# Chapter 15
# Pollution Remediation by Way of Using Genetically Modified Plants (GMPs)


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## 15.1 Introduction: Biotechnology and Phytoremediation

Environmental contamination with some harmful organic and inorganic contaminants is a consequence of some human economic activities that generate dangerous wastes (e.g., the ones from mine exploitation; petroliferous, fabric, and pharmaceutical industries; the agricultural use of herbicides) and pose a serious concern. These pollutants are hard to eliminate from nature and can cause serious damages to human and other forms of life. Among the organic ones, it is possible to highlight chlorinated solvents, halogenated hydrocarbons, and nitrogen compounds commonly present in explosives. Among the inorganic ones are the heavy metals and other elements such as the radioactive uranium (Jafari et al. 2013; Mendes et al. 2019; Pesantes et al. 2019; Pu et al. 2019; Rosculete et al. 2019; Vázquez-Luna and Cuevas-Díaz 2019; Zhang et al. 2019).

Heavy metal pollution is among the most serious environmental problems nowadays; once it is capable of bioaccumulating in living systems, is difficult to eliminate from contaminated water and soil, presents toxicity being able to cause poisoning and oxidative stress and also presents high carcinogenic potential (Alkorta et al. 2004; Ali et al. 2019). It is necessary to develop ways to extract them from contaminated environments, and the genetic manipulation of plants to perform this task is an elegant solution. In fact plants are more suitable to act in this sense once microorganisms can only convert metals into a less toxic form instead of removing them from a contaminated environment (Garbisu et al. 2002; Ojuederie and Babalola 2017).

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K. R. Hakeem et al. (eds.), *Bioremediation and Biotechnology*,
https://doi.org/10.1007/978-3-030-35691-0_15

Transgenic plants are the ones that underwent DNA manipulation with the intention to introduce a new trait to the organism, which does not occur naturally in the species. So, by applying methodologies to genetically modify plant DNA, it is possible to develop transgenic organisms to express or overexpress genes related to metal (or other contaminants) uptake/transportation/metabolization and apply these genetically modified organisms to perform phytoremediation with efficiency. By doing that it becomes possible to remove these contaminants from the environment, minimizing the risk they consist in our lives. Heavy metals were the first target of genetic manipulation of plants to perform remediation of contaminated soil (Misra and Gedamu 1989).

Plants offer some interesting characteristics that make its use advantageous when compared to the use of microorganisms for remediation. Working with plants is easier, especially when it comes to nutrient input due to the fact that as autotrophic systems they manage to provide their own nutrient sources. It is also an interesting feature the fact that plants can be controlled to avoid undesirable spreading, maintaining in situ remediation and also preventing the dispersion of contaminants. An eco-friendly system can be developed avoiding erosion, being suitable to application in a broad range of remediation sites, and presenting low costs associated with this renewable phytoremediation technology (Suresh and Ravishankar 2004; Abhilash et al. 2009; Lee 2013; Wan et al. 2016).

Transgenesis in plants for remediation commonly aims to insert or overexpress genes that codify proteins related to the uptake and/or sequestration of pollutants (Shukla et al. 2013; Mani and Kumar 2014; Das et al. 2016), for example, binding proteins and transporters (Table 15.1).

Among transporters it is interesting to highlight the members of ATP-binding cassette (ABC) family (proteins related to not only plant detoxification but also to important ion regulation process) (Martinoia et al. 2002), cation diffusion facilitator (CDF) family (e.g., the metal tolerance/transport protein (MTP) involved in metal storage) (Ricachenevsky et al. 2013), and metal ion transporters like the ones responsible for cytoplasmic transport of zinc and iron from the family of ZRT/IRT-related proteins (ZIP) (Ducic and Polle 2005).

Metals are important pollutants in the environment especially heavy ones, and it is common that plants possess genes related to their transportation to the organism's inner part as they consist in essential microelements for these organisms (Williams et al. 2000). Metal phytoremediation can be performed through different technologies such as phytoextraction (removing metals from soils and concentrating them in the shoots), phytostabilization (accumulating metals in roots or minimizing their mobility by causing their precipitation in rhizosphere), and phytovolatilization (Nascimento and Xing 2006).

