Tobacco (Nicotiana tabacum) a Potent Phytoremediator

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ABSTRACT

The low-cost, plant-based phytoremediation technique has often been described as a promising technique to remediate agricultural land contaminated with organic and inorganic pollutants. The plants used, have to meet certain requirements, which are fulfilled by tobacco (Nicotiana tabacum). It is a fast growing plant with a high biomass, which is easily harvested. Its propagation is simple, as each plant generates thousands of seeds. It can prosper everywhere between the 50th latitude north and the 40th latitude south and has no demanding requirements on temperature, humidity and soil conditions. Tobacco has also revealed a high tolerance for various organic and inorganic pollutants. It can accumulate heavy metals in relatively high-levels, especially Cd, in comparison to other species and has also shown not be susceptible to various organic pollutants, such as polychlorinated biphenyls (PCB) and trinitrotoluene (TNT). Its rapid growth, high leave biomass and its high disposition for transformation has made tobacco an optimal plant for genetic engineering. It has not only been applied in the field of medicine, e.g. production of antibodies, but also in the area of phytoremediation. Metal chelator, metal transporter, metallothionein (MT), and phytochelatin (PC) genes have been transferred to plants for improved metal uptake and sequestration. Also the expression of bacterial enzymes has enabled the reduction of phytoxicity or the concentration reduction of various organic pollutants.

Keywords: heavy metals, organic pollutants, phytoremediation

Abbreviations: EDDS, ethylene diamine disuccinate; EDTA, ethylene diamine tetraacetate; MT, metallothionein; PCB, polychlorinated biphenyls; PCP, pentachlorophenol; PETN, pentaerythritol tetranitrate; PC, phytochelatin; PCB, polychlorinated biphenyls; TF, translocation factor; TNT, trinitrotoluene

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INTRODUCTION

Over the centuries, human activities, such as extensive mining, agriculture, industry and military operations have released huge amounts of toxic compounds, contaminating large areas in both developed and developing countries. These contaminants have negative effects on the ecosystems and other natural resources, and, moreover pose great danger to public health, as pollutants can enter food via agricultural products or leach out into drinking water (Commission of the European Communities 2002; European Environmental Agency 2003). The European Environment Agency has estimated the total costs for the clean-up of contaminated sites in Europe to be between EUR 59 and 109 billion (Commission of the European Communities 2002). World wide about 2 000 million ha of soil, equivalent to 15 per cent of the Earth’s land area (an area larger than the United States and Mexico combined), have been degraded (i.e. erosion, contamination) through human activities (UNEP 2002).

There are two major classes of contaminants: inorganic and organic. Inorganic compounds include heavy metals, such as cadmium (Cd), lead (Pb) and mercury (Hg), and non-metallic compounds, such as arsenic (As), and radionuclides, such as uranium. Organic contaminants include different compounds, such as petroleum hydrocarbons, chlorinated solvents, halogenated hydrocarbons, such as trichloroethylene, and explosives, such as trinitrotoluene (TNT).

The clean up of contaminated land by traditional physiochemical methods, including soil excavation and land filling, soil washing and immobilisation or extraction can be very costly, and, in addition, destructive to the soil. Phytoremediation, the use of green plants to remove pollutants from the environment or to render them harmless (Raskin et al. 1997), with its lower cost and environmental friendly nature, has received increasing attention in the last decades (Salt et al. 1998; Garbisu and Alkorta 2001). This emerging low-cost technology can be applied to both inorganic and organic pollutants, present in solid substrates (e.g. soil), liquid substrates (e.g. water), and the air.

