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Review

Novel roles for genetically modified plants in environmental protection

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Transgenic plants of environmental benefit typically consist of plants that either reduce the input of agrochemicals into the environment or make the biological remediation of contaminated areas more efficient. Examples include the construction of species that result in reduced pesticide use and of species that contain genes for either the degradation of organics or the increased accumulation of inorganics. Cutting-edge approaches, illustrated by our own work, focus on the applicability of genetically modified (GM) plants that produce insect pheromones or that are specifically tailored to the phytoremediation of cadmium or PCBs. This paper discusses the role that the next generation of GM plants might play in preventing and reducing chemical contamination and in converting contaminated sites into safe agricultural or recreational land.

Introduction: the need for (new) GM plants

With the rapid growth in the global population making it increasingly difficult to provide sufficient amounts of food [1], one potential solution is the use of genetically modified (GM) organisms, which might support starving populations through increased crop yield. However, the launch of GM foodstuffs has been impeded, in particular, by the reluctance of different regional jurisdictions to permit the application of GM plants [2].

Another solution, therefore, might be to use remediation techniques to convert contaminated areas* into suitable agricultural land and thereby increase the sites available for food production. Phytoremediation using conventional plants (grasses, sunflower, corn, hemp, flax, alfalfa, tobacco, willow, Indian mustard, poplar, etc.) (Figure 1) shows good potential, especially for the removal of pollutants from large areas with relatively low concentrations of unwanted compounds: areas for which it is not cost-effective to use traditional physical or chemical methods.

Thus far, with traditional dig-and-dump methods being much faster, widespread use of phytoremediation has been limited by the relatively long period of time plants require to reduce contaminant levels [3,4]; several harvest periods generally prove insufficient.

However, gene transfer has already led to the production of GM crop varieties on hundreds of millions of hectares [5]. This irreversible fact, together with recently improved attitudes towards GM plants (even within the EU [6,7], where GM food has traditionally been viewed with distrust), has resulted in calls for the large scale implementation of transgenic plants that can prevent or remove contamination more effectively.

Using GM plants in environmental protection

The generation of transgenic plants for environmental protection involves the two quite separate fields of pollution prevention and pollution removal, with specifically tailored plants already existing for both purposes. Pollution-preventing GM plants can significantly reduce the

Glossary

Allelochemicals: compounds formed and released by one species with the aim of influencing its surroundings (e.g. other, sensitive plant species and their rhizospheres).
Desaturase: enzyme introducing a carbon–carbon double bond, in this case into a fatty acid in a specific position.
GMO (genetically modified organism): an organism with some specific gene(s) introduced or removed artificially.
Herbicides: compounds that are toxic for some plants and used to protect crops against weeds.
Insecticides: compounds toxic to some insects.
Pesticides: compounds toxic to some pests.
Phytoremediation: use of plants to accumulate, remove or render harmless toxic compounds contaminating the environment.
Phytostabilisation: process in which plants are exploited to prevent migration of environmental contaminants to sites where they may pose a danger to human health.
Phytovolatilisation: process by which plants volatilise through their leaf surface some environmental contaminants taken up by their roots.
PCBs (polychlorinated biphenyls): a group of recalcitrant organic compounds that differ in their chlorine substitution on the biphenyl ring. Until the late 1960s, when they were banned because of their toxicity, they were widely used in paints, heat transfer media, electric devices, plastics, etc. because of their technological properties (inflammability, chemical stability and their dielectric constant).
Rhizoremediation: exploitation of microorganisms within the root zone of plants to remove contaminants from the environment.
Sexual pheromone: a compound for chemical communication between females and males within one species.
Transgenic plant: GMO, plant with some specific gene(s) introduced or removed artificially.

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* Typically, such areas have been contaminated by: the spillage of fuels and their additives; the results of industrial accidents; the long-term accumulation of contaminants; the leakage of mine tailings; the long-term treatment of agricultural land with low quality fertilisers; sewage sludge; excess pesticide use; the use of land as army training areas; and pollutant deposits accrued from many years of industrial activity.