Plants can be engineered to increase the accumulation of heavy metal in its shoots (phytoextraction); *Brassica juncea*, *Nicotiana tabacum*, *Arabidopsis thaliana,* and plants from *Populus* gender have already been modified with this purpose. Cd and Pb increased accumulation and tolerance in *B. juncea* shoots can be achieved by the overexpression of the ATP-binding cassette (ABC) family transporter AtATM3 (Bhuiyan et al. 2011); a transporter from the same family (in transgenic plants, the

**Table 15.1** Examples of GMP developed to perform phytoremediation

| Source of transgene | Transgene or desired gene product | Genetically modified plant (GMP) species generated | Desired characteristic presented by the GMP | Reference |
|---|---|---|---|---|
| *Arabidopsis thaliana* | *ATM3* | *Brassica juncea* | Cd and Pb increased accumulation and tolerance | Bhuiyan et al. (2011) |
| *Saccharomyces cerevisiae* | *YCF1* | *Populus tremula* and *Populus alba* | Cd, Zn, and Pb increased accumulation | Shim et al. (2013) |
| *Psychotria gabriellae* | *IREG1* | *Arabidopsis thaliana* | Ni tolerance and accumulation increased | Merlot et al. (2014) |
| *Pseudomonas* sp. | *copC* | *Arabidopsis thaliana* | Cu tolerance and accumulation increased | Rodríguez-Llorente et al. (2012) |
| *Arabidopsis thaliana* | *CAX2* and *CAX4* | *Nicotiana tabacum* | Increased biomass when grown in the presence of heavy metals and higher accumulation of Cd, Mn, and Zn | Korenkov et al. (2007) |
| *Oryza sativa* | *MTP1* | *Nicotiana tabacum* | Cd increased accumulation | Das et al. (2016) |
| *Astragalus bisulcatus* | *SMT* | *A. thaliana* and *B. juncea* | Se increased volatilization and tolerance | LeDuc et al. (2004) |
| *Arabidopsis thaliana* | *IRT1* | *Arabidopsis thaliana* | Cd and Zn increased accumulation | Connolly et al. (2002) |
| *Noccaea caerulescens* | ZNT1 | *Arabidopsis thaliana* | Zn and Cd increased accumulation | Lin et al. (2016) |
| *Escherichia coli* | *gshI* | *Brassica juncea* | Cd, Cr, Cu, Pb, and Zn increased uptake | Zhu et al. (1999a, b) |
| *Allium sativum and Saccharomyces cerevisiae* | *PCS1 / GSH1* | *Arabidopsis thaliana* | Cd and As increased accumulation | Guo et al. (2008) |
| *Thlaspi caerulescens* | *PCS1* | *Nicotiana glauca* | Cd, Zn, and Pb increased accumulation | Martinez et al. (2006) |
| *Arabidopsis thaliana* | *PCS1* | *Brassica juncea* | Cd and As increased tolerance | Gasic and Korban (2007) |
| *Elsholtzia haichowensis* | *MT1* | *Nicotiana tabacum* | Cu increased tolerance and accumulation | Xia et al. (2012) |
| *Sedum alfredii* | *MT2* | *Nicotiana tabacum* | Cu increased tolerance and accumulation | Zhang et al. (2014) |
| *Brassica campestris* | *MT1* and *MT2* | *Arabidopsis thaliana* | Cd and Cu increased tolerance | Lu et al. (2015) |
| *Oryza sativa* | *MT2c* | *Arabidopsis thaliana* | Cu increased tolerance | Liu et al. (2015) |

(continued)

