Chapter 2
Hairy Roots as a Source for Phytoremediation

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Abstract "Phytoremediation," application of green plants to process and regulate the waste materials in soil, water, and the air is an important part of the new field of ecological engineering. Phytoremediation has been addressed as among the promising and eco-friendly processes for the decontamination of several environmental pollutants. In recent years, in vitro plant cultures play an important role in the phytoremediation process. This chapter focuses on the development of hairy root clones by using *Agrobacterium rhizogenes* in various plant species and their application in the remediation process. It is well-known that enzymes that are expected to be necessitated in the detoxifying process of lethal compounds. In view of the ease of employing this in vitro culture method as a transgenic arena, and interdependent the huge progress in functional genomics studies, it is required to develop novel hairy root cultures that capable to express, provoke, and metabolize additional genes in a greater efficiently under in vitro and in vivo. The present chapter summarized the differences between how hairy roots are more helpful in converting toxic to nontoxic forms comparatively with normal roots. This chapter also reports the most up-to-date achievements of using hairy root cultures in the phytoremediation process. This information is essential for assessing the feasibility of a remediation process prior to its filed uptake.

Keywords Hairy roots · Enzymes · Phytoremediation · *Agrobacterium rhizogenes*

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J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_2

1 Introduction

The stages of environmental contamination by the use of pesticides, heavy metals (HMs), leather tannery effluents, phenols, organic matter, explosives, azo dyes have increased significantly over the last few decades, mostly due to industries and tanneries (Perotti et al. 2020). Tannery effluents contain large quantities of both inorganic and organic contaminants, such as phenols and hexavalent chromium (Cr (VI)). It is well known that toxicity depends upon the redox state. In the case of chromium, for example, Cr (VI) is highly toxic compared to Cr (III) due to its high solubility, availability, and mobility in the soil as well as through biological membranes. Based on the facts, the removal of toxic compounds from the environment is of high relevance for a safe environment. In this sense, many biological methods have been proposed to remove these harmful substances from the water bodies and soil (Chen et al. 2012). Phytoremediation, using plant vegetation to degrade the contaminated environments, is a green and eco-friendly technology that has gained importance concerning traditional decontamination methods (Flocco and Giulietti 2007).

In recent decades, a research area in the area of phytoremediation experienced increasing interest, mainly in Europe and the United States, but also in developing countries. Even though it was primarily implemented for the degradation of different kinds of inorganic impurities from soil samples, the phytoremediation approach has increasingly proving to be proficient for the processing of organic contaminants additionally. These facts are making it necessary to acquire discernment into the machinery essential for the decontamination process. Prior to the implementation of a phytoremediation protocol to field conditions, it is necessary to conduct laboratory-scale studies, for which the plant experimental models systems are essential. These model systems permit the control and reproducibility in experimental conditions necessary for conducting basic phytoremediation research.

In vitro plant cultures especially hairy root cultures (HRCs) are a useful alternative methodology for decontamination of PCBs, trinitrotoluene (TNT), textile dyes, phenolics, HMs, and radioactive nuclides (Agostini et al. 2013). Hairy roots (HRs) are originated by the infection of explants with *Agrobacterium rhizogenes* strain, gram-negative soil bacteria by transfers of the transfer DNA comprising the loci between the T_R and T_L region of the root-inducing plasmid (Chandra 2012; Lal 2020; Sujatha et al. 2013). Concomitantly, as the genetic and biochemical properties of HRs were exposed, scientific community started focussing on its exploitation for research benefits. The main reason behind this is the major property that has attracted scientists the most was the equal or sometimes higher potential of HRs to produce important bioactive compounds, and the HRs biotechnology podium has proved to be valuable systems for studying key aspects of pollutants phytoremediation (Georgiev et al. 2012). Even plant root cultures also provoke the breakdown of harmful substances in the soils through the secretion of root exudates and oxido-reductive enzymes that are mainly involved in the degradation of organic pollutants (Jha et al. 2020).

Though the research on *A. rhizogenes* transformed HRs is available for several years for enhancing the secondary metabolites (SMs) but during the past few years' research on HRCs is increasing. Mainly, during the last decade research on HRs podium is helping in understanding the mechanism of the phytoremediation process. In the present chapter, we focus on the source of HRs from different medicinal plants used to remediate the contaminants and tabulated in Table. 2.1. It is therefore unsurprising that the major objective of current research is to diversify the research to understand the plausible reason how HRs are superficial in comparison with normal roots.

