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Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants

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Phytoremediation has emerged as an attractive methodology to deal with environmental pollution, which is a serious worldwide problem. Although important advances have been made in this research field, there are still some drawbacks to become a widely used practice, such as the limited plant's metabolic rate and their difficulty to break down several organic compounds or to tolerate/accumulate heavy metals. However, biotechnology has opened new gateways in phytoremediation research by offering the opportunity for direct gene transfer to enhance plant capabilities for environmental cleanup. In this context, hairy roots (HRs) have emerged as an interesting model system to explore the potential of plants to remove inorganic and organic pollutants. Besides, their use in rhizoremediation studies has also been explored. In this minireview we will discuss the most recent advances using genetic engineering for enhancing phytoremediation capabilities of plants and HRs.

Contents

Introduction

Phytoremediation, using plants to clean up contaminated environments, is a green and an eco-friendly technology which has gained importance over traditional decontamination methods that are more disruptive [1]. The concept of using plants for biological remediation emerged few decades ago when it was demonstrated their capability of accumulating high quantities of toxic metals and/or metabolizing organic compounds in their tissues or organs. Moreover, this technology allows the restoration

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of polluted environments with low costs and low collateral impacts. Phytoremediation includes a wide range of processes such as phytoextraction, phytostabilization, phytotransformation, phytovolatilization, rhizofiltration and phytostimulation. Although it was first applied for the removal of inorganic pollutants, it has gradually proved to be efficient for the treatment of organic pollutants [2]. In other words, plants can take up, accumulate and/or metabolize both kinds of contaminants, as well as they can support growth and performance of microbial degraders of pollutants [3].

Regarding inorganic pollutants, roots are usually able to take them up by a process known as phytoextraction, which promote cleanup of soil or wastewater [4]. This process depends greatly on the redox state and chemical speciation besides the plant species. Unlike many organic contaminants, inorganics cannot be degraded chemically or biologically [5]. They are taken up via specific membrane transporter proteins (in the case of metals which are either nutrient themselves) or they are incorporated inadvertently, using other protein transporters, because of the chemical similarity with plant nutrients [6]. Once inorganic pollutants have been chelated, they are stored in the vacuole or exported to the shoot via the xylem to avoid cytoplasmic accumulation and toxicity, but in many cases they still cause oxidative stress and essential nutrients unbalance [7]. In this sense, metal hyperaccumulating plant species are of particular interest for phytoremediation, since they are capable of taking and storing high concentrations of heavy metals without developing toxicity symptoms.

Meanwhile, the efficient removal of organic pollutants should consider the sorption (binding to the root surface and cell walls) [8–11] and the uptake by plant cells driven by simple diffusion since specific transporters for xenobiotics in cell membranes have not been described. Transformation of organic pollutants into less toxic compounds (often just less phytotoxic) by plants is called phytotransformation, in some cases even phytodegradation occurs. It is important to mention that once the organic compounds are taken up, the phytotransformation generally involves three transformation phases [12]: (I) conversion or activation, (II) conjugation, and (III) compartmentalization [13,14]. Plant enzymes that typically catalyze the reactions of phase I, which is considered rate-limiting in the metabolism of xenobiotics in plants, are cytochrome P450 monooxygenases, carboxylesterases, peroxidases, and laccases [15,16]. The phase II involves conjugation with GSH, sugars, or amino acids, catalyzed by glutathione, glucosyl, and malonyl transferases, resulting in more soluble, polar compounds [17]. The phase III of plant metabolism is compartmentalization and storage of soluble conjugates in vacuoles or in the cell wall as well as in the apoplast. Because of the similarity between the detoxification mechanisms of xenobiotics in plants and the reactions in the animal liver [18] the '*green liver*' concept was introduced.

Even though important progress has been made in the understanding of plants metabolism, the ability to exploit them for environmental remediation is frequently restricted by the limited understanding of metabolic pathways, the full range of enzymes involved and tolerance mechanisms. In this context, hairy roots (HRs) have proved to be valuable systems for studying key aspects of pollutants phytoremediation [19,20]. They have also contributed to our knowledge of the complex biochemical and molecular mechanisms involved in this process.

Other limitations in the use of plant-based technologies are related with the fact that plants are autotrophs and not always suited for the metabolism and breakdown of certain organic compounds [21]. On the contrary, bacteria and mammals possess the enzymatic machinery necessary to achieve a complete mineralization of organic molecules; thus their degradative enzymes can be used to complement the metabolic capabilities of plants [22]. Consequently, an important way to improve their performance is through the obtainment of genetically modified plants or plant systems derived from them, tailored for phytoremediation purposes [23]. Knowledge about plant genomics, proteomics and metabolomics has helped to manipulate plant metabolism and to change or enhance their ability to remediate different xenobiotics [24–27, and references therein].

In this minireview we will illustrate how far the uses of plant-based technologies have evolved to make them more efficient, reliable and safer for their worldwide application.

