyens et al., 2009). Indole acetic acid as a sub-group of auxins together with nitric oxide are produced in plant shoot transported

to root tips and consequently enhance cell elongation, root growth, root surface area and development of lateral roots (Dimkpa et al., 2009).

2. Inoculation with non-pathogenic rhizobacteria can induce signaling cascades and plant systemic resistance, alter the selectivity for Na, K and Ca ions resulting in higher K/Na ratios and change in membrane phospholipid content as well as the saturation pattern of lipids (Dimkpa et al., 2009).
3. Bacteria may produce osmolytes, such as glycine betaine, act synergistically with plant osmolytes, accelerating osmotic adjustment (Dimkpa et al., 2009).
4. PGPR containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity reduces ethylene level within the plant and consequently facilitates plant growth under stress conditions (Dimkpa et al., 2009; Glick, 2004; Kang et al., 2010). The possible mechanisms described by Kang et al. (2010) accordingly. "ACC synthesized in plant tissues by ACC synthase is thought be exuded from plant roots and be taken up by neighboring bacteria. Subsequently, the bacteria hydrolyze ACC to ammonia and 2-oxobutanoate. This ACC hydrolysis maintains ACC concentrations low in bacteria and permits continuous ACC transfer from plant roots to bacteria. Otherwise, ethylene can be produced from ACC and then cause stress responses including growth inhibition." (Kang et al., 2010).

4.1.2 Mechanisms underlying abiotic stress tolerance in the rhizosphere

They are as follows:

1. Rhizobacterial with the capability of nitrogen fixation can positively influence on host plant growth by increasing nitrogen availability (Dimkpa et al., 2009; Kang et al., 2010; Rajkumar et al., 2009). Therefore they can act as a biofertilizer which affect plant growth (Gerhardt et al., 2009).
2. The mobility of heavy metals in contaminated soils can be significantly reduced through root zone bacteria which finally cause precipitation of metals as insoluble compounds in soil and sorption to cell components or intracellular sequestration (Dimkpa et al., 2009).
3. Bacterial migration from the rhizoplane to the rhizosphere plays a role in reducing plant uptake of some metals (e.g. Cd) in biologically unavailable complex forms (Dimkpa et al., 2009).
4. Iron–chelating siderophores complexes can be taken up by the host plant, resulting in a higher fitness (Dimkpa et al., 2009). They can also form complexes with other non-soluble metals (e.g. Pb) and enhancing their ability to uptake by hyperaccumulators such as *Brassica napus* (Rajkumar et al., 2009).
5. Bacterial exopolysaccharides lead to the development of soil sheaths around the plant root, which reduces the flow of sodium into the stele (Dimkpa et al., 2009).
6. Root zone bacteria can influence pH and redox potential in the rhizosphere, for instance, through the release of organic acids. This can have positive effects on the availability of nutrients (e.g. phosphorous) for the plant (Dimkpa et al., 2009; Weyens et al., 2009; Rajkumar et al., 2009).
7. Under abiotic stress such as high concentration of environmental pollutants causing plant less tolerant in response to biotic stress (e.g. disease, pathogens), PGPR can act as biocontrol agents which mitigate the effect of pathogenic organisms (Gerhardt et al., 2009)

4.2 Plant-fungi symbiosis

One of the approaches to enhance phytoremediation efficiency is using of plant-fungi association. In this regard using of arbuscular mycorrhizal fungi (AMF) that are naturally present in the roots of most plant species where they form a mutualistic association, as well as endophytic fungi which live systemically within the aerial portion of many grass species, can improve plant tolerance to biotic and abiotic stresses (Hildebrandt et al., 2007; Kuldau & Bacon 2008; Lingua et al., 2008; Soleimani et al., 2010a). The role of these groups of fungi on reducing abiotic stresses is mentioned in the following.

