Biotechnology Advances 27 (2009) 474–488

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics

P.C. Abhilash, Sarah Jamil, Nandita Singh *

Eco-Auditing Group, National Botanical Research Institute, Council of Scientific & Industrial Research, Rana Pratap Marg, Lucknow 226 001, Uttar Pradesh, India

A R T I C L E I N F O

Article history:
Received 14 October 2008
Received in revised form 26 March 2009
Accepted 3 April 2009
Available online 14 April 2009

Keywords:
Phytoremediation
Cytochrome P450s
Glutathione S-tranferases
Bacterial enzymes
Transgenic plants
Metabolism
Organic xenobiotics

A B S T R A C T

Phytoremediation — the use of plants to clean up polluted soil and water resources — has received much attention in the last few years. Although plants have the inherent ability to detoxify xenobiotics, they generally lack the catabolic pathway for the complete degradation of these compounds compared to microorganisms. There are also concerns over the potential for the introduction of contaminants into the food chain. The question of how to dispose of plants that accumulate xenobiotics is also a serious concern. Hence the feasibility of phytoremediation as an approach to remediate environmental contamination is still somewhat in question. For these reasons, researchers have endeavored to engineer plants with genes that can bestow superior degradation abilities. A direct method for enhancing the efficacy of phytoremediation is to overexpress in plants the genes involved in metabolism, uptake, or transport of specific pollutants. Furthermore, the expression of suitable genes in root system enhances the rhizodegradation of highly recalcitrant compounds like PAHs, PCBs etc. Hence, the idea to amplify plant biodegradation of xenobiotics by genetic manipulation was developed, following a strategy similar to that used to develop transgenic crops. Genes from human, microbes, plants, and animals are being used successfully for this venture. The introduction of these genes can be readily achieved for many plant species using *Agrobacterium tumefaciens*-mediated plant transformation or direct DNA methods of gene transfer. One of the promising developments in transgenic technology is the insertion of multiple genes (for phase 1 metabolism (cytochrome P450s) and phase 2 metabolism (GSH, GT etc.) for the complete degradation of the xenobiotics within the plant system. In addition to the use of transgenic plants overexpressed with P450 and GST genes, various transgenic plants expressing bacterial genes can be used for the enhanced degradation and remediation of herbicides, explosives, PCBs etc. Another approach to enhancing phytoremediation ability is the construction of plants that secrete chemical degrading enzymes into the rhizosphere. Recent studies revealed that accelerated ethylene production in response to stress induced by contaminants is known to inhibit root growth and is considered as major limitation in improving phytoremediation efficiency. However, this can be overcome by the selective expression of bacterial ACC deaminase (which regulates ethylene levels in plants) in plants together with multiple genes for the different phases of xenobiotic degradation. This review examines the recent developments in use of transgenic-plants for the enhanced metabolism, degradation and phytoremediation of organic xenobiotics and its future directions.

© 2009 Elsevier Inc. All rights reserved.

Contents

1. Introduction . . . 475
2. Transgenic plants for environmental remediation . . . 476
3. Degradation pathways in plants . . . 476
4. Cytochrome P450s: environmental perspectives . . . 477
5. Insertion of CYP450 in higher plants for enhanced xenobiotic metabolism . . . 477
   5.1. Transgenic plants expressing human P450s for herbicide metabolism . . . 477
      5.1.1. Transgenic rice . . . 479
      5.1.2. Transgenic tobacco and potato plants . . . 479
   5.2. Transgenic plants expressing human P450s for halogenated hydrocarbon metabolism . . . 479
      5.2.1. Transgenic poplars. . . . 479
   5.3. Plant cell suspension cultures as expression system for human P450 isoenzymes . . . 480

* Corresponding author. Tel./fax: +91 522 2205847.
E-mail addresses: pcabhilash@gmail.com (P.C. Abhilash), nanditasingh8@yahoo.co.in (N. Singh).

0734-9750/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.biotechadv.2009.04.002

6. Glutathione S-transferases (GSTs): environmental perspectives . . . 480
7. Transgenic plants overexpressing GSTs for enhanced degradation/conjugation of organic xenobiotics . . . 480
8. Transgenic plants for the enhanced remediation of explosives. . . . 481
9. Transgenic plants for the rhizoremediation of organic xenobiotics . . . 482
10. Transgenic plants to be developed for the phytoremediation of some other priority pollutants . . . 483
11. Conclusions and future directions . . . 484
Acknowledgements . . . 485
References . . . 485

1. Introduction

Environmental pollution with organic xenobiotics (pesticides, pharmaceuticals, petroleum compounds, PAHs, PCBs etc.) is a global problem, and the development of inventive remediation technologies for the decontamination of impacted sites are therefore of paramount important. Physical, chemical and biological methods can all be used for the remediation of contaminated sites (Lee and Huffman, 1989; Felsot and Dzantor, 1995; Johnson et al., 1997; Hatakeda et al., 1999; Wirtz et al., 2000; Kummling et al., 2001; Perrin-Ganier et al., 2001; Matsunaga and Yashuhara, 2003; Gao et al., in press; Yang et al., 2007), however, phytoremediation has long been recognized as a cost effective method for the decontamination of soil and water resources (Salt et al., 1998; Macek et al., 2000; Meagher, 2000; Eapen and D'Souza, 2005). Further, a variety of pollutant attenuation mechanisms possessed by plants makes their use in remediating contaminated land and water more feasible than physical and chemical remediation (Glick, 2003; Huang et al., 2004, 2005; Greenberg, 2006; Gerhardt et al., 2009). As a result of their sedentary nature, plants have evolved diverse abilities for dealing with toxic compounds in their environment. Plants act as solar-driven pumping and filtering systems as they take up contaminants (mainly water soluble) through their roots and transport/translocate them through various plant tissues where they can be metabolized, sequestered, or volatilized (Cunningham et al., 1996; Greenberg et al., 2006; Abhilash, 2007; Doty et al., 2007).

When microorganisms are used for remediation of xenobiotics, both inoculation of microorganisms and nutrient application are essential for their maintenance at adequate levels over long periods (Eapen et al., 2007). Besides, the microbes which show highly efficient biodegradation capabilities under laboratory conditions may not perform equally well at actual contaminated sites (Goldstein et al., 1985; Macek et al., 2008). The potential of genetic engineering to accelerate the bioremediation of xenobiotics has been recognized since the early 1980s, with initial attempts being focused on microorganism (Rugh et al., 1998; Rosser et al., 2001; Sung et al., 2003; Rugh, 2004; Doty, 2008; Singleton, 2007). However, there are two main problems with the introduction of transgenic microorganisms: the bureaucratic barriers blocking their release into the environment and the poor survival rate of those engineered strains that have been introduced into the contaminated soil. Phytoremediation for removal of xenobiotics can be an alternate/supplementary method, since plants are robust in growth, are a renewable resource and can be used for in situ remediation (Cunningham and Berti, 1993; Cunningham et al., 1995, 1996; Cunningham and Ow, 1996; Suresh and Ravishankar, 2004; Parameswaran et al., 2007). It has almost certain public acceptance. Further, plants may survive higher concentrations of hazardous wastes than many micro-organisms used for bioremediation. Phytoremediation increases the amount of organic carbon in the soil, which can stimulate microbial activity and augment the rhizospheric degradation of the pollutants. Phytoremediation also yields other benefits including carbon sequestration, soil stabilization, and the possibility of biofuel or fiber production. The development of phytoremediation technologies for the plant-based clean-up of contaminated soils is therefore of significant interest (Hooker and Skeen, 1999; Dietz and Schnoor, 2001; Eapen and D'Souza, 2005).

With respect to their direct roles in remediation processes, plants use several different strategies for dealing with environmental chemicals: phytoextraction, phytodegradation, phytovolatilization, and rhizodegradation (Fig. 1) (Schnoor, 1997). Phytoextraction involves the removal and subsequent storage of contaminants by the plant and is often applied to the exclusion and storage of metals that may undergo speciation in plants, but cannot be metabolized. However, certain organic chemicals may also be treated in this manner due to inherent resistance to degradation. Conversely, phytodegradation describes processes in which plants metabolize the contaminants they take up. Components of this mechanism are often utilized by plants exposed to herbicides and thus have been researched extensively. The metabolic processes involved in phytodegradation have strong similarities to those used by animals for modification and degradation of drugs and other toxins. This has given rise to a conceptual model for phytodegradation known as the "green liver" model (Sanderman, 1994). A further attenuation mechanism, referred to as phytovolatilization, involves the release of contaminants to the atmosphere following their uptake from the soil or water. This mechanism has been observed for both organic and heavy metal contaminants, including trichloroethylene (TCE), which has been observed in the off-gas from plant leaves in the laboratory and field (Compton et al., 1998), and in the production of volatile, elemental mercury by genetically-engineered *Arabidopsis thaliana* grown in the presence of ionic mercury (Rugh et al., 1996; Bizily et al., 1999). An indirect mechanism, rhizodegradation refers to the transformation of contaminants by resident microbes in the plant rhizosphere (i.e., the microbe-rich zone in intimate contact with the root vascular system). As mentioned above, the presence of plants on contaminated sites can drastically affect soil redox conditions and organic content (often through the secretion of organic acids from roots), as well as soil moisture. Rhizodegradation is the dominant mechanism in the removal of total petroleum hydrocarbons from soil by deep-rooted trees (Carman et al., 1998), as well as annual species (Schwab and Banks, 1994).

Although much research has been done to demonstrate the success of phytoremediation, resulting in its use on many contaminated sites, (Aprill and Sims, 1990; Gunther et al., 1996; Binet et al., 2000; Liste and Alexander, 2000; Mattina et al., 2000; White, 2000, 2001, 2002; Fismes et al., 2002; Li et al., 2002; Maila and Cloete, 2002; Yoon et al., 2002; Singh and Jain, 2003; Sung et al., 2003; Sunderberg et al., 2003; Trapp et al., 2003; White, 2003; Gao and Zhu, 2004; Ma et al., 2004; Mattina et al., 2004; Suresh et al., 2005; Parrish et al., 2006; Mills et al., 2006; Aslund et al., 2007; Kobayashi et al., in press) the method still lacks wide application. Further, detoxification of organic pollutants by plants is often slow, leading to the accumulation of toxic compounds in plants that could be later released into the environment (Aken, 2008). There are also concerns over the potential for introduction of contaminants into the food chain. The question of how to dispose of plants that accumulate organic pollutants is also a serious concern. A direct method for enhancing the effectiveness of phytoremediation is to overexpress in transgenic plants the genes involved in metabolism, uptake, or transport of specific pollutants (Shiota et al., 1994; Rugh, 2004; Cherian and Oliveira, 2005; Kramer, 2005; Eapen et al., 2007; Macek et al., 2008; Aken, 2008; Doty, 2008). If the plants are able to degrade the xenobiotics to non-toxic metabolites or completely mineralized into carbon dioxide, nitrate, chlorine etc., there is no apprehension over hazardous waste

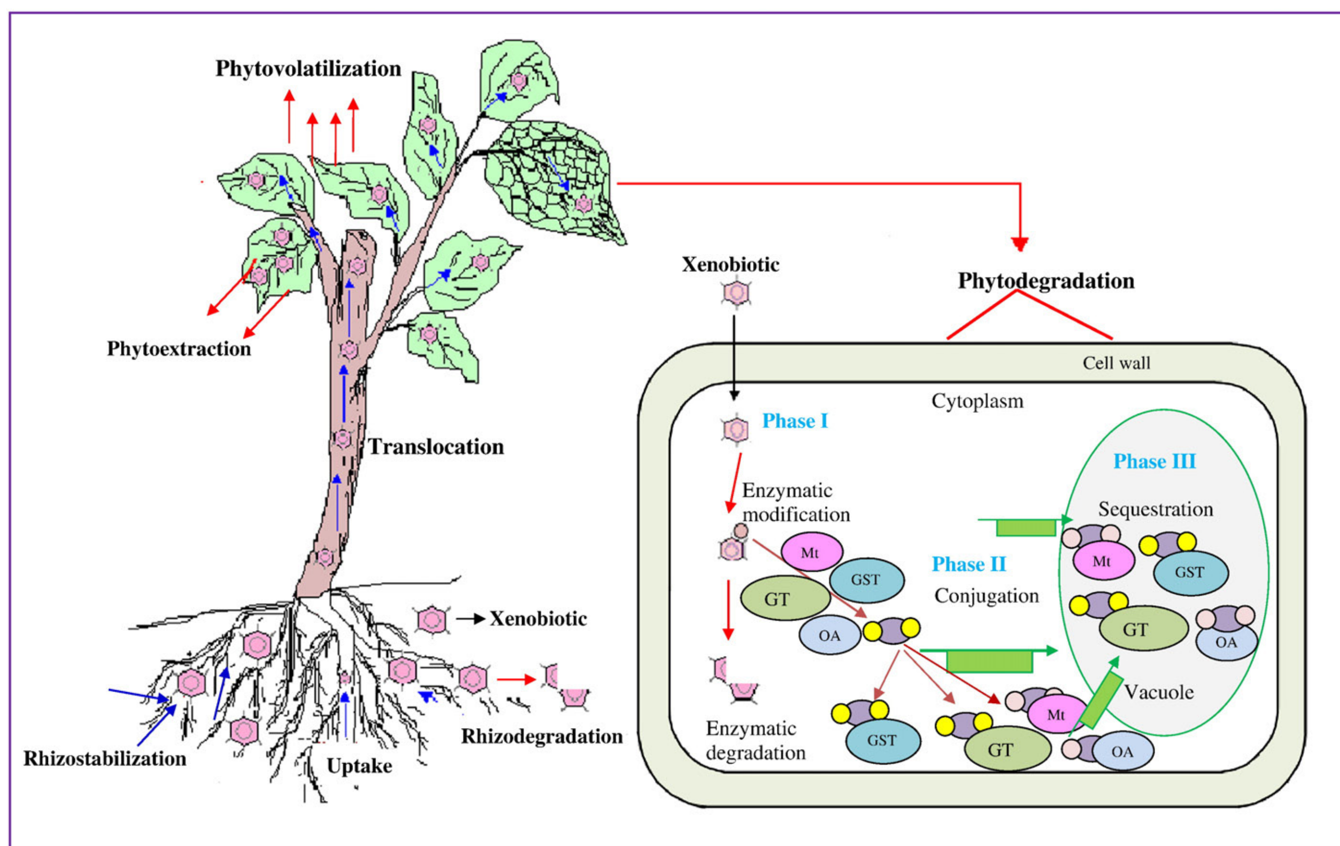

Fig. 1. Typical attenuation mechanism possessed by plants against xenobiotics. The xenobiotics can be stabilized or degraded in the rhizosphere, adsorbed or accumulated in to the roots and transported to the aerial parts, volatilized or degraded inside the plant tissue. Plant detoxification generally involves conversion or enzymatic modification (phase I) followed by conjugation (phase II) followed by active sequestration (phase III). Active transporters are marked in green boxes (GST = glutathione *S*-transferases; GT = glucosyltransferases; Mt = Malonyltransferases; OA = organic acids (Newman and Reynolds, 2004; Pilon-Smits, 2005). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

management strategies for disposing of harvested plants. The purpose of this review is to provide recent advances in development of transgenic plants overexpressing catabolic genes including bacterial and human cytochrome P450 for the enhanced degradation and mineralization of xenobiotic pollutants.