**Table 15.1** (continued)

| Source of transgene | Transgene or desired gene product | Genetically modified plant (GMP) species generated | Desired characteristic presented by the GMP | Reference |
|---|---|---|---|---|
| *Bacillus megaterium* | *TnMERI1* | *Arabidopsis thaliana* | Cd and Pb increased accumulation and tolerance | Hsieh et al. (2009) |
| *Enterobacter cloacae* | *Onr* | *Nicotiana tabacum* | TNT and GTN increased tolerance | French et al. (1999) |
| *Enterobacter cloacae* | *NfsI* | *Nicotiana tabacum* | TNT increased tolerance | Hannink et al. (2007) |
| *Arabidopsis thaliana* | *743B4, 73C1* | *Arabidopsis thaliana* | TNT increased tolerance | Gandia-Herrero et al. (2008) |
| *Rhodococcus rhodochrous* | *XplA, XplB* | *Arabidopsis thaliana* | RDX phytoremediation | Jackson et al. (2007) |
| *Escherichia coli* | *NfsA* | *Arabidopsis thaliana* | TNT increased tolerance | Kurumata et al. (2005) |
| *Homo sapiens* | *CYP2E1* | *Arabidopsis thaliana* | Capacity to deal with residues of TCE | Doty et al. (2000) |
| *Homo sapiens* | *CYP1A1, CYP2B6,* and *CYP2C19* | *Oryza sativa* | Phytoremediation of the herbicides atrazine and metolachlor | Kawahigashi et al. (2006) |
| *Homo sapiens* | *CYP2C9, CYP1A1, CYP2B6,* and *CYP2C19* | *Solanum tuberosum* | Phytoremediation of herbicides including sulfonylureas | Inui and Ohkawa (2005) |
| *Pseudomonas* sp. | Modified bacterial *atzA* gene | *Medicago sativa* and *Nicotiana tabacum* | Atrazine-enhanced metabolism | Wang et al. (2005) |
| *Zea mays* | *gstI-6His* | *Nicotiana tabacum* | Phytoremediation of the herbicide alachlor | Karavangeli et al. (2005) |

ABC transporters are commonly localized in the tonoplast, sequestrating metals in the vacuolar lumen (Song et al. 2014), the yeast cadmium factor 1 (YCF1), can be expressed in transgenic *Populus tremula* and *Populus alba* to increase the shoots' accumulation of Cd and Zn (Shim et al. 2013); *A. thaliana* can have its shoots' nickel and copper tolerance and accumulation increased by being genetically engineered to express, respectively, the metal transporter PgIREG1 (a gene originally expressed, e.g., in the hyperaccumulator shrub *Psychotria gabriellae*) (Merlot et al. 2014) and the copper-resistant protein (from *Pseudomonas* sp.) (Rodríguez-Llorente et al. 2012); *N. tabacum* can be genetically modified to overexpress the rice metal tolerance protein OsMTP1 increasing its capacity of Cd accumulation in shoots by high level of generation of thiol compounds that can chelate metals sequestrating them into vacuoles (Das et al. 2016). The *A. thaliana* CAX2 and CAX4 (low-affinity $Ca^{2+}$, heavy metal cation/H+ antiporters) when expressed in *N. tabacum* results in organisms with

an increased biomass when grown in the presence of heavy metals and higher accumulation of Cd, Mn, and Zn (Korenkov et al. 2007).

*N. tabacum* and *A. thaliana* can be genetically modified to perform Hg phytovolatilization. Bacterial gene from the reductase merA and organomercurial lyase gene merB are interesting tools to achieve this goal. MerA transgenic plants can uptake $Hg^{2+}$ through roots and convert it into $Hg^{0}$: a less toxic and volatile form. Mer B plants can convert the uptaken methylmercury into sulfhydryl-bound $Hg^{2+}$. And transgenic plants expressing both genes are able to convert not only $Hg^{2+}$ but also methylmercury into the volatile form (Rugh et al. 1996, 1998, 2000; Heaton et al. 1998). The selenocysteine methyltransferase from *Astragalus bisulcatus* when overexpressed in *A. thaliana* and *B. juncea* leads to an increase in Se volatilization and tolerance (LeDuc et al. 2004).