The area of phytoremediation focusing on the removal
of inorganic pollutants from soil (phytoextraction) is based on a natural propensity of plants to take up metals. Certain plant species, known as hyperaccumulators, such as Thlaspi, Urtica, Chenopodium, Polygonum sachalessa and Alyssum have shown the ability to extract, accumulate and tolerate high levels of heavy metals. By definition, a hyperaccumulator must accumulate at least 100 mg kg\(^{-1}\) (0.01% dry wt.) Cd, As and some other trace metals, 1000 mg kg\(^{-1}\) (0.1% dry wt.) cobalt (Co), copper (Cu), chrome (Cr), nickel (Ni) and Pb and 10,000 mg kg\(^{-1}\) (1% dry wt.) manganese (Mn) and Ni (Watanabe 1997; Reeves and Baker 2000). However, their potential for application in bioremediation is limited by the fact that they are slow growing and have a small biomass (Mulligan et al. 2001; Puschenreiter et al. 2001). Another option is the use of high biomass plants which are usually not considered to have metal-specific affinity and contain only low to average heavy metal concentrations, but which compensate for this by their high biomass, as for example tobacco (Keller et al. 2003).

The concept of using plants to remediate soils contaminated with organic pollutants is a recent development, based on observations that the disappearance of organic chemicals is accelerated in vegetated soils as compared to that of surrounding non-vegetated bulk soils. In addition to the direct uptake and metabolism of organic pollutants, plants can exudates from their roots that enhance microbial bioremediation in the rhizosphere, which has been termed phytoremediation (Slat et al. 1995).

The remedial capacity of plants can be significantly improved by genetic manipulation and plant transformation technologies (Cherian and Oliveira 2005). Over the past years, a range of different plant systems such as Arabidopsis thaliana, Indian mustard (Brassica juncea), canola (B. napus) and tomato (Lycopersicon esculentum Mill. cv. ‘Pera’) have been developed to increase the uptake and the detoxification of inorganic and organic pollutants (Oller et al. 2005; Farwell et al. 2006; Li et al. 2006; Banuelos et al. 2007). The choice of system depends on many factors, but the intrinsic efficiency and the suitability for scale-up, storage and downstream processing are particularly important. Tobacco (Nicotiana tabacum) is a leafy crop which generates a large amount of biomass and allows rapid scale-up due to the immense number of seeds produced in each generation. In addition, tobacco is long established as a model system for plant transformation, and therefore benefits from simple gene transfer, regeneration procedures and optimised vector systems. These characteristics make tobacco an excellent candidate for the production of transgenic plants in the field of phytoremediation (Stoger et al. 2005). However, the potential for using these genetic resources in transgenic phytoremediation approaches has been poorly explored. Inadequate data is available on the performance of these transgenic plants on soil substrate, or under field conditions. Most transgenic plants have so far only been tested only in hydroponic or agar-based media containing trace elements. In this medium, the concentration of competing ions and the speciation of trace elements is highly reproducible and the plant exposure to elements is high. On contaminated field sites, the soil composition, trace element bioavailability and the chemical speciation are site-specific and subject to considerable spatial and temporal variations within one site (Sappin-Didier et al. 2005).

**INORGANIC CONTAMINANTS**

**Phytoextraction**

The uptake capability can vary greatly among the tobacco species (Doroszewska and Berbec 2004). The study of Mench et al. (1989) revealed that N. tabacum extracted a significant higher Cd amount than N. rustica as verified in the higher Cd concentration in the leaves and greater biomass. Such differences have been reported even amongst N. tabacum varieties, although these differences appear less important than external factors, such as soil characteristics (Lugon-Moulin et al. 2004). Tobacco (N. tabacum) can accumulate Cd at relatively high levels compared to other species (Davis 1984; Mench et al. 1989; Wagner 1993; Kayser et al. 2000; Wenger et al. 2002; Keller et al. 2003; Evangelou et al. 2004, 2007b) as summarised and compared to other crop plants in Table 1. Concentrations in field-grown tobacco leaves usually range from <0.5 to 5 mg Cd kg\(^{-1}\), although higher values can also be found (van der Hoorn et al. 2004). In studies by Kayser et al. (2000), Cd concentrations in N. tabacum were only 50% lower than in the hyperaccumulators, T. caerulescens and A. murale and were respectively 6-, 7- and 2.5-fold higher than in the crop plants, corn (Zea mays) sunflower (Helianthus annuus) and Indian mustard (B. juncea). In the same study, in the case of zinc (Zn) and Cu the concentrations in tobacco showed no significant difference to the crop plants. The hyperaccumulators, T. caerulescens and A. murale however reached Zn and Cu concentrations in shoots which were 20- and 2.5-fold respectively, higher compared to the concentrations achieved by N. tabacum. The findings of Mench et al. (1989) support the results of Kayser et al. (2000), as N. tabacum reached significantly higher Cd concentrations than Z. mays (21.7 mg kg\(^{-1}\)). The studies of Jiang et al. (2003) and Meers et al. (2004) showed that plants grown at a Cd soil concentration of 200 mg kg\(^{-1}\) in B. juncea. In these studies however the Cd soil concentration was 200 mg kg\(^{-1}\) which is 10-fold higher than any study conducted with N. tabacum. N. tabacum as seen in Table 1 can achieve high concentrations in the case of Zn whereas the uptake of Cu and Pb is in the same order of magnitude as the other listed crop plants.