2. Transgenic plants for environmental remediation

The concept that plants can degrade xenobiotics emerged in 1940s, when plants were shown to metabolize pesticides (Sanderman, 1994). Since then, the development of genomics, proteomics, and metabolomics has contributed much to enhance or manipulate the plant metabolism of many xenobiotic pollutants (Raskin, 1996; Eapen et al., 2007; Aken, 2008). Although phytoremediation has first applied for the removal of inorganic pollutants from soil, this technology has gradually proven to be efficient for the treatment of organic pollutants including chlorinated solvents, polyaromatic hydrocarbons and explosives (Pilon-Smits, 2005; Salt et al., 1998). The first generation of commercially available transgenic plants were produced to reduce the loss of crop yield caused by insect damage at the same time as reducing the amount of pesticides required (e.g. plants expressing Bt toxin). However, transgenic plants for phytoremediation were first developed for remediating heavy metal contaminated soil sites; for example, *Nicotiana tabaccum* expressing a yeast metallothionein gene for higher tolerance to cadmium, or *Arabidopsis thaliana* overexpressing a mercuric ion reductase gene for higher tolerance to mercury (Misra and Gedama, 1989; Rugh et al., 1996). The first attempt to develop engineered plants for phytoremediation of organic pollutants targeted explosives and halogenated organic compounds in tobacco plants (French et al., 1999; Doty et al., 2000). The efficiency of transgenic plants to degrade chlorinated solvents, explosives, phenolics etc. have been extensively acknowledged in the literature (McCutcheon and Schnoor, 2003; Mackova et al., 2006; Meagher, 2000; Eapen et al., 2007; Doty, 2008; Macek et al., 2008). These plants have been developed to contain either transgenes responsible for the metabolization of xenobiotics or transgenes that result in the increased resistance of pollutants.

3. Degradation pathways in plants

Xenobiotic metabolism in human, animals and higher plants usually happen through three main biochemical processes; conversion or transformation (phase I), conjugation (phase II), and compartmentalization (phase III) (Schmidt et al., 2006a,b). During phase I, hydrophobic pollutants are converted to less hydrophobic metabolites through N-, O-, and S-dealkylation, aromatic and aliphatic hydroxylation, epoxidation, peroxidation, oxidative desulfuration, sulfoxidation or reduction by cytochrome P450s. Reactions catalyzed by cytochrome P450s are initial vital steps leading to detoxification, inactivation and excretion (Schmidt et al., 2006a,b). This conversion usually produces less toxic metabolites. In phase II, organic pollutants or their phase I metabolites are directly conjugated with glutathione, sugars, or aminoacids to produce hydrophilic compounds. Finally, in phase III, conjugated metabolites are deposited in vacuoles or cell walls (Hatzioz, 1997). Recently, the last phase of metabolism has been categorized into two independent phases, one confined to transport

and storage in the vacuole, and a second one taking final reactions (cell wall bindings or excretion) (Theodoulou, 2000; Schroder, 2007).

4. Cytochrome P450s: environmental perspectives

Cytochrome P450s enzymes comprise a superfamily of heme proteins crucial for the oxidative, peroxidative, and reductive metabolism of a diverse group of compounds, including endobiotics, such as steroids, bile acids, fatty acids, prostaglandins, and leukotrienes, and xenobiotics, including most of the therapeutic drugs and environmental pollutants (Klingenberg, 1958; Nelson et al., 1996; Kreuz et al., 1996). The first report on the existence of a CYP enzyme or a microsomal carbon monoxide-binding pigment was published in 1958 by Klingenberg et al (Klingenberg et al., 1958). This enzyme gave a unique 450-nm optical absorption peak, and when its hemoprotein nature was recognized, it was given the name cytochrome P450 (Omura and Sato, 1962, 1964; Omura, 1999). In almost all living organisms, these enzymes are present in more than one form, thus forming one of the largest families of enzymes. The enzyme system is located in microsomes and consists of several cytochrome P450 isoforms and a nonspecific NADPH-cytochrome P450 oxidoreductase. The notable diversity of CYP enzymes has given rise to a systematic classification of individual forms into families and subfamilies. The protein sequences within a given gene family are at least 40% identical (e.g. CYP2A6 and CYP2B6), and the sequences within a given subfamily are >55% identical (e.g. CYP2A6 and CYP2A7) (Nelson et al., 1996). However, the number of families and enzymes varies among different organisms. The numbers of different CYP families and genes discovered so far in few representative groups are shown in Table 1.

Although cytochrome P450 (P450 or CYP) monooxygenases in higher plants play an important role in the oxidative metabolism of endogenous and exogenous liphophilic compounds (Inui et al., 2000; Eapen et al., 2007; Doty, 2008), molecular information on P450 species metabolizing xenobiotics in plants is quite limited. On the other hand, there are a number of P450 species metabolizing xenobiotics in the microsomes of human liver (Inui et al., 2000). Humans have been estimated to have at least 53 different *CYP* genes and 24 pseudogenes (Nelson, 1999). So far, it has been reported that 11 P450 species (Table 2) in human liver are involved in over 90% P450-dependent metabolism of drugs (Funae et al., 1998; Inui et al., 2000; Kawahigashi et al., 2006a,b; 2007;2008). These P450s oxidize carbon and nitrogen, usually resulting in the formation of a hydroxyl group and occasionally the subsequent removal of alkyl groups, which is similar to the metabolic processes in plants (Kawahigashi et al., 2008). A study of 11 human P450s in the CYP1, 2, and 3 families using a recombinant yeast expressing system showed that they can metabolize 27 herbicides and 4 insecticides (Inui et al., 2001). Further, another study conducted by same research group found that human CYP1A1 metabolized 16 herbicides, including triazines, ureas, and carbamates, and CYP2B6 metabolized more than 10 herbicides, including chloroacetanilides, oxyacetamides, and 2,6-dinitroanilines, three insecticides, and two industrial chemicals (Inui et al., 2001).

Table 1
Number of CYP families and species found in selected groups.

Taxonomical groups	No of CYP families	No. of sequences
Animals	99	2279
Plants	97	1932
Fungi	282	1001
Bacteria	177	621
Protists	51	210
Archaea	5	8

Table 2
Human CYP families and their main functions (Graham-Lorence and Petersen, 1999a,b).

Cytochrome family	Main functions
CYP1	Xenobiotic metabolism
CYP2	Xenobiotic metabolism Arachidonic acid metabolism
CYP3	Xenobiotic and steroid metabolism
CYP4	Fatty acid hydroxylation
CYP5	Thromoboxane synthesis
CYP7	Cholesterol 7α-hydroxylation
CYP8	Prostacyclin synthesis
CYP11	Cholesterol side-chain cleavage Steroid 11β-hydroxylation Aldosterone synthesis
CYP17	Steroid 17α-hydroxylation
CYP19	Androgen aromatization
CYP21	Steroid 21-hydroxylation
CYP24	Steroid 24-hydroxylation
CYP26	Retinoic acid hydroxylation
CYP27	Steroid 27-hydroxylation
CYP39	Unknown
CYP46	Cholesterol 24-hydroxylation
CYP51	Sterol biosynthesis

CYP2C19 metabolized sixteen herbicides, including triazines, ureas, and carbamates (Inui et al., 2001).

5. Insertion of CYP450 in higher plants for enhanced xenobiotic metabolism

During the last two decades, numerous experiments were conducted on the overexpression of human and mammalian (e.g. rat, mouse, rabbit) CYP450 isoenzymes (CYP1, CYP1, CYP3) in higher plants such as *Nicotiana tabaccum*, *Solanum tuberosum*, *Oryza sativa* or *Arabidopsis thaliana.* The introduction of these genes can be readily achieved for many plant species using *Agrobacterium tumefaciens*-mediated plant transformation or direct DNA gene transfer (Doty, 2008). Microsomes containing selected human P450s are commercially available which are produced by means of bacterial (*Escherichia coli*) or baculovirus expression system (Schmidt et al., 2006a,b). The integration and expression of this transgene can be confirmed by southern, northern and western blot analysis (Kawahigashi et al., 2007). The primary objective of these genetic manipulations were the production of either herbicide resistant plant (e.g. tolerance towards atrazine, simazine) or plants capable for enhanced metabolization of xenobiotics (herbicides or volatile halogenated hydrocarbons) and their subsequent removal from contaminated soil and ground water. Many of these plants were overexpressed with a single (e.g. *CYP1A1* or *CYP2E1*) or several P450 genes (e.g. *CYP1A1*, *Cyp2B6*, and *CyP2C19*). Partially, plants were additionally modified with NADPH-cytochrome P450 reductase, or P450 and reductase were expressed as fusion enzyme (Shiots et al., 2000; Schmidt et al., 2006a,b). Due to the broad substrate specificity of human and mammalian P450s, the transgenics showed remarkable improvement of metabolic degradation towards single or multiple xenobiotics (Table 3).

5.1. Transgenic plants expressing human P450s for herbicide metabolism

Herbicides are economically important because they prevent losses in crop yield due to weed infestation (Lockhart et al., 1990; Kawahigashi et al., 2008). However, the overuse and repeated use of same herbicide can lead to the development of herbicide resistant weeds. According to the Weed Science Society of America, over 310 biotypes of herbicide resistant weeds have been reported in agricultural fields and gardens worldwide. As a result of these herbicide tolerance, larger amount of herbicides are needed to kill these weeds, so that residues contaminate the soil and nearby water bodies (Kawahigashi et al., 2006a,b; 2007). Plants used for decontamination of these contaminated system should be resistant to herbicides. The two primary strategies in agricultural corps against

Table 3
Transgenic plants for enhanced phytoremediation of xenobiotics.

Target plant	Gene (s)	Enzymes	Source	Transgene effects	Reference
Oryza sativa	*CYP1A1*	Cytochrome P450 monoxygenase	Human	Enhanced metabolism of chlorotoluron, norflurazon	Kawahigashi et al., 2007, 2008
Nicotiana tabaccum	*onr*	Pentaerythritol tetranitrate reductase (PETN)	*Enterobacter cloaceae*	Enhanced denitration of glycerol trinitrate (GTN) and TNT	French et al., 1999
N. tabaccum	*NfsI*	Nitroreductase	*E. cloaceae*	The transgenic plants removed high amount of TNT from the test solution and reduction of TNT to 4-hydroxylamino-2, 6-dinitrotoluene.	Hannink et al., 2001, 2007
A. thaliana	*NfsA*	Nitroreductase	*E. coli*	The plants showed higher nitroreductase activity and 7-8 times higher uptake compared with wild plants.	Kurumata et al., 2005.
A. thaliana	*XplA and XplB*	Cytochrome P450 monoxygenase	*Rhodococcus rhodochorus*	Enhanced degradation of RDX	Jackson et al., 2007
Hybrid aspen (*P. tremula x P. tremuloides*)	*pnrA*	Nitroreductase	*Pseudomonas putida*	The transgenic aspen (hybrid) was shown to tolerate and take up greater amounts of TNT from contaminated water and soil.	Van Dillewijn et al., 2008
Hybrid poplar (*Populus tremula x populous alba*)	*CYP450 2E1*	Cytochrome P450 monoxygenase	Rabbit	Increased removal of TCE, vinyl chloride, carbon tetrachloride, benzene and chloroform from hydroponic solution and air	Doty et al., 2007
O. sativa	*CYP1A1*	Cytochrome P450 monoxygenase	Human	Remediation of atrazine and simazine	Kawahigashi et al., 2005b
O. sativa	*CYP1A1, CYP2B6 and CYP2C19*	Cytochrome P450 monoxygenase	Human	Phytoremediation of atrazine and metolachlor	Kawahigashi et al., 2006a
Solanum tuberosum, O. sativa	*CYP1A1, CYP2B6,* and *CYP2C19*	Cytochrome P450 monoxygenase	Human	Resistance to sulfonylurea and other herbicides	Inui and Ohkawa, 2005.
N. tabaccum	*CYP105A1*	Cytochrome P450 monoxygenase	*Streptomyces griseolus*	Resistance to sulfonylurea	O'keefe et al., 1994.
O. sativa	*CYP2C9*	Cytochrome P450 monoxygenase	Human	Tolerance to sulfonylurea	Hirose et al., 2005.
N. tabaccum	*CYP450 2E1*	Cytochrome P450 monoxygenase	Human	Oxidation of TCE and ethylene dibromide	Doty et al., 2000
O. sativa	*CYP2B6*	Cytochrome P450 monoxygenase	Human	Metabolism of ethofumesate and benfuresate	Kawahigashi et al., 2005c
O. sativa	*CYP2B6*	Cytochrome P450 monoxygenase	Human	Remediation of metachlor	Kawahigashi et al., 2005a
O. sativa	*CYP2B22, CYP2C49*	Cytochrome P450 monoxygenase	*Sus scrofa*	Tolerance to several herbicides	Kawahigashi et al., 2005c
N. tabaccum, A. thaliana	*CYP71A10*	Cytochrome P450 monoxygenase	*Glycine max*	Tolerance to phenyl urea herbicide	Siminszky et al., 1999
N. tabaccum	*tpx1*and *tpx2*	Peroxidases (Px)	*Lycopersicon esculentum*	Hairy cultures of transgenic tobacco showed enhance removal of phenol	Alderete et al., 2009
N. tabaccum	*CYP76B1*	Cytochrome P450 monoxygenase	*Helianthus tuberosus*	Tolerance to herbicide	Didierjean et al., 2002.
N. tabaccum	*GstI-6His*	Glutathione S-transferases (GST I)	Maize	Higher tolerance to alachlor	Karavangeli et al., 2005.
N. tabaccum	*bphc*	2,3, dihydroxybiphenyl-1, 2-dioxygenase	PCB degrading bacteria	Enhanced degradation of PCBs	Chrastilova et al., 2007
Lycopersicon esculentum	*tpxI*	Peroxidases (Px)	Roots of *L. esculentum*	The overexpression of *tpxI* gene in transgenic tomato hairy roots resulted in the enhanced removal of phenol.	Oller et al., 2005
Alfalfa N. tabaccum	*atzA*	Atrazine chlorohydrolase	Bacteria	Enhanced metabolic activity against atrazine	Wang et al., 2005
A. thaliana N. tabaccum	*Mn* peroxidase gene	Peroxidases	*Coriolus versicolor*	Enhanced removal of pentachlorophenol (PCP)	Iimura et al., 2002
O. sativa	*Protox*	Protoporphyrinogen IX oxidase	*Bacillus subtilis*	Tolerance to diphenyl ether herbicide oxyflufen	Jung et al., 2008
A. thaliana	*743B4, 73C1*	Glycosyltransferases (UGTs)	*A. thaliana*	Overexpression of UGTs genes resulted in the enhanced detoxification of TNT and enhanced root growth.	Gandia-Herrero et al., 2008
N. tabaccum	*ophc2*	Organophosphorus hydrolase (OPH)	*Pseudomonas pseudoalcaligenes*	Enhanced degradation of organophosphorus (methyl parathion).	Wang et al., 2008
Brassica juncea	*γ-ECS, GS*	γ-Glutamycysteine synthetase; Glutathione synthetase	*Brassica juncea*	Overexpression of ECS and GS resulted in enhanced tolerance to atrazine, 1-chloro-2, 4-dinitrobenzene, phenanthrene, metolachlor.	Flocco et al., 2004
Populus trichocarpa	*γ-ECS*	γ-Glutamycysteine synthetase	Poplar	Overexpression of *γ-ECS* resulted in increased tolerance to chloroacetanilide herbicides.	Gullner et al., 2001
N. tabaccum	*CYP450E1*	Cytochrome P450 monoxygenase	Human	Enhanced degradation of anthracene and chloropyriphos	Dixit et al., 2008
	GST	Glutathione-*S*-transferase	*Trichoderma virens*		
Transgenic plants for enhanced rhizodegradation					
A. thaliana	*LACI*	Root specific laccase	Cotton	Secretes laccase to the rhizosphere and have shown enhanced resistance to phenolic alleleochemicals and enhanced tolerance to 2, 4, 6,-trichlorophenol.	Wang et al., 2004