Phytostabilization can be achieved by limiting the uptaken heavy metal transportation from plant's roots to the shoots. In transgenic *N. tabacum* expressing AtHMA4, the Cd transport is restricted by apoplastic barrier (Siemianowski et al. 2014); in *Manihot esculenta* the overexpression of the transporters AtZIP1 and AtMTP1 make possible to achieve Zn accumulation in the roots with restrict transportation to the shoots (Gaitán-Solís et al. 2015); in *A. thaliana* the overexpression of the metal transporter IRT1 can induce an increase in accumulation of Cd and Zn by the plant (Connolly et al. 2002), and the overexpression of Zn transporter ZNT1 from *Noccaea caerulescens* can increase in the transgenic plant the accumulation of Zn and Cd (Lin et al. 2016); and in the *Populus* gender species mentioned before, the same gene YCF1 when expressed also increases the accumulation of Pb in the plant roots (Shim et al. 2013).

When it comes to remediation of heavy metals by genetically modifying plants to express or overexpress binding proteins, it is necessary to highlight metal chelators like the peptides phytochelatins (Hirata et al. 2005), metallothioneins (Tripathi et al. 2015), and mercuric ion binding protein proteins (MerPs) (Huang et al. 2003).

When it comes to transgenic plants producing phytochelatins, the genetic modifications involve mainly two important enzymes that play a key role in their synthesis: phytochelatin synthase and *c*-glutamylcysteine synthetase (Hirata et al. 2005). *Brassica juncea* can extract more Cd, Cr, Cu, Pb, and Zn than wild plants when modified to overexpress γ-glutamylcysteine synthetase and glutathione synthetase (proteins involved in phytochelatin synthesis) (Zhu et al. 1999a, b). Bacterial and yeast glutathione synthetase expression in *A. thaliana* leads to increased accumulation of Cd and As in the transgenic organism (Guo et al. 2008). *Nicotiana glauca* genetically modified to overexpress TaPCS1 gene (from which product is a phytochelatin synthase) can accumulate high levels of Cd, Zn, and Pb (Martinez et al. 2006). The expression of phytochelatin synthase from *Arabidopsis* in *Brassica juncea* increases the transgenic tolerance not only to Cd but also to As (Gasic and Korban 2007).

Metallothionein genes can be introduced in target plant species to enhance heavy metal tolerance. These proteins rich in cysteine amino acid residues possess high affinity to cationic metals (Singh et al. 2003). *N. tabacum* can be modified using the EhMT1 (Xia et al. 2012) or SaMT2 (Zhang et al. 2014) gene to increase the Cu tolerance and accumulation; *A. thaliana* can be engineered using BcMT genes to increase

the tolerance to Cd and Cu accumulating the latter in shoots (Lu et al. 2015); Cu tolerance can also be increased by OsMT2c gene (Liu et al. 2015).

A MerP from *Bacillus megaterium* strain MB1 transposon TnMERI1 when expressed in cell membrane and vesicles of transgenic *A. thaliana* can induce the increased accumulation and tolerance to Hg, Cd, and Pb (Hsieh et al. 2009).

In 1999 tobacco plants (*N. tabacum*) were engineered to also remediate other pollutants than heavy metals. By introducing the sequence responsible for codifying pentaerythritol tetranitrate reductase from *Enterobacter cloacae* into this plant species DNA makes it possible for it to increase the tolerance to TNT (2,4,6-trinitrotoluene) and glyceryl trinitrate (GTN); plants can also be engineered to remediate explosive residues that are persistent environmental cytotoxic pollutants (French et al. 1999). The attempts to reprogram plants to degrade toxic nitro-substituted compound continued allowing the development of other transgenic tobacco plant variants to remove TNT residues by using, for example, *E. cloacae* nitroreductase *NfsI* gene (Hannink et al. 2007); transgenic *A. thaliana* is able to deal well with TNT residues by overexpressing its own bifunctional O- and C-glucosyltransferases (Gandia-Herrero et al. 2008) or by being genetically modified to express *Escherichia coli* nitroreductase (Kurumata et al. 2005). *A. thaliana* can also be engineered to eliminate residues of the military explosive RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by using genes of cytochrome P450 monooxygenases from *Rhodococcus rhodochrous* (Jackson et al. 2007).