4.2.1 AMF and plant abiotic stress

Environmental stresses such as organic and inorganic pollutants trigger oxidative stress commonly showed by increasing content of malondialdehyde in plant. Mycorrhizal fungi are important factors which have the ability to regulate oxidative stress (i.e. reducing the amount of malondialdehyde), as a general strategy, to protect plants from abiotic and biotic stresses (Bressano et al., 2010). Several researches have revealed using AMF as a useful method showing more beneficial effects of phytoremediation, especially in metal contaminated soils (Bressano et al., 2010; Jiang et al., 2008; Lingua et al., 2008; Schützendübe & Polle, 2002). The enhancement of phytoextraction efficiency of *Brassica juncea* L. inoculated with Acacia-associated fungi reported by Jiang et al. (2008). It has been confirmed that mycorrhizal fungi in association with poplars are suitable for phytoremediation purposes underscore the importance of appropriate combinations of plant genotypes and fungal symbionts (Lingua et al., 2008). Furthermore, some fungi have the potential to degrade organic pollutants via extracellular or intracellular oxidation using various enzymes such as laccase, peroxidase, nitroreductase and transferases (Harm et al., 2011), and thereafter reduce stress of organic compounds in soil.

AMF can reduce metal stress in host plants or improve plant growth and development via several ways. Production and excretion of organic acids (e.g. citrate and oxalate) may increase dissolution of primary minerals containing phosphate which is one of the main nutrients for plant (Harm et al., 2011). Furthermore, release of siderophores can enhance iron uptake by plant and boost the growth. In the other hand, increasing the metal solubility or metal-complexing through acidification of the mycosphere could enhance metal uptake by plants which is important in phytoextraction. Extra-hyphal immobilization may occur through the complexation of metals by glomalin (i.e. metal-sorbing glycoproteins excreted by AMF) and biosorption to cell wall constituents such as chitin and chitosan (Harm et al., 2011). This process could be important in phytostabilization of heavy metals in contaminated soils considering that fungal mycelia and glomain could only increase soil aggregate stability against wind and water erosion (Harm et al., 2011). Methalothionin is another protein excreted by some mycorrhizal fungi which can also be important to reduce heavy metal stress in plants (Schutzendubel & Polle, 2002). It would be possible for heavy metals to storage in vacuoles or complex by cytoplasmic metallothioneins in fungi cells or volatilize via metal transformation (Harm et al., 2011). To alleviate heavy metal stress in plants associated with AMF, several genes encoding proteins (e.g. metallothionein, 90 kD heat shock protein, Glutathione-S-transferase) potentially involved in metal tolerance are expressed which are varied in their response to different heavy metals (Hildebrandt et al., 2007). However, improvement of plant mineral nutrition and health and also detoxification of metals in plants associated with AMF could be important to use them in phytoremediation of soil and water contaminated with heavy metals and/or organic pollutants (Lingua et al., 2008).

4.2.2 Endophytic fungi and plant abiotic stress

Endophytic fungi are a group of fungi that live their entire life cycle within the aerial portion of many grass species, forming nonpathogenic, systemic and usually intercellular associations (Soleimani et al., 2010a). Endophytes induce mechanisms of drought avoidance (morphological adaptations), drought tolerance (physiological and biochemical adaptations), and drought recovery in infected grasses (Malinowski and Belesky, 2000). In response to phosphorous deficiency, root morphology of host plant is altered or exudation of phenolic-like compounds may modify the rhizosphere conditions (Malinowski and Belesky, 2000). Aluminium toxicity mainly in acidic soils can be reduced on root surface of endophyte-infected plants through Al sequestration which appears to be related to exudation of phenolic-like compounds with Al-chelating activity (Malinowski and Belesky, 2000). Besides, drought and light stress as well as salt stress could be reduced in endophyte-infected plants via release of some proteins (e.g. dehydrins) and phenolic-like compounds in the rhizosphere (Kuldau & Bacon, 2008; Malinowski and Belesky, 2000). Several researches have also demonstrated the positive effect of endophytic fungi on phytoremediation of heavy metals as well as organic pollutants such as petroleum hydrocarbons (Soleimani et al., 2010a, 2010b). However, there is a lack of information regarding the effect of endophytic fungi on plant tolerance in response to stress of pollutants, especially organic pollutants, in both laboratory and field conditions.

4.3 Transgenic plants

Plants absorb toxic elements by the same pathways they take up essential elements. There should be a vast investigation on the processes involved in metal uptake, transport and storage by hyperaccumulating plants. Improving the number of absorption sites, changing specificity of uptake system to decrease competition by unwanted cations and enhancing intracellular binding sites should be considered to improve heavy metal accumulation in plants (Eapen & D'Souza, 2005). Modification of plant characteristics through the genetic engineering to enhance metal uptake, transport and accumulation as well as plant tolerance to abiotic stresses is a new approach for phytoremediation (Karenlampi,et al., 2000).