2 Hairy Roots in Phytoremediation

Plant cell cultures have been employed for the biotransformation of various organochlorine, organophosphorus pesticides, phenols, PCBs, and explosives like GTN (Anees et al. 2020; Doran 2009; Scheel and Sandermann 1977). Over the last 30 years, HRs have been exploited for different kinds of beneficial intents, distinguishing from metabolic engineering, and industrially important recombinant protein synthesis to conduct an assessment of phytoremediation (Praveen et al. 2014; Wilson and Roberts 2012). The advantage of HRs system could allow variations to carry out the organization of phyto-molecules that cannot realistically be generated by chemical synthesis. The molecular basis of genetic transformation of HRCs by using *A. rhizogenes* strains is well known and recently detailed in many studies (Gutierrez-Valdes et al. 2020; Mehrotra et al. 2020; Rency et al. 2019; Satish et al. 2019). More recently, HRs technology has been established as a biotechnological concept in a majority of plant root systems. HRs, fine fibrous structures can be grown in large mass in culture media in a controlled environment and can, therefore, be subjected to various physiological assays such as phytoremediation that are formed on plant tissues infected by *A. rhizogenes* (Georgiev et al. 2007). Plants do not necessarily cause the same types of metabolic reactions that bacteria or fungi do, rather than mineralizing a contaminant the way a bacterium would, plants typically interact with a xenobiotic compound in three phases. The process of phytoremediation encompasses three detoxification phases (Abhilash et al. 2009; Betts 1998; Schröder et al. 2007).

Level – I: Transformation, including oxidation, reduction and hydrolysis, and the catalysis to speedup the ratio of virtually all the chemical reactions through enzymes for example P450 monooxygenases, reductases, peroxidases, dehydrogenases, and esterases.

Level – II: For better solubility, the process initiated with the conjugation of contaminants with endogenous compounds such as mono-, oligo- and polysaccharides, peptides, proteins, amino acids.

Table 2.1 Phytoremediation of environment pollutants by hairy root culture of different plant species

Heavy metals	Hairy roots	References
Cadmium	*Alyssum bertoloni*	Boominathan and Doran (2003)
Nickel	*Thlaspi caerulescens*	Boominathan and Doran (2003)
	Alyssum murale	Vinterhalter et al. (2008)
	A. bertolonii	Ibañez et al. (2016)
Copper	*Rubia tinctorum*	Maitani et al. (1996)
	Hyptis capitata	Malik et al. (2016)
Zinc	*Solanum nigrum*	Subroto et al. (2007)
Uranium	*Daucus carota*	Straczek et al. (2009)
	Armoracia rusticana	Soudek et al. (2011)
	Brassica juncea	Flocco and Giulietti (2007)
	Chenopodium amaranticolor	
Arsenic	*N. tabacum*	Talano et al. (2014)
	V. zizanioides	Moogouei (2018)
Chromium	*B. napus* HR and *Pantoea* sp. FC 1	Ontanon et al. (2014)
Cadmium and lead	*B. juncea*	Eapen et al. (2007)
Cadmium	*(M. oleifera T. latifolia C. proxmus)1*	Ghada et al. (2017)), Flocco and Giulietti (2007), Malik et al. (2016)
	(Beta vulgaris Nicotiana tabacum Solanum nigrum Thlaspi caerulescens)2	
	Adenophora lobophylla	
	A. potaninii	
Cr(VI)	*Lemna minuta Kunth*	Paisio et al. (2018)
Nitrate	*V. zizanioides*	Moogouei (2018)
Metformin and cesium	*A. chlorostachys*	Moogouei (2018)
Zinc and nickel	*B. juncea*	Ismail and Theodor (2012)
Copper	*N. tabacum*	Perez-Palacios (2015)
Chromium	*B. napus*	Perotti et al. (2020)
Phenol and its derivatives		
Phenol	*B. juncea*	Singh et al. (2006)
	Raphanus sativus	
	Azadirachta indica	
	Beta vulgaris	
	B. napus	Coniglio et al. (2008)
	Lycopersiconesculentum	Gonzalez et al. (2006); Oller et al. (2005)
	Armoracia lapathifolia A. lapathifolia	Flocco and Giulietti (2007)
	Tomato hairy roots Dacus carota	González et al. (2006)

