Transgenic plants for enhanced phytoremediation of toxic explosives
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Phytoremediation of organic pollutants, such as explosives, is often a slow and incomplete process, potentially leading to the accumulation of toxic metabolites that can be further introduced into the food chain. During the past decade, plants have been genetically modified to overcome the inherent limitations of plant detoxification capabilities, following a strategy similar to the development of transgenic crop. Bacterial genes encoding enzymes involved in the breakdown of explosives, such as nitroreductase and cytochrome P450, have been introduced in higher plants, resulting in significant enhancement of plant tolerance, uptake, and detoxification performances. Transgenic plants exhibiting biodegradation capabilities of microorganisms bring the promise of an efficient and environmental-friendly technology for cleaning up polluted soils.

Introduction
Phytoremediation, or the use of plants for cleaning up pollution, is a potentially attractive technology for the treatment of soil contaminated by toxic chemicals, such as nitro-substituted explosives and energetic compounds [1,2]. Phytoremediation arose a few decades ago from the recognition that plants were capable of metabolizing toxic pesticides [3,4]. Today, bioremediation using plants has acquired the status of a proven technology for the treatment of heavy metal and organic-contaminated soils [1,2]. However, unlike bacteria and mammals, plants are autotrophic organisms that lack the enzymatic machinery necessary for efficiently metabolizing organic compounds, often resulting in slow and incomplete remediation performance [5**]. This led to the idea to modify plants genetically by the introduction of bacterial or mammalian genes involved in the breakdown of toxic chemicals. Although transgenic plants have not yet been used in field applications, new advances, such as extension to more plant species, discovery of more catabolic genes, and innovative transformation strategies, are likely to deliver a technology that will improve phytoremediation efficiency and help control soil pollution [5**,6,7]. This review summarizes recent advances in developing plants specifically engineered for enhancing phytoremediation of explosives and energetic compounds.

Explosives as environmental contaminants
Best known for their explosives properties, nitro-substituted compounds, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and Glycercer trinitrate (GTN), are also toxic and persistent environmental pollutants contaminating numerous military sites in Europe and North America (Figure 1). Manufacture of explosives, testing and firing on military ranges, and decommission of ammunition stocks have generated toxic wastes, leading to large-scale contamination of soils and groundwater [8,9]. From laboratory studies, most nitro-substituted explosives were found to be toxic for all classes of organisms, including bacteria, algae, plants, invertebrates, and mammals [10–15]. TNT and RDX are listed as ‘priority pollutants’ and ‘possible human carcinogens’ by the U.S. Environmental Protection Agency (EPA) [11,16]. Physicochemical properties, biodegradation, and toxicity of nitro-substituted explosives have been extensively reviewed in the literature [8,9,17–19].

Traditional remediation of explosive-contaminated sites requires soil excavation before treatment by incineration or landfilling, which is costly, damaging for the environment, and, in many cases, practically infeasible owing to the range of contamination [18]. As one of the ‘top ten technologies for improving human health’ [20], bioremediation using microorganisms or plants represents an attractive alternative for the treatment of explosive-contaminated sites. Different classes of microorganisms, including bacteria and fungi, are capable of transforming energetic pollutants, which can be used as energy, carbon, and/or nitrogen sources [8,9,19,21]. As a highly oxidized molecule, TNT is easily reduced to form toxic amino derivatives or a hydride Meisenheimer complex, further transformed with the release of ammonium or nitrite (Figure 2) [8,9]. However, except with white-rot fungi that secrete powerful peroxidases, no significant mineralization of TNT has been reported in biological systems.
Initial biotransformation of RDX frequently involves either direct enzymatic cleavage or single or two-electron reduction of the nitro groups to form unstable nitroso and hydroxylamino derivatives. In contrast to TNT, whose limiting degradation step is the aromatic ring fission, a change in the molecular structure of RDX results in ring collapse and the generation of small aliphatic metabolites (Figure 2) [8,9,17].

Phytoremediation: clean-up of pollution with plants

First developed for removal of heavy metals, phytoremediation has been used successfully for the treatment of a variety of organic compounds, including chlorinated solvents, polyaromatic hydrocarbons, and explosives [1,2]. The main advantages of phytoremediation over other remediation options include low installation and maintenance costs and no damaging impact on the environment. In addition, phytoremediation offers potential beneficial side effects, such as carbon sequestration and biofuel production [22,23]. As autotrophic organisms, plants use sunlight and carbon dioxide as energy and carbon sources and can be seen as natural, solar-powered, pump-and-treat systems for cleaning up contaminated environments [5**]. Being exposed to a variety of natural allelochemicals and xenobiotic compounds through the root system, plants have developed diverse detoxification mechanisms and have for long been recognized as capable of metabolizing organic compounds [3,4,24].