The first transgenic plants for phytoremediation were developed to enhance heavy metal tolerance including tobacco plants (*Nicotiana tabacum*) with a yeast metallothionein gene that gives tolerance to cadmium, and *Arabidopsis thaliana* that overexpressed a mercuric ion reductase gene for higher tolerance to mercury. Since each heavy metal may have a specific mechanism for uptake, therefore it is important to design suitable strategies for developing transgenic plants specific for each metal (Eapen & D'Souza, 2005).

There are different possible areas for genetic manipulation to create a suitable transgenic plant for phytoremediation. Generally, genes can be transferred from any living source to develop efficient transgenic plants for phytoremediation. Storage and detoxification of metals in some metal accumulating plants is due to metal storage in epidermal cells. Hence, genes can be inserted and/or overexpressed to produce metallothioneins, phytochelatins and metal chelators to improve plant tolerance and metal accumulation, thus play a role in detoxification of metals in plants (Eapen & D'Souza, 2005; Hassan et al., 2011). Genetic manipulation of metal transporters can be effective in modification of metal tolerance/accumulation in plants. One of the key essential features of metal hyperaccumulators is root-to-shoot translocation of ions. A strong metal sink in the shoots and improved xylem loading and repressed metal sequestration in root vacuoles are possible ways to enhance the root-to-shoot translocation (Hassan et al., 2011). Different metabolic pathways from various organisms can be presented into plants for hyperaccumulation or phytovolatization resulting in plants being more tolerant to heavy metals. Alteration of enzymes which are involved in oxidative stress may also produce an altered metal tolerance in plants. Since having highly branched root systems with large surface area is important for efficient uptake of toxic metals, introduction of genes affecting root biomass can improve rhizofiltration of heavy metals in some hyperaccumulator plants (Eapen & D'Souza, 2005). Besides, the phytoremediation potential of most hyperaccumulating plants is limited because of their low biomass and slow growth and close association with a special habitant. Therefore, biomass of hyperaccumulator plants can be changed by introduction of genes affecting phytohormone synthesis resulting in enhanced biomass (Eapen & D'Souza, 2005; Kotrba et al., 2009). Transgenic plants expressing bacterial ACC deaminase genes can decrease ethylene level which is a major problem that reduces phytoremediation efficiency in plants exposed to abiotic stresses (Kawahigashi, 2009).

Genetically engineered plants can offer new characteristics that may not be met in normal plants (Table 1). Transgenic plants for phytoremediation presenting new or improved characteristics are engineered by the introduction and/or overexpression of genes taken from other organisms, such as bacteria or mammals. Bacteria and mammals are heterotrophs and have the enzymes necessary for achieving complete mineralization of organic pollutants; therefore bacterial and mammalian degradative enzymes can complete the metabolic efficiencies of plants (Van Aken, 2008).

Tolerance to toxic elements is a key factor in bioremediation. Plants which are more persistent in a harsh environment tend to maintain a high biomass and fast growth rate in regions unfavorable for growth and have more time for accumulating metals from the soil. (Eapen & D'Souza, 2005). Metal tolerance can significantly be increased by over-expression of proteins involved in intracellular metal sequestration but may not be utilized for metal accumulation. Tolerance and accumulation are highly independent traits, therefore they should both be manipulated to obtain a suitable plant for phytoremediation. (Eapen & D'Souza, 2005; Karenlampi et al., 2000).

Increasing the plant's ability to convert a toxic element into a less toxic form can improve its tolerance to excess amount of that toxic trace element. Typically, such a plant could be able to accumulate higher amounts of the detoxified form. In order to create a model system for phytoremediation of heavy metals a MerP protein was expressed in transgenic *Arabidopsis*. The transgenic *Arabidpsis* showed higher tolerance and accumulation capacity for mercury, cadium and lead when compared with the control plant (Hsieh et al., 2009). Organomercury can be converted to metallic Hg volatized from the leaf surface in plants with capability to produce bacterial mercuric reductase and organomercurial lyase (Kotrba, et al., 2009). Besides, volatiziation of selenium compounds could be promoted via overexpressing genes encoding enzymes involved in production of gas methylselenide species (Kotrba, et al., 2009). Some of the genetically engineered plants and sources of the genes involved in the process are mentioned in Table 1.