The ability of tobacco to achieve high heavy metal concentration in the shoots, combined with high biomass, makes it possible to reach high heavy metal outputs. In Keller et al. (2003) N. tabacum had significantly higher Cd outputs (g ha\(^{-1}\)) than B. juncea and Z. mays, and similar outputs contrasted to willow (Salix viminalis), but lower than T. caerulescens. In the case of Cu N. tabacum achieved significantly higher outputs than all the other plants mentioned above, with the exception of Z. mays. In Wenger et al. (2002), although N. tabacum reached higher Zn concentrations than Z. mays, the biomass was lower and, therefore, the removal of Zn was higher by Z. mays than by N. tabacum.

In this review the effect of added chelating agents and their effect on the uptake of heavy metals will not be examined as it has been recently elsewhere reviewed by Evangelou et al. (2007). However, the tolerance or susceptibility of N. tabacum to various chelating agents applied to soil and leaves has been briefly described. Ethylene diamine tetraacetate (EDTA) displayed a similar degree of toxicity towards H. annuus (Meers et al. 2005) and a higher one towards Z. mays and white bean (Phaseolus vulgaris) (Luo et al. 2005) compared to N. tabacum. Meers et al. (2005) applied 1.77 mmol kg\(^{-1}\) EDTA and observed no toxicity symptoms, whereas Luo et al. (2005) applied 5 mmol kg\(^{-1}\) EDTA and the dry weight decreased by approximately 55% in comparison to the control. With respect to ethylene diamine tetraacetate (EDTA), it showed a similar degree of toxicity towards B. juncea (Epstein et al. 1999; Wu et al. 2004) and H. annuus (Meers et al. 2005). Regarding EDDS, this was not the case with N. tabacum. It was no more susceptible to chelating agents than other plant species. The degree of toxicity is in concurrence with present literature on the subject.

Besides achieving a high heavy metal concentration in the shoots, the translocation factor (TF), the ratio of metal (loid) concentration in shoots to that in roots, is very important. The TF can be used to evaluate the capacity of a plant to transfer metals from roots to shoots (TF is usually >1 (or ≥1) in (hyper)accumulators and <1 in excluders (McGrath and Zhao 2003), thus revealing if the harvestable part of the plant is the component with the highest metal concentration. In the study of Keller et al. (2003), N. tabacum was the only species which showed TF >1 for Cd, Cu and Zn. Zea mays

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**Table 1**

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Metal</th>
<th>Concentration (mg kg(^{-1}))</th>
<th>TF</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zea mays</td>
<td>Cd</td>
<td>21.7</td>
<td>&gt;1</td>
<td>Jiang et al. (2003)</td>
</tr>
<tr>
<td>B. juncea</td>
<td>Cd</td>
<td>200</td>
<td>&gt;1</td>
<td>Meers et al. (2003)</td>
</tr>
<tr>
<td>N. tabacum</td>
<td>Cd</td>
<td>100</td>
<td>&gt;1</td>
<td>Keller et al. (2003)</td>
</tr>
<tr>
<td>Z. mays</td>
<td>Cu</td>
<td>5.0</td>
<td>&gt;1</td>
<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>H. annuus</td>
<td>Cu</td>
<td>5.0</td>
<td>&gt;1</td>
<td>Meers et al. (2005)</td>
</tr>
<tr>
<td>N. tabacum</td>
<td>Cu</td>
<td>100</td>
<td>&gt;1</td>
<td>Keller et al. (2003)</td>
</tr>
<tr>
<td>N. tabacum</td>
<td>Pb</td>
<td>10,000</td>
<td>&gt;1</td>
<td>Evangelou et al. (2004)</td>
</tr>
<tr>
<td>Z. mays</td>
<td>Pb</td>
<td>1,000</td>
<td>&gt;1</td>
<td>Evangelou et al. (2007)</td>
</tr>
</tbody>
</table>