Table 3 (*continued*)

Target plant	Gene (s)	Enzymes	Source	Transgene effects	Reference
N. tabaccum	*LAC*	Fungal laccase	*Coriolus versicolar*	Secretion of laccase into the rhizosphere and remove the pollutants bisphenol A and PCP.	Sonoki et al., 2005
N. tabaccum	Biphenyl dioxygenase gene	Biphenyl dioxygenase	*Burkholderia xenovorans*	Catalyse the oxygenation of 4-chlorobiphenyls.	Mohammadi et al., 2007
A. thaliana	*DbfB*	Aromatic-cleaving extradiol dioxygenase	*Terrabacter* sp.	Enhanced detoxification of 2, 3-dihydroxybiphenyl (2, 3-DHB)	Uchida et al., 2005
N. tabaccum	*DhaA*	Haloalkane dehydrogenase	*Terrabacter* sp.	Enhanced detoxification of 1-chlorobutane in rhizosphere	Uchida et al., 2005
N. tabaccum	*bphC*	Biphenyl catabolic enzymes	*Pandoraea pnomenusa*	Enhanced degradation of PCBs	Francova et al., 2003; Novakova et al., 2009

herbicide tolerance are (i) modification of target sites and (ii) development of enhanced detoxification (Putwain, 2005). Among the various enzymatic group, cytochrome P450 and glutathione S-transferase play major roles in the enhanced degradation of herbicides (Ohkawa et al., 1999). Molecular information on plant P450 related to organic pollutant metabolism is limited, however, many P450-dependent oxidations in plant microsomes have been reported (Kawahigashi et al., 2007), including oxidation of chlorotoluron in maize (Fonne-Pfister and Kreuz, 1990) and wheat (Mougin et al., 1990); linuron in wheat (Frear, 1995) and maize (Moreland et al., 1993); atrazine in tulip (*Tulipa generiana* L.); and isoproturon in yam bean (Belfrod et al., 2004). Although numerous cytochrome genes are reported in plants, only some herbicide metabolizing P450 genes have been cloned and characterized, such as *CYP73A1* and *CYP76B1* from Jerusalem artichoke (*Helianthus tuberosus*) (Pierrel et al., 1994; Robineau et al., 1998), *CYP71A11* from tobacco (*Nicotiana tabaccum*) (Yamada et al., 2000), and *CYP71A10* from soy bean (*Glycine max*) (Siminszky et al., 1999). Fischer et al. (2001) reported sixteen cytochrome P450 species responsible for the herbicide detoxification and cross-tolerance (De Prado et al., 2005) in *Lolium rigidum*.

5.1.1. Transgenic rice

Rice is the staple food grain in many of the most populous countries like Bangladesh, China, Egypt, India and Indonesia (Kawahigashi et al., 2007) and it is predicted that the demand for rice will grow by 25% in the next 25 years (Smill, 2004). To increase rice production, the insertion in plants of P450 transgenes involved in xenobiotic metabolism is considered to be a useful technique for producing rice with cross-resistance to various herbicides (Ohkawa et al., 1999). Further, engineered rice plants expressing human cytochrome P450 genes are expected to be resistant to herbicides and be able to clean up residual agrochemicals (Ohkawa et al., 1999; Kawahigashi et al., 2007). Thus, many researchers introduced a human gene for *CYP1A1* in rice plant through agrobacterium mediated transformation (Kawahigashi et al., 2003). Transgenic rice plants showed normal morphological and physiological features compared with their respective controls, except for the enhanced degradation due to the introduced transgenes (*CYP1A1*). Further, this transgenic rice showed increased tolerance to 10 out of 13 tested herbicides belonging to different chemical families (aryloxyphenoxypropionate (quizalofopethyl); benzamide (isoxaben), carbamate (chlorpropham); dinitroaniline (pendimethalin); oxyacetamide (mefenacet); phosphoamidate (amiprophos-methyl); pyridazinone (norflurazon); thiocarbamate (pyributicarb), and urea (chlorotoluron and diuron) (cross tolerance; Kawahigashi et al., 2008) and should be useful for degrading herbicides and thus decreasing the environmental load (Kawahigashi et al., 2008).

5.1.2. Transgenic tobacco and potato plants

Shiota et al. (1994, 1996) found that transgenic tobacco plants expressing the fused enzyme between rat CYP1A1 and yeast NADPH-Cytochrome P450 oxidoreductase metabolized the herbicide chlorotoluron through N-demethylation and ring-methyl hydroxylation, giving rice to resistance to herbicides. Further, Inui et al. (1999) developed transgenic potato plants expressing human CYP1A1, which showed remarkable cross-tolerance towards atrazine, chlorotoluron and pyriminobac methyl. Later, they accomplished to co-express three human P450 species, *CYP1A1*, *CYp2B6* and *CYP2C9* in transgenic potato plants. These transgenic potato plants exhibited remarkable cross-tolerance toward photosynthesis-inhibiting herbicides (atrazine), chlorotoluron, metabenathiazuron, the lipid biosynthesis-inhibiting herbicides acetochlor and metolachlor and the carotenoid biosynthesis-inhibiting herbicide norflurazon, probably by co-operative herbicide metabolism of the three P540 species (Inui and Ohkawa, 2005).

5.2. Transgenic plants expressing human P450s for halogenated hydrocarbon metabolism

Halogenated volatile hydrocarbons such as trichloroethylene (TCE), vinyl chloride, carbon tetrachloride, benzene and chloroform etc. are the common environmental pollutants that pose serious health effects. They all are proven or probable human carcinogens, neurotoxins and hepatotoxins (Doty et al., 2007). TCE is heavily used as a degreaser throughout the industrialized world and can be persistent in the environment for decade. Vinyl chloride is a derivative of TCE produced from TCE contaminated sites due to microbial dehalorespiration. Another halogenated hydrocarbon, ethylene dibromide (EDB), was used as a soil fumigant to kill nematodes and as a gasoline additive. Chloroform, a byproduct of the disinfection process used to treat drinking water all over the world. Benzene, another proven human carcinogen, is a common pollutant associated with petroleum (Doty et al, 2007). CYP2E1 is an extensively studied mammalian cytochrome that oxides many of these compounds, including styrene, 1, 2-dichloropropane and others. Doty et al. (2000) engineered transgenic tobacco plants overexpressing a human cytochrome P4502E1 for the enhanced metabolism of trichloroethylene (TCE). The preliminary results showed that transgenic tobaccos metabolized TCE 640-fold faster than the wild type. The initial metabolic product of TCE oxidation by P4502E1 is chloral (2, 2, 2-trichloroacetaldehyde), which is further metabolized to trichloroethanol or trichloroacetic acid. Similarly, EDB metabolized to bromoacetaldehyde and bromide ion. The increased metabolism of chlorinated hydrocarbons by transgenic tobacco suggests that introduction of P450 2E1 into deep-rooted, fast growing trees could result in a significantly increased uptake and destruction of these pollutants (Doty et al., 2000). However, thorough study of transgenic plants will be required to verify that toxic intermediates are not released into the environment. Consumption of leaves from non-transgenic poplar exposed to TCE has no harmful effects on herbivores insects (Doty et al., 2000). Later, the same researchers reported the introduction of a rabbit cytochrome P450 in transgenic hairy root cultures of *Atropa belladonna*, which also exhibited a faster metabolism of TCE (Banerjee et al., 2002).

5.2.1. Transgenic poplars

Although much genetic manipulation have been experimented in laboratory model plants like *N. tabaccum* and *A. thaliana*, their small

biomass and shorter life span might not be suitable for onsite remediation. Hence, there is a particular interest in the genetic manipulation of trees bestowed with robust growth, extensive root system and larger biomass like poplars and willows. However, *A. tumefaciens*-mediated genetic transformation of forest trees is a challenging task, which explains why there have been only a few reports about the transgenic modification in tree species (Han et al., 2000; Aken, 2008). Enhanced metabolism of organic pollutants in transgenic trees is associated with a faster uptake, which can be explained by a steeper concentration gradient inside plant tissues (French et al., 1999; Doty et al., 2000; Aken, 2008). Recently, Doty et al. (2007) developed a hybrid poplar plants (*Populus tremula x Populus alba*) overexpressing rabbit CYP2E1 for removing volatile hydrocarbons from hydroponic solutions. The transgenic poplar trees showed increased metabolism of TCE, vinyl chloride, carbon tetrachloride, chloroform and benzene. Among the various transgenic lines developed, line 78, expressed CYP2E1 at a higher level (3.37 to 4.6 fold) and exhibited the highest level of TCE metabolism (>100-fold higher than non-transgenic controls). Transgenic poplars were also shown effective for removing volatile pollutants from air. Transgenic poplars removed TCE from air at a higher rate than non-transgenic poplars (79% TCE (none removed by controls); 49% of vinyl chloride (compared with 29% by non-transgenic controls); and ~40% of benzene (compared with 13% by controls).

5.3. Plant cell suspension cultures as expression system for human P450 isoenzymes

Plant cell suspension cultures have been increasingly used for investigating the phytotoxicity and metabolic fate of xenobiotics, especially of pesticides in plants. The results were compared with those of the intact plants grown under aseptic conditions. The advantages of cell suspension culture system over intact plants are (i) lack of interfering photochemical and microbial transformation of the pollutants, (ii) results may obtained more quickly, (iii) absence of chlorophyll and other plant pigments facilitates complete extraction and identification of metabolites, (iv) plant cells can survive at moderate concentrations of xenobiotics without any cellular damage, (v) plant cell suspensions can be easily cultured in scaled-up assays (up to 50 g fresh weight) and air-lift-fermenters (~500 g fresh weight) and (vi) in vitro culture of root and shoot cells allow indefinite propagation and experimentation using tissues derived from the same plant, avoiding the risks of variability among species (Suresh and Ravishankar, 2004; Schmidt et al., 2006a,b). Schmidt et al. (2006a,b) introduced various P450 genes (*CYP1A1*, *CYP2A2*, *CYP3A4*) in tobacco cell cultures to study the degradation and detoxification of various insecticides (carbaryl, DDT, diflubenzuron, dimethoate, imidacloprid, and methoxychlor), herbicides (atrazine, fluometuron, and metamitron) and other xenobiotics (pyrene and n-nonylphenol) (Joußen et al., 2008; Bode et al., 2004a,b; Berger et al., 2005;). All these studies are again confirming the broad substrate specificity of human P450s and their subsequent incorporation with transgenic plants for xenobiotic remediation.

6. Glutathione S-transferases (GSTs): environmental perspectives

In addition to P450 oxidation, glutathione conjugation is an important mechanism for xenobiotic detoxification. Glutathione *S*-transferases (GSTs) (EC. 2.5.1.18) are a family of multifunctional enzymes involved in the cellular detoxification and excretion of many physiological and endogenous substances (Wilce and Parker, 1994), which are found in animals, plants and microorganisms (Santos et al., 2002). In addition, studies on GSTs are further characterizing their role in xenobiotic metabolism. Under normal conditions, glutathione is predominantly present in its reduced form (GSH), with only a small proportion present in its fully oxidized state (GSSG) (Dixon et al., 1998a,b). GSTs catalyze the nucleophilic addition of the thiol of reduced glutathione (γ-glutamyl-cysteinyl-glycine) to electrophonic centers in organic compounds. The glutathione conjugates so-formed are more hydrophilic, thus facilitating their exclusion. Thus GSTs catalyzed transformation is one of the early steps along the mercapturic acid pathway in which hydrophobic xenobiotics are detoxified and eliminated from the organisms (Habig et al., 1974). Subsequently, an ATP dependent efflux pump that mediates the export of glutathione conjugates from cells (Hayes and Wolf, 1990; Ishikawa, 1992) (Fig. 2).