Genetically engineered plants

Even though plants have the natural ability to decontaminate inorganic and organic pollutants, many efforts have been made to obtain genetically modified plants with improved phytoremediation abilities [23,27]. The purpose of introducing a new trait to the plant which does not occur naturally is not only to provide advantages against abiotic stresses, like the presence of a pollutant, but also to improve shelf life and yield. It should be noticed that obtaining a transgenic plant with improved phytoremediation capacity depends not only on the availability of gene sequences and enzymes involved in pollutants removal, but also on several other factors including: (1) the presence of a reproducible and highly efficient gene transfer technique, (2) the choice of explants which can easily regenerate, (3) the presence of a reliable regeneration method for the plant species, into which the novel gene is desired to be introduced, and (4) the availability of an effective screening and selection method for the recovery of transformants. The choice of a promoter sequence is no less important for proper transgene expression in transgenic plants.

Regarding genes involved in multistep degradation pathways of pollutants, transport and sequestration, they can be isolated from bacteria, fungi, animals or plants and introduced into candidate plants. Therefore, three main strategies have been employed: (a) transformation with genes from other organisms; (b) transformation with genes from other plant species and (c) over expression of genes from the same plant species [29]. Since the first work describing transgenic plants modified for pollutants metabolism [30], a lot of research has been performed in this way. However, reports dealing with inorganic contaminants have always been more abundant, as compared to those on organic contaminants, while there is scarce amount of papers addressing simultaneously with both kind of pollutants [29]. This could be related to the fact that heavy metal stress is one of the most strongly yield- and productivity-reducing factors in agriculture. It is therefore unsurprising that a major objective of current research is to identify ways of improving crops by making them resistant to heavy metal exposure [31].

Phytoremediation related genes

Plants possess a unique genetic inheritance, gained in the long process of evolution, which consists especially of characters adapting them for suboptimal environmental conditions including high temperature, cold, dehydration, high salt concentration, xenobiotics, and so on. Thus, the identification and analysis of genes that might be useful for phytoremediation is desirable to deal with some challenges such as how to increase the total uptake of pollutants or their biotransformation/bioaccumulation, or translocation, among others.

The main objective of inorganic's remediation is to enhance metal accumulation and tolerance in transgenic plants by overexpressing genes involved in homeostasis, metabolism, uptake and/or translocation of toxic elements [32]. Thus, at present, there are two main biotechnological approaches to reduce the impact of heavy metals toxicity in nature. One involves enhancing heavy metals uptake capacity of certain plant species, which would increase their utility in phytoextraction and, thus, in the phytoremediation of contaminated areas. The second involves improving plant resistance to heavy metal toxicity, which would prevent the inhibition of plant growth and productivity. Genetic manipulation of the expression, activity and localization of heavy metal ion transporters is a promising approach, since these proteins can directly control the uptake, distribution and accumulation of various elements in plants [31]. In this sense, genetically modified plants expressing metal chelators, metal transporters, metallothioneins (MTs), and phytochelatins (PCs) genes are commonly tried. For example, Morel *et al*. [33] and Verret *et al*. [34] described the function of two *Arabidopsis thaliana* P1B-type ATPases AtHMA3 and AtHMA4 that are responsible *in planta* for vacuolar sequestration and xylem loading (and therefore root-to-shoot translocation of heavy metals), respectively. Another member of bacterial P-ATPase superfamily, PbtA from haloaromate-degradative soil bacterium *Achromobacter xylosoxidans* A8 was also studied. It was demonstrated that this transmembrane transporter localized in tonoplast and plasma membrane increased Pb and Zn resistance of heavy metal-sensitive *Saccharomyces cerevisiae* strains and enhanced Pb/Zn/Cd accumulation, making it a very promising target for alteration of plant heavy metal-associated phenotype [35]. Rodriguez-Llorente *et al*. [36] engineered a Cu-hyperaccumulator plant by expressing the *copC* gene from *Pseudomonas* sp. Az13, encoding a periplasmic Cu-binding protein, in *A. thaliana*. The transgenic lines showed up to 5-fold increased Cu accumulation in roots and shoots compared to untransformed plants, over the limits established for Cu-hyperaccumulators.

Regarding organic pollutants two main strategies have been pursued: (1) the manipulation of metabolic activities to enhance *in planta* degradation rates or to impart a novel metabolic activity and (2) the enhanced secretion of enzymes from roots leading to accelerated *ex planta* degradation of organic contaminants [37]. These approaches could lead, in an optimal case, to the complete mineralization of the pollutants or the formation of less-toxic metabolites than those produced by the existing pathway [38]. In contrast to inorganic pollutants, increases in organic contaminants uptake by transgenic plants have not been reported since, as previously described, mechanisms by which they enter plant roots are poorly understood [37].