(continued)

Table 2.1 (continued)

Heavy metals	Hairy roots	References
2,4-Dichlorophenol	*B. napus*	Agostini et al. (2003)
	Nicotiana tabacum	Angelini et al. (2014)
N-acetyl-4-aminophenol	*Armoracia rusticana*	Huber et al. (2009)
Polychlorinated biphenyls	*Solanum nigrum*	Mackova et al. (1997)
Tetracycline and oxytetracycline	*Helianthus annuus*	Gujarathi et al. (2005)
Dichloro-diphenyl-trichloroethane	*B. juncea*	Suresh et al. (2005)
	Cichorium intybus	
Guaiacol, catechol, phenol, 2-chlorophenol, and 2,6-dichlorophenol	*Daucus carota*	De Araujo et al. (2002), De Araujo et al. (2006)
	Ipomoea batatas	
	Solanum aviculare	
TNT	*M. aquaticum*	Hughes et al. (1996)
	M. spicatum	
	Catharanthus roseus	
TNT	*Catharanthus roseus*	Bhadra et al. (1999)
Pesticides		
Dichloro-diphenyl-trichloroethane (DDT)	*B. juncea*	Suresh et al. (2005)
	Cichorium intybus	
Explosives		
Tri-nitro-toluene (TNT)	*Catharanthus roseus*	Hughes et al. (1996)
Explosives (DNT, TNT; ADNTs; DANTs)	*A. rusticana*	Nepovím et al. (2004)
RDX and HMX	*Catharanthus roseus*	Malik et al. (2016)
TCE	*Atropa belladonna*	Malik et al. (2016)
Azo dyes		
Reactive red 198 (RR198)	*Tagetes patula*	Patil et al. (2009)
Reactive green 19 A HE4BD	*Sesuvium portulacastrum*	Lokhande et al. (2015)
Reactive black 8	*Physalis minima*	Jha et al. (2014)
Acid red 114 (AR114)	*Ipomoea carnea*	Jha et al. (2016)
Reactive red 120	*Helianthus annuus*	Srikantan et al. (2018)
Methyl Orange	*B. juncea*	Telke et al. (2011)
Green 19A- HE4BD	Sesuvium *portulacastrum*	Lokhande et al. (2015)

Abbreviations from the table: *TNT* Trinitrotoluene, *TCE* Trichloroethylene, *RDX* Royal demolition explosive, *HMX* High melting explosive

Level – III: In this phase, transportation and accumulation of soluble contaminations will be done into vacuole organelle or they can bound to cell wall and diminished.

The HRCs perhaps also be deliberated as prescreening for plants with improved potential for phytoremediation (Suza et al. 2008). So many applications including the capability of rapidly growing under microbe-free culture conditions, with an increased surface area of contact in the middle of contaminant and plant tissues, and genetically and metabolic consistency as compared to wild type have been provided for HRs applications in phytoremediation process (Gujarathi et al. 2005; Georgiev et al. 2007). Transformed roots are amenable to genetic modifications and may advance the characterization of genes that regulate the phytoremediation potentials of plants. In addition, the expression of appropriate genes in plant root system improves the rhizo-degradation of extremely recalcitrant compounds, for example, PAHs and PCBs, etc. (Abhilash et al. 2009; Gerhardt et al. 2009).

Another advantage of using HRs for studying about the phytoremediation system is their capability to develop large quantities of root exudates that are consisting of enzymes and few kinds of metal-chelating substances that may detoxify or cutoff the adverse organic and inorganic pollutants in the soil (Bais et al. 2006; Doty 2008; Gujarathi et al. 2005). Flocco et al. (1998) reported in *A. lapathifolia* and concluded that roots comprise major levels of peroxidase enzymes (E.C. 1.11.1.7) that are recognized to be concerned with the detoxification process of phenols and some other aromatic molecules. Among all the toxic compounds that contaminate the water and agricultural soils, polychlorinated biphenyls (organic chlorine molecules) and some inorganics, in particular, HMs and radionuclides (an atom that has excess nuclear energy) have been the important issues in phytoremediation investigation. These important characteristics made HRCs have been referred to as an exceptional experimental model to remediate organic and inorganic pollutants because of their biochemical and genetic stability through cost-effective phytoremediation treatments as phytostabilization, phytovolatilization, rhizofiltration, phytodegradation, and phytoextraction (Upadhyay et al. 2019; Majumder and Jha 2012). Several reports have concluded and reported that HRs originated from various kinds of plant species can be exploited for the treatment of different organic and inorganic pollutants including heavy metals, dyes, ions, pesticides, excess nutrients, and solvents (Fig. 2.1).