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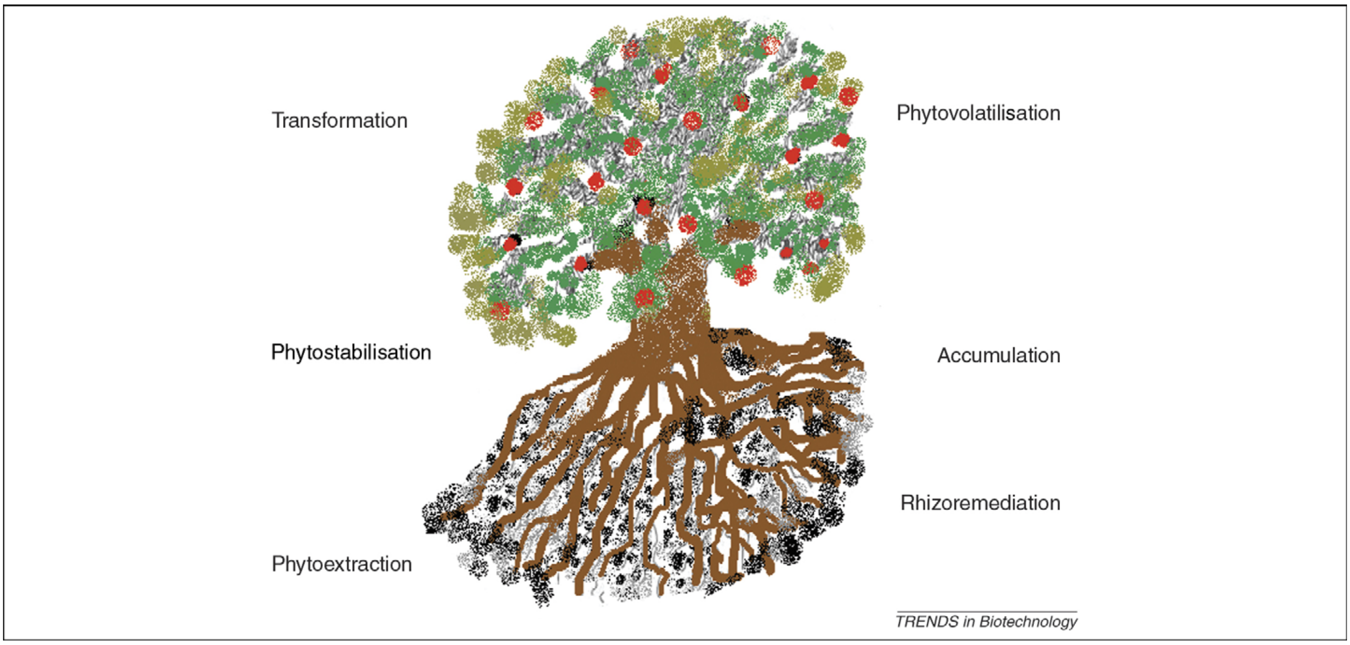

Figure 1. Growing plant roots explore the soil particles and take up water, nutrients, trace elements and other compounds, thus playing an important role in biological remediation. Plant roots can extract contaminants from soil and accumulate, transform and transport them into the parts of the plant that are above-ground. In leaves, fruits or stems, many compounds are stored, transformed or volatilised. Such processes are known as phytoremediation. Via exudate and root turnover, many plant products enter the root zone. Some of these compounds supply soil microorganisms with energy, some act as a carbon source and some can even serve as inducers of degradative pathways. Growing roots also help to spread microorganisms within the soil, thereby supporting rhizoremediation.

amount of agrochemicals needed for crops, thus reducing environmental pollution. Examples include Roundup Ready soya, which enables the use of more environmentally-friendly herbicides, as well as *Bacillus thuringiensis* (Bt)-corn and Bt-cotton, which minimise pesticide use[†]. Recently, however, a new approach to pest management has been developed, based on the construction of plants that produce and emit insect pheromones [8,9]. Pollution-removing GM plants, which deal with contaminations caused by explosives, chlorinated solvents, mercury, selenium, phenolics, etc. [3,4,10,11], have been extensively reviewed in the literature [12–17]. These plants have been developed to contain either transgenes responsible for the metabolisation of organic compounds (thereby leading to the accumulation of less toxic or less recalcitrant compounds) or transgenes that result in the increased accumulation of inorganic compounds. Once optimised, this approach should lead to the accumulation of pollutants in harvestable parts [18,19] and thus either enable their removal or prevent their migration to sites where they may pose a danger to human health [3,17].