As it can be seen from the examples found in the literature, the development of transgenic plants for organic compounds remediation is mainly based on overexpression of genes coding for enzymes involved in xenobiotic transformation (phase I) or conjugation (phase II) [37]. To the best of our knowledge, there are not genetic engineering studies involving sequestration or compartmentalization (phase III). Moreover, genetic engineering of plants is important since they rarely mineralized hazardous organic compounds, because only few plant enzymes are able to catalyze ring opening reactions, in contrast to the degradative metabolism of microorganisms [1,12,39]. In this regard, Jha *et al*. [40] showed that catechol appeared as a metabolite of phenol biodegradation along with *cis–cis* muconic acid and fumaric acid as a result of the ring cleavage, using *Helianthus annuus* L. HRs (that will be described in sections below). Thus, obtaining transgenic plants able to catalyze the complete detoxification of xenobiotics is a goal of great interest in phytoremediation technologies [29]. For instance, the bacterial *bph*C gene encoding for 2,3-dihydroxybiphenyl-1,2-dioxygenase, that cleaves polychlorinated biphenyls (PCBs), was used to obtain transgenic plants with enhanced capability for PCBs degradation [41–43].

Plant species used in transgenic studies

Regarding plant species used for transformation experiments, *A. thaliana* and *N. tabacum* are among the most common model organisms due to well-developed and efficient protocols for DNA delivery and recovery of transformants [44]. However, as it was shown in Table 1, in the past few years the use of transgenic plants include not only the transformation of model plants and laboratory tests to clarify the roles and functions of genes but also the transformation of plants effectively useful for phytoremediation in the field [45]. It is well known that some plants do not develop a high biomass and therefore the total uptake of non-nutrient substances is low [46]. Thus, combining an efficient uptake and tolerance with increased biomass of vegetable species is one of the focuses of ongoing research. In addition, such plants would have some other suitable properties like fast-growing, deep roots and wide distribution since they could grow in different climatic and soil conditions. For example, poplars and willows are trees bestowed with robust growth, extensive root system and high biomass. Enhanced metabolism of organic pollutants in these tree species is associated with a faster uptake, which can be explained by a steeper concentration gradient inside plant tissues [22]. However, one of the obstacles in their use is related with difficulties in genetic transformation mediated by *Agrobacterium tumefaciens* [22]. In this context, a transgenic hybrid aspen (*P. tremula* × *P. tremuloides* var. Etropole) expressing a nitroreductase gene from *Pseudomonas putida*, *PnrA*, was shown to tolerate and take up greater amounts of 2,4,6-trinitrotoluene (TNT) from contaminated waters and soil compared with wild type plants [47].

Other interesting species for phytoremediation purposes are the members of the *Brassicaceae*, also called *Cruciferae* plant family. Among *Cruciferae*, in addition to *Arabidopsis* species, there are other genus such as *Brassica*, *Hirschfeldia*, *Capsella*, *Thlaspi* and *Lepidium* that have also been reported for their key role in phytoremediation since many wild crucifer species tolerate the toxic effects of a wide range of metals and hyperaccumulate them. Besides many of these species are well adapted to a range of

TABLE 1

Examples of transgenic plants for enhanced remediation of inorganic and organic pollutants.

Chemical nature of the contaminant	Gene transferred	Gene function	Origin	Target plant species	Effect	Reference
Inorganic	*ScMTII*	Metallothionein	*Saccharomyces cerevisiae*	*N. tabacum*	Higher Cd accumulation	[107]
	AtHMA4	Export protein (Zn/Cd root to shoot translocation)	*A. thaliana*	*N. tabacum*	Decreased Cd uptake/ accumulation in roots and shoots. Induction of cell wall lignification and enhanced H_2O_2 accumulation	[108]
	AtACBP1 and AtACBP4	Acyl-CoA-binding proteins (ACBPs)	*A. thaliana*	*Brassica juncea*	Increased accumulation of Pb in roots but the transgenic plants were not Pb tolerant	[109]
	IlMT2b	Metallothionein	*Iris lactea var. chinensis*	*A. thaliana*	Increased Cu concentration and reduced H_2O_2	[110]
	gshI	γ-Glutamylcysteine synthetase	*Escherichia coli*	*Populus tremula* × *P. alba*	Higher Cd uptake and accumulation in aerial parts. Lower concentrations of $O_2^{\cdot -}$ and H_2O_2, higher concentrations of glutathione (GSH) and oxidized GSH in roots and/or leaves	[111]
	StGCS-GS	γ-Glutamylcysteine synthetase-glutathione synthetase	*Streptococcus thermophilus*	*Beta vulgaris L.*	Increased Cd, Zn and Cu concentrations in shoots, higher GSH and PC levels. Tolerance to different metal combinations with greater translocation to shoots	[112]
Organic	*bphC*	2,3-Dihydroxybiphenyl-1,2-dioxygenase	Bacterial	*N. tabacum*	Enhanced tolerance to the commercial PCBs mixture Delor 103	[42]
	gshI	γ-Glutamylcysteine synthetase	*E. coli*	*Populus* × *canescens*	Increased GSH content under paraquat exposure	[113]
	P450 CYP1A2	Cytochrome P450 monooxygenase	Human	*A. thaliana*	Resistance to the phenylurea herbicide chlortoluron	[114]
	nidA, nidB, NahAa and *NahAb*	Naphthalene dioxygenase (*nid A* and *nidB*). Flavoprotein reductase and ferredoxin (*NahAa* and *NahAb*)	*Mycobacterium vanbaalenii PYR-1* *Pseudomonas putida G7*	*A. thaliana*	Enhanced tolerance towards 2–4 rings PAHs, faster assimilation of PAHs and lesser accumulation *in vivo*	[115]
	NahAa, NahAb, NahAc, and *NahAd*	Flavoprotein reductase, ferredoxin, subunits of terminal dioxygenase, respectively.	*P. putida G7*	*A. thaliana* and *Oryza sativa*	Improved uptake of phenanthrene and lower concentrations in shoots and roots	[116]
	CYND (*gi1167514*) and *FDH* (*gi145358030*)	Cyanidase and formate dehydrogenase	Bacterial cyanidase and plant formate dehydrogenase	*A. thaliana*	Enhanced cyanide metabolism and tolerance	[117]
	CsGSTU1 and *CsGSTU2*	Gluthatione transferase	*Citrus sinensis*	*N. tabacum*	Fluorodifen tolerance	[118]
	dBphC	2,3-Dihydroxybiphenyl-1,2-dioxygenase	Soil metagenomic library	*Medicago sativa* L. *cv. Gongnong*	Increased tolerance towards of PCBs/2,4-DCP. Stronger dissipation of PCBs and high removal efficiency of 2,4-DCP in a short time.	[43]