2.1 Phytoremediation for Heavy Metal Degradation

HMs are characterized as metallic components that have comparatively highly thick contrary to water (Tchounwou et al. 2012). There are 59 elements categorized as HMs on the terrestrial crust, which are discharged into the environment through man-made activities or by natural constituents. Among those five HMs viz., copper, chromium, cadmium, zinc, and lead are believed to be extremely harmful (Shaban et al. 2016). HMs may cause the brain to injure and also creates several other disorders in living beings. These HMs cannot be humiliated easily and their remediation from the environment is very essential. In this situation, the application of advanced biological methods especially HRCs is the only alternative and gives more attention since they are believed to be renewable, eco-friendly, and produce valuable credentials for enzymatic reactions (Jadhav et al. 2009).

HMs contamination of soils is one of the particularly relevant environmental issues throughout the world (Doumett et al. 2008; Nouri et al. 2006). Consequently,

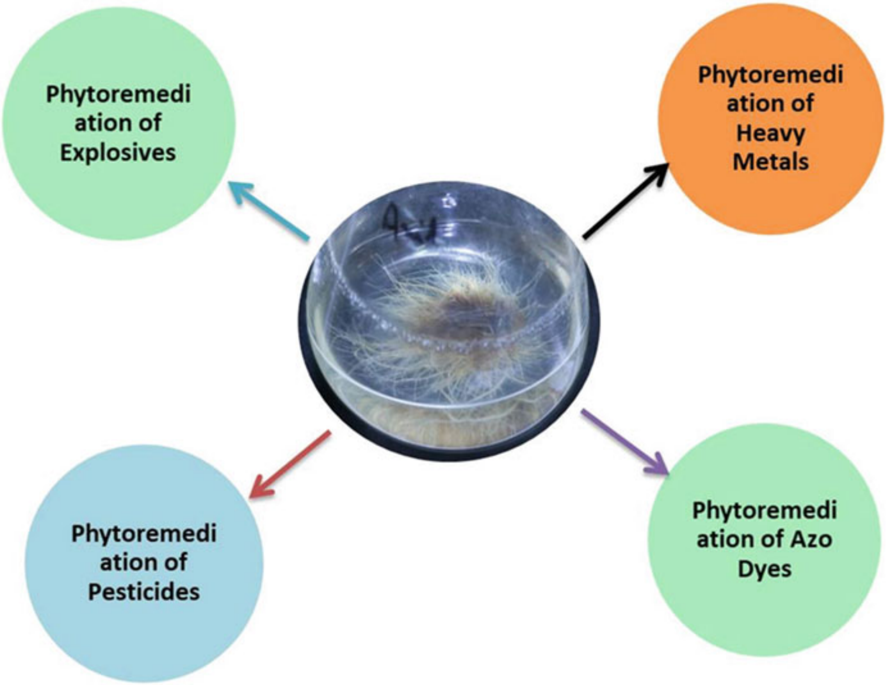

Fig. 2.1 Source of hairy roots for Phytoremediation of various organic and inorganic pollutants

the direct discharge of recycling wastewater for horticultural and agricultural usage and is considered as an impersonating potential threat to the health of the population (Yapoga et al. 2013). Reverse osmosis, chemical oxidation, coagulation-flocculation, filtration, adsorption, photodegradation, and advanced oxidation methods have been reported for dye decolorization and degradation, but high cost, generation of high-risk by-products, and high amounts of energy demand, have restricted the application of these approaches (Joo et al. 2007; Bizani et al. 2006; Crini 2006). In order to study the physiological mechanisms, research on different plant species has been carried out on the toxicity of cadmium to HRs. In all these research studies, HRCs demonstrate an admirable ability to absorb HM cadmium and to eradicate the contagiousness of this compound (Shi et al. 2012; Subroto et al. 2007; Vinterhalter et al. 2008).