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**References**

- Sappin-Didier et al. 2005
- Doroszewska and Berbec 2004
- Kayser et al. 2000
- Mench et al. 1989
- Wagner 1993
- Davis 1984
- Meers et al. 2005
- Luo et al. 2005
- Evolution et al. 2007b
showed a TF >1 only for Zn, while B. juncea showed a TF >1 for Cd and Zn as did T. caerulescens. Although Chiang et al. (2006) in their study of N. benthamiana and H. annuus reported a TF <1 for Cd, this does not contradict the results of Keller et al. (2003), as another tobacco species was used.

### Transgenic tobacco and phytoextraction

A large number of genes involved in acquisition, allocation and detoxification of trace elements have been identified and characterised from a variety of organisms. Genetic engineering methodologies have made it possible to transfer such appropriate genes into high biomass plants. An acquisition strategy would entail in using genes involved in the biosynthesis of metal chelators, such as citrate, malate, histidine or in root exudation of protons (Hall 2002). An allocation strategy would examine the modification of the metal transport across the plasma membrane. Several transporters (i.e., the heavy metal (CPx-type) ATPases, the natural resistance-associated macrophage protein family, members of the cation diffusion facilitator family) (Williams et al. 2000) and N. tabacum calmodulin-binding protein (Arazi et al. 1999) involved in the transport of metals could be over expressed in order to increase metal uptake and detoxification. A detoxification approach could be the over expression of peptides involved in the metal homeostasis of plants such as metallothioneins (MT), phytochelatines (PC) and glutathione (Cobbett and Goldsborough 2002), the reduction of free metal concentration in the cytoplasm by chemical transformation (Bibilly et al. 1999; Rugh et al. 1999) or the over expression of superoxide dismutase to reduce active oxygen species (Boominathan and Doran 2006). An overview of the methods applied to transform tobacco and their uptake and tolerance enhancement are displayed in Table 2.

Most of the studies have focused on Cd as tobacco has a natural propensity to accumulate Cd. In order to confer resistance to toxic levels of heavy metals, Misra et al. (1989) introduced a chimeric human MT gene into N. tabacum cells. The transgenic plants displayed a 6-fold higher tolerance to Cd than the control plants. The dry weight of the control dropped from 28.6 g to 5 g owing to the Cd treatment (0.1 mM CdCl₂), whereas the dry weight of the transformed N. tabacum plants remained at approximately 28 g. de Borne et al. (1998) used tobacco plants which expressed the mammalian MT gene, and observed that the leaf Cd levels were decreased by 73% compared to the control plants. The Cd concentration in control leaf lamina, 4.44 mg kg⁻¹ was reduced to 1.8 mg kg⁻¹. However, the decrease in leaf Cd was correlated with an increase in Cd in the roots and stems, thus leaving a large part of the Cd in the non harvestable part of the plant. In controls, 70-80% of the Cd was translocated to the leaves, whereas 40-50% was translocated in MT1 plants. Significantly Macek et al. (2002) introduced an additional small metal binding domain, a polyy
histidine chain, to the metal binding protein, yeast MT, and observed a 90% increased Cd accumulation (27.0 mg kg⁻¹) combined with a reduction of Cd content in the roots, by 55% (9.0 mg kg⁻¹), compared to the control which accumulated 15.8 and 9.0 mg kg⁻¹ Cd respectively. With the same construct Pavlíková et al. (2004b) investigated transgenic N. tabacum and their study indicated 110% higher Cd levels compared to the control plants, while Nagata et al. (2006) increased the tolerance of N. tabacum to Hg (2-fold higher biomass and root length) by over expressing the bacterial polyphosphate kinase. Rumeau et al. (2004) increased Zn content by 30% in transplastomic tobacco plants expressing a polyhistidine-tagged Rubisco large subunit however it had no effect on the uptake of Cu and Fe. Kawashima et al. (2004) increased Cd, Se and Ni tolerance by the over expression of cysteine synthase in the cytosol and chloroplast of N. tabacum. N. tabacum plants expressing Arabidopsis antiporter CAX2 (calcium exchanger 2) were not only more tolerant to elevated Mn²⁺ levels but also accumulated more Ca²⁺ (20%), Cd²⁺ (15%), and Mn²⁺ (20%) (Hirschi et al. 2000). Yazaki et al. (2006) inserted the human multidrug resistance-associated protein, one of the most intensively studied ABC transporters, into N. tabacum cv. ‘Samsun NN’ and achieved a higher Cd tolerance compared to the control plants.