Pollution prevention

The first generation of commercially available transgenic plants (e.g. plants expressing the Bt toxin) were able to reduce the loss of crop yield caused by insect damage at the same time as reducing the amount of pesticide required. As both these and herbicide-resistant plants have been the subject of numerous reviews, and their advantages or disadvantages discussed extensively [2,5,20], we will focus on plants that produce and emit insect pheromones (Figure 2). Grown close to or around, for example, a field of food crops requiring protection, this type of GM plant emits a pheromone that attracts male moth pests, thereby reducing their ability to mate effectively. In such cases, the protected crop does not itself need to be transgenic (see Box 1). We tested this novel approach by constructing tobacco plants that produce an insect sexual pheromone from their own fatty acid pool. This was achieved by inserting the gene encoding acyl-CoA-delta^{11}-(Z)-desaturase (from the cabbage looper moth), which is responsible for the production of the sexual pheromone in female moths [21,22]. The transformed plants were examined for fatty acid content showing substantial presence of the precursor,

Box 1. Transgenic plants emitting insect sexual pheromones

Transgenic plants capable of synthesising and releasing pheromones into the environment are not intended to destroy entire pest populations, but rather to limit their ability to mate effectively in a field containing a protected crop and thereby to reduce the pest population. In this scenario, GM plants would be planted in the vicinity of the protected crop to disrupt the ability of damaging pests to communicate chemically or to concentrate them in another, desired, location as part of an integrated pest management (IPM) system. Via this method, neither the transgene itself nor its products would be able to enter the human food chain, thus eliminating the possible health risks associated with genetically modified plant (GMP) consumption. Moreover, such an approach means that non-targeted insect life remains unaffected, and insect-resistant strains, such as those been reported with the use of the *Bacillus thuringiensis* (Bt) toxin in transgenic crops, do not develop. Moreover, the synthesis of pheromones, or their intermediates, in GMPs could also be used as an alternative to traditional chemical methods for pheromone production.

[†] It is hard to find good scientific reasons why GM technology has not been universally embraced. All of the widely publicized objections, whether they be the supposed threat to Monarch butterflies or the 'risk' of the inadvertent introduction of allergens into the food chain, have been soundly rebutted and relegated to the status of 'urban myths' [2].

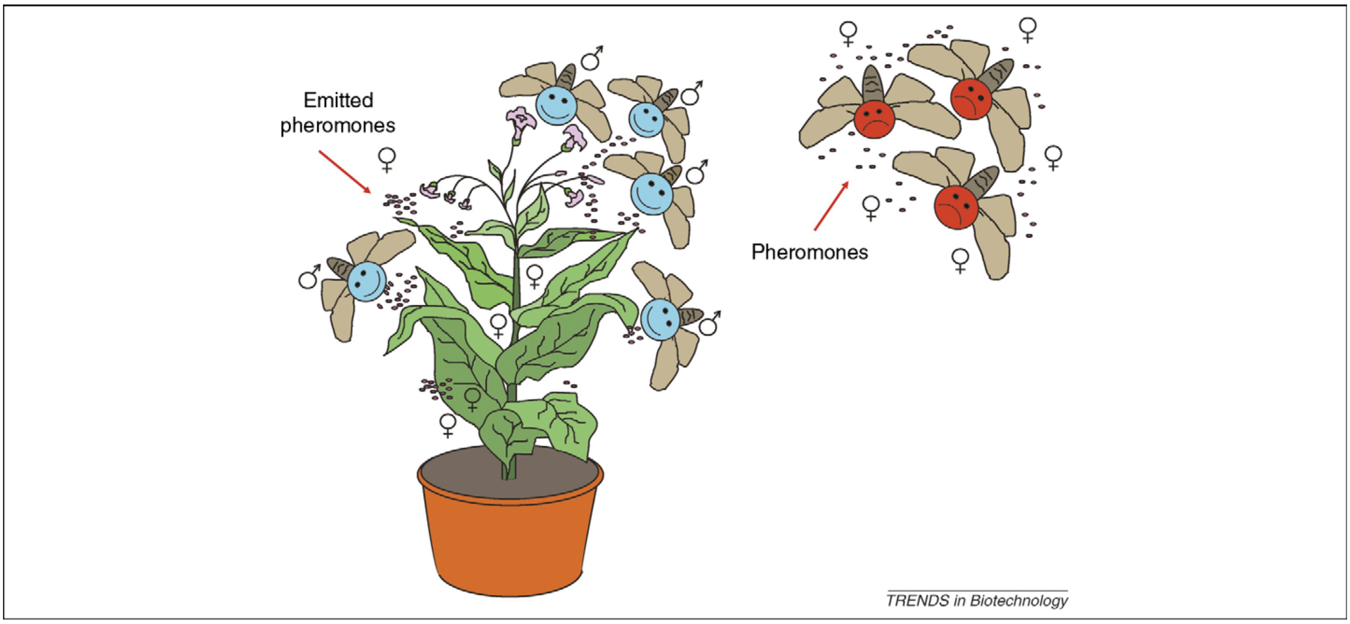

Figure 2. Plants emitting specific insect sexual pheromones compete with female moths in attracting males of the same species, therefore lowering the effectivity of their mating and resulting in a decrease in moth population. This approach cannot eradicate the pest totally, but it will lower the losses of plants that are to be protected.