2.2 *Phytoremediation of Phenols*

HRCs have been investigated for phytoremediation of different kinds of pollutants like dichlorophenol (a chlorinated by-product of phenol) by *Brassica napus* (Agostini et al. 2003), dichloro-diphenyl-trichloroethane (DDT) by *B. juncea* and

Cichorium intybus (Suresh et al. 2005), phenol by *Helianthus annuus* (Jha et al. 2013). Phenol is the most important natural contaminant in environment through coal conversion process, petroleum refineries, pesticides, and petrochemical products (Jha et al. 2020). As individuals learn about the dangers of the contaminants, they can share their knowledge with others. However, the lack of resources available to people in less developed countries can impact significantly how much of a difference education can make. Often, individuals in less developed areas are more focused on surviving day-to-day than on the possible long-term effects of hazardous chemicals (Russell 2005). Moreover, the proficiency of plants to metabolize harmful substances will rely on the biochemical properties of metabolizing enzymes and different defensive mechanisms that may extend the plant tissue survival rate. In fact, the results from a comparative study of peroxidase enzymes from HRs of *Daucus carota*, *Ipomoea batatas*, and *Solanum aviculare* evidenced an interspecific divergence in the priority for chlorophenol and phenol among the peroxidases (de Araujo et al. 2004). Also, peroxidase isozymes engaged in the degradation of phenol compounds within a species may indicate the difference in substrate selection and the efficiency catalytic activity of phenol metabolism (Coniglio et al. 2008). This is considering that these investigations are essential in creating awareness about the enzymatic mechanisms of pollutant remediation for choosing selective enzymes that might be developed in large quantities and used as catalysts for breakdown of the contaminants (Gonzalez et al. 2006).

2.3 Phytoremediation of Xenobiotic Compounds

The HRCs are known for their fast growth, high metabolic activity, and genetic as well as biochemical stability have been exploited for studies on biotransformation of various xenobiotics and were proven to be very effective (Giri and Lakshmi Narasu 2000). The detonating materials viz., TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine are extensive environmental pollutants frequently identified as sub-contaminants in the army training fields (Rylott et al. 2011). Degradation of the exploding TNT to further components through *Catharanthus roseus* HRCs was reported by Hughes et al. (1996). Similarly, the biotransformation of anthracene by HRCs of *Medicago sativa* and found that the root concentration factors were higher than that of whole plants (Paul and Campanella 2000). The well-organized nature of HRCs offers additional benefits, making them further susceptible for growing in large-scale levels using bioreactors to understand the mechanisms in detail. Phytoremediation of DDT to DDD and DDE has been claimed using the cell-suspension cultures of *Glycine max* and *Triticum aestivum* (Arjmand and Sandermann Jr 1985; Scheel and Sandermann 1977). The common remediation pathway of DDT involves subtractive dechlorination to DDD followed by dihydrochlorination to DDE. The DDE was evidenced to be further humiliated to DDMU through a dechlorination reaction (Hay and Focht 1998; Quensen et al. 1998). According to Suresh et al. (2005), *Cichorium intybus* and *B. juncea* HRCs are stable for the phytoremediation of insecticide DDT and concluded that DDT did not cause growth inhibition in HRCs of *C. intybus and B. juncea* at the added concentration. These cultures confirmed that the growth pattern, biomass, and differentiation are comparable to those of nontreated controls. Similarly, Scheel and Sandermann (1977) reported in parsley and soybean and observed rapid uptake of DDT. It has been suggested that the transformation of examined pesticides in the plants mostly occurs via direct oxidation and subsequent conjugation pathways (Kurashvili et al. 2016). It is estimated and reported by the US department of defense that, between 16 billion to 160 billion needed for clean-up of unexploded munitions (U.S. GAO 2014).