In 2000 tobacco plants were also engineered to deal with residues of halogenated organic compound trichloroethylene (TCE), an industrial solvent. This substance can be metabolized up to 640-fold faster than in wild tobacco plants after receiving the DNA information to express mammalian cytochrome P450 2E1 enzyme. It also makes possible for the transgenic plant to increase not only the uptake but also the debromination of ethylene dibromide (Doty et al. 2000).

Herbicides are other important target for phytoremediation. These chemicals are applied to protect crop yields from weed; however to combat the resistant organisms it is necessary to increase the amount of chemicals used. By doing that the residues that remain on soil and water next to the plantations are worrying pollutants. The family of proteins most commonly used as tools to remediate this kind of residues is P450 family (Abhilash et al. 2009). By genetically modifying *Oryza sativa* to express human CYP1A1, CYP2B6, and CYP2C19, it is possible to program rice plants to phytoremediate the herbicides atrazine and metolachlor (Kawahigashi et al. 2006), and by using these genes and also CYP2C9, not only transgenic rice but also transgenic potato plants can be developed to deal with herbicide residues. CYP1A1, CYP2B6, and CYP2C19 make potato plants resistant to several herbicides, and transgenic rice plant expressing CYP2C9 presents resistance to sulfonylureas being both suitable for environment phytoremediation (Inui and Ohkawa 2005). Atrazine-enhanced metabolism can also be achieved by modifying plants to express bacterial atrazine chlorohydrolase; this commonly used herbicide can be efficiently degraded by transgenic *Medicago sativa* and *N. tabacum* (Wang et al. 2005). The chloroacetanilide herbicide alachlor can be efficiently remediated by using genetically modified tobacco plants overexpressing maize enzyme glutathione S-transferase I (Karavangeli et al. 2005).

## 15.2 Main Strategies of Plant Transgenesis

In order to manipulate the DNA from plants, there is a wide range of techniques that can be applied; these strategies can be divided in two main groups: biological and nonbiological methodologies.

The nonbiological genetic modification techniques most commonly applied include biolistics, gene delivery performed by different delivery vehicles (e.g., polymers, nanomaterials, and liposomes), electroporation, and microinjection. Biolistics consists in particle incorporated in a desirable DNA bombardment to deliver this DNA to plant target cells even in intact tissue fragment or to microspores. Proposed in the late 1980s (Sanford et al. 1987) and also known as gene gun and particle bombardment, it commonly uses tungsten particles of low cost or gold particles that offer higher efficiency in the process. Loaded particles accelerated by pressurized helium can penetrate cell efficiently to deliver the DNA making it possible to transform not only the nuclear genome but also the mitochondrial and plastidial ones; it is the most popular nonbiological technique to produce transgenic plants (Southgate et al. 1995; Baltes et al. 2017; Cunningham et al. 2018). Electroporation causes temporary opening of pores in cell membrane to allow DNA entrance into cells by submitting the sample to strong electric field pulses; it is commonly performed in protoplasm as target (Weaver 1995; Keshavareddy et al. 2018). Microinjection consists in injecting, with a glass microcapillary-injection pipette, the DNA sequence into protoplasts commonly immobilized by low melting point agarose (Mohanty et al. 2016). Polymers (Bart et al. 2006), nanoparticles (Cunningham et al. 2018), and liposomes (Wordragen et al. 1997) can also serve as gene delivery vehicles to plant transgenesis having as target most frequently the protoplasm.

When it comes to biological techniques, the most commonly applied is the use of *Agrobacterium tumefaciens* (most commonly or *Agrobacterium rhizogenes*), but it is also possible to use viral vectors (DNA or RNA virus) to deliver the transgene to target cells (Zaidi and Mansoor 2017). The use of *A. tumefaciens* to generate transgenic plants started in the 1970s and is up to date the most widely used method with this purpose. This soil bacterium naturally infects dicots, inserting in a stable way its DNA inside host's DNA causing crown gall disease. So, by engineering bacterial plasmid DNA replacing virulence genes by transgenes of interest, it is possible to generate transgenic plants by using this prokaryote (Cunningham et al. 2018) (Fig. 15.1).