According to Dixon et al. (1998a), all plant GSTs have native relative masses of around 50 kDa and are composed of two similarly signed (~25 kDa) sub units. Furthermore, each subunit contains a kinetically independent active site with distinct binding domains for glutathione and co-substrates (Marrs, 1996). The sub-untis may be identical, giving rise to homodimers, or distinct but related, resulting in heterodimers, with each distinct sub-unit encoded by a different gene (Dixon et al., 1998a). Sine plants contain complex multigene families of GSTs; the various subunits may be able to producing multiple homo and hetro-dimeric GST isoenzymes (Dixon et al., 1998a;b). The presence of a large number of isoenzymes with a differential, overlapping substrate selectivity, affords the organism the possibility to detoxify a wide range of reactive xenobiotics, by catalyzing their conjugation with GSH, or by 'trapping' them through non-covalent or covalent binding. The extent to which detoxification and/or activation will occur depends on the number and amount of specific isoenzymes present in a tissue (Vos and Bladeren, 1990). Therefore, the overexpression of these genes in suitable plant species is essential to enhance their catabolic potential.

7. Transgenic plants overexpressing GSTs for enhanced degradation/conjugation of organic xenobiotics

Poplar plans were transformed to overexpress the bacterial gene encoding γ-glutamyl-cysteine-synthetase (γ-ECS), which is the rate limiting regulatory enzyme in the biosynthesis of GSH (Noctor and Foyer, 1998). The transformed plants showed enhanced levels of GSH and its precursor γ-ECH (Noctor et al., 1996). The increased production of GSH contributes to the antioxidative protection of plant cells against oxidative stress caused by various environmental factors (Noctor and Foyer, 1998). Gullner et al. (2001) reported that increased levels of glutathione have been shown to increase the resistance to chloroacetanilide herbicides in transgenic poplar plants expressing γ- glutamyl-cysteine synthetase. Karavangeli et al. (2005) developed transgenic tobacco plants overexpressing maize glutathione *S*-transferase I for enhanced phytoremediation of chloroacetanilide herbicide. The isoenzyme GST I from maize exhibits significant catabolic activity for the chloroacetanilide herbicide alachlor and appears to be involved in its detoxifying process. The transgenic plants showed substantially higher tolerance to alachlor compared to non-transgenic plants in terms of growth and development (Karavangeli et al., 2005). Recently, Schroder et al. (2008) reported the glutathione dependent detoxification (conjugate detoxification) of organic xenobiotics (acetyl salicylic acid, lamotrigin, paracetamol, 1-chloro-2, 4-dinitrobenzene (CDNB), fenoxaprop, and propachlor) in *Phragmites australis* [(Cav.) Trin. Ex. Steud.]. A study of Brenter et al. (2008) showed that expression of glutathione *S*-tranferases in poplar trees (*Populus trichocarpa*) resulted in a significant increase of gene expression to two GST, peaking at levels of 25 and 10 fold the expression level of non-exposed plants after 24 h of each of the GST genes, respectively (Brenter et al., 2008). *Brassica juncea* overexpressing γ-glutamyl-cysteine-synthetase and glutathione synthetase also have shown enhanced tolerance to atrazine, CDNB, metolachlor and phenanthrene (Flocco et al., 2004). Recently, Dixit et al. (2008), introduced human P4502E1 and GST from fungus *Trcihoderma virens* in *N. tabacum*. The transgenic plant has shown enhanced degradation of anthracene and chloropyriphos. Thus, it is expected that the transgenic

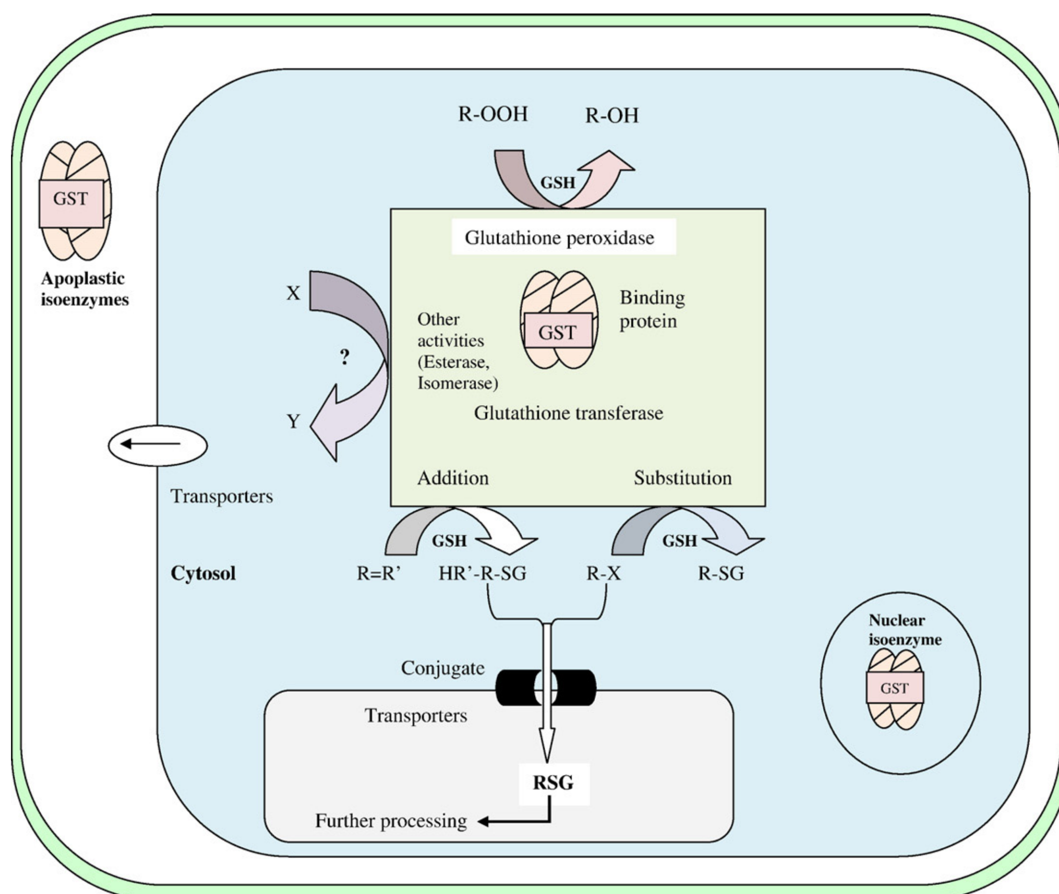

Fig. 2. Schematic representation of the role of GSTs in xenobiotic detoxification and endogenous metabolism. It is a proven fact that true detoxification reactions in Phase II performed by GSTs, rendering the compound under consideration less toxic because of conjugation (usually in a substitution reaction but occasionally as an addition reaction), and then a set of further reactions that include cleavage, rearrangement, secondary conjugation etc. Majority of the plant GSTs are supposed to be cytosolic, however, there is evidence for the existence of apoplastic and nuclear isoenzymes (Dixon et al, 1998a,b). The conjugated molecules (R-SG) are then transported to the vacuoles for further processing. The alternative activities of GSTs include glutathione peroxidase, esterase, isomerase and binding activities, which may play additional roles in endogenous metabolism (modified from Dixon et al, 1998a,b).

expression of both human P450 and glutathione conjugation enzymes in plants will provide enhanced detoxification and therefore improved remediation of organic xenobiotics.

8. Transgenic plants for the enhanced remediation of explosives

Contamination of soil and water with residues of explosives and related compounds due to intensive military activities is a widespread environmental concern (Richman, 1996). Contamination sources are mainly associated with their manufacture, use, loading, storage and disposal processes (Aken, 2009). There are three main groups of explosives; nitrate esters, nitroaromatics and nitramines (Rylott and Bruce, 2009). The main nitrate esters are glyceroltrinitrate (nitroglycerine, GTN) and pentaerythritotetranitrate (PETN). Nitroaromatics explosives contain an aromatic ring with multiple nitro groups (e.g. 2, 4, 6-trinitrotoluene (TNT), dinitrotoluenes (DNT), aminodinitrotoluenes (ADNT), diaminonitrotoluene, and nitrobenzens). Nitroamines contain *N*-nitro groups. Among the various groups, the most important military high explosive currently used is RDX. Their occurrence in the environment even in low concentrations, not only poses the risk of explosion but also a hazard to biological systems and human health because of their toxic and mutagenic effects (Bruns-Nagel et al., 1996; Honeycutt et al., 1996). Under ambient environmental conditions, most explosives exhibit a resistance to natural attenuation processes including volatilization, biodegradation and hydrolysis, resulting in persistence in soil and groundwater (Twibell et al., 1984). Although the microbial catabolic pathways leading to the complete mineralization of explosives are yet to be revealed, it is generally accepted that these compounds can be transformed into various intermediates in wide range of microorganisms by various enzymes (Esteve-Nunez et al., 2001; Ramos et al., 2005). The best known of these enzymes are flavonitroreductase belonging to the β/α old yellow enzyme (OYE) family, such as *XenA* and *XenB* in *Pseudomonas*, pentaerythritol tetranitrate (*PETNr*) reductase in *Enterobacter cloacae*, *NemA* in *Escherichia coli*, morphinone reductase in *Pseudomonas putida* M10, and OYE in *Saccharomyces cerevisie* (Blehert et al., 1999; Khan et al., 2002, 2004; Orville et al., 2004a;b; Ramos et al., 2005).

Vanek et al. (2003) studied the phytoremediation of PETN in model systems of plant tissue cultures (*Rheum palmatum*, *Saponaria officinalis and Populus simonii*). The work of Huges et al. (1997) on aquatic plants demonstrated that TNT can be metabolized in the absence of microorganisms (Hughes et al., 1997). Both poplar and willow have been used in munitions remediation research (Doty, 2008). The application, however, may be limited by the fact that the indigenous biodegradability of plants is less effective than those of adapted bacteria. As explosives are phytotoxic, phytoremediation of these pollutants is very difficult. This limitation might be overcome by incorporating bacterial nitroreductase genes into the plant genomes (French et al., 1999; Hannink et al., 2001; Rosser et al., 2001). When bacterial genes involved in the degradation of explosives were overexpressed in transgenic plants, the plants became more tolerant of the pollutant and could more readily remove it. As an initial attempt, French et al. (1999) introduced pentaerythritol tetranitrate (*PETNr*; a monomeric flavin mononucleotide (FMN)-containing protein) reducatse into *N. tabacum*, resulting in increased tolerance

to TNT. Furthermore, tobacco plants expressing PETNr were able to germinate and grow naturally on solid media containing 1 mM GTN, a concentration that would be lethal to non-transgenic plants (French et al., 1999). *PETNr* sequentially reduces two of the four nitro groups of PETN to yield pentaerythritol dinitrate, which is subsequently oxidized to the dialdehyde (Binks et al., 1996). Later studies proved that *PETNr* also possesses activity towards nitroaromatics. It has been shown to catalyze the reductive transformation of TNT. Reductive transformation of TNT to 2- or 4-HADNT via a nitrosointermediate is the most common catabolic pathway for TNT transformation in bacteria under aerobic conditions (Rylott and Bruce, 2009). The catabolic fingerprinting in TNT degrading bacterium *Enterobacter cloacae* reveals that this step was shown to be catalyzed by a FMN-containing, nitroreductase enzyme (NR). This NR enzyme can transform TNT significantly faster than PETNr, and when expressed in transgenic plants, NR also confers greater tolerance to TNT than PETNr (French et al., 1998, 1999; Hannink et al., 2001; Rylott and Bruce, 2009). The overexpression of this *NR* gene in transgenic tobacco resulted in the enhanced tolerance to TNT contamination (Hannink et al., 2001). An analysis of metabolic derivatives in NR-expressing transgenic tobacco plants revealed that Nr-expressing plant produced predominantly 4-HADNT and 4-ADNT isomers, indicating that NR favours reduction of nitro group from the 4 position of the aromatic ring (Hannink et al., 2007; Rylott and Bruce, 2009). Using a similar approach as that used for developing transgenic plant against TNT, Rylott et al. (2006) isolated genes from an RDX-utilizing bacterium and over expressed in *Arabidopsis* plants. The transgene consisted of an unusual microbial P450 system with two components: a flavodoxin reductase (*xplB*) and a fused flavodoxin cytochrome P450 (*xplA*). Recently, Van Dillewijn et al. (2008) developed a transgenic aspen incorporated with a nitroreductase, *pseudomonas nitroreductase A* (*pnrA*), isolated from the bacterium *Pseudomonas putida* for the enhanced degradation of TNT (Fig. 3). When compared with the non-transgenic plants, the transgenic trees were able to take up higher levels of TNT from liquid culture and soil (Van Dillewijn et al., 2008). Latest studies revealed that overexpression of two of the uridine diphosphate (UDP) glycosyltrasferases (UGTs) (*743B4* and *73C1* isolated from *Arabidopsis thaliana*) genes in *Arabidopsis thaliana* resulted in increased conjugate production, and enhanced root growth in 74B4 overexpression seedlings grown in liquid culture containing TNT (Gandia-Herrero et al., 2008). Among the seven characterized UGTs, six of the recombinantly expressed UGTs conjugated the TNT-transformation products 2-and 4-hydroxylaminodinitrotoulene substrates, exhibiting individual bias for either 2- or the 4- isomer (Gandia-Herrero et al., 2008) and two monoglucose conjugate products were observed for both 2- and 4-hydroxylamino-dinitrotoulene substrates (Gandia-Herrero et al., 2008).

Fig. 3. Transgenic Aspen expressing bacterial nitroreductase have been shown to exhibit increased tolerance to TNT (Ramose et al., 2005). Recent studies using transgenic aspen overexpressed with a nitroreductase, *pseudomonas nitroreductase A* (*pnrA*), isolated from the bacterium *Pseudomonas putida* have shown higher accumulation of TNT from liquid culture and soil, compared with the non-transgenic plants. Furthermore, the tolerance limit towards TNT was also significantly higher than for non-transgenic plants (Van Dillewijn et al., 2008). Reproduced, with permission (© 2004 Elsevier Ltd.).