which was further converted to alcohol by the enzyme normally present in tobacco plants. Our experiments constitute the first example of a GM plant producing measurable amounts of a moth sex pheromone, thereby potentially adding a new method to the existing battery of integrated pest management approaches. Furthermore, we confirmed that these GM plants emit compounds identical to the natural sex pheromone of the African rice borer moth, *Chilo zacconius*, making this moth an ideal candidate for field-testing the application of this system.

Leaves of the same GM tobacco plants also served as a 'cell factory' for the production of monoenic lipids that were chemically converted into the pheromone mixture of another insect, the cabbage moth (*Mamestra brassicae*). This moth exploits a mixture of acetates, rather than the alcohols used by *C. zacconius*. In this case, the GM plants supply the starting material, thereby removing the need for certain synthetic steps and enabling the correct pheromone mixture to be prepared by means of a simple one-pot reaction [8]. The effectiveness of our semi-synthetically prepared mixture has since been successfully trialled in field tests in northern Bohemia.

Pollution removal: phytoremediation and rhizoremediation

Phytoremediation [3,10,11] is not solely a function of plants (Figure 3) but must always be considered in combination with the effect of rhizospheric microorganisms [4,23]. Although they have an inherent ability to detoxify some xenobiotics (i.e. to make them non-phytotoxic), plants, compared with microorganisms [12], generally lack the mechanisms necessary for the complete degradation/mineralisation of toxic compounds.

The potential of genetic engineering to enhance the biodegradation of xenobiotics has been recognised since the early 1980s, with initial attempts being focused on microorganisms. However, there are two main problems with the introduction of GM microorganisms: the legislative barriers blocking their release into the environment and the poor survival rate of those engineered strains that have been introduced into real contaminated soil. The latter problem reflects the inadequate level of knowledge that currently exists about the consortia of microorganisms present in real soil and the ways in which they interact. The survival rate of introduced bacterial species might, however, be improved by the use of strains that have a selective advantage over others, such as strains supported by plants: for example, root colonisers [24].

The use of plants, rather than microorganisms, as genetically engineered environmental cleanup biosystems might also help to overcome the legislative barriers. However, some species, for as yet unknown reasons, are simply more sensitive to contamination than others, so not all plants are equally well suited to metabolise or accumulate pollutants. For remediation purposes, besides their ability to take up, accumulate or metabolise the xenobiotics, one of the most important criteria is the ability of the plant to selectively support the metabolism and survival of degrading bacteria in the rhizosphere [25–27]. Only recently developed methods of detection, such as stable isotope probing, have enabled us to obtain a deeper insight into the effect of pollutants and plants on microorganisms [4,26,28]. Metagenomics, for example, has brought new insights into the presence and activity of degrading microorganisms within rhizosphere consortia, enabling the tracking of responses to compounds released by plants [4,29].

The genetic modification of microorganisms to improve their performance in the rhizosphere represents a challenging possibility that should not be abandoned simply because their release into the environment is currently restricted. The ability of degrading bacteria to colonise roots may be manipulated by improving symbiotic microorganisms. One such example is the rhizoremediation of