2.4 Phytoremediation of Azo Dyes

Rapid industrial improvement particularly from pharmaceutical, fabric, food processing units, leather, and agricultural-based industries is a matter of serious concern for causing environmental pollution. Almost 10,000 different textile dyes such as reactive, disperse, basic, etc. are used for coloring and printing purposes by processing industries (Jha et al. 2020). Mainly, 90% of the dyeing process involves from the overall textile-released liquid effluents, which consists of a mixture of various pollutants viz., organochlorines, surfactants acids or bases, HMs lead, salts, phthalates, suspended solids, dyes, and several other chemicals (Zaharia and Suteu 2013). The main dye used frequently in textile is azo dye as a result of their superior features, durability against microbial decomposition and higher photolytic strength, and risk to the environment and human health in view of their renowned issues such as mutagenicity, carcinogenic effects, and toxicity (Forss and Welander 2011; Mansour et al. 2011). Taking all of this into account, HRs technology is an alternative to physical and conventional methods for detoxification (Golob et al. 2005). Textile dyes degradation was successfully reported with *Tagetes patula* and *Physalis minima* HRCs by inducting the enzymes DCIP reductase and azo reductase (Jha et al. 2015; Patil et al. 2009). The phytotoxicity experiments demonstrated that the nonhazardous quality of degraded dyes by using HRs of *P. minima* and *Sesuvium portulacastrum* (Jha et al. 2014; Lokhande et al. 2015). Jha et al. (2016) reported in *Ipomoea carnea* that synergetic activity of oxidative and reductive enzymes discharged from the HRCs may be the reason for the phytoremediation of the dyes. In another dye degradation report, the dye remediation was very effective with reactive red 120 as inducer in HRCs of *H. annuus* with unique attention on the effect of light on adsorption equilibrium and dye-degradation kinetics (Srikantan et al. 2018). It has been reported that *B. juncea* HRCs increase the decolonization of methyl orange to 92% within 4 days of the incubation period. The enzyme assay of HRs obtained after the decolorization of methyl orange indicated considerable intracellular laccase activity.

2.5 Hairy Roots in Other Environmental Applications

In view of the increased demand for SMs, particularly as drug precursors, there is a need to develop new strategies for production. Production of genetically stable HRCs from many plant species has opened up the possibilities for fundamental studies and a lot of feasible applications of biotechnology. Since the main obstacle with in vitro cell cultures is that the transformants are genetically unstable from generation to generation, tend to produce low yields of SM and time-consuming process (Giri and Narasu 2000). HRCs importance for SMs in pharmaceutical, cosmetics, and various fields is increased. In this respect, hairy or transformed root cultures have plenty of advantages compared to the normal ones such as genetic and biochemical stability and need only regular media (Pitta–Alvarez et al. 2000). The SMs formed by HRCs are the same as those usually synthesized in mother plants with a higher yield (Karuppusamy 2009). Many reports are available on the importance of HRCs in SMs production and other applications (Boobalan and Kamalanathan 2020; Makhzoum et al. 2013; Moola and Diana 2019; Shi et al. 2020). The plausible mechanism of HRs to study uptake, metabolism, and removal of organic pollutants was described in Figs. 2.2 and 2.3.

Another important aspect of using HRs is biofuel, it was the renewable energy produced from animal fats and vegetable oils. Biofuels and bio-based chemicals produced from renewable resources have come into existence as a reliable technology for the degradation of fossil-fuel-dependent resources, which report on concerns about their global environment pollution (Habibi et al. 2017). It helped to decrease environmental pollution than fossil-fuel. To improve the content of the oil and

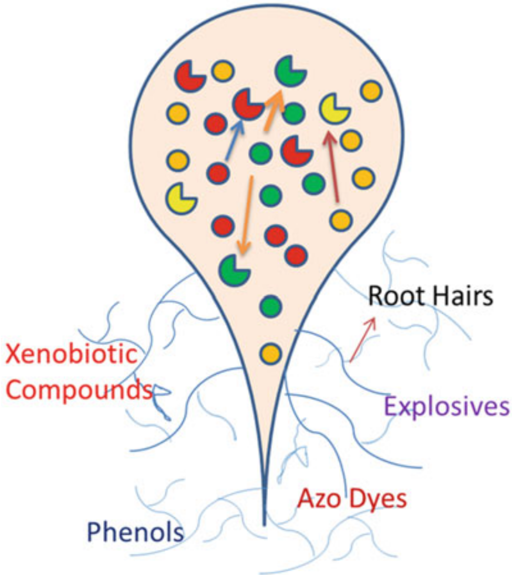

Fig. 2.2 The hypothesis of environmental contaminants (red, green, and yellow) may initiate the reactive oxygen species in hairy roots; it will help in the enhanced production of metabolizing enzymes that can be used for decontamination