Nitro-substituted explosives are efficiently taken up and partially metabolized inside plant tissues [5**,22,25]. The detection of TNT metabolites, such as aminodinitrobenzene, aminodinitrobenzoate, aminodinitrotoluene, and aminodinitrobenzene, suggests that plants can carry out both reductive and oxidative transformation of nitroaromatic compounds [21]. Identified RDX transformation products include reduction derivatives, such as hexahydro-1,3,5-trinitro-1,3,5-triazine, and a range of breakdown products, such as 4-nitro-2,4-diazabutanal, formaldehyde, and nitrous oxide [26,27]. Experiments with [U-14C]RDX even showed a partial light-dependent mineralization by poplar plants [27]. The detection of oxidation and reduction metabolites, as well as large molecular weight products and non-extractable fractions [28**,29**], suggests that plants metabolize explosives according to the ‘green liver’ model [4,30]. As in the liver of mammals, detoxification by plants typically involves three different phases: actication of the initial toxic compound (phase I), conjugation with a molecule of plant origin (phase II), and sequestration in plant organelles or structures (phase III) (Figure 3).

Despite these promising observations, phytoremediation suffers serious limitations, such as slow removal of pollutants from soil and the absence of significant detoxification, possibly leading to accumulation of toxic metabolites inside plant tissues [5**,31]. As a corollary of their autotrophic metabolism – exogenous organic compounds do not serve as electron donors – plants lack the catabolic enzymes necessary to achieve full mineralization of organic molecules, potentially resulting in the accumulation of toxic metabolites [5**]. It was demonstrated that incomplete transformation of explosives in poplar plants resulted in the release of toxic compounds.
from plant tissues, potentially threatening the food chain [32].

Transgenic plants for phytoremediation of explosive compounds

There is great promise that transgenic plants expressing bacterial or mammalian genes involved in xenobiotic metabolism will improve the efficiency and safety of phytoremediation, leading to a wider application in the field [22]. Microbes and mammals are heterotrophic organisms that possess the enzymatic machinery necessary to achieve a complete mineralization of organic molecules, complementing the metabolic capabilities of plants [5]. Although the first transgenic plants developed for phytoremediation aimed to improve metal tolerance [33], the strategy has been extended to enhance the plant metabolism of organic pollutants, such as explosives and halogenated compounds [34,35]. The use of transgenic plants for phytoremediation applications has been reviewed recently in [5,6,18,34,36,37,38]. Transgenic plants developed for the remediation of explosives are listed in Table 1.

In a pioneer work, French et al. [35] designed the first transgenic plants specifically engineered for the phytoremediation of organic compounds: tobacco plants (Nicotiana tabacum) were modified by the introduction of a bacterial pentaerythritol tetranitrate (PETN) reductase, an enzyme involved in the degradation of nitrate esters and nitroaromatic explosives, and derived from an Enterobacter cloacae previously isolated from an explosive-contaminated soil [39]. PETN reductases are bacterial flavonitroreductases that catalyze TNT degradation by either sequential reduction of nitro groups or aromatic hydride addition, resulting in denitration and, eventually, detoxification of TNT [21] (Figure 2).

Figure 2

Simplified representation of the microbial degradation of the explosives, 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) [8].
Transgenic tobacco seeds were able to germinate and grow in the presence of high concentrations of GTN (1 mM) and TNT (0.5 mM), inhibiting for wild-type seeds. In addition, transgenic plants exposed to 1 mM GTN showed enhanced denitrification compared with wild-type plants [35].

Together with flavonitroreductases, a large variety of nitroreductases, including NfsA and NfsB of Escherichia coli, PnrA of Pseudomonas putida, and NfsI from E. cloacae, catalyzes the sequential reduction of TNT nitro groups to hydroxylamino and amino derivatives, potentially followed by the release of ammonium through a Bamberger-like rearrangement (Figure 2). Hanninck et al. [40] showed that transgenic tobacco constitutively expressing the nitroreductase NfsI from E. cloacae removed high amounts of TNT from the solution (100% of 0.25 mM after 72 h), while only negligible amounts were removed by wild-type plants. Transgenic plants were also able to tolerate higher TNT concentrations, up to 0.5 mM, which was lethal for wild-type seedlings. Recently, the same transgenic system showed a reduction of TNT into 4-hydroxylamino-2,6-dinitrotoluene and conjugation to plant macromolecules to a greater extent in modified plants as compared with the wild type [41]. Following a similar strategy, Kurumata et al. [42] transformed Arabidopsis thaliana plants by the introduction of an E. coli nitroreductase, NfsA, with activity against different nitroaromatic compounds [42]. Plants expressing the bacterial transgene exhibited a 20-time higher nitroreductase activity and 7–8 times higher TNT uptake compared with wild-type plants. In addition, modified plants grew on inhibiting TNT concentration (0.1 mM) and showed in-tissue reduction of TNT into 4-amino-2,6-dinitrotoluene, which was not observed with wild-type organisms [42]. These examples demonstrate that the introduction of bacterial catabolic genes into plants significantly improved their capability to take up and metabolize the toxic explosive TNT.