environmental conditions, are well suited to *in vitro* culture techniques and to genetic manipulation, which make them attractive candidates for the introduction of genes aimed to improve phytoremediation capabilities [48]. In this context, *B. juncea* expressing a bacterial glutathione reductase gene showed enhanced Cd tolerance at the chloroplast level [49] whereas the overexpression of a γ-tocopherol methyl transferase (γ-TMT) gene produced an increase in heavy metals tolerance, among other stresses [50].

Alfalfa (*M. sativa* L.) is one of the most important perennial forage crops all over the world with a variety of elite characteristics, such as highly productive biomass, drought tolerance, fast growing, and availability in large amount during several months of the year. Therefore, alfalfa has been proposed as an ideal natural resource for remediation of contaminated soils. A recent study showed that transgenic alfalfa had strong ability to decontaminate heavy metals and organic pollutants-contaminated soil. It was found that transgenic alfalfa co-expressing glutathione S-transferase and human P450 2E1 showed enhance ability to remediate mixed heavy metal (Hg or Cd)/organic pollutants (trichloroethylene, TCE) compared to wild type [43 and references therein].

On the other hand, genetic transformation of cereals and monocotyledonous plants using routine methods is challenging due to difficulties in regeneration of the target plant species as well as in DNA delivery. In this sense, the method of choice for DNA delivery into dicotyledonous plants mediated by *A. tumefaciens* was originally considered as not suitable for cereals, as they are not infected by *Agrobacterium* spp. in nature. However, recent advances led to efficient DNA delivery into wheat and barley using this method and consequently to the improvement of tolerance to environmental stresses in these plant species [28]. Of great economic interest is the genetic transformation of *Oryza sativa*, since rice is the main food for people in many parts of the world; it is the predominant dietary energy source for countries in Asia and the Pacific, countries in North and South America and countries in Africa [51,52]. This crop is considered as a model monocotyledon and is an example of species easy to transform. A widely used strategy for producing rice with cross resistance to various herbicides was the insertion of P450 transgenes involved in xenobiotic metabolism in plants [53–55]. More examples of transgenic rice are listed in [13].

Besides different plant species used for phytoremediation studies, other model systems derived from them have been developed for studying key aspects of various plant-based remediation strategies, like hairy roots (HRs). They became increasingly attractive, cost-effective options that have also shown great potential for pollutants removal. In the sections below we will highlight HRs contributions in the progress of phytotechnologies and the new insights related with their use.

Hairy roots: general characteristic and applications

HRs have been obtained from many plant species, mainly from those which exhibit susceptibility to *A. rhizogenes* infection such as dicotyledonous [19]. However, different strategies are being continually developed and probed in order to induce HRs from new plant species, even from recalcitrant species, like monocotyledonous [56]. HRs have several advantages such as genotype and phenotype stability, as well as fast and indefinite *in vitro* growth in absence of phyto-hormones under sterile conditions. They have been traditionally used to study root physiology along with biosynthetic pathway elucidation and for secondary metabolites production. Due to HRs biosynthetic capacity to produce secondary metabolites similar to the native plant roots or even higher they have been called as 'phytochemical factories' [57]. In this aspect, the key has been the great advance in HRs bioreactors design and optimization for large scale production [58]. At present, a wide range of commercially important high-value bioactive molecules such as pharmaceutical and cosmetic products are being produced by HRs from various plant species at industrial level [57]. The Swiss company ROOTec Bioactives AG manufactures high-value plant-derived compounds with rapid set-up of production process compared to field cultivation and chemical synthesis, as well as competitive cost compared with conventional production [59]. One important objective of this company is to bring accessibility to compounds from rare and/or unexplored plants, avoiding exploitation and promoting their protection, in line with the Convention on Biological Diversity in Rio (1992) and Nagoya (2010). This is an important aspect since it is in accordance with biodiversity conservation of plant kingdom.