9. Transgenic plants for the rhizoremediation of organic xenobiotics

One of the most promising approaches to enhancing the phytoremediation technology is the insertion of xenobiotics degrading genes into the root system of suitable plant species for the enhanced rhizospheric secretion and the subsequent degradation of pollutants (Glick, 2003; Gerhardt et al., 2009; Kawahigashi, 2009). The advantage of this method is that the plants do not need to take up the pollutants in order to detoxify them; instead, the secreted enzymes can degrade the pollutants in rhizospheric zone (Kawahigashi, 2009). The rhizosphere is the soil in the immediate vicinity of a root that is affected by root processes. It comes into being when a root tip enters a volume of soil and disappears some time after the root has died and decomposed (Darrah et al., 2006). Many studies demonstrate significantly enhanced dissipation and or mineralization of persistent organic pollutants at the root–soil interface (Anderson et al., 1993; Anderson and Coats, 1995; Kuiper et al., 2004; Chaudhry et al., 2005; Abhilash and Singh, 2009b). This rhizosphere effect is generally attributed to an increase in microbial density, diversity and or metabolic activity due to the release of plant root exudates, mucigel and root lysates (enzymes, amino acids, carbohydrates, low-molecular-mass carboxylic acids, flavonones and phenolics; Curl and Truelove, 1986; Kidd et al., 2008). Further, plants can also increase the physical and chemical properties of the contaminated soil, and increase contact between the root-associated microorganisms and the soil contaminants (Fig. 4).

Transgenic plants that secrete detoxifying enzymes can be useful for the rhizoremediation of wide range of hydrophobic chemicals. Wang et al. (2004), developed transgenic Arabidopsis plants secrete a root-specific laccase (LAC1) from cotton plants to the rhizosphere and have shown enhanced resistance to phenolic allelochemicals and 2, 4, 6-trichlorophenol. Similarly, Sonoki et al. (2005) transformed tobacco plants with an extracellular fungal laccase from *Coriouls versicolar* for the rhizoremediation of bisphenol A and PCP. Furthermore, the overexpression of a bacterial biphenyl dioxygenase (BPDO) from *Burkholderia xenovorans* LB400 resulted in the enhanced oxygenation of 4-chlorobiphenyls (Mohammadi et al, 2007). BPDO catalyzes a stereospecific oxygenation of biphenyl, producing *cis*-2,3-dihydro-2,3-dihydroxybiphenyl-2,3-dehydrogenase. 2,3-dihydroxybiphenyl is then cleaved by the 2, 3-dihydroxybiphenyl-1,2-dioxygenase (2, 3-DHBD) and the resulting 2-hydroxy-6-oxo-6-phenyl-hexa-2,4-dienoic acid (HOPDA) is hydrolysed producing benzoic and pentatonic acids

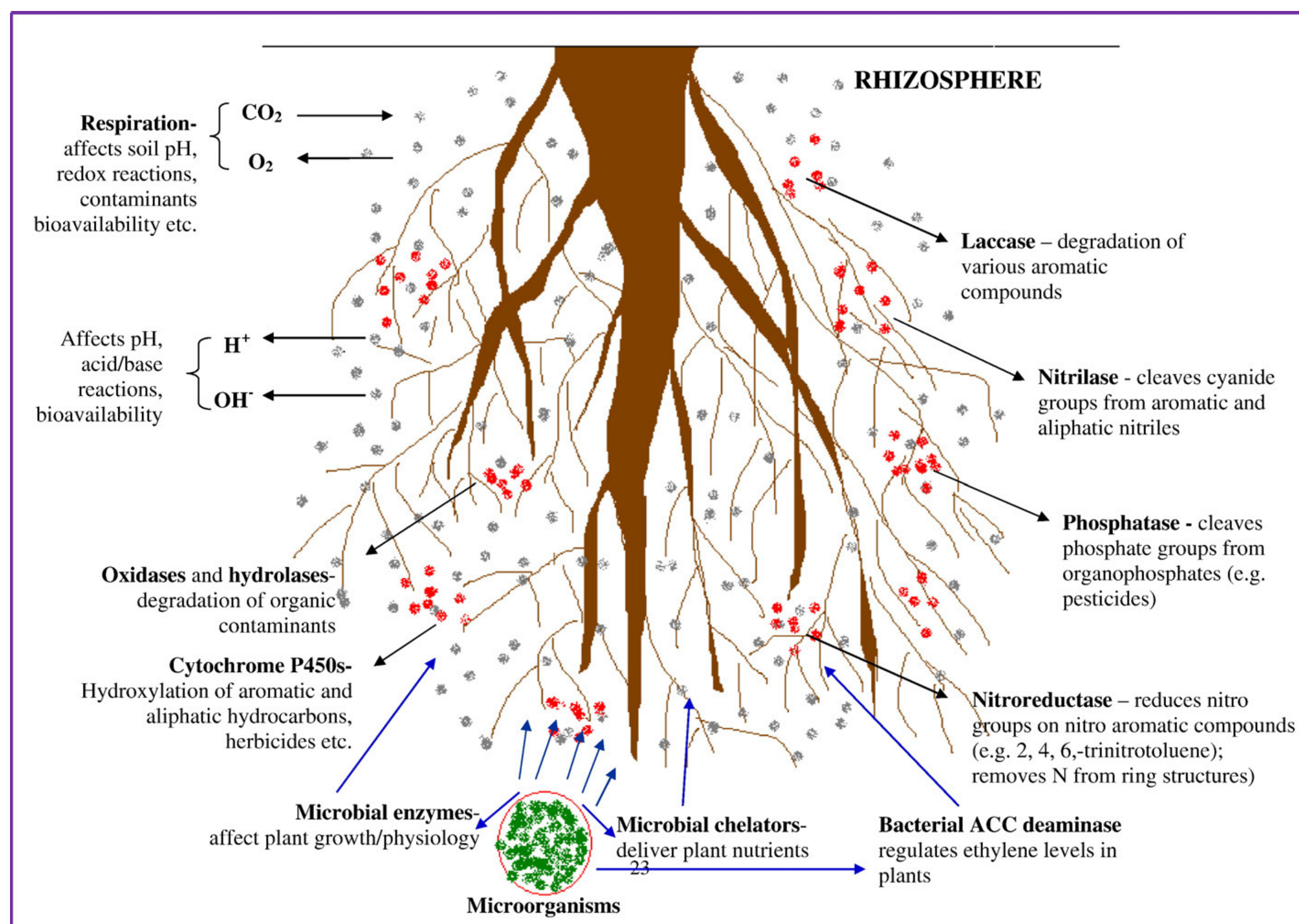

Fig. 4. Schematic representation of the enzymatic and microbial activities responsible for the enhanced remediation in rhizospheric zone. One of the prosing approaches to using transgenic plants for the enhanced phytoremediation of organic xenobiotics is to construct plants that secrete pollutant-degrading enzymes into the rhizosphere (Doty, 2008). Furthermore, the incorporation of bacterial ACC deaminase (ACC) will reduce the ethylene stress to the plants and therefore the healthy growth of roots; which will ultimately results in enhanced rhizodegradation of pollutants (Glick, 2005; Gerhardt et al., 2006; Arshad et al., 2007).

(Sylvestre et al., 2009). The encoding genes for PCB degradation in *Burkholderia xenovorans* LB400 are *bphA* (BphAE α subunit), *bphE* (BphAE β subunit), *bphF* (BphF) and *bphG* (BphG) (Sylvestre et al., 2009). Gene *bphC* from *Pandoraea pnomenusa* B-356 was successfully cloned in tobacco plants for the enhanced remediation of PCB (Francova et al., 2003; Novakova et al., 2009). Transgenic Arabidopsis plants expressing the aromatic-cleaving extradiol dioxygenase (DBfB) resulted in the enhanced degradation of 2, 3-dihydroxybiphenyl (2, 3-DHB) (Uchida et al., 2005). Similarly, transgenic tobacco plants expressing haloalkane dehydrogenase (DhaA) accelerated the detoxification of 1-chlorobuatne in rhizospheric zone (Uchida et al., 2005).

Apart from the presence of catabolic genes, the success of phytoremediation depends upon the morphological and intrinsic properties of the plant species itself. Therefore, the choice of plants is likely to impact on the success of the rhizoremediation technology (Sylvestre et al., 2009). A plant species with large above ground biomass is crucial for the phytoextraction, whereas the plant species with extensive root system or belowground biomass is important for the rhizoremediation. However, the growth of the plants in contaminated sites is normally hindered by the pollutants. Accelerated ethylene production in response to stress induced by pollutants is known to inhibits the root growth and is considered a major obstacle to phytoremediation (Kawahigashi, 2009). Previous studies proved that bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase regulates ethylene levels in plants by metabolizing its precursor ACC into α-ketobutyric acid and ammonia (Bernard, 2005; Arshad et al., 2007). Interestingly, this ACC deaminase has been detected in some plant growth-promoting bacteria (PGPR), and thus regulates the biosynthesis of ethylene in inoculated plant roots (Glick et al., 1998; Glick, 2005). Transgenic plants that express ACC deaminase genes can reduce ethylene levels, resulting in a more extensive root system (Arshad et al., 2007). It is expected that the resultant increase in root growth provided by ACC deaminase might enhance the rhizoremediation potential. Furthermore, incorporation of multiple genes related to the different phases of xenobiotics degradation, together with ACC deaminase may improve the remediation potential of transgenic plants (Kawahigashi, 2009).

10. Transgenic plants to be developed for the phytoremediation of some other priority pollutants

Although, the transgenic technology has progressed from single gene engineering to multigene engineering and successfully demonstrated for the enhanced degradation and remediation of many persistent hazardous pollutants, the application of this technology yet to be exploited for the phytoremediation of some priority pollutants like hexachlorocyclohexane isomers (HCH). HCH is one of the highly toxic and persistent organochlorine pesticides that have caused serious environmental problems since it began to be produced at the beginning of the 1940s. The extensive and indiscriminative use of

lindane (γ-HCH) and technical HCH over the last few decades has led to the widespread contamination of its four major isomers (α-, β-, γ- and δ-HCH) into various environmental compartments (Abhilash and Singh, 2009a). It is estimated that 1.6 to 4.8 million tons of HCH residues were dumped or landfilled close to the respective production sites worldwide (Vijgen et al., 2006; Weber et al., 2008). Although the use of HCH has been discontinued for a considerable period of time in many countries, the residues continue to have a significant impact on a number of ecosystems (Abhilash, 2009). Recently, α-, β- and γ-HCH have been nominated by the POPs Reviewing Committee for inclusion into the Stockholm Convention to address the HCH contamination on a global level. Therefore, there is an urgent need to stop the production of lindane and remediate the contaminated soil sites.

Abhilash and Singh (2008) identified some plant species growing in an industrial premise capable of accumulating HCH isomers (*Solanum torvum*, *Withania somnifera*, *Lantana camera*, *Achyranthes aspera*, *Dalbergia sisso*, *Calotropis procera*, *Erinathus munja*). A glass house study proved that *W somnifera* enhanced the dissipation of lindane (73%) in treated soil (20 mg kg^{-1}) (Abhilash and Singh, 2009b) (Fig. 5). Therefore, the remediation potential of this species, especially the rhizoremediation will be exploited for the decontamination of lindane and other HCH contaminated soil sites. Microbial catabolic profiling in *Sphingomonas paucimobilis* B90 reveals that *LinA1* and *LinA2* of a dehydrochlorinase catalyzes the first and second steps in the metabolism of hexachlorocyclohexanes (Suar et al., 2005; Lal et al., 2008). Many previous works reported that two bacterial strains (*Sphingomonas paucimobilis* UT26 and *S. paucimobilis* B90A) can degrade both γ- and α-HCH isomers (Sahu et al., 1999; Imai et al., 1991; Johri et al., 1996). Nagata et al. (1993) purified *LinA* gene from *S. paucimobilis* UT26, a dehydrochlorinase catalyzing the dehydrochlorination of α-HCH, γ-HCH, δ-HCH, γ-pentachlorocyclohexane (γ-PCCH) and α-pentachlorocyclohexane (α-PCCH); whereas β-HCH is not dehydrochlorinated. Later, Trantirek et al. (2001) depicted the mechanistic information and the stereochemistry of the reactions mediated by *LinA*. As an initial step, γ-HCH is enantioselectively converted to (3R, 4S, 5S, 6R)- 1,3,4,5,6-PCCH, which is then converted to 1,2,4-trichlorobenzene (1, 2,4-TCB) via the presumed but instable intermediate (3R, 6R)-1,3,4,6-tetrachlorocyclohexa-1, 4-diene (Trantirek et al., 2001; Suar et al., 2005). On the amino acid level, *Lin A1* and *Lin A2* were 88% identical to each other, and *Lin A2* was 100% identical to *LinA* of S. paucimobilis UT26 (Suar et al., 2005). The additional molecular fingerprinting of these genes will elucidate the details of their catabolic potential against lindane and other HCH isomers. One of the possible strategies to exploit the catabolic potential of these genes is their possible insertion into suitable HCH resistant/ accumulating plant species. Based on our extensive monitoring studies on HCH accumulation capabilities of plants species growing in a lindane producing industrial premise and on glass house experiments, we have suggested two plant species (Fig. 4) for the insertion of HCH degrading microbial genes. However, protocols for genetically transforming *W. somnifera* and *J. curcas* need to be developed and strategies for gene containment will need to be evaluated. Furthermore, the potential of this transgenic technology for the phytoremediation of HCH contaminated sites must be conducted.

11. Conclusions and future directions

Although effectual under controlled conditions, the majority of the transgenic plants developed in different countries in the last decade never have been used in a real contaminated site. Furthermore, although less hindered by regulatory framework than transgenic microbial-based remediation, onsite introduction of transgenic plants are possible only with a considerable amount of bureaucracy. Major concerns over field release of such genetically manipulated plants include increased invasiveness and decreased genetic variability of native plants due to interbreeding. Dearth of knowledge with regard to detoxification mechanisms used by plants to cope up with xenobiotics is a major procedural constriction for focused engineering approach. Such enzymological knowledge for xenobiotics provides informed decisions on which genes to engineer. It has been suggested that increased understanding of the enzymatic process involved in

Fig. 5. Plant species suitable for the phytoremediation of hexachlorocyclohexane (HCH). Recent studies reveal that *a LinA1* and *LinA2* gene (dehydrochlorinase) in *Sphingomonas paucimobilis* catalyzes the first and second steps in the metabolism of HCH (Kumari et al., 2002; Suar et al., 2005). The insertion of these genes in promising species like (A) *Withamina somnifera* Dunal (previously shown to accumulate HCH isomers from an HCH contaminated site (Abhilash and Singh, 2008; Abhilash, 2009; Abhilash and Singh, 2009a) (B) *Jatropha curcas* L (Abhilash, 2009) will enhance the phytoremediation potential of these species.

plant tolerance and detoxification of xenobiotics will provide new directions for manipulating plant with superior remediation potential. Further, some of the engineered plants are unsuitable for field application because of its small biomass and growth rates. However, in spite of these scruples, researchers continued to trail the development of transgenic plants bestowed with finer qualities (enhanced growth rate and biomass, deep root system, increased metabolism etc.). The uses of sterile clones have been suggested as a solution to invasiveness and interbreeding. However, as this new technology develops, the limitations accountable for the delay in its successful application will be overcome. The ecological paybacks offered by phytoremediation provide the impetus for pursuing its extensive execution.

