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.

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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].