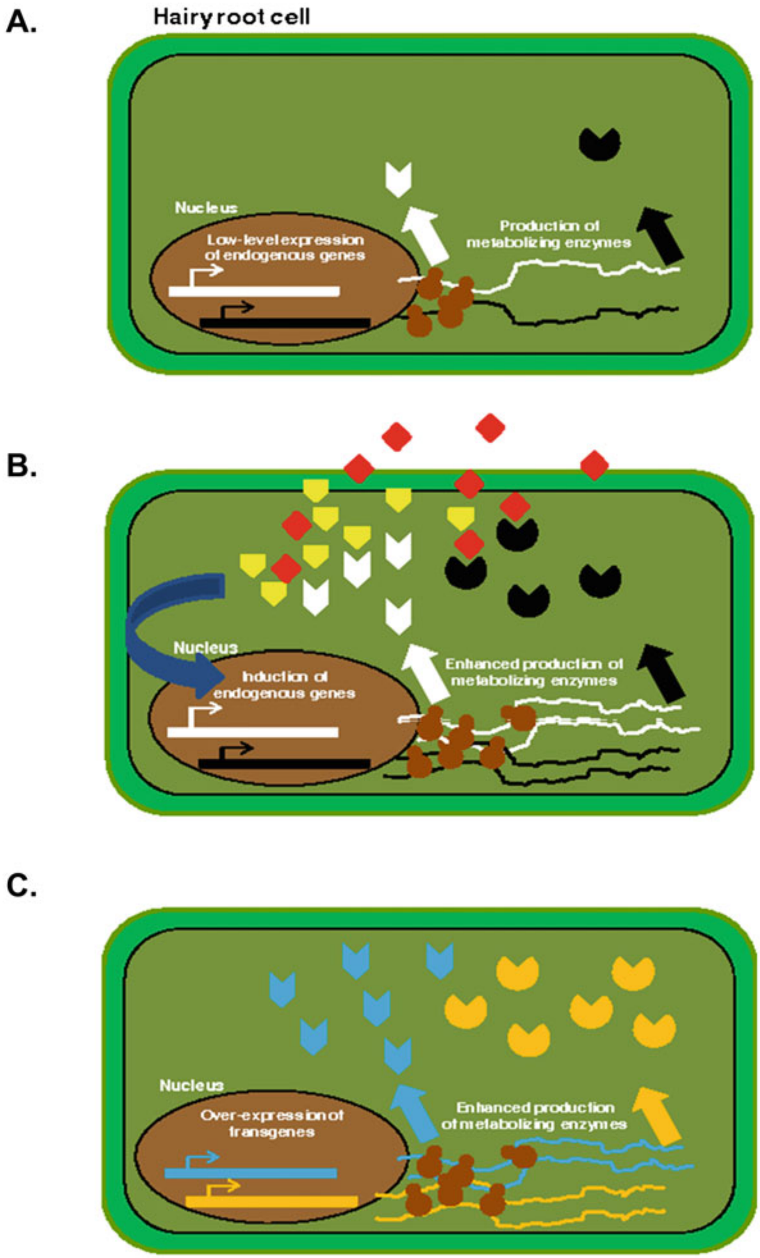

Fig. 2.3 Metabolism of environmental contaminants by hairy root cells
(**a**) a cartoon depiction of a hairy root cell expressing contaminant metabolizing enzymes (white chevron and black pie) at basal levels; (**b**) environmental contaminants (red diamonds) may promote the production of reactive oxygen species (yellow pentagon), the enhanced production of ROS scavenging enzymes and antioxidants (white chevron), and/or contaminant metabolizing enzymes (black pie); (**c**) the expression of transgenes of animal or plant origin may also result in the enhanced production of contaminant metabolizing enzymes (blue chevron and orange pie) and phytoremediation capacity of plants (Suza et al. 2008)

economic properties of plants, genetic engineering is the most powerful tool to develop plants for achieving this purpose. *Jatropha curcas* is a potential biodiesel crop that contains toxic compounds such as curcin, phorbol esters, trypsin inhibitors, lectin, and phytate (Annarao et al. 2008). The explants of *J. curcas* (leaf, cotyledons, and embryonic axes) were treated with *A. rhizogenes* and gene transformed cultures were undergoing to direct or indirect organogenesis. After the organogenesis, the foreign genes were integrated into transformed plant shoots (Habibi et al. 2017). Only a few reports are focused on developing plant varieties for biofuel production, researchers may focus on HRs for increasing the biodiesel production.