Acknowledgements

Authors are thankful to Director, National Botanical Research Institute, for providing facilities. Thanks are also due to Prof. Brian Dixon, Department of Biology, University of Waterloo, Canada for his valuable suggestions. P.C. Abhilash is thankful to University Grants Commission, Govt. of India for providing Doctoral fellowship (UGC-JRF-SRF).

References

Abhilash PC. Phytoremediation: an innovative technique for ecosystem clean up. Our Earth 2007;4:7-12.
Abhilash PC. Monitoring of organochlorine pesticide (Lindane) in soil–plant system of a contaminated environment and its phytoremediation/bioremediation. Ph D thesis, University of Lucknow, India, 2009.
Abhilash PC, Singh N. Distribution of hexachlorocyclohexane isomers in soil samples from a small scale industrial area of Lucknow, North India, associated with lindane production. Chemosphere 2008;73:1011–5.
Abhilash PC, Singh N. Seasonal variation of HCH isomers in open soil and plant–rhizospheric soil system of a contaminated environment. Environ Sci Pollut Res 2009a. doi:10.1007/s11356-009-0133-5.
Abhilash PC, Singh N. *Withania somnifera* Dunal mediated dissipation of lindane from simulated soil: implications for rhizoremediation of contaminated soils. J. Soils Sediments 2009b. doi:10.1007/s11368-009-0085-x.
Aken BV. Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. Trends Biotechnol 2008;26:225–7.
Aken BV. Transgenic plants for enhanced phytoremediation of toxic explosives. Curr Opin Biotechnol 2009. doi:10.1016/j.copbio.2009.01.011.
Alderete LGS, Talano MA, Ibannez SG, Purro S, Agostini E. Establishment of transgenic tobacco hairy roots expressing basic peroxidases and its application for phenol removal. J Biotechnol 2009;139:273–9.
Anderson TA, Coats JR. Screening rhizosphere soil samples for the ability to mineralize elevated concentrations of atrazine and metachlor. J Environ Sci Health B 1995;30:473–84.
Anderson TA, Guthrie EA, Walton BT. Bioremediation in the rhizopshere: plant roots and associated microbes clean contaminated soil. Environ Sci Technol 1993;24:885–9.
Aprill W, Sims RC. Evaluation of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere 1990;20:253–65.
Arshad M, Saleem M, Hussain S. Perspecives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 2007;25:356–62.
Aslund MLW, Zeeb BA, Rutter A, Reimer KJ. In situ phytoextraction of polychlorinated biphenyl-(PCB) contaminated soil. Sci Total Environ 2007;374:1-12.
Banerjee S, Shang QT, Wilson AM, Moore AL, Strand SE, Gordon MP, et al. Expression of active mammalian P450 2E1 in hairy root cultures. Biotechnol Bioeng 2002;77: 462–6.
Belfrod EJ, Dorfler U, Stampfl A, Schroder P. Microsomal detoxification enzymes in yam bean [*Pachyrhizuz erosus* (L.) urban]. Z Naturforsch [C] 2004;59:693–700.
Berger A, Ruß AS, Schuphan I, Schmidt B. Metabolism of 4-n-nonylphenol by non-modified and CYP1A1- and CYP1A2-transgenic cell cultures of tobacco. Z Naturforsch 2005;60c:883–92.
Bernard RG. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Let 2005;251:1–7.
Binet P, Portal JM, Leyval C. Dissipation of 3-6-ring polycyclic aromatic hydrocarbons in the rhizosphere of rye grass. Soil Biol Biochem 2000;32:2011–7.
Binks PR, French CE, Nicklin S, Bruce NC. Degradation of pentaerythritol tetranitrate by Enterobacter cloacae PB2. Appl Environ Microbiol 1996;62:1214–9.
Bizily S, Rugh CL, Summer AO, Meagher RB. Phytoremediation of methylmercury pollution: *mer*B expression in *Arabidopsis thaliana* confers resistance to organomercurials. Proc Natl Acad Sci USA 1999;96:6808–13.
Blehert DS, Fox BG, Chambliss GH. Cloning and sequence analysis of two *Pseudomonas* flavoprotein xenobiotic reductases. J Bacteriol 1999;181:6254–63.
Bode M, Haas M, Faymonville T, Thiede B, Schuphan I, Schmidt B. Biotransformation of metamitron by human P450 expressed in transgenic tobacco cell cultures. J Environ Sci Health Part B 2004a;41:201–22.
Bode M, Stöbe B, Thiede B, Schuphan I, Schmidt B. Biotransformation of atrazine in transgenic tobacco cell culture expressing human P450. Pest Manag Sci 2004b;60:49–58.
Brenter LB, Mukherji ST, Merchie KM, Yoon JM, Schnoor JL. Expression of glutathione S-tranferases in poplar trees (*Populus trichocarpa*) exposed to 2,4,6-trinitrotoluene (TNT). Chemosphere 2008;73:657–62.
Bruns-Nagel D, Breitung J, von Low E, Steinbach K, Gorontzy T, Kahl M, Blotevogel K, Gemsa D. Microbial Transformation of 2,4,6-Trinitrotoluene in Aerobic Soil Columns. Appl Environ Microbiol 1996;62:2651–6.
Carman E, Crossman T, Gatliff E. Phytoremediation of no. 2 fuel–oil contaminated soil. J Soil Contam 1998;7:455–66.
Chaudhry Q, Blom-Zandstra M, Gupta S, Joner EJ. Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ Sci Pollut Res 2005;12:34–48.
Cherian S, Oliveira MM. Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 2005;39:9377–90.
Chrastilova Z, Mackova M, Novakova M, Macek T, Szekeres M. Transgenic plants for effective phytoremediation of persistent toxic organic pollutants present in the environment. Abstracts/ J Biotechnol 2007;131S:S38.
Compton HR, Harosi DM, Hirsch SR, Wrobel JG. Pilot-scale use of trees to address voc contamination. In: Wickramanayake GB, Hinchee RE, editors. Bioremediation and phytoremediation of chlorinated and recalcitrant compounds. Columbus: Battelle Press; 1998.
Cunningham SD, Berti WR. Remediation of contaminated soils with green plants: an overview. In Vitro Cell Dev Biol 1993;29:207–12.
Cunningham SD, Ow DW. Promise and prospects of phytoremediation. Plant Physiol 1996;110:715–9.
Cunningham SD, Berti WR, Huang JW. Phytoremediation of contaminated soils. Biotechnol 1995;13:393–7.
Cunningham SD, Anderson TA, Schwab AP, Hsu FC. Phytoremediation of soil contaminated with organic pollutants. Adv Agron 1996;56:55-114.
Curl EA, Truelove B. The rhizosphere. Springer-Verlag: Heidelberg; 1986.
Darrah PR, Jones DL, Kirk GJD, Roose T. Modeling the rhizosphere: a review of methods for up scaling to the whole plant scale. Eur J Soil Sci 2006;57:13–25.
De Prado JL, Osuna MD, Heredia A, Dee Prado R. *Lolium rigidum*, a pool of resistance mechanisms to ACCase inhibitor herbicides. J Agric Food Chem 2005;53:2185–91.
Didierjean L, Gondet L, Perkin R, Lau SM, Schaller H, O'Keefe, et al. Engineering herbicide metabolism in tobacco and *Arabidopsis* with CYP76B1, a cytochrome P450 enzyme from Jerusalem artichoke. Plant Physiol 2002;130:179–89.
Dietz A, Schnoor JL. Advances in phytoremediation. Environ Health Perspect 2001;109: 163–8.
Dixon DP, Cumminus I, Cole DJ, Edwards R. Glutathione-mediated detoxification system in plants. Curr Opin Plant Biol 1998a;1:258–66.
Dixon DP, Cole DJ, Edwards R. Purification, regulation and cloning of a glutathione transferase (GST) from maize resembling the auxin-inducible type-III GSTs. Plant Mol Biol 1998b;36:75–87.
Dixit P, Singh S, Mukherjee PK, Eapen S. Development of transgenic plants with cytochrome P450E1 gene and glutathione-S-transferase gene for degradation of organic pollutants. AbstractsJ Biotechnol 2008;136S:S692–3.
Doty SL. Enhancing phytoremediation through the use of transgenic plants and entophytes. New Phytol 2008;179:318–33.
Doty SL, Shang QT, Wilson AM, Moore AL, Newman LA, Strand SE, Gordon MP. Enhanced metabolism of halogenated hydrocarbons in transgenic plants contain mammalian P450 2E1. Proc Natal Acad Sci USA 2000;97:6287–91.
Doty SL, Shang QT, Wilson AM, Moore AL, Newman LA, Strand SE, et al. Enhanced metabolism of halogenated hydrocarbons in transgenic plants contain mammalian P450 2E1. Proc Natl Acad Sci USA 2007;97:6287–91.
Eapen S, D'Souza SF. Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 2005;23:97-114.
Eapen S, Singh S, D'Souza SF. Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol Adv 2007;25:442–51.
Esteve-Nunez A, Caballero A, Ramos JL. Biological degradation of 2, 4, 6, -trinitrotoluene. Microbiol Mol Biol Rev 2001;65:335–52.
Felsot AS, Dzantor EK. Effect of alachlor concentration and an organic amendment on the soil dehydrogenase activity and pesticide degradation rate. Environ Toxicol Chem 1995;14:23–8.
Fischer TC, Klattig JT, Gierl A. A general cloning strategy for divergent plant cytochrome P450 and genes and its application in *Lolium rigidum* and *Ocimum basilicum*. Theor Appl Genet 2001;103:1014–21.
Fismes J, Perrin-Ganier C, Empereur-Bissonnet P, Morel JL. Soil-to-root transfer and translocation of polycyclic aromatic hydrocarbons by vegetables grown on industrial contaminated soils. J Environ Qual 2002;31:1649–56.
Flocco CG, Lindblom SD, Smits EAHP. Overexpression of enzymes involved in glutathione synthesis enhances tolerance to organic pollutants in *Brassica juncea*. Int J Phytoremediat 2004;6:289–304.
Fonne-Pfister R, Kreuz K. Ring-methyl hydroxylation of chlorotoluron by an inducible cytochrome P450 dependent enzyme from maize. Photochemistry 1990;29:2793–6.
Francova K, Sura M, Macek T, Szekeres M, Bancos S, Demnerova K, et al. Preparation of plants containing bacterial enzyme for degradation of poly chlorinated biphenyls. Fresenius Environ Bull 2003;12:309–13.
French CE, Nicklin S, Bruce NC. Aerobic degradation of 2, 4, 6-trinitrotoluene by Enterobacter cloacae PB2 and by pentaerythritol tetranitrate reductase. Appl Environ Microbiol 1998;64:2864–8.
Frear DS. Wheat microsomal cytochrome P450 monooxygenases: characterization and importance in the metabolic detoxification and selectivity of wheat herbicides. Drug Metab Drug Interac 1995;12:329–57.

French CJ, Rosser SJ, Davies GJ, Nicklin S, Bruce NC. Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase. Nat Biotechnol 1999;17:491–4.

Funae Y, Obata N, Kirigami S. Multiple function of cytochrome P450. Kan Tan Sui 1998;37:91.

Gandia-Herrero F, Lorenz A, Larson T, Graham IA, Bowles J, Rylott EL, et al. Detoxification of the explosive 2,4,6- trinitrotoluene in *Arabidopsis*: discovery of bifunctional *O*- and *C*-glucosyltransferases. Plant J 2008;56:963–74. doi:10.1111/j.1365-313X.2008.03653.x.

Gao Y, Zhu L. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 2004;55:1169–78.

Gao X, Wang W, Liu X. Low-temperature dechlorination of hexachlorobenzene on solid supports and the pathway hypothesis. Chemosphere (2008);71:1093–9.

Gerhardt KE, Greenberg BM, Glick BR. The role of ACC deaminase in facilitating the phytoremediation of organics, metals and salts. Curr Trends Microbiol 2006;2:61–72.

Gerhardt KE, Huang XD, Glick BR, Greenberg BM. Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 2009;176:20–30.

Glick BR. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 2003;21:239–44.

Glick BR. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 2005;251:1–7.

Glick BR, Penrose DM, Li J. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J Theor Biol 1998;190:63–8.

Goldstein RM, Mallory LM, Alexander M. Reasons for possible failure of inoculation to enhance bioremediation. Appl Environ Microbiol 1985;50:977–83.

Graham-Lorence S, Petersen JA. How similar are P450s and what can their differences teach us? Arch Biochem Biophys 1999a;369:24–9.

Graham-Lorence S, Petersen JA. P450s: structural similarities and functional differences. FASEB J 1999b;10:206–14.

Greenberg BM. Development and field tests of a multi-process phytoremediation system for decontamination of soils. Can Reclam 2006;1:27–9.

Greenberg BM, Hunag XD, Gurska Y, Gerhardt KE, Wang W, Lampi MA, et al. Successful field tests of a multi-process phytoremediation system for decontamination of persistent petroleum and organic contaminants. Proceedings of the twenty-ninth Artic and Marine Oil Spill Program (AMOP). Technical Seminar vol. 1. Environment Canada; 2006. p. 389–400.

Gullner G, Komives T, Rennenberg H. Enhanced tolerance of transgenic poplar plants overexpressing gamma-glutamylcysteine synthetase towards chloroacetanilide herbicides. J Exp Bot 2001;52:971–9.

Gunther T, Dornberger U, Fritsche W. Effects of rye grass on biodegradation of hydrocarbons in soil. Chemosphere 1996;33:203–15.

Habig WH, Pabst MJ, Jacoby WB. Glutathione s-transferase. The first step in mercapturic and formation. J Biol Chem 1974;249:7130–9.