It was quite unpredictable, from the 1980 years until now, that the gradual progressions in HRs studies would lead to the current research platform [60]. Moreover, research involving HRs have established different intra- and interdisciplinary work areas, have progressed, and diverged to provide various applications of HRs cultures that lead to the 'Hairy Root Biotechnology Podium' of the present days [60]. This development includes the application of HRs for (a) basic research, like secondary metabolites and recombinant proteins production coupled with productivity enhancement strategies and (b) functional research, including generation of agroeconomical traits in plants and molecular breeding, production of high value as well as less hazardous molecules through biotransformation/molecular farming. Also, HRs have been applied for phytoremediation assays with interesting findings, as well as rhizoremediation, a less explored area in which the contribution of associated microorganisms is considered [61]. In addition, transgenic HRs expressing foreign genes are useful since they can contribute with the remediation of mixed contaminants, bringing new enzymatic capabilities to plant tissues and providing the additional advantage of fewer regulatory restrictions and higher public acceptance for being a confined system.

HRs as model plant systems for phytoremediation purposes

HRs are frequently applied in phytoremediation research as model plant system, since they allow examining the intrinsic metabolic capabilities of plant cells and their abilities to tolerate toxic compounds without the interference of soil matrix and microbes [62,63]. Thus, HRs have contributed to the knowledge of the complex biochemical and molecular mechanisms involved in phytoremediation. In this sense, a better understanding of the intrinsic roots metabolism is allowed since there is not translocation of the pollutant or its intermediates to aerial parts. Besides, HRs are physiologically closer to real roots compared with undifferentiated cell suspension cultures and thus, the first reactions against a xenobiotic take place in the same way as in plant roots. Among other advantages of HRs, they have a short sub-cultivation period, stable biomass and prolific root growth independent on the season, providing a more reliable and reproducible experimental system over time, that is required to increase the effectiveness of phytoremediation processes [63]. As they grow in an aseptic environment, they can also be used to differentiate the responses and capabilities of plant tissues from those of microorganisms [64]. For instance, they could be efficiently used to study the ability to remove organic xenobiotics and to accumulate heavy metals in a proper scale avoiding the use of high volumes of contaminated solutions.

In this context, since the early experiments [65], HRs have proved to be effective tools for studying the mechanisms of metal uptake, accumulation and tolerance [66]. For example, the development of HRs from metal hyperaccumulators alpine pennycress (*Thlaspi caerulescens*), *Alysum* sp., Indian mustard (*B. juncea*) and sunflower (*H. annuus*), greatly contribute to the knowledge about the role of roots in the accumulation of metals and radionuclides [67–70]. Moreover, they have not only been used as a model system to investigate physiological and biochemical processes

TABLE 2

Examples of the more recently obtained transgenic plants for enhanced remediation of inorganic and organic pollutants.

Chemical nature of the pollutant	Nature of HR	Xenobiotic/pollutant	HR species	Reference
Inorganic	Wild type	Nickel	*Alyssum bertolonii*	[71]
			A. murale	[119]
		Zinc	*Solanum nigrum*	[120]
		Uranium	*Daucus carota*	[121]
			A. rusticana	[122]
		Arsenic	*N. tabacum*	[123]
		Chromium	*B. napus* HR and *Pantoea* sp. FC 1	[93]
		Cadmium and lead	*B. juncea*	[124]
		Zinc and nickel	*B. juncea* L.	[125]
	Transgenic	Copper	*N. tabacum*	[77]
Organic	Wild type	PCB	*S. nigrum* SNC-9O	[72,73]
		Explosives (DNT, TNT; ADNTs; DANTs)	*A. rusticana*	[126]
		Tetracycline and oxytetracycline	*Hellianthus annuus*	[127]
		N-acetyl-4-aminophenol (paracetamol)	*A. rusticana*	[128]
		Reactive Red 198 dye	*Tagetes patula* L.	[129]
		Textile dye: Methyl orange	*B. juncea* L.	[130]
		Textile dye: Reactive green 19A- HE4BD	*Sesuvium portulacastrum* (L.) L	[131]
		Phenol and chlorophenols	*D. carota, Ipomoea batatas* and *Solanum aviculare*	[9]
			B. juncea L.	[132]
			Solanum lycopersicum	[133,134]
			B. napus HR	[10]
			N. tabacum	[135]
			B. napus HRs	[136]
			H. annuus L.	[40]
			B. napus inoculated with two rhizobacteria	[61]
		Phenol	*B. napus* inoculated with *Pantoea* sp. FC 1	[93]
	Transgenic	TCE	*Atroppa belladonna* expressing a rabbit P4502E1 enzyme	[78]
		Phenol	*Solanum lycopersicum* overexpressing *tpx1* gene	[79]
			N. tabacum expressing *tpx1* and/or *tpx2* genes	[11,137]
			N. tabacum expressing *tpx1* gene + AMF	[92]

