Research review paper

Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics

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ABSTRACT

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.

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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; Hatakeeda et al., 1999; Wirtz et al., 2000; Kummeling et al., 2001; Perrin-Ganier et al., 2001; Matsunga 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 (Glück, 2003; Huang et al., 2004, 2005; Greenberg, 2006; Gerhardt et al., 2006). 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, sequesetrated, 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 microorganism: 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, pyrrolvolatilization, and rhizodegradation (Fig. 1) (Schnoor, 1997). Phytoremediation 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 pyrovolatilization, 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 ofionic 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 in many contaminated sites, (April and Sims, 1990; Gunther et al., 1996; Binet et al., 2000; Liste and Alexander, 2000; Mattina et al., 2000; White, 2000, 2001, 2002; Fisnmes 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; Astlund 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, chloride etc., there is no apprehension over hazardous waste.
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 tabacum 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). During 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 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) monoxygenases in higher plants play an important role in the oxidative metabolism of endogenous and exogenous lipophilic compounds (Inui et al., 2000; Epen 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 removal of another wheel 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 some 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.

<table>
<thead>
<tr>
<th>Taxonomical groups</th>
<th>No of CYP families</th>
<th>No of sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>99</td>
<td>2279</td>
</tr>
<tr>
<td>Plants</td>
<td>97</td>
<td>1932</td>
</tr>
<tr>
<td>Fungi</td>
<td>282</td>
<td>1001</td>
</tr>
<tr>
<td>Bacteria</td>
<td>177</td>
<td>621</td>
</tr>
<tr>
<td>Protists</td>
<td>51</td>
<td>210</td>
</tr>
<tr>
<td>Archaea</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cytochrome family</th>
<th>Main functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1</td>
<td>Xenobiotic metabolism</td>
</tr>
<tr>
<td>CYP2</td>
<td>Xenobiotic metabolism</td>
</tr>
<tr>
<td>CYP3</td>
<td>Arachidonate acid metabolism</td>
</tr>
<tr>
<td>CYP4</td>
<td>Fatty acid hydroxylation</td>
</tr>
<tr>
<td>CYP5</td>
<td>Thromboxane synthase</td>
</tr>
<tr>
<td>CYP7</td>
<td>Cholesterol 7x-hydroxylation</td>
</tr>
<tr>
<td>CYP8</td>
<td>Prostacyclin synthesis</td>
</tr>
<tr>
<td>CYP11</td>
<td>Cholesterol side-chain cleavage</td>
</tr>
<tr>
<td>CYP17</td>
<td>Steroid 11β-hydroxylation</td>
</tr>
<tr>
<td>CYP19</td>
<td>Androgen aromatization</td>
</tr>
<tr>
<td>CYP21</td>
<td>Steroid 21-hydroxylation</td>
</tr>
<tr>
<td>CYP22</td>
<td>Steroid 24-hydroxylation</td>
</tr>
<tr>
<td>CYP26</td>
<td>Retinoic acid hydroxylation</td>
</tr>
<tr>
<td>CYP27</td>
<td>Steroid 27-hydroxylation</td>
</tr>
<tr>
<td>CYP39</td>
<td>Unknown</td>
</tr>
<tr>
<td>CYP46</td>
<td>Cholesterol 24-hydroxylation</td>
</tr>
<tr>
<td>CYP51</td>
<td>Sterol biosynthesis</td>
</tr>
</tbody>
</table>

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 tabacum, Solanum tuberosum, Orzsa 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 was the production of either herbicide resistant plant (e.g. tolerance towards atrazine, simazine) or plants capable for enhanced metabolism 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.