### Table 2: Genes and methods used for the development of transgenic tobacco plants to cope with inorganic pollutants, as well as the effect resulting from the transformation.

<table>
<thead>
<tr>
<th>Targeted Metal</th>
<th>Gene</th>
<th>Method</th>
<th>Effect (compared to control)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>Human MT-II processed gene (hMT-II) with CaMV 35S promoter</td>
<td>Agrobacterium tumefaciens (Ti-plasmid)</td>
<td>6-fold better tolerance</td>
<td>Misra et al. 1989</td>
</tr>
<tr>
<td>Pb</td>
<td>Wheat gene encoding phytochelatin synthase (TaPCS1)</td>
<td>Agrobacterium tumefaciens (Ti-plasmid)</td>
<td>15% higher Cd concentration</td>
<td>Kawashima et al. 2004</td>
</tr>
<tr>
<td>Cu</td>
<td>CUP1 MT gene (Saccharomyces cerevisiae) with CaMV 35S promoter</td>
<td>A. tumefaciens (plasmid PBII121)</td>
<td>Up to 3-fold higher Cd concentration</td>
<td>Thomas et al. 2003</td>
</tr>
<tr>
<td>Hg</td>
<td>Bacterial arsenate reductase gene (arsC)</td>
<td>A. tumefaciens</td>
<td>Higher fresh weight and 50% higher Cd concentration</td>
<td>Dhanikher et al. 2003</td>
</tr>
</tbody>
</table>

In contrast to the control plants, while Nagata et al. (2006) increased the tolerance of N. tabacum to Hg (2-fold higher biomass and root length) by over expressing the bacterial polyphosphate kinase. Rumeau et al. (2004) increased Zn content by 30% in transplastomic tobacco plants expressing a polyhistidine-tagged Rubisco large subunit however it had no effect on the uptake of Cu and Fe. Kawashima et al. (2004) increased Cd, Se and Ni tolerance by the over expression of cysteine synthase in the cytosol and chloroplast of N. tabacum. N. tabacum plants expressing Arabidopsis antiporter CAX2 (calcium exchanger 2) were not only more tolerant to elevated Mn²⁺ levels but also accumulated more Ca²⁺ (20%), Cd²⁺ (15%), and Mn²⁺ (20%) (Hirschi et al. 2000). Yazaki et al. (2006) inserted the human multidrug resistance-associated protein, one of the most intensively studied ABC transporters, into N. tabacum cv. ‘Samsun NN’ and achieved a higher Cd tolerance compared to the control plants.

The effect of a transformation is not only correlated with the target metal but also to where the construct is placed in the genome. This is clearly demonstrated in the study by Thomas et al. (2003) where the introduced construct increased the tolerance of N. tabacum but did not have any effect on the uptake of Zn and Ni.