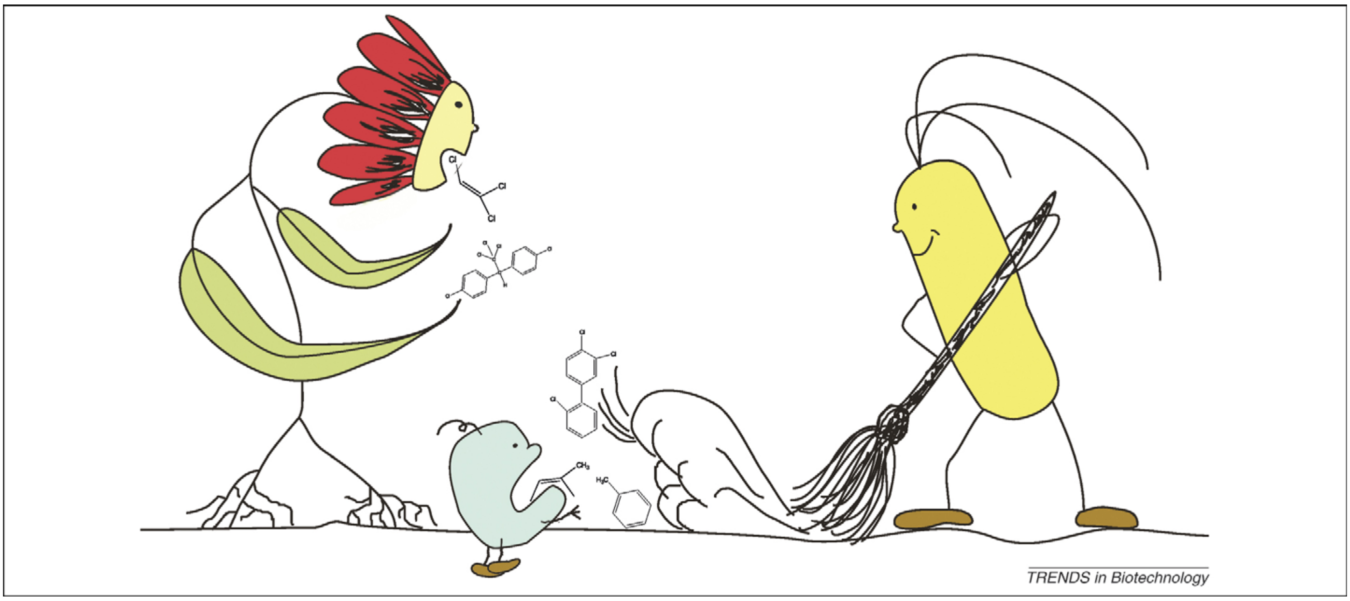

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Figure 3. In bioremediation, the roles of the different organisms that are present in the soil cannot always be clearly separated. Some bacterial species (shown in blue and yellow) might degrade selected compounds only to some extent and their end-product can then be further metabolised by other species. Plants can take up the pollutants and the pollutant metabolites formed by other organisms and convert them further or detoxify them. Products of plant metabolism become available to other parts of the complex bioremediation system via leaf fall or root turnover. Therefore, to develop an effective remediation system, all participating components need to be considered.

PCBs by *Pseudomonas fluorescens*, in which biphenyl degradation is regulated using a system that responds to signals from alfalfa roots [24]. The introduction of such GM microorganisms ensures that any changes are limited to the consortia of native bacteria in the rhizosphere and not introduced into the surrounding soil [30].

Another rather promising approach appears to be the development of engineered endophytic bacteria that improve the phytoremediation of water-soluble, volatile organic compounds [31]. Trichloroethylene (TCE)-degrading bacteria have been proven to protect host plants against the phytotoxicity of TCE and to contribute to a significant decrease in TCE evapotranspiration.

Plants with an enhanced ability to accumulate heavy metals

Plants exploit their natural metabolic mechanisms to take up essential trace metals. Cations or oxyanions must either be accumulated in harvestable parts or transformed into less-toxic forms. Although hyperaccumulators, such as *Thlaspi caerulescens*, can uptake sufficient levels of metals to make harvesting and metal recovery economic, they are often limited by their small biomass [10,32]; the amount of pollutant they can remove from soil is a function of their tissue concentration multiplied by the quantity of biomass formed. Despite this, and despite the fact that no universal phytoremediation plant exists, plants that are selective and only capable of accumulating certain elements, are already being used in the cleanup of a broad spectrum of hazardous elements.

In terms of the development of GM plants with improved metal detoxification abilities, we will only discuss the most promising approaches, some of which have been described in more detail elsewhere [13,33–36]. For example, the introduction of bacterial genes is one strategy, which, in US trials in contaminated fields, has already been shown to result in the reduction of toxic organomercurials, as well as in the storage of mercury in its non-toxic form [10,37]. Another promising approach to enhancing metal uptake employed the nicotianamine synthase gene [38] involved in the formation of phytosiderophore, the metal-binding amino acid [39] that increases the bioavailability of metals to plants.

However, the most common strategy involves targeting the proteins involved in metal homeostasis (metallothioneins, phytochelatins, glutathione) for genetic manipulations [40,41]. Although such approaches typically involve the manipulation of plant enzymes responsible for the formation of phytochelatins and related compounds (e.g. overexpression of glutathione synthetase [42], gamma-glutamylcysteine synthetase [43], phytochelatin synthase [44]), manipulations with other enzymes have also been successful. For example, field trials have shown that the overexpression of ATP sulfurylase facilitates increased selenium reduction and its storage in less toxic form [45,46]: a bonus being that such plants also accumulate the potent anticancerogenic compound, methylselenocysteine.