3 Hairy Roots Versus Normal Roots

A. rhizogenes is established for the HRs induction of plants that helps to produce plentiful adventitious roots (Largia et al. 2016). The HRs can be developed in vitro, without the rhizogenic bacterium, and they usually grow greater than regular roots of the identical plant. Plant roots help mainly in phytoextraction process by acting as a channel for the absorption of contaminants, which is then translocated through the vascular system and concentrated in plant harvestable tissues (Doty 2008). Since, biotransformation is a technique of altering the functional groups of xenobiotics to produce specific products, the use of enzymes to remediate the contaminants was reported by Krings and Berger (1998). Prior to entering the pollutants and at the later stage to the root system, the contaminants may turn into the target for decontamination through internal plant metabolites in a method known as phytodegradation (Boominathan et al. 2004). Since the root system is the key source for up taking the contaminants either in the soil or water, it may provide a key point for the assessment of the phytoremediation potential (Suza et al. 2008). Investigations by many researchers effectively investigated the mechanism (Table 2.1) for accumulation, absorption, distribution, tolerance, and detoxification of lethal compounds through HRCs. Talano et al. (2020) reported that bioremediation study using HR system will be beneficial to measure the potential of certain plant species and to estimate the outcome of natural roots that are indirectly attached to ground soil and its hazardous pollutants. They have advantages like a wider area of exterior contact between pollutants and tissues, rapid in vitro growth, and greater genetic as well as metabolic balance with respect to its wild type roots. In addition, HRs have the ability to take up a high concentration of toxins without any adverse effects in comparison with normal roots. More differences between wild type and HRs were tabulated in Table 2.2. Most of the reports about HRs induction through *A. rhizogenes* proved that the transformed HRCs showed higher root biomass contrary to normal root biomass, and the ratio of lateral root formation is very rapid in HRCs than normal cultures on hormone-free culture media (Rency et al. 2019). Normal roots also can induce SMs, however, the HRCs show much more growth kinetics concerning its

Table 2.2 Difference between tap, adventitious, and hairy root system

Tap root system	Adventitious root system	Hairy root system
It develops from the radical of the embryo	It develops from part of the plant other than the radical or not derivatives	It can be developed by using *A. rhizogenes* transformation
Genetically metabolically less stable and not comparative	Genetically metabolically not stable	Genetically metabolically high stable and cellular differentiation
It has a persistent primary root known as the taproot	The primary root is short-lived	Root has the ability to grow rapidly
The system grows deep into the soil	If underground, do not grow into the soil	Root system does not follow the geotropism
The tap root system is always underground	The adventitious root system may be underground or aerial	Hairy root system never be underground
The main root called as taproot and lateral branches called as secondary roots this in-turn produce tertiary roots and so on	A number of main roots developed at one spot and all roots are similar thicknesses	A number of main roots developed at different spots where the bacteria were wounded and all roots are similar thicknesses.
Not able to produce a large number of exudates/enzymatic machinery	Ability to produce a minimum quantity of exudates/enzymatic machinery but there is a variation	Ability to produce a large number of exudates/enzymatic machinery but there is a variation

rapid growth, root biomass generation, higher lateral root forming, and improved accumulation of bioactive compounds (Chandra and Chandra 2011; Mukundan and Hjortso 1990).

4 Conclusion and Way Forward

Considering these backgrounds, it is concluded that, HRs, enzymes, or metabolites of HRCs provides complementary information to understand the industrial cleanup process. However, the broad research on HRs, and the complete transformation of toxic to nontoxic is still unknown. The use of HRs extracts or enzymes definitely can be a substitute to pure enzymes since these are highly expensive to acquire and might be deactivated more promptly in the course of reaction with a subsequent decrease of its activity. These results will not only help in understanding about the enzymatic process that involved in phytoremediation of phenols, HMs, explosives, and dyes but also helps in the understanding of the complex interaction between toxic chemicals, plant cells, and microorganisms to clean up the environment and to design the novel transgenic plants with improved remediation traits.

In order for their future application, it is important and necessary to select an optimal condition to obtain the most effective removal by considering the economy of the process. In addition, transgenic plants also help in mineralize major complex compounds and also, it is expected that considering the recent advances in genetics, proteomics, and metabolomics and novel detoxifying enzymes could be identified and expressed into plants allowing the host plant to have a wider range of phytoremediation capabilities. Even, phyto-technologies have contributed significantly to mitigate and control environmental pollution. Still, more research has to be done and even many challenges have to overcome on the enhancement of pollution uptake and decontamination process which is an important challenge for current research.

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