Ferredoxin, a stress-sensitive protein, was replaced in tobacco chloroplasts by an isofunctional protein, a cyanobacterial flavodoxin that usually exist in photosynthetic microorganisms such as algae and bacteria and is missing in plants. The resulting transgenic plants showed tolerance to some abiotic stresses such as drought, chilling, oxidants, heat and iron starvation (Zurbriggen et al., 2008).

There are specific genes called "pollutant-responsive elements" (PRE) that can be induced by the presence of particular toxic chemicals in the environment. They should be identified

and characterized so that they can be fused with reporter genes and be introduced into plants. For example the promoter of the barley gene HvhsplT which is expressed in the presence of some heavy metals had been fused to the reporter gene. This new gene combination was used to make a transformed tobacco plant which could be used as a bioindicator for monitoring heavy metal pollution (Mociardini, et al., 1998).
A combined use of transgenic plants and bacteria in the rhizosphere could improve phytoremediation of contaminated environments and may overcome the current limitations of phytoremediation such as low detoxification and absorption efficiency. The combination of plants for removing or degrading toxic pollutants and rhizospheric microorganisms for improving the availability of hydrophobic compounds can be effective in breaking down

Gene	Target plant	Effect
gshI	*Brassica juncea*	Cd tolerance , Cd, Zn, Cu and Pb accumulation
gshI and gshII	*Arabidopsis thaliana and Brassica juncea*	As and Cd tolerance and accumulation
GSH1 and AsPCS1	*Arabidopsis thaliana*	As and Cd tolerance and accumulation
OAS-TL	*N. tabacum*	Cd and Ni tolerance
APS1	*Brassica juncea*	Se tolerance and accumulation ,Cd accumulation
SMT	*Brassica juncea*	Se accumulation
SMT and APS1	*Brassica juncea*	Se accumulation
merP	*Arabidopsis thaliana*	Hg accumulation and tolerance
ADC	*Oryza sativa*	Heavy metal tolerance
CUP1	*Brassica oleracea Nicotiana tabacum*	Cd tolerance , Cu accumulation
TaPCS1	*Nicotiana. glauca*	Pb and Cd tolerance Pb, Cd, Zn, Cu and Ni accumulation
FRE1 and FRE2	*Arabidopsis thaliana Nicotiana tabacum*	Fe accumulation
merApe9 and merA18	*Arabidopsis thaliana L.tulipifera*	Hg and Au resistance
HisCUP1	*Nicotiana tabacum*	Cd accumulation , Cd tolerance
Ferritin	*Oryza sativa*	Fe accumulation
NtCBP4	*Arabidopsis thaliana*	Ni tolerance, Pb and Ni accumulation
MT-I and MT-II	*Nicotiana tabacum*	Cd tolerance
ZAT	*Arabidopsis thaliana*	Zn tolerance
AtPCS1	*Nicotiana tabacum Brassica juncea*	Cd accumulation, Cd and As tolerance
SOS1	*Oryza sativa*	Heavy metal tolerance
SOD	*Nicotiana tabacum and Zea mays*	Heavy metal tolerance
SAMDC	*Oryza sativa*	Heavy metal tolerance

Table 1. Genes involved in plant genetic engineering for phytoremediation of heavy metals (Hsieh et al., 2009; Karenlampi et al., 2000; Kotrba et al., 2009; Kolodyazhnaya et al., 2009).

many types of toxic chemicals (Kawahigashi, 2009). Special bacterial genes which encode enzymes involved in the breakdown of explosives, such as cytochrome P450 and nitroreductase have been also used in manipulating higher plants to enhance plant tolerance, uptake, and detoxification of contaminated environments (Van Aken, 2009).

Therefore, using transgenic plants or combined use of them with microorganisms in the rhizosphere could be mentioned as a promising technique to reduce abiotic stresses in plants which are used in phytoremediation. Future researches, especially in the field conditions, can distinguish the efficiency of this approach.

5. Conclusion

Abiotic stresses, especially due to high level of organic and inorganic pollutants, are major limiting factors which could adversely affect phytoremediation. To reduce the effects of these stresses in plants which are used in phytoremediation, using bacteria- or fungi symbiosis as well as plant genetic engineering could be a promising way to enhance remediation efficiency. In practical point of view, it should be considered that combined enhanced-phytoremediation approaches are possible to use too. Finally, understanding the involved mechanisms in the mentioned enhancing methods would be a useful tool to extend use of phytoremediation based on these approaches. Since most of researches have been carried out in laboratory conditions, field trials are needed to perform in contaminated sites.

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