Many papers deal with the expression of metallothioneins in plants [47–49], but our work has focused on improving a plant's ability to accumulate metals by introducing (into the implemented protein) additional metal-binding domains with a high affinity to heavy metals [19]. Such a fusion product with a histidine anchor [18,50] was tested in real contaminated soil, and transfer factors were estimated for cadmium, zinc and nickel [51,14]. In our trials, transgenic tobacco accumulated twice the amount of cadmium in above-ground biomass than did the controls.

A possible enhancement to this approach, currently being tested, involves the cloning of short (cysteine-rich) metal-binding sequences [52] into plants to improve their metal-binding properties. This approach was followed up by quantum chemical studies of the interactions of metal ions with biologically relevant functional groups: studies that suggested further possible developments in the metal-binding capacities of fusion proteins. Subsequently, theoretical combinatorial chemistry was applied to the complexation and selectivity of metal ions in model sites [53,54], resulting in the design of highly selective combinations of metal-binding sites that might be merged into one polypeptide chain.

Plants with an enhanced ability to detoxify persistent organic compounds

To cope with organic xenobiotics, plants use a mechanism developed to fight allelochemicals, which are toxic compounds produced by other species competing for their resources [10,55,56]. Because the use of plants in the removal of organic compounds has been widely discussed in both reviews [10,12,16,57] and books [3,4], we again will concentrate on only the newest approaches.

To increase their natural abilities, different P450 cytochromes have been introduced into plants. These enzymes are considered to be responsible for the first phase in plant detoxification, the activation reaction of recalcitrant compounds in plants [56]. One illustrative example of this is the enhanced metabolism of halogenated hydrocarbons in transgenic plants containing cytochrome P450 2E1 [58]. Intriguingly, however, overexpression of a basic peroxidase in tomato [59] resulted in increased phenol phytoremediation, thereby supporting the hypothesis that, apart from P450 cytochromes, peroxidases are also involved in this first phase [60,61].

Chloroacetanilide herbicides and explosive compounds [62] have been the focus of other studies. The development of GM tobacco, which overexpresses glutathione-S-transferase for the phytoremediation of chloroacetanilide herbicides [63], addresses the second phase in plant detoxification: namely, the conjugation of the activated compound.

The biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase [64] is the classic example of the exploitation of a bacterial gene for phytoremediation. More recently, plants have been constructed that express bacterial enzymes capable of TNT transformation and RDX (hexahydro-1,3,5-trinitro-1,3,5 triazine, an explosive nitroamine widely used in military and industrial applications) degradation [65].

To achieve the aerobic degradation of ubiquitous persistent PCBs, they must first be activated by hydroxylation. The vital missing step in the efficient degradation of PCBs by plant cells is the opening of the biphenyl ring by the bacterial enzyme *bphC*, which is responsible for the cleavage of hydroxylated PCB derivatives, even those formed by plants [66]. Therefore, we have thoroughly studied the cooperation of plants and bacteria in PCB degradation [24–26,28,30]. In particular, we have described the generation of tobacco plants carrying the *bphC* gene [67], subsequently testing the seeds for their ability to germinate in high concentrations of PCB [14]. Improved substrate specificity has since been achieved by the expression of bacterial biphenyl-chlorophenyl dioxygenase genes in tobacco [68].

In addition to these techniques, phytoremediation can even proceed *ex planta*, as shown in the case of trichlorophenol and phenol allelochemicals phytoremediated via an engineered secretory laccase [69].

Conclusion

With most of the GM plants prepared in the EU in the last decade never having reached a real contaminated site, it is results from other regions, primarily the US, that show us the potential for cost-effective commercial applications of the GM approach [34]. Hopefully, therefore, in the near future GM plants will be widely used, not only to significantly reduce pesticide use in agriculture (in particular, reducing the organophosphates and organocarbonates that possess substantial mammalian toxicity), but also to actively remove the residues of agrochemical, industrial and accidental contaminations of the environment. In the great environmental cleanup required, the future lies in tailored phytoremediation-specific plants able to support microbial activities in the rhizosphere. However, to exploit these possibilities on a large scale, it will first be necessary to achieve changes in the existing legislation, overcome regulatory barriers and educate the public into improving their opinion of GM plants.

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