PCB: polychlorinated biphenyls; DNT: 2,4-dinitrotoluene; TNT: 2,4,6-trinitrotoluene; ADNTs: aminodinitrotoluenes; DANTs diaminonitrotoluenes; TCE: trichloroethylene; AMF: arbuscular mycorrhizal fungi.

in plants but also constitute a way to generate a metal-enriched product from the harvested plant biomass. This procedure might be useful for processing metal-enriched plant material harvested from phytomining operations [71].

Regarding organic contaminants, HRs were successfully applied for transforming/removing PCBs, explosives, antibiotics and other pharmaceutical products, textile dyes, phenol and chlorophenols, TCE (Table 2). It is important to note that using HRs it was possible to study particular enzymes and to elucidate metabolic pathways [72–74], as well as signalling events, cellular antioxidant responses and other tolerance mechanisms related to the removal process. Moreover, as it was mentioned before, using HRs cultures [40] showed for the first time plant cell ability to cleave the phenolic ring. Therefore, this model system has contributed to a deepening knowledge of phytoremediation process.

Transgenic HRs for the improvement of removal process

As it was already mentioned, understanding the physiological and biochemical processes, types of enzymes and genes involved in the metabolism of a particular compound, allows the obtainment of genetically modified plants with the purpose of improving the efficiency of pollutants removal or transformation. Furthermore, encouraging results have been obtained using transgenic HRs (Table 2). For inorganic compounds, subcellular targeting of metal-binding proteins to cytoplasmic membrane or to desired cell organelles could enhance metal accumulation [75,76]. As it was mentioned before, Cu hyperaccumulating *A. thaliana* plants were generated by expressing Cu-binding periplasmic protein CopC [36]. This novel strategy has been also applied to obtain transgenic HRs, which accumulated high Cu concentrations by expressing CopC protein either in the cytoplasm of cells or by targeting it to the vacuole [77]. Regarding organic compounds, it was demonstrated that *A. belladonna* HRs expressing a rabbit P4502E1 enzyme were able to metabolize TCE [78] whereas transgenic tomato and tobacco HRs expressing a basic peroxidase were capable for removing phenol with higher efficiency than wild type HRs [79,11].

The new challenge of phytoremediation research should be directed to find/develop suitable plants and/or HRs cultures derived from them with ability to remediate complex mixture of contaminants since the majority of polluted sites contain both inorganic and organic compounds. Simultaneous metal resistance/accumulation and organic xenobiotics detoxification by plants could be improved through different biotechnological approaches [80–82]. Thus, the obtainment of 'supertransgenic' plants or HRs by a multitransgene strategy, could contribute with an increased phytoremediation of mixed polluted environments [23].