<table>
<thead>
<tr>
<th>Target plant</th>
<th>Gene (s)</th>
<th>Enzymes</th>
<th>Source</th>
<th>Transgene effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oryza sativa</td>
<td>CYP1A1</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Enhanced metabolism of chlorotoluron, norflurazon</td>
<td>Kawahigashi et al., 2007, 2008</td>
</tr>
<tr>
<td>Nicotiana tabaccum</td>
<td>onr</td>
<td>Pentaoxytritol tetrat rate (PET)</td>
<td>Enterobacter cloacae</td>
<td>Enhanced denitrification of glycerol trinitrate (GTN) and TNT</td>
<td>French et al., 1999</td>
</tr>
<tr>
<td>N. tabaccum</td>
<td>NfiI</td>
<td>Nitroreductase</td>
<td>E. coli</td>
<td>The transgenic plants removed high amount of TNT from the test solution</td>
<td>Hannink et al., 2001, 2007</td>
</tr>
<tr>
<td>A. thaliana</td>
<td>NfiA</td>
<td>Nitroreductase</td>
<td>E. coli</td>
<td>4-hydroxylamine-2, 6-dinitrotoleone.</td>
<td>Kurumata et al., 2005, 2006</td>
</tr>
<tr>
<td>A. thaliana</td>
<td>XplA and XplB</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Rhodococcus rhodoch us</td>
<td>The transgenic plants showed higher nitroreductase activity and 7-8 times higher uptake compared with wild plants.</td>
<td>Jackson et al., 2007</td>
</tr>
<tr>
<td>Hybrid aspen (P. tremula x P. tremuloides)</td>
<td>pmA</td>
<td>Nitroreductase</td>
<td>Pseudomonas putida</td>
<td>Enhanced degradation of RDX</td>
<td>Van Dilewijn et al., 2008</td>
</tr>
<tr>
<td>Hybrid poplar (Populus tremula x populus alba)</td>
<td>CYP450 2E1</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Rabbit</td>
<td>Increased removal of TCE, vinyl chloride, carbon tetrachloride, benzene and chlorof orm from hydroponic solution and air</td>
<td>Doty et al., 2007</td>
</tr>
<tr>
<td>O. sativa</td>
<td>CYP1A1</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Remediation of atrazine and simazine</td>
<td>Kawahigashi et al., 2005b</td>
</tr>
<tr>
<td>O. sativa</td>
<td>CYP1A1, CYP2B6</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Phytoremediation of atrazine and metolachlor</td>
<td>Kawahigashi et al., 2006a</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>CYP1A1, CYP2B6</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Resistance to sulfonamide and other herbicides</td>
<td>Imui and Ohkawa, 2005, 2006</td>
</tr>
<tr>
<td>O. sativa</td>
<td>CYP2C9</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Streptomyces griseol</td>
<td>Resistance to sulfonamide and other herbicides</td>
<td>O’keefe et al., 1994, 1997</td>
</tr>
<tr>
<td>N. tabaccom</td>
<td>CYP450 2E1</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Helianthus tuberosus</td>
<td>Tolerance to sulfonylurea</td>
<td>Hirose et al., 2005, 2007</td>
</tr>
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<td>O. sativa</td>
<td>CYP2B6</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Oxidation of TCE and ethylene dibromides</td>
<td>Doty et al., 2000</td>
</tr>
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<td>O. sativa</td>
<td>CYP2B6</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Metabolism of ethofumesate and benfuresate</td>
<td>Kawahigashi et al., 2005c</td>
</tr>
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<td>O. sativa</td>
<td>CYP2B6</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Remediation of metamitox</td>
<td>Kawahigashi et al., 2005a</td>
</tr>
<tr>
<td>N. tabaccom, A. thaliana</td>
<td>CYP71A10</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Glycine max</td>
<td>Tolerance to phenyl urea herbicide</td>
<td>Siminszky et al., 2005, 2006</td>
</tr>
<tr>
<td>N. tabaccom</td>
<td>tpx1 and tpx2</td>
<td>Peroxidases (Ppx)</td>
<td>Lycopersicon esculentum</td>
<td>Hairy cultures of transgenic tobacco showed enhanced removal of phenol</td>
<td>Alderete et al., 2009</td>
</tr>
<tr>
<td>N. tabaccom</td>
<td>CYP76B1</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Helianthus tuberosus</td>
<td>Tolerance to herbicide</td>
<td>Didierjean et al., 2002, 2003</td>
</tr>
<tr>
<td>N. tabaccom</td>
<td>Gatf-6His</td>
<td>Glutathione S-transferases (GST1)</td>
<td>Maize</td>
<td>Higher tolerance to alachlor</td>
<td>Karavangeli et al., 2005, 2006</td>
</tr>
<tr>
<td>N. tabaccom</td>
<td>bphc</td>
<td>2,3-dihydroxybiphenyl-1, 2-dioxygenase</td>
<td>PC2B2 decoder</td>
<td>Enhanced degradation of PCBs</td>
<td>Chrastilova et al., 2007</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>tpxl</td>
<td>Peroxidases (Ppx)</td>
<td>Roots of L. esculentum</td>
<td>The overexpression of txt gene in transgenic tomato hairy roots resulted</td>
<td>Oller et al., 2005, 2006</td>
</tr>
<tr>
<td>Alfalfa N. tabaccum</td>
<td>atzA</td>
<td>Atrazine chlorohydrolyase Peroxidases</td>
<td>Coriaria versicolor</td>
<td>Enhanced metabolic activity against atrazine</td>
<td>Wang et al., 2005, 2006</td>
</tr>
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<td>N. tabaccum</td>
<td>Mun peroxidase gene</td>
<td>Protex</td>
<td>Protoporphyrinogen IX oxidase</td>
<td>Bacillus subtillis</td>
<td>Enhanced removal of pentachlorophenol (PCP)</td>
</tr>
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<td>A. thaliana</td>
<td>743B4, 73C1</td>
<td>Glucosyltransferases (UGTs)</td>
<td>A. thaliana</td>
<td>Overexpression of UGT genes resulted in enhanced detoxification of TNT and root growth.</td>
<td>Gandia-Herrero et al., 2008, 2009</td>
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<tr>
<td>N. tabaccum</td>
<td>ophe2</td>
<td>Organophosphorus hydrolyase (OHPH)</td>
<td>Pseudomonas pseudocaldigenes</td>
<td>Enhanced degradation of organophosphorus (methyl parathion).</td>
<td>Wang et al., 2008, 2009</td>
</tr>
<tr>
<td>Brassica juncea</td>
<td>γ-ECS, GS</td>
<td>γ-Glutamylcysteine synthetase; Glutathione synthetase</td>
<td>Brassica juncea</td>
<td>Overexpression of ECS and GS resulted in enhanced tolerance to atrazin, 1-chloro-2, 4-dinitrobenzene, phenantherne, metolachlor, phenoxy herbicides.</td>
<td>Flocco et al., 2004, 2006</td>
</tr>
<tr>
<td>Populus trichocarpa</td>
<td>γ-ECS</td>
<td>γ-Glutamylcysteine synthetase</td>
<td>Poplar</td>
<td>Overexpression of γ-ECS resulted in increased tolerance to chloroacetanilide herbicides.</td>
<td>Gullner et al., 2001, 2002</td>
</tr>
<tr>
<td>N. tabaccum</td>
<td>CYP450E1</td>
<td>Cytochrome P450 monooxygenase</td>
<td>Human</td>
<td>Enhanced degradation of atracene and chloroxygen</td>
<td>Dixit et al., 2008, 2009</td>
</tr>
<tr>
<td>LACI</td>
<td>A. thaliana</td>
<td>Root specific laccase</td>
<td>Cotton</td>
<td>Secrtes laccase to the rhizosphere and have shown enhanced resistance to phenolic allelochemicals and enhanced tolerance to 2, 4, 6-trichlorophenol.</td>
<td>Wang et al., 2004, 2005</td>
</tr>
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</table>