## 15.3 Difficulties Associated with Phytoremediation and New Molecular Biology Strategies in Plant Transgenesis Field

There are plant species that can naturally hyperaccumulate metals such as some members of Brassicaceae family, and before plant genetic modification techniques fully developed, phytoremediation was performed using these species: able to uptake

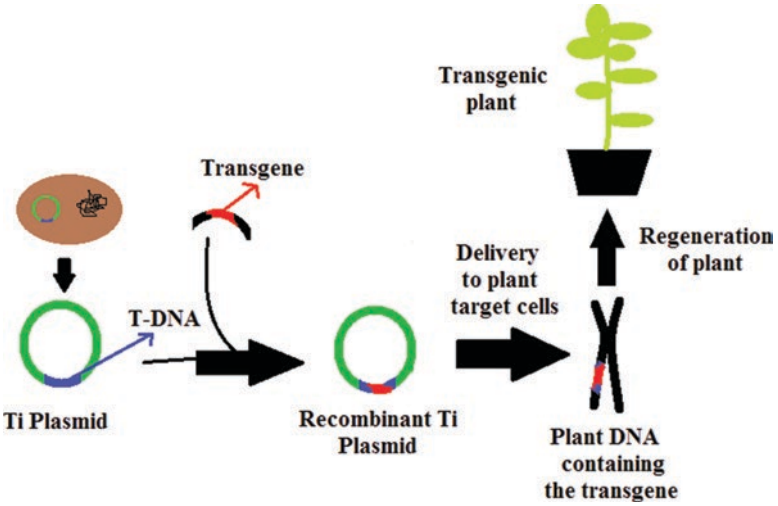


**Fig. 15.1** Biological method of producing a transgenic plant using bacterium from *Agrobacterium* gender as transgene's delivery vehicle. The tumor-inducing plasmid (Ti-plasmid) from *A. tumefaciens*, for example, can receive the DNA sequence, in a site called T-DNA, which should be delivered to target plant cell to develop the transgenic organism. The recombinant plasmid containing the transgene can be processed and guided to the host genome. So, the desirable sequence can be integrated to the host's DNA making it possible to obtain the transgenic plant

a great amount of heavy metals and transport them from the root to the shoot. However some of these species, especially in the presence of a high level of contaminants, presented slow growth turning the decontamination process very time-consuming (Jafari et al. 2013).

The plant transgenesis presented solution to this kind of problem and other difficulties faced in this field of expertise. It made possible to implant desirable characteristics to some plant species and change undesirable ones. For example, plants that grow fast but were unable to survive in toxic environment or were unable to accumulate high levels of contaminants could be converted, for example, using genes from hyperaccumulators, into organisms suitable for phytoremediation (Ali et al. 2013). For example, as already mentioned, *Brassica juncea* (the Indian mustard) grows fast, and by adding to its DNA the codifying sequence for a selenocysteine methyltransferase from *Astragalus bisulcatus* (a selenium hyperaccumulator) made it possible to obtain a *B. juncea,* able to accumulate more Se, and tolerate it better than the wild type and also perform this element volatilization (e.g., suitable feature for soil decontamination) (LeDuc et al. 2004).

However inserting genes inside an organism DNA is not always an easy task. There are mainly three types of difficulties associated with plant genetic manipulation: undesirable effects associated with insertion (the transgene's insertion can occur in target cells' genome in a place different to the one previously planned resulting in undesirable results such as mutation and loss of function of important genes that can be interrupted by the insertion process), position (depending on the insertion place the regulatory sequences nearby can cause, e.g., unexpected transgene silencing), and

somaclonal events (plant in vitro manipulation commonly induces somaclonal variation, impacting plant exhibited characteristics, changing some of them) (Ziemienowicz 2010). And these undesirable events become even more important when not only one gene insertion is aimed.