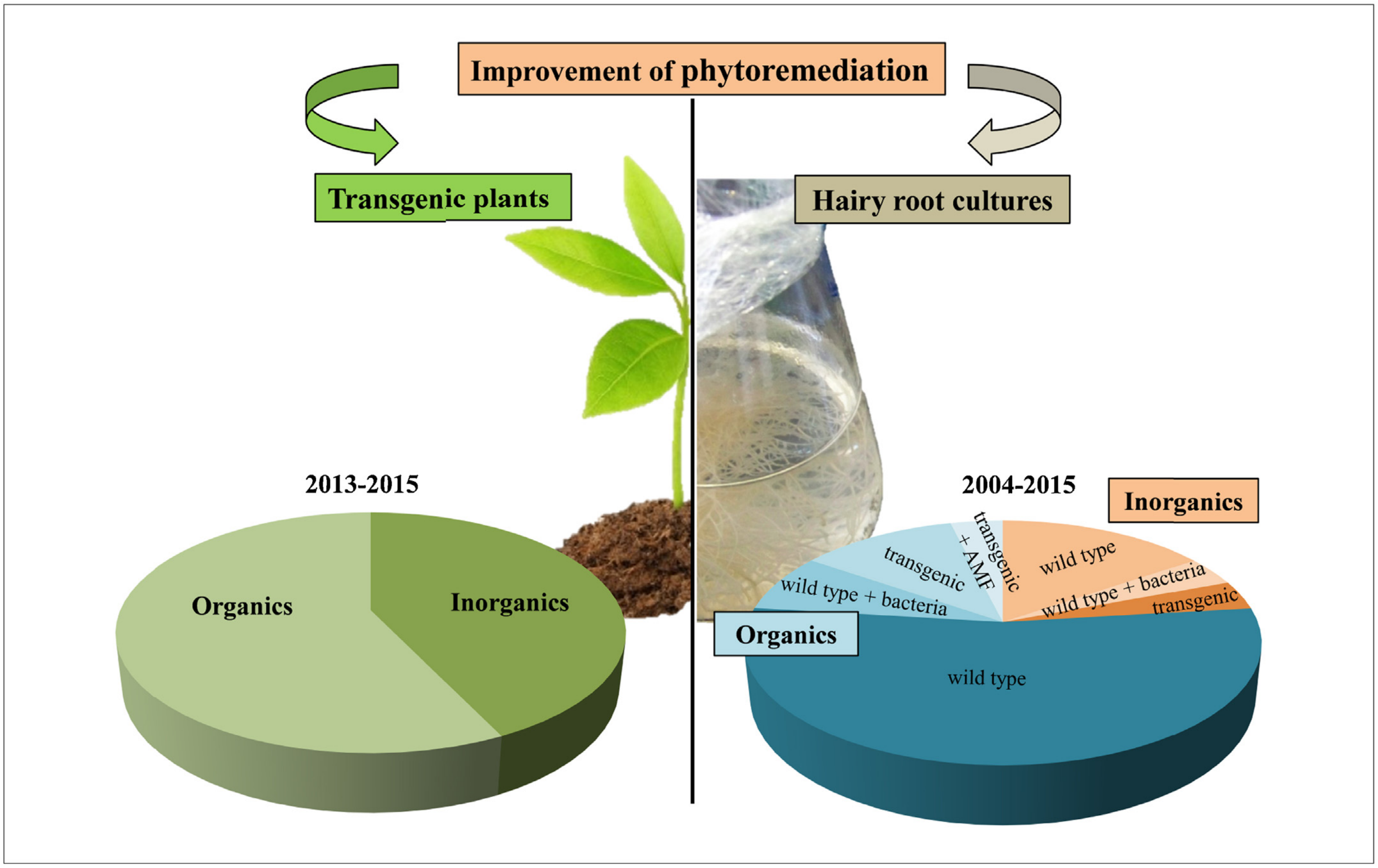

FIGURE 1

Schematic representation of the minireview content. Transgenic plants and HRs have been suggested as useful tools to improve phytoremediation and to deepen the knowledge about contaminant removal process. The last reports (2013–2015) related to transgenic plants were mainly involved in the improvement of organic compounds remediation. Besides, in order to show different alternatives to improve phytoremediation using HRs we have selected publications included from 2004 to 2015, since they are scarce, involving wild type HRs, other HRs inoculated with bacteria or arbuscular mycorrhizal fungi (AMF) and examples of transgenic HRs as different alternatives for the removal of organic and inorganic compounds.

Plant-microorganism interactions for phytoremediation: studies using HRs

Phytoremediation process is greatly affected by the interaction of plants with endophytic or rhizospheric communities of free living or symbiotic microorganisms, including bacteria and fungi in the surrounding polluted rhizosphere [83–88]. Since the metabolic capacity of microorganisms is extremely diverse, their combination with plants would be a better approach to improve the remediation of contaminated environments. This process called rhizoremediation is an emerging field that offers important advantages, such as a simplified environment that allows the study of a specific association avoiding other positive or negative microbe interactions (Table 2). In this sense, arbuscular mycorrhizal fungi (AMF) associated with plant roots have been used to enhance phytoremediation of toxic compounds. It has been shown that the interaction of *Pteris vittata*-AMF can increase arsenic uptake in this hyperaccumulating fern [89] while other AMF/plants partnerships have shown higher Cd resistance [90]. Regarding HRs/AMF associations, carrot HRs inoculated with *Glomus intraradices* alleviated growth inhibition induced by toxic concentrations of Cd, compared to the non-inoculated control [91]. The effect of arbuscular mycorrhiza (AM) on the growth and Cd uptake of transgenic tobacco with increased ability to accumulate Cd was assessed [91]. In a similar way, colonization of transgenic tobacco HRs expressing *tpx1* gene with *G. intraradices* efficiently protected roots against phenol-induced oxidative damage [92]. Therefore, this kind of plant-AMF systems could be used to study the complex interactions between both partners and also to predict their responses when they are exposed to different xenobiotics.

Regarding microbe assisted-phytoremediation, the association between rhizospheric microorganisms and HRs can also positively contribute for the improvement of phytoremediation process. In particular, *B. napus* HRs inoculated with *Burkholderia kururiensis* KP 23 and *A. rhizogenes* LBA 9402, showed enhanced phenol degradation compared to *B. napus* HRs alone [61]. Moreover, synergistic effect on rhizoremediation of two types of contaminants (phenol or Cr) has been obtained using *B. napus* HRs inoculated with a resistant bacteria strain isolated from an industrial polluted soil [93]. This microorganism (*Pantoea* sp. FC 1) was able to use phenol as sole carbon source and reduce CrVI to the less toxic form (CrIII). In addition, it exhibited some plant-growth promoting properties, such as Indole Acetic Acid (IAA) production, synthesis of siderophores and ability to solubilise inorganic phosphates. These results demonstrated the usefulness of HRs and bacteria interactions for removing inorganic as well as organic compounds. However, more research is needed to gain a deeper insight of these complex interactions and to apply this synergistic system for removing several mixtures of pollutants.