**merA** and **merB** genes have been isolated from bacteria,
and when combined may produce elementary Hg from methyl-Hg, which can be introduced into plants. *A. thaliana* was first transformed with modified *merA* and *merB* genes, and produced transgenic plants Hg-resistant and volatile (Rugh et al. 1996; Bizily et al. 1999). Since then several plants including tobacco, have been transformed. Rugh et al. (2000) showed that *merA/merB* tobacco plants displayed an enhanced Hg-tolerance, while *merA* tobacco plants removed 3- to 4-fold more Hg from hydroponic medium than untransformed controls (Meagher et al. 2000). Heaton et al. (1998), He et al. (2001), Ruiz et al. (2003) and Heaton et al. (2005) have also worked with similar constructs and have achieved encouraging results. In Detail: In Heaton et al. (1998) the transformed tobacco plants displayed up to 3.5-fold higher fresh weight and up to 80% lower Hg concentration, owing to the volatilisation of Hg, compared to control. He et al. (2001) observed a higher germination rate and a 5-fold higher Hg release from the leaves. Ruiz et al. (2003) achieved 2-fold higher biomass and 2-fold higher chlorophyll content with the introduction of the construct. The *merA* construct introduced by Heaton et al. (2005) increased the transpiration of the transformed tobacco plants by 2-fold and reduced the Hg concentration by 75% in comparison to the wild type tobacco plants.

**ORGANIC CONTAMINANTS**

**Phytodegradation**

As organic compounds are usually man-made and xenobiotic in plants, there are no transporters for their uptake. The usual mechanism of uptake is by simple diffusion (passive uptake). When organic contaminants come into contact with roots, they may be sorbed to the root structure. The hydrophobic or hydrophilic nature of the organic compounds also determines their possible uptake (Cherian and Oliveira 2005).

Organic pollutants can be degraded or mineralised by plants, either independently or in association with microorganisms. According to a study by Ryslava et al. (2003), the degradation rate of organic pollutants, such as polychlorinated biphenyls (PCB) by rhizospheric microbial activity depends on the plants used. *N. tabacum* sufficiently degraded PCB, whereas black nightshade (*Solanum nigrum*) and alfalfa (*Medicago sativa*) owing to their rhizospheric microbial community displayed significantly lower degradation rates. Moreover, *N. tabacum* showed significantly higher PCB contents in its shoots compared to the other two species used.

Plants have significant metabolic activity in both roots and shoots, and some of the enzymes involved in these metabolic processes, namely nitrreductases, dehalogenases, laccases, peroxidases, etc., are useful in the remediation process. The process though, in native plants is often incomplete and inefficient for the vast amount of different organic pollutants in the environment, and thus genetic engineering can be viewed as a useful tool in coping with these challenges.

**Transgenic tobacco and phytodegradation**

Genetic engineering has several targets in the process of phytotransformation where it can be introduced and used to increase its effect. Phytotransformation is a process by which plants take up organic pollutants and, subsequently, metabolise or transform them into less toxic metabolites. Once taken up and translocated the organic chemicals generally undergo three transformation stages: (a) chemical modification (oxidations, reductions, hydrolysis); (b) conjugation (with glutathione, sugars, amino acids); and (c) sequestration or compartmentation (conjugates are converted to other conjugates and deposited in plant vacuoles or bound to the cell wall and lignin) (Cherian and Oliveira 2005). An overview of the methods applied to transform tobacco and their effectiveness are displayed in Table 3.

Typical plant enzymes which catalyse the first phase of the reactions are P450 monoxygenases, and these are a good candidate for enhancing the phytoremediation potential. Bode et al. (2004) introduced two species of human P450 cDNA in tobacco cells by *Agrobacterium*-mediated transformation, and these were tested against atrazine metabolism. Transgenic cultures were able to produce larger amounts of nonphytotoxic (primary oxidised) metabolites than non-transformed cultures. In another study, Didierjean et al. (2002), introduced Jerusalem artichoke (*H. tuberosus*) xenobiotic inducible cytochrome P450, CYP76B1, into *N. tabacum* and reported an increase in the tolerance to various herbicides, such as linuron (20-fold), isoproturon and chlorotoluron (10-fold). Transgenic tobacco expressing mammalian cytochrome P450 monooxygenase has been shown to harbor oxygenating activity for organic compounds, such as the herbicide chlorotoluron, 7-ethoxycoumarin, benzo[a]pyrene, and halogenated hydrocarbons (Shiota et al. 1994; Doty et al. 2000).

The potential of *Nicotiana tabacum* as a phytoremediation plant. Evangelou et al.