Han KH, Meilan R, Ma C, Strauss SH. An Agrobacterium tumefaciens transformation protocol effective on a variety of cottonwood hybrids (genus Populus). Plant Cell Reports 2000;19:315–20.

Hannink N, Rossser SJ, French CE, Basran A, Murray JAH, Nicklin S, et al. Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. Nat Biotechnol 2001;19:1168–72.

Hannink NK, Subramanian M, Rosser SJ, Basran A, Murray JAH, Shanks JV, et al. Enhanced transformation of TNT by tobacco plants expressing a bacterial nitroreductase. Int J Phytoremediat 2007;9:385–401.

Hatakeda K, Ikushima Y, Sato O, Aizawa T, Saito N. Supercritical water oxidation of polychlorinated biphenyls using hydrogen peroxide. Chem Eng Sci 1999;54: 3079–84.

Hatzioz KK. Regulation of enzymatic systems detoxifying xenobiotics in plants: a brief overview and directions for future research. Dordrecht: Kluwer Academic Publishers; 1997.

Hayes JD, Wolf CR. Molecular mechanisms of drug resistence. Biochem J 1990;272:281–95.

Hirose S, Kawahigashi H, Ozawa K, Shiota N, Inui H, Ohkawa H, et al. Transgenic rice containing human CYP2B6 detoxifies various classes of herbicides. J Agric Food Chem 2005;53:3461–7.

Honeycutt ME, Jarvis AS, McFarland VA. Cytotoxicity and mutagenicity of 2,4,6-trinitrotolulene and its metabolites. Ecotoxicol Environ Saf 1996;35:282–7.

Hooker BS, Skeen RS. Transgenic phytoremediation blasts onto the scene. Nat Biotechnol 1999;17:428.

Huang XD, El-Alawi YS, Penrose D, Glick BR, Greenberg BM. A multiprocess phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ Pollut 2004;130:465–76.

Huang XD, El-Alawi YS, Gurska J, Glick BR, Greenberg BM. A multiprocess phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem J 2005;81:139–47.

Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R. Transformation of TNT by aquatic plants and plant tissue cultures. J. Chromatogr 1997;518:361–4.

Ishikawa T. The ATP-dependent glutathione S-conjugate export pump. Trends Biochem Sci 1992;17:463–8.

Iimura Y, Ikeda S, Sonoki T, Hayakawa T, Kajita S, Kimbara K, et al. Expression of a gene for Mn-peroxidase from *Coriolus versicolor* in transgenic tobacco generates potential tool for phytoremediation. Appl Microbiol Biotechnol 2002;59:246–51.

Imai R, Nagata Y, Fukuda M, Takagi M, Yano K. Molecular cloning of a *Pseudomonas paucimobilis* gene encoding a 17-kilodalton polypeptide that eliminates HCH molecules from γ-hexachlorocyclohexane. J. Bacteriol 1991;173:6811–9.

Inui H, Ohkawa H. Herbicide resistance plants with mammalian P450 monooxygenase genes. Pest Manag Sci 2005;61:286–91.

Inui H, Ueyama Y, Shiota N, Ohkawa Y, Ohkawa H. Herbicide metabolism and cross-tolerance in transgenic potato plants expressing human CYP1A1. Pestic Biochem Physiol 1999;64:33–46.

Inui H, Kodama T, Ohkawa Y, Ohkawa H. Herbicide metabolism and cross tolerance in transgenic potato plants co-expressing human *CYP1A1*, *CyP2B6*, and *CYP2C19*. Pestic Biochem Physiol 2000;66:116–29.

Inui H, Shiota N, Motoi Y, Ido Y, Inoue T, Kodama T, et al. Metabolism of herbicides and other chemicals in human cytochrome P450 species and in transgenic potato plant co-expressing human *CYP1A1*, *CYP2B6* and *CYP2C19*. J Pestic Sci 2001;26:28–40.

Jackson EG, Rylott EL, Fournier D, Hawari J, Bruce NC. Exploring the biochemical properties and remediation applications of the unusual explosive-degrading P450 system XplA/B. Proc Natl Acad Sci USA 2007;104:16822–7.

Johnson AC, Worall F, White C, Walker A, Besien TJ, Williams RJ. The potential of incorporated organic matter to reduce pesticide leaching. Toxicol Environ Chem 1997;58:47–61.

Johri AK, Dua M, Tuteja D, Saxena R, Saxena DM, Lal R. Genetic manipulations of microorganisms for the degradation of hexachlorocyclohexane. FEMS Microbial Rev 1996;16:69–84.

Joußen N, Heckel DG, Haas M, Schuphan I, Schmidt B. Metabolism of imidacloprid and DDT by P450 CYP6G1 expressed in cell cultures of *Nicotiana tabacum* suggests detoxification of these insecticides in Cyp6g1-overexpressing strains of *Drosophila melanogaster*, leading to resistance. Pest Manag Sci 2008;64:65.

Jung S, lee HJ, Lee Y, Kang K, Kim YS, Grimm B, et al. Toxic tetrapyrrole accumulation in protoporphyrinogrn IX oxidase overexpressing transgenic rice plants. Plant Mol Biol 2008;67:535–46.

Karavangeli M, Labrou NE, Clonis YD, Tsaftaris A. Development of transgenic tobacco plants overexpressing glutathione *S*-transferase I fro chloroacetanilide herbicides phytoremediation. Biomolec Eng 2005;22:121–8.

Kawahigashi H. Transgenic plants for phytoremediation of herbicides. Curr Opin Biotechnol 2009. doi:10.1016/j.copbio.2009.01.010.

Kawahigashi H, Hirose S, Inui H, Ohkawa H, Ohkawa Y. Transgenic rice plants expressing human CYP1A1 exudes herbicide metabolites from their roots. Plant Sci 2003;165:373–81.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Transgenic rice plants expressing human *CYP2B6*. J Agric Food Chem 2005a;53:9155–60.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Transgenic rice plants expressing human *CYP1A1* remediate the triazine herbicides atrazine and simazine. J Agric Food Chem 2005b;53:8557–64.

Kawahigashi H, Hirose S, Ozawa K, Ido Y, Kojima M, Ohkawa H, et al. Analysis of substrate specificity of pig CYP2B22 and CYP2C49 towards herbicides by transgenic rice plants. Transg Res 2005c;14:907–17.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Phytoremediation of herbicide atrazine and metolachlor by transgenic rice plants expressing human *CYP1A1*, *CYP2B6* and *CYP2C19*. J Agric Food Chem 2006a;54:2985–91.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Broad range of herbicide tolerance of glutinous upland rice variety 'Yumenoha-tamochi' carrying human cytochrome P450 genes. Plant Biotechnol Adv 2006b;25:75–85.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Herbicide resistance of transgenic rice plants expressing human CYP1A1. Biotechnol Adv 2007;25:75–85.

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Transgenic rice plants expressing human P450 genes involved in xenobiotic metabolism for phytoremediation. J Mol Microbiol Biotechnol 2008;15:212–9.

Khan H, Harris RJ, Barna T, Craig DH, Bruce NC, Munro AW, et al. Kinetic and structural basis of reactivity of pentaerythritol tetranitrate reductase with NADPh, 2-cyclohexenone, nitroesters, and nitroaromatic explosives. J Biol Chem 2002;277: 21906–12.

Khan H, Barna T, Harris RJ, Bruce NC, Barsukov I, Munro AW, et al. Atomic resolution behavior of enzyme-substrate complexes of *Enerobacter cloacae* PB2 pentaerythritol tetranitrate reductase. Multiple conformational states and implications for the mechanism of nitroaromatic explosive degradation. J Biol Chem 2004;279: 30563–72.

Kidd PS, Prieto-Fernandez A, Monterroso C. Rhizosphere microbial community and hexachlorocyclohexane degradative potential in contrasting plant species. Plant Soil 2008;302:233–47.

Klingenberg M. Pigments of rat liver microsomes. Arch Biochem Biophys 1958;75:376–86.

Kobayashi T, Navarro RR, Tatsumi K, Iimura Y. Influence of compost amendment on pyrene availability to two subspecies of *Cucurbita pepo*. Sci Total Environ (2008);404:1–9.

Kramer U. Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotechnol 2005;16:133–41.

Kreuz K, Tommasini R, Martinoia E. Old enzyme for a job. Plant Physiol 1996;111:349–53.

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ. Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact 2004;17:6-15.

Kummling KE, Gray DJ, Power JP, Woodland SE. Gas phase chemical reduction of hexachlorocyclohexane and other chlorinated compounds: waste treatment experience and applications. 6th International HCH and Pesticides Forum, 20–22 March; 2001. Poznan, Poland.

Kumari R, Subudhi S, Suar M, Dhingra G, Raina V, Dogra C, lal S, Van der Meer JR, Holliger C, Lal R. Cloning and characterization of lin genes responsible for the degradation of hexachlorocyclohexane isomers by *Sphingomonas paucimobilis* strain B90. Apppl Environ Microbiol 2002;68:6021–8.

Kurumata M, Takahashi M, Sakamotoa A, Ramos JL, Nepovim A, Vanek T, et al. Tolerance to and uptake and degradation of 2, 4, 6-trinitrotoluene (TNT) are enhanced by the expression of a bacterial nitroreductase gene in Arabidopsis thaliana. Z Naturforsch [C] 2005;60:272–8.

Lal R, Dadhwal M, Kumari K, Sharma P, Singh A, Kumari H, Jit S, Gupta SK, Lal D, Verma M, Kaur J, Bala K, Jindal S. *Pseudomonas* sp to *Sphingobium indicum*: a journey of microbial degradation and bioremediation of hexachlorocyclohexane. Indian J Microbiol 2008;48:3-18.

Lee CC, Huffman GL. Innovative thermal destruction technologies. Environ Prog 1989;8:190–9.

Li H, Sheng G, Sheng W, Xu O. Uptake of trifluralin and lindane from water by ryegrass. Chemosphere 2002;48:335–41.

Liste HH, Alexander M. Plant promoted pyrene degradation in soil. Chemosphere 2000;40:7-10.

Lockhart JAR, Samuel A, Greaves MP. The evolution of weed control in British agriculture. In: Hance R, Holly K, editors. Weed control handbook: principles. Oxford: Blackwell; 1990. p. 43–74.

Ma X, Andrew AR, Burken JG, Albers S. Phytoremediation of MTBE with hybrid poplar trees. Int J Phytoremed. 2004:4;157–67.

Macek T, Mackova M, Kas J. Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 2000;18:23–34.

Macek T, Kotrba P, Svatos A, Novakova M, Demnerova K, Mackova M. Novel roles for genetically modified plants in environmental protection. Trends Biotechnol 2008;26:146–52.

Maila MP, Cloete TE. Germination of *Lepidium sativum* as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil. Int Biodeterior Biodegrad 2002;50:107–13.

Mackova M, Barriault D, Francova K, Sylvestre M, Möder M, Vrchotova B, Lovecka P, Najmanová J, Demnerova K, Novakova M, Rezek J, Macek T. Phytoremediation of polychlorinated biphenyls. In: Mackova M, Dowling D, Macek T, editors. Phytoremediation and rhizoremediation. Theoretical background. Series: Focus on Biotechnology. Springer; 2006.

Marrs KA. The functions and regulation of glutathione S-transferases in plants. Annu Rev Plant Physiol Plant Mol Biol 1996;47:127–58.

Matsunaga A, Yashuhara A. Complete dechlorination of 1-chloronaphthalene by electrochemical reduction with naphthalene radical anion as mediator. Environ Sci Technol 2003;37:3435–41.

Mattina MI, Iannucci-Berger W, Dykas L. Chlordane uptake and its translocation in food crops. J Agric Food Chem 2000;48:1909–15.

Mattina MI, Eitzer BD, Iannucci-Berger W, Lee WY, White JC. Plant uptake and translocation of highly weathered, soil-bound technical chlordane residues: data from field and rhizotron studies. Environ Toxicol Chem 2004;23:2756–62.

McCutcheon SC, Schnoor JL. Phytoremediation: transformation and control of contaminant. John Wiley & Sons Inc, New Jersey, USA. 2003.

Meagher RB. Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 2000;3:153–62.

Mills T, Arnold B, Sivakumaran S, Northcott G, Vogeler I, Robinson B, et al. Phytoremediation and long-term site management of soil contaminated with pentachlorophenol (PCP) and heavy metals. J Environ Manag 2006.

Misra S, Gedamu L. Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. Theor Appl Genet 1989;78:161–8.

Mohammadi M, Chalavi V, Novakova-Sura M, Laiberte JF, Sylvestre M. Expression of bacterial biphenyl-chlorobiphenyl dioxygenase genes in tobacco plants. Biotechnol Bioeng 2007;97:496–505.

Moreland DE, Corbin FT, McFarland JE. Oxidation of multiple substrates by cron microsomes. Pestic Biochem Physiol 1993;47:206–14.

Mougin C, Cabanne F, Canivenc MC, Scalla R. Hydroxylation and N-demethylation of chlorotoluron by wheat microsomal enzymes. Plant Sci 1990;66:195–203.

Nagata Y, Hatta T, Imai R, Kimbara K, Fukuda M, Yano K, et al. Purification and characterization of γ-hexachlorocyclohexane (γ-HCH) dehydrochlorinase (LinA) from *Pseudomonas paucimobilis*. Biosci Biotechnol Biochem 1993;57:1582–3.

Nelson DR. Cytochrome P450 and the individuality of species. Arch Biochem Biophys 1999;369:1-10.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 1996;6:1-42.

Newman LA, Reynolds CM. Phytodegradation of organic compounds. Curr Opin Biotechnol 2004;15:225–30.

Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 1998;49:249–79.

Noctor G, Strohm M, Jouanin L, Kunert KJ, Foyer CH, Rennenberg H. Synthesis of glutathione in leaves of transgenic poplar overexpressing [gamma]-glutamylcysteine synthetase. Plant Physiol 1996;112:1071–8.

Novakova M, Mackova M, Chrastilova Z, Viktorova J, Szekeres M, Demnerova K, et al. Cloning of the bacterial *bphC* gene into *Nicotiana tabacum* to improve the efficiency of PCB phytoremediation. Biotechnol Bioeng 2009;102:29–37.

Ohkawa H, Tsujii H, Ohkawa Y. The use of cytochrome P450 genes to introduce herbicide tolerance in crops. Pestic Sci 1999;55:1–8.