Transgenic plants for enhanced rhizodegradation

Table 3
5.1. 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 genes has been cloned and characterized, such as CYP73A1 and CYP76B1 from Jerusalem artichoke (Helianthus tuberosus) (Pierrle et al., 1994; Robineau et al., 1998), CYP71A11 from tobacco (Nicotiana tabacum) (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

Engineered rice plants expressing human cytochrome P450 genes have been developed to confer resistance to herbicides and be able to clean up residual compounds, including styrene, 1,2-dichloropropane and others. Doty et al. (2007) 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-trichloroacetalddehyde), 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 rice should be expected to be resistant 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 towards photosynthesis-inhibiting herbicides (atrazine), chlorotoluron, metabenziazuron, 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).

5.1.2. 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 towards photosynthesis-inhibiting herbicides (atrazine), chlorotoluron, metabenziazuron, 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).
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 plant (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 Ravishanker, 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 electrophilic 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-units 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). Since plants contain complex multigene families of GSTs; the various subunits may be able to producing multiple homo and hetero-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 catalytic 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 enzyme GST I from maize exhibits significant catalytic 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 (CDN), fenoxaprop, and propachlor) in Phragmites australis (Cav.) Trin. Ex. Steud.). A study of Brenter et al. (2008) showed that expression of glutathione S-transferases 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). Brasica juncea over-expressing γ-glutamyl-cysteine-synthetase and glutathione synthetase also have shown enhanced tolerance to atrazine, CDN, metolachlor and phenthanthrene (Flocco et al., 2004). Recently, Dixit et al. (2008), introduced human P4502E1 and GST from fungus Trichoderma virens in N. tabacum. The transgenic plant has shown enhanced degradation of anthracene and chloropyriphos. Thus, it is expected that the transgenic
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), diamoninitrotoluene, 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 flavinreductase belonging to the β/α old yellow enzyme (OYE) family, such as XenA and XenB in Pseudomonas, pentaerythritol tetranitrate (PETNr) reductase in Enterobacter cloaceae, NemA in Escherichia coli, morphinone reductase in Pseudomonas putida M10, and OYE in Saccharomyces cerevisiae (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 Hughes 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; Hammink 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) reductase into N. tabacum, resulting in increased tolerance...
the rhizosphere effect is generally attributed to an increase in microbial processes. It comes into being when a root tip enters a volume of soil and the soil in the immediate vicinity of a root that is affected by root secretion and mineralization of persistent organic pollutants at the root rhizospheric zone (Kawahigashi, 2009). The rhizosphere is also possesses activity towards nitroaromatics. It has been shown to catalyze the reductive transformation of TNT. Ructive 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 (xpβ) and a fused flavodoxin cytochrome P450 (xpA). 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) glycosyltransferases (UGTs) (74284 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-hydroxylaminodinitrotoluene 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-hydroxylaminodinitrotoluene substrates (Gandia-Herrero et al., 2008).

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 root exudates, mucigel and root lyases (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 Corioli versicolor 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-dihydroxibiphenyl-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.
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-chlorobutane 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 inhibit 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 indiscriminate 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 camara, Achyranthes aspera, Dalbergia siso, 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-PCHC) and α-pentachlorocyclohexane (α-PCHC); 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, 6S)-1,3,4,5,6-pentachlorocyclohexane (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) Withania 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 trial 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.

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