<table>
<thead>
<tr>
<th>Targeted organic pollutant</th>
<th>Gene</th>
<th>Method</th>
<th>Effect (compared to control)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td>Human CYP1A1 and human CYP1A2 with CaMV 35 S promoter</td>
<td>Agrobacterium <em>tumefaciens</em></td>
<td>transgenic plants transformed 100% of atrazine to metabolites, control plants only 20%</td>
<td>Bode et al. 2004</td>
</tr>
<tr>
<td>Linuron, Isoproturon, Chlorotoluron</td>
<td>CYP76B1 from <em>Helianthus tuberosus</em> with GAL10-CYC1 promoter</td>
<td>A. <em>tumefaciens</em> (plasmid RK2013)</td>
<td>20-fold tolerance increase to linuron and 10-fold to isoproturon and chlorotoluron</td>
<td>Didierjean et al. 2002</td>
</tr>
<tr>
<td>7-ethoxycoumarin benzo[a]pyrene</td>
<td>Fusion of rat CYP1A1 and yeast NADPH-cytochrome P450 oxidoreductase</td>
<td>A. <em>tumefaciens</em> (plasmid pAFCRI and plasmid pTF2)</td>
<td>10-fold higher activity toward both 7-ethoxycoumarin and benzo[a]pyrene</td>
<td>Shiota et al. 1994</td>
</tr>
<tr>
<td>TNT</td>
<td>Nitroreductase(<em>nfsI</em>) from <em>E. cloacae</em></td>
<td>A. <em>tumefaciens</em> (plasmid pW1)</td>
<td>Up to 2-fold higher dry weight, higher TNT removal</td>
<td>Hannink et al. 2001</td>
</tr>
<tr>
<td>GTN, Atrazine</td>
<td>PETN-reductase (one) with CaMV 35 S promoter modified bacterial <em>aiz</em> gene with CsVMV promoter</td>
<td>A. <em>tumefaciens</em> (plasmid pW1)</td>
<td>2-fold faster GTN metabolism</td>
<td>Gisbert et al. 2003</td>
</tr>
<tr>
<td>PCP</td>
<td>Mn-peroxidase of <em>Coriolus versicolor</em> with CaMV 35 S promoter</td>
<td>A. <em>tumefaciens</em> (plasmid pTF1)</td>
<td>38-fold higher atrazine tolerance</td>
<td>Wang et al. 2005</td>
</tr>
<tr>
<td>PCP, BCP</td>
<td>laccase of <em>Coriolus versicolor</em> with CaMV 35 S promoter</td>
<td>A. <em>tumefaciens</em> (plasmid pTF1)</td>
<td>2-fold higher PCP removal</td>
<td>Himura et al. 2002</td>
</tr>
<tr>
<td>PCB</td>
<td>Bacterial gene <em>pbgHC</em> with CaMV 35 S promoter</td>
<td>A. <em>tumefaciens</em> (plasmid pTF1)</td>
<td>Up to 6-fold higher reomoval</td>
<td>Sonoki et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-fold higher germination capability</td>
<td>Macek et al. 2005</td>
</tr>
</tbody>
</table>
Besides assisting the natural phytotransformation, new compounds from various organisms can be introduced into plants to increase their phytoremediation potential for organic pollutants. For instance, as 2,4,6-trinitrotoluene (TNT), pentaoxyriholite tetranitratre (PETN) and nitroglycerin, are useful because as stated by Meagher (2006) mineralisation of TNT by native (that is, not genetically engineered) plants is inefficient and generally incomplete. To engineer plants to attract to TNT, two bacterial enzymes (PETN reductase and nitroreductase), able to reduce TNT into less harmful compounds, were over expressed in N. tabacum cv. 'Xanthi’. The two genes, pmtA (encoding PETN reductase) and nfr1 (encoding nitroreductase), under the control of a constitutive promoter provided the transgenic plants with increased tolerance to TNT at concentrations that severely affected the development of wild type plants (French et al. 1999). 