Besides TNT, plants were also transformed for improving performances against RDX, which is today the most widely used military explosive. A. thaliana plants were engineered to express a bacterial gene, XplA, encoding a RDX-degrading fused flavodoxin-cytochrome P450-like enzyme [43,44]. The donor strain, Rhodococcus rhodochrous strain 11Y, originally isolated from RDX-contaminated soil, was capable of achieving a 30%-mineralization of [U-14C]RDX in pure culture [44]. Rhodococcal bacteria have been shown to degrade RDX by denitrification, leading to ring cleavage and the release of small aliphatic metabolites (Figure 2) [43]. Liquid cultures of A. thaliana expressing XplA removed 32–100% of RDX (initial concentration 180 μM), while less than 10% was removed by wild-type plants. When growing on RDX-contaminated soil, the transgenic lines showed no phytotoxic effect up to 2000 mg kg⁻¹, while significant inhibition was observed in wild type at 250 mg kg⁻¹. Conversely, RDX analysis in exposed plants showed significantly higher concentrations in wild-type plants, suggesting that transgenic lines were capable of more efficient transformation of RDX.

Although A. thaliana and tobacco are well characterized, laboratory model plants, they are not well adapted for phytoremediation applications, given their small stature.
and shallow root system. Poplar and aspen (Populus sp.), on the contrary, are widely distributed, fast-growing, high biomass plants ideal for phytoremediation applications [45]. Recently, a transgenic hybrid aspen (P. tremula × P. tremuloides) expressing a nitroreductase gene from P. putida. PnrA, was shown to tolerate and take up greater amounts of TNT from contaminated waters and soil compared with wild-type plants [46*]. Using [U-ring 13C]TNT, the authors showed a rapid adsorption of TNT on the root surface, followed by a slow entrance into the plant, with most [13C]carbon remaining in the roots.

The few examples above demonstrate that introduction of bacterial genes involved in explosive degradation improves phytoremediation performances by at least three different ways: increasing degradation, plant tolerance, and uptake of toxic compounds. The later effect, observed with explosives and chlorinated solvents, is likely to be related to a reduced phytotoxicity and a steeper gradient of pollutant concentration inside plant tissues [34,35].

Introduction of exogenous genes involved in xenobiotic transformation can induce unexpected side effects, such as an enhancement of the rhizosphere microfloral activity. Travis et al. [47**] have recently reported that transgenic tobacco plants overexpressing type I nitroreductase gene from E. cloacae were able to detoxify TNT in soil, resulting in increased microbial biomass and metabolic activity in the rhizosphere of transgenic plants as compared with wild-type plants.

Conclusions
Enhancing phytoremediation through genetic transformation of plants has become a proven technology that will help overcome the major limitation inherent to the process, that is, the threat that accumulated toxic compounds would contaminate the food chain. Further developments will involve the introduction of broad-substrate, natural or engineered, catabolic genes, such as mammalian cytochrome P450 or fungal peroxidase, for the simultaneous remediation of a variety of pollutants, as commonly found in contaminated sites [6]. Similarly, the introduction of multiple transgenes involved in different bioremediation processes, for example, uptake by roots and metabolic phases of the ‘green liver’ model, will bring further promises of an efficient and environmental-friendly technology for cleaning up polluted soils. Another important barrier to field application of transgenic plants for bioremediation arises from the true or perceived risk of horizontal gene transfer to related wild or cultivated plants. Therefore, it is likely that the next generation of transgenic plants will involve systems preventing such a transfer, for instance by the introduction of transgenes into chloroplast DNA or the use of conditional lethality genes [48].

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This excellent review focuses on recent developments of transgenic plants for remediation of organic pollutants, including explosives, chlorinated compounds, petroleum products, PCBs, and pesticides. The paper includes tables summarizing the phytoremediation of organic pollutants by both natural and engineered plants.


This review examines recent advances in enhancing phytoremediation of organic pollutants and heavy metals through both the development of transgenic plants and the use of symbiotic endophytic microorganisms within plant tissues.


This paper describes the overexpression of glutathione S-transferases in poplar plants exposed to the explosive TNT. In addition, evidence of conjugation of TNT with glutathione was witnessed in vitro using a mammalian glutathione S-transferase.


This paper describes the discovery of glycosyltransferases genes overexpressed in Arabidopsis thaliana plants exposed to TNT. Recombinantly expressed glycosyltransferases were shown to catalyze the conjugation of TNT with glucose, suggesting the implication of the enzymes in TNT detoxification by plants.


This paper highlights new developments of transgenic plants for general environmental applications, including pollution protection (e.g. reduced pesticide use) and phytoremediation.


This paper describes the transformation of Arabidopsis thaliana plants by the introduction of an Escherichia coli NfsA nitroreductase, with activity against different nitroaromatic compounds. Transgenic plants showed higher nitroreductase activity, TNT tolerance, and TNT uptake compared with wild-type plants. The paper also showed a reduction of TNT into 4-amino-2,6-dinitrotoluene, which was not observed with wild-type organisms.


This paper shows that transgenic tobacco plants overexpressing a bacterial nitroreductase gene detoxified soil contaminated with TNT, resulting in significantly increased microbial biomass and activity in the rhizosphere compared with wild-type plants.