Sometimes not only one gene is necessary to be inserted in a plant genome in order to transform it into an efficient organism for phytoremediation (e.g., for Hg remediation). And the task of inserting multiple genes in nuclear genome can be laborious, elevate the study costs and may not lead to the desirable result. However, when multiple genes are involved, the target of genetic manipulation is commonly the chloroplast genome and the approach of homologous recombination to insert the transgene into DNA reduces problems related to off-target insertion (Hussein et al. 2007; Martret et al. 2011).

With advances in molecular biology field, more precise strategies for targeted genome editing have been developed (such as CRISPR/Cas9), and they are already being applied to obtain transgenic plants (Petolino 2015; Forsyth et al. 2016; Malzahn et al. 2017; Borrelli et al. 2018).

And it is also possible to make plants' present desirable characteristics without inserting an exogenous gene inside of their genome or without inserting more gene copies from the same species. RNA silencing can also be applied to obtain organism optimized to perform phytoremediation. Rice OsNRAMP5 Cd transporter, for example, can have its mRNA degraded by silencing methodology, resulting in enhanced cadmium translocation to the shoots, intensifying its pollutant phytoextraction from contaminated soil (Takahashi et al. 2014).

As genome projects from plant species continue to be performed and the identification of genes' function investigated, new possible sequences to be used to generate transgenes with desirable characteristics to phytoremediation continue to appear. But other important opportunity that genetic engineering of plants presents regarding dealing with environmental persistent pollution is the possibility to reduce the use of toxic chemical in crops, for example. It is possible to produce, for example, transgenic corn based on *Bacillus thuringiensis* as natural bioinsecticide (inducing, for example, the production of Cry protein endotoxins), offering a reduction of 56 million kilograms of insecticide use in USA from 1996 to 2011 (Benbrook 2012).

When it comes to transgenic plants developed to perform phytoremediation, there are few biosafety concerns once they are designed for one specific purpose (i.e., removing contaminants from the environment) and will not serve as food for human beings and animals. The major concerns would be related to gene flow from the transgenic plants used for phytoremediation in the environment to wild plants naturally present in that area (which chloroplast modification instead of nuclear DNA modification would help to avoid) and potential loss of diversity once transgenic plants would possess advantageous characteristics to survive in contaminated soil (Kotrba et al. 2009). However when it comes to the abovementioned strategies to reduce the use of chemicals in crops, as the use of insecticide in corn fields, concerns regarding biosafety stand out. Corn is used not only in human but also animals' food. So, food safety and allergenicity of new proteins produced in the transgenic plant, among other risks related to resistance genes introduced in the ecosystem, should receive attention.

## 15.4 Future Perspectives

The development of transgenic plants for phytoremediation is a promising and important tool in plant biotechnology field to deal with persistent and highly toxic environment pollution and also offers the opportunity to increase the knowledge regarding plant genomes and DNA manipulation, metabolism of heavy metals, and some organic substances that can be environmental contaminants.

Naturally, as the researches advance, advances also the comprehension over: metal uptake and elimination by plants, genetic manipulation of plant nuclear and chloroplastic genome, interaction plant-other forms of life and with the environment (also in contaminated areas) specially in rhizosphere, species that can be useful in phytoremediation providing genes to development of transgenic organisms or performing contaminants neutralization, and strategies to deal with mixed contamination in polluted sites. It is also expected that the numbers of field trials increase to enhance the understanding of transgenic plants' interaction and effects on the ecosystem and to evaluate the possible occurrence of negative economic and biological impacts.

Therefore it is expected that new techniques involving plant species can be developed by teams of researchers from diverse fields of expertise to offer interesting solutions to phytoremediation.

## 15.5 Conclusion

GMPs are an important tool when it comes to dealing with the increasingly evident global problem of pollution, especially the one related to heavy metals, explosives, industrial solvents, and herbicides. The transgenic plants make it possible to achieve, at low cost, removal of contaminant residues from the environment. Therefore, it is expected that new techniques involving plant species continue to be developed by teams of researchers from diverse fields of expertise to offer interesting and safe innovative solutions to phytoremediation.

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