Other organic compounds, such as atrazine, pentachlorophenol (PCP), bisphenol A (BPA) and PCBs have also become targets for genetic engineering in the field of phytoremediation. The biodegradation of atrazine in transgenic N. tabacum expressing a modified bacterial atrazine chlorohydrolase (atZ) gene was increased, and was accompanied by an increased atrazine tolerance compared to the control plants (Wang et al. 2005). In 2002 Limura et al. reported that manganese peroxidase-expressing transgenic tobacco was able to reduce PCP effectively. In a study by Sonoki et al. (2005) the laccase of Coriolus versicolor, an extracellular fungal enzyme, was introduced into N. tabacum cv. ‘Samson NN” and the transgenic plants were able to remove BPA and PCP from an aqueous solution, by secreting laccase into the rhizosphere. Tobacco plants expressing the bacterial enzyme responsible for cleaving PCBs, coded by the gene bphC from the bacterial biphenyl operon, displayed a higher PCB tolerance than the control plants (Maek et al. 2005).

As in the case for genetic engineering in the field of heavy metal phytorextraction the effect of a transformation is correlated to the over expression of the construct introduced. In a study by Uchida et al. (2005), crude leaf extracts of transgenic plants expressing tobacco expressing haloalkane dehalogenase, which catalyses hydrolytic dechlorination of 1-chlorobutane, cytoplasmic enzymes showed 76.4-fold higher xenobiotic-degrading activity than did leaves expressing apoplastic-targeted enzymes.

ROOTS AND DEPTH OF CONTAMINATION

Before selecting the plant to be applied on the contaminated field different parameters have to be taken into account. These include the initial level of contamination, the type and the use of the soil to remediate, the area and depth of soil concerned. Indeed, plants have to be able to reach the metal to be removed, which means that their root system must develop within the contaminated zone. According to Keller et al. (2003) N. tabacum is able to extract metals only from the upper part of the contaminated layer (0.2 m) unlike Z. mays and S. virnalis which could extract metals from depths up to 0.75 m. Consequently, tobacco is suitable only for the phytorextraction of areas where the contaminated soil does not have a great depth.

FUTURE OF TRANSGENIC AND WILD TYPE TOBACCO IN THE FIELD OF PHYTOREMEDIATION

In the last decades transgenic tobacco plants with enhanced capacities to chelate or metabolise toxic metals have been tested in the field (Yeorgan et al. 1992; Brandle et al. 1993). Although the future is promising for transgenic plants, there is much concern about their use. Questions not dissimilar to those surrounding genetically modified food plants have been raised: Will they spread uncontrollably? Will they decrease genetic variability by interbreeding with wild plants? Will they rob the soil of its nutrients as well the toxin? Will they somehow find their way into the food chain and harm human and animal health (Watanabe 2001)? The last question, though, is not applicable to tobacco, as it is a non-food/non-feed crop. However, transgenic plants will probably not be applicable in the near future owing to restrictive laws and low public acceptance in several countries.

Tobacco is a self pollinating plant; however it produces extensive amounts of pollen which could be distributed by wind. In order to prevent the transfer of transgenic pollen to wild populations various legislation organs have adopted several laws. In the case of transgenic tobacco in the field of phytoremediation, harvesting before bloom would minimise the risk of hybridisation. During bloom tobacco biomass increases only very slowly, thus it does not extract high amounts of various elements from the soil as a consequence the most effective part for phytoremediation has been completed. Harvesting in that point in time would therefore, not significantly reduce the phytoremediation efficiency.

Therefore, the use of the less efficient wild type tobacco plants could be combined with profit making operations, such as bioenergy. Depending on the family of N. tabacum 30-40% of the seed is in average oil (Umarov et al. 1991; Giannelos et al. 2002) and is composed of linoleic acid (71.63%), oleic acid (13.46%) and palmitic acid (8.72%) (Mukhtar et al. 2007). Although tobacco seed oil is a non-edible vegetable oil, it can be utilised for biodiesel production as a new renewable alternative diesel engine fuel (Usta 2005; Veljkovic et al. 2006). For this purpose research has to focus on the amounts of inorganic and organic pollutants accumulated in the seeds during the phytoremediation process. Tobacco is a plant, which fulfils all the characteristics for a suitable phytoremediation plant and combined with the production of bioenergy it could become one of the main plants used in the field of phytoremediation.

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