In the last few years, phytoremediation research has also focused in the interaction between plants and endophytic bacteria [94]. Several reports confirm endophyte ability to remove heavy metals from solution and to degrade xenobiotic compounds [95–97]. For instance, the presence of naturally recruited or genetically engineered endophytic bacteria in plant tissues can result in manifold increases in the capacity to degrade xenobiotic compounds compared with uninoculated plants [96,98]. However, to our knowledge, there are no reports describing the contribution of endophytic bacteria using HRs for phytoremediation purposes.

Finally, it is important to point out that HRs, as plant model systems, would provide useful information to facilitate the transition of this phytotechnology into plants suitable for rhizoremediation applications in field studies. Figure 1 summarizes different strategies using transgenic plants or HRs to improve phytoremediation abilities for organic and inorganic pollutants removal.

Hairy roots versus plants for phytoremediation studies

HRs have proved to be useful for phytoremediation researches providing basic information related on the capability of plant cells to tolerate, detoxify, metabolize, and store a wide variety of organic and inorganic pollutants. Particularly, many aspects of the primary interaction roots-pollutants have been elucidated taking advantage of the physiologic similarity between HRs and real roots [38,63]. Recently, omic technologies have allowed to deepen our knowledge about the HRs biotechnological potential and they have revealed the main similarities and differences between HRs and their mother plants [99–101]. Thus, it is expected that these technologies would be useful tools to clarify some aspects related with phytotransformation of recalcitrant xenobiotics, which still remain unclear. In addition, transgenic HRs give the possibility to study genes functionality and the role of some key proteins and enzymes involved in several metabolic pathways used by plant cells to tolerate and detoxify environmental pollutants. As it was pointed out, HRs have an undeniable potential regarding to the use of plants for *in vitro* phytoremediation studies. However, some limitations, mostly associated with their application in a real scenario should be considered. In this sense, the difficulty of HRs for adapting to the constant environmental fluctuations, load of pollutants, hydraulic conditions and presence of undesirable microorganisms constitute their greatest disadvantages [102]. Moreover, most of the studies using HRs in bioreactors to remove pollutants have remained at laboratory scale [103], and their industrial and/or large-scale implementation still remains scarce [102]. Thus, there is an urgent need to look for better choices to remove high volumes of polluted wastewater.

On the other hand, whole plants growing in hydroponic conditions were also used for phytoremediation studies. This kind of growing conditions takes the advantages of *in vitro* culture to develop basic studies, minimizing interferences and providing a homogeneous system. Thus, this strategy allows analyzing plant abilities to remove/transform pollutants and, also, the optimal conditions to do it, which is of valuable importance for further pilot scale applications and/or for *in situ* remediation strategies,for example, constructed wetlands [102]. It is important to note that plant hydroponic cultures have been used as a successful strategy to evaluate the efficiency of transgenesis procedures for improving the phytoremediation of structurally complex compounds [80,104,105].

Considering these backgrounds, it is clear that using both, HRs and plants, gives complementary information to understand the complex nature of pollutants metabolism and may help to achieve high removal efficiencies. The first ones, give a versatile system to explore basic aspects of phytotransformation in relatively short time whereas the second ones provide a biological tool to extent this knowledge to field conditions. Moreover, genetic engineering is contributing with the elucidation of proteins function in the removal process as well as the improvement of removal efficiency in phytoremediation.

Conclusion

Although phytotechnologies have greatly contributed to reduce and control environmental pollution, there are still many challenges to overcome. The extensive knowledge available on genes and enzymes involved in pollutants removal allowed plants or HRs to be genetically modified to enhance their tolerance and remediation capabilities. However, public acceptance on the use of transgenic organisms is a key aspect that limits their great potential for field applications. It is noteworthy that although these genetically modified plants and HRs will not be intended as human and animal food they still have a number of drawbacks to be widely used. Thus, scientific community should make an effort to address the most important questions that limit their application. Moreover, considering that the use of transgenic HRs, a confined plant system, contributes not only with the knowledge of metabolic processes related to pollutants removal and the functionality of foreign genes, but also constitutes an alternative to remediate high volumes of contaminated effluents or water. Either way, for a more intensive use of HRs, a considerable development of appropriate and efficient bioreactors with cheap operational costs is needed. In this context, the combined use of rhizospheric microorganisms along with HRs became also in an attractive alternative aimed to enhance the efficiency of phytoremediation, especially in recent years in the light of widening knowledge of the until now non-culturable microorganisms obtained due to metagenomic studies [106]. Furthermore, these interactions demonstrated to be a successful approach that allows obtaining added value from both partner overcoming some of the limitations that have each of these technologies (bioremediation-phytoremediation) separately.

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