O'keefe DP, Tepperman JM, Dean C, Leto KJ, Erbes DL, Odell JT. Plant expression of a bacterial cytochrome P450 that catalyzes activation of a sulfonylurea pro-herbicide. Plant Physiol 1994;105:473–824.

Oller ALW, Agostini E, Talano MA, Capozucca C, Milrad SR, Tigier HA, et al. Overexpression of a basic peroxidase in transgenic tomato (*Lycopersicon esculentum Mill.* cv. Pera) hairy roots increases phytoremediation of phenol. Plant Sci 2005;169:1102–11.

Omura T. Forty years of cytochrome P450. Biochem Biophys Res Commun 1999;266:690–8.

Omura T, Sato R. A new cytochrome in liver microsomes. J Biol Chem 1962;237:1375–6.

Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemeprotein nature. J Biol Chem 1964;239:2370–8.

Orville AM, Manning L, Blehart DS, Fox BG, Chambliss GH. Crystallization and preliminary analysis of xenobiotic reductase B from *Pseudomonas fluorescens* I-C. Acta Crystallogr D Biol Crystallogr 2004a;60:1289–91.

Orville AM, Manning L, Blehert DS, Studts JM, Fox B, Chambliss GH. Crystallization and preliminary analysis of xenobiotic reductase A and ligand complexes from *Pseudomonas putida* II-B. Acta Crystallogr D Biol Crystallogr 2004b;60:957–61.

Parameswaran A, Leitenmaier B, Yang M, Kroneck PM, Welte W, Lutz G, Papoyan A, Kochian LV, Kupper H. A native Zn/Cd pumping P (1B) ATPase from natural overexpression in a hyperaccumulator plant. Biochem Biophys Res Commun 2007;36:51-6.

Parrish ZD, White JC, Isleyen M, Gent MPN, Iannucci-Berger W, Eitzer BD, et al. Accumulation of weathered polycyclic aromatic hydrocarbons (PAHs) by plant and earthworm species. Chemosphere 2006;64:609–18.

Perrin-Ganier C, Schiavon F, Morel JL, Schiavon M. Effect of sludge-amendment or nutrition addition on the biodegradation of the herbicide isoproturon in soil. Chemosphere 2001;44:887–92.

Pierrel MA, Batard Y, Kazimaier M, Mignotte-Vieux C, Durst F, Werck-Reichhart D. Catalytic properties of the plant cytochrome P450 CYP73 expressed in yeast: substrate specificity of a cinnamate hydroxylase. Eur J Biochem 1994;224:835–44.

Pilon-Smits E. Phytoremediation. Annu Rev Plant Biol 2005;56:15–39.

Putwain PD. The resistance of insecticide and herbicide on the biodiversity and productivity of aquatic communities. Ecol Appl 2005;15:618–27.

Ramos JL, Gonzalez-Perez MM, Caballero A, van Dillewijn P. Bioremediation of polynitrated aromatic compounds: plants and microbes put up a fight. Curr Opin Biotechnol 2005;16:275–81.

Raskin I. Plant genetic engineering may help with environmental clean-up. Proc Natl Acad Sci USA 1996;93:3164–6.

Richman M. Terrestrial plants tested for cleanup of radionuclides, explosives' residue. Water Environ Technol 1996;8:17–8.

Robineau T, Batard Y, nedelkina S, Cabello-Hurtado F, LeRet M, Sorokine O, et al. The chemically inducible plant cytochrome P450 CYP76B1 actively metabolizes phenylureas and other xenobiotics. Plant Physiol 1998;118:1049–56.

Rosser SJ, French CE, Bruce NC. Engineering plants for the phytoremediation of explosives. In Vitro Cell Dev Biol Plant 2001;37:330–3.

Rugh CL. Genetically engineered phytoremediation: one man's trash is another man's transgene. Trends Biotechnol 2004;22:496–8.

Rugh CL, Wilde D, Stack NM, Thompson DM, Summer AO, Meagher RB. Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *merA* gene. Proc Natl Acad Sci USA 1996;93:3182–7.

Rugh CL, Senecoff JF, Meagher RB, Merkle SA. Development of transgenic yellow poplar for mercury phytoremediation. Nat Biotechnol 1998;16:925–8.

Rylott EL, Bruce NC. Plants disarm soil: engineering plants for the phytoremediation of explosives. Trends Biotechnol 2009;27:73–81.

Rylott EL, Jackson RG, Edwardsj J, Womack GL, Seth-Smith HMB, Rathbone DA, Strand SE, Bruce NC. An explosive-degrading cytochrome P450 activity and its targeted application for the phytoremediation of RDX. Nature Biotechnol 2006;24:216–9.

Sahu SK, Patnaik KK, Sharmila M, Sthunathan N. Degradation of α-hexachlorocyclohexane , β-hexachlorocyclohexane, and γ-hexachlorocyclohexane by a soil bacterium under aerobic conditions. Appl Environ Microbiol 1999;56:3620–2.

Salt DE, Smith RD, Raskin I. Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 1998;49:643–68.

Sanderman Jr H. Higher plant metabolism of xenobiotics: the 'green liver' concept. Pharmacogenetics 1994;4:225–41.

Santos PM, Mignogna G, Heipieper HJ, Zennaro E. Occurrence and properties of glutathione S-transferases in phenol-degrading *Pseudomonas* strains. Res Microbiol 2002;153:89–98.

Schmidt B, Faymonville T, Gembé E, Joußen N, Schuphan I. Comparison of the biotransformation of the 14C-labelled insecticide carbaryl by non-transformed and human CYP1A1, CYP1A2-, and CYP3A4-transgenic cell cultures of *Nicotiana tabacum*. Chem Biodiv 2006a;3:878–96.

Schmidt B, Joußen N, Bode M, Schuphan I. Oxidative metabolic profiling of xenobiotics by human P450s expressed in tobacco cell suspension cultures. Biochem Soc Trans 2006b;34:1241–5.

Schnoor JL. Phytoremediation. Pittsburgh: National Environmental Technology Applications Center; 1997. Technology Evaluation Report TE-97-01.

Schroder P. Exploiting plant metabolism for the phytoremediation of organic xenobiotics. In: Willey N, editor. Phytoremediation: methods and reviews. Totowa, NJ: Humana Press Inc.; 2007.

Schroder P, Daubner D, Maier H, Neustifter J, Debus R. Phytoremediation of organic xenobiotics-Glutathione dependent detoxification in Phragmites plants from European treatment sites. Bioresour Technol 2008;99:7183–91.

Schwab AP, Banks MK. Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In: Anderson TA, Coats JR, editors. Bioremediation through rhizosphere technology. Washington DC: American Chemical Society; 1994.

Shiota N, Nagasawa A, Sakakai T, Yabusaki Y, Ohkawa H. Herbicide-resistant tobacco plants expressing the fused enzyme between rat cytochrome P4501A1 (CYP1A1) and yeast NADPH-cytochrome P450 oxidoreductase. Plant Physiol 1994;106:17.

Shiota N, Inui H, Ohkawa H. Metabolism of the herbicide chlortoluron in transgenic tobacco plants expressing the fused enzyme between rat cytochrome P4501A1 and yeast NADPH-cytochrome P450 oxidoreductase. Pestic Biochem Physiol 1996;54:190–8.

Shiots N, Kodama S, Inui H, Ohkama H. Expression of cytochrome P450 1A1 and P450 1A2 as fused enzymes with yeast NADPH-cytochrome P450 oxidoreductase in transgenic tobacco plants. Biosci Biotechnol Biochem 2000;64:2025–33.

Siminszky B, Corbin FT, Ward ER, Fleischmann TJ, Dewey RE. Expression of a soybean cytochrome P450 monooxygenase cDNA in yeast and tobacco enhances the metabolism of phenylurea herbicides. Proc Natl Acad Sci USA 1999;96:1750–5.

Singh OV, Jain RK. Phytoremediation of toxic aromatic pollutants from soil. Appl Microbiol Biotechnol 2003;63:128–35.

Singleton GL. Genetic analysis of transgenic plant for enhanced phytoremediation. Seattle, WA, USA: University of Washington; 2007.

Smill V. Feeding the world: how much more rice do we need? World Rice Research Conference 2004, Tsukuba Japan, Japanese Committee For International Year of Rice; 2004. p. 1–3.

Sonoki T, Kajita S, Ikeda S, Uesugi M, Tatsumi K, Katayama Y, et al. Transgenic tobacco expressing fungal laccase promotes the detoxification of environmental pollutants. Appl Microbiol Biotechnol 2005;67:138–42.

Suar M, Hauser A, Poiger T, Buser HR, Muller MD, Dogra C, et al. Enantioselective transformation of α-hexachlorocyclohexane by the dehydrochlorinases LinA1 and LinA2 from the soil bacterium *Sphingomonas paucimobilis* B90A. Appl Environ Microbiol 2005;71:8514–8.

Sunderberg SE, Ellington JJ, Evans JJ, Keys DA, Fisher JW. Accumulation of perchlorate in tobacco plants: development of a plant kinetic model. J Environ Monit 2003;5:505–12.

Sung K, Munster CL, Rhykerd R, Drew MC, Corapcioglu MY. The use of vegetation to remediate soil freshly contaminated by recalcitrant contaminants. Water Res 2003;37:2408–18.

Suresh B, Ravishankar GA. Phytoremediation — a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 2004;24:97-124.

Suresh B, Sherkhane PD, Kale S, Eapen S, Ravishankar GA. Uptake and degradation of DDT by hairy root cultures *Cichorium intybus* and *Brassica juncea*. Chemosphere 2005;61:1288–92.

Sylvestre M, Macek T, Mackova M. Transgenic plants to improve rhizoremediation of polychlorinated biphenyls (PCBs). Curr Opin Biotechnol 2009. doi:10.1016/j.copbio.2009.01.006.

Theodoulou FL. Plant ABC transporters. Biochim Biophys Acta 2000;1465:79-103.

Trantirek L, Hynkova K, Nagata Y, Murzin A, Ansorgova A, Sklenar V, et al. Reaction mechanism and stereochemistry of γ-hexachlorocyclohexane dehydrochlorinase LinA. J Biol Chem 2001;276:7734–40.

Trapp S, Yu X, Mosbaek H. Persistence of methyl tertiary butyl ether (MTBE) against metabolism by Danish vegetation. Environ Sci Pollut Res 2003;10:357–60.

Twibell JD, Home JM, Smalldon KW, Higgs DG. The persistence of military explosives on hands. J Forensic Sci 1984;29:284–90.

Uchida E, Ouchi T, Suzuki Y, Yoshida T, Habe H, Vamaguchi I, et al. Secretion of bacterial xenobiotic-degrading enzymes from transgenic plants by an apoplastic expressional system: an applicability for phytoremediation. Environ Sci Technol 2005;39:7671–7.

Vaněk T, Nepovím A, Podlipná R, Zeman S, Vágner M. Phytoremediation of selected explosives. Water Air Soil Pollut 2003;3:259–67.

Van Dillewijn P, Couselo JL, Corredoira E, Delgado E, Wittich RM, Ballester A, et al. Bioremediation of 2, 4, 6-trinitrotoluene by bacterial nitroreductase expressing transgenic aspen. Environ Sci Technol 2008;42:7405–10.

Vijgen J, Li YF, Martin F, Lal R, Weber R. The legacy of lindane and technical HCH production. Organohalog Compd 2006;68:899–904.

Vos RME, Bladeren PJV. Glutathione S-tranferases in relation to their role in the biotransformation of xenobiotics. Chem Biol Interact 1990;75:241–65.

Wang GD, Li QJ, Luo B, Chen XY. Ex planta phytoremediation of trichlorophenol and phenolic allelochemicals via engineered secretory laccase. Nat Biotechnol 2004;22:893–7.

Wang L, Samac DA, Shapir N, Wackett LP, Vance CP, Olszewski NE, et al. Biodegradation of atrazine in transgenic plants expressing a modified bacterial atrazine chlorohydrolase (*atzA*) gene. Plant Biotechnol J 2005;3:475–86.

Wang XX, Wu NF, Guo J, Chu XY, Tian J, Yao B, et al. Phytodegradation of organophosphorus compounds by transgenic plants expressing a bacterial organophosphorus hydrolase. Biochem Biophys Res Commun 2008;365:453–8.

Weber R, Gaus C, Tysklind M, Johnston P, Forter M, Hollert H, Heinisch H, Holoubek I, Lloyd-Smith M, Masunaga S, Moccarelli P, Santillo D, Seike N, Symons R, Torres JPM, Verta M, Varbelow G, Vijgen J, Watson A, Costner P, Woelz J, Wycisk P, Zennegg M. Dioxin- and POP-contaminated sites — contemporary and future relevance and challenges. Environ Sci Pollut Res 2008;15:363–93.

White JC. Phytoremediation of weathered *p,p*'-DDE residues in soil. Int J Phytoremediat 2000;2:133–44.

White JC. Plant-facilitated mobilization of weathered 2,2-bis(*p*-chlorophenyl)-1, 1-dichloroethylene (*p,p*'-DDE) from an agricultural soil. Environ Toxicol Chem 2001;20:2047–52.

White JC. Differential bioavailability of field weathered p,p'-DDE to plants of the *Cucurbita peppo* and *Cucumis* genera. Chemosphere 2002;49:143–52.

White JC, Mattina MJI, Lee WY, Eitzer BD, Iannucci-Berger W. Role of organic acids in enhancing the desorption and uptake of weathered *p,p*'-DDE by *Cucurbita pepo*. Environ Pollut 2003;124:71–80.

Wilce MCJ, Parker MW. Structure and function of glutathione S-transferases. Biochem Biophys Acta 1994;1205:1-18.

Wirtz M, Klucik J, Rivera MJ. Ferredoxin-mediated electrocatalytic dechlorination of haloalkanes by cytochrome P450 cam. J Am Chem Soc 2000;122:1047–56.

Yamada T, Kambara Y, Imaishi H, Ohkawa H. Molecular cloning of novel cytochrome P450 species induced by chemical treatments in cultured tobacco cells. Pestic Biochem Physiol 2000;68:11–25.

Yang H, Cho Y, Eum H, Kim E. Destruction of chlorinated organic solvents in a two stage molten salt oxidation reactor system. Chem Eng Sci 2007;68:5137–43.

Yoon JM, Oh BT, Just CL, Schnoor JL. Uptake and leaching of octahydro-1, 3, 5, 7,-tetranitro-1, 3, 5, 7-tetrazocine by hybrid poplar trees. Environ Sci Technol 2002;36:4649–55.