Hairy Root Culture: A Biotechnological Approach to Produce Valuable Metabolites

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Abstract

Hairy root (HR) cultures are attracting more attention due to their unique ability in degrading different pollutants and production of metabolites with therapeutic or industrial applications. This specific type of plant cell culture is derived from explants that are infected by *Agrobacterium rhizogenes*. The HR cultures are categorized by their growth rate as well as their genetic and biochemical stability. Progress in design of innovative bioreactors and process intensification for HR growth will allow successful industrial production of metabolites. This chapter will present advances in work on HR cultures related to the detoxification of pollutants, production of valuable metabolites, and their cultivation in large-scale intensified bioreactors.

Keywords

Hairy root • Metabolite • Scale up • Bioreactor • Process intensification

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V.S. Meena et al. (eds.), *Agriculturally Important Microbes for Sustainable Agriculture*, DOI 10.1007/978-981-10-5589-8_7

7.1 Introduction

Plants are able to produce a wide range of primary and secondary metabolites (Yazaki et al. 2008; Sharma et al. 2013). However, the large-scale production of these valuable compounds has been limited by low growth rates, climate dependency, restricted cultivation areas, plant diseases, pests, overharvesting, and intense labor requirement (Sharma et al. 2013; Meena et al. 2013a; Bahadur et al. 2014; Maurya et al. 2014; Jat et al. 2015; Kumar et al. 2016b; Rates 2001). Moreover, the chemical synthesis of plant-derived metabolites is not a feasible choice due to their complex structures and their specific stereochemical requirements (Sharma et al. 2013; Namdeo 2007). All these issues emphasize the need for developing new methods and protocols for the industrial-level fabrication of plant-derived metabolites.

Suspension culture of plant cells has been considered as another promising source for biosynthesis of valuable secondary metabolites (Sharma et al. 2013). More than 25% of the available pharmaceuticals are either based on originally found compounds in plants or are extracted from them (Giri and Narasu 2000). Production of secondary metabolites by using suspended plant cell culture is usually a challenging task as these compounds produce at distinct developmental stages. Therefore, in vitro studies of differentiated and organized tissues (mainly the roots) have been developed and were reported to be a more predictable approach as compared to cell suspension cultures (Sharma et al. 2013; Kumar et al. 2015; Ahmad et al. 2016; Meena et al. 2016a; Parewa et al. 2014).

The plant roots are suitable for large-scale production since they are the key point for synthesis and/or storage of certain chemicals. The biotechnological fabrication of wide range of valuable secondary metabolites by using plant cultures can be seen as an alternative to the extraction of whole plant material (Namdeo 2007). Several strategies have been investigated in order to further enhance the production of secondary metabolites from medicinal plants. Some of these include high yielding cell line screening, media modification, elicitation, precursor feeding, large-scale cultivation system, plant cell immobilization, hairy root culture, biotransformation, and others (Rao and Ravishankar 2002; Vanisree et al. 2004).

Recently, hairy root (HR) culture has been developed in order to inhibit the use of large volumes of plants that are needed to be purified. Totipotency is among the major characteristics of plant cells; therefore, HRs could successfully produce primary and secondary metabolites similar to intact roots (Giri and Narasu 2000; Qaderi et al. 2016). HR culture is a tool that makes use of soil bacterium *Agrobacterium rhizogenes* ability to transfer genes to the genome of the host plant (Sharma et al. 2013; Thwe et al. 2016). This technique was developed as the innovative path for bulky production of secondary metabolite and phytochemicals which allows developing large amount of roots and secondary metabolites in short time for continuous supply of improved value products (Korde et al. 2016). These HRs have also been used for root physiology and biosynthetic pathway (Giri and Narasu 2000), regeneration of whole plants with desirable phenotypes, and phytoremediation of toxic substances and reactive dyes (Talano et al. 2012; Prakash and Verma

2016; Meena et al. 2015a, 2016b; Priyadharsini and Muthukumar 2016; Kumar et al. 2017). Finally, HR cultures include other aspects such as molecular metabolic engineering, bioreactor design, and optimization (Ono and Tian 2011). In this chapter, we present advances in work on HR cultures related to the detoxification of pollutants, production of valuable metabolites, and fabrication in large-scale intensified bioreactors. A suggestion to overcome current challenges and emerging trends for future progression of research has also been provided.

7.2 Definition and Basic Features of HRs

HR production is carried out through the plant tissue culture technique in order to study the plant metabolic processes or to manufacture precious secondary metabolites with the use of plant genetic engineering. HR culture is also called as transformed root culture from gram-negative soil bacterium *A. rhizogenes* that contains root-inducing plasmids (Ri plasmids) (Korde et al. 2016; Pistelli et al. 2010). It infects roots of dicot, and some monocot plants cause them to produce the opines which is a type of unusual amino acids (octopine, agropine, nopaline, mannopine, and cucumopine). Such opines are used by the bacterium as a carbon, nitrogen, and energy source (Ferdosi and Kashefi 2014).

The morphology of HRs is significantly different from the normal roots as they are much more branched and have much lateral meristematic growth, which will lead to higher biomass. The abnormal roots however are easier to grow in artificial media without hormone, and they are neoplastic in nature, with hazy growth. Fabricated HRs by infection of *A. rhizogenes* have a high growth rate as well as genetic and biochemical makeup (Korde et al. 2016).

However, new techniques are developed in order to make HRs by the use of new plant species (Georgiev et al. 2011). HRs have numerous advantages such as indefinite and fast in vitro growth even in the absence of phytohormones as well as high genotype and phenotype stability (Ono and Tian 2011). HR culture is among the main cultures that are used to investigate metabolic processes of plants, secondary metabolites production, recombinant proteins, plant genetic engineering, phytoremediation, artificial seed production, biofortification, and biopharmaceuticals. Applications of these efficient technologies also include several aspects as metabolic engineering, bioreactor design, and process optimization (Raghavendra et al. 2016; Zahedi 2016; Meena et al. 2015b; Rawat et al. 2016; Kumar et al. 2016a).

7.3 Mechanism of HR Cultures

The interaction between plants and *A. rhizogenes* in HR establishment involves a complex series of events. *A. rhizogenes* is responsible for a neoplastic outgrowth of fine roots at the infection site, and infected plants show reduced vitality. These symptoms came to be known as the hairy root disease. Roots arising at the site of infection can be cultured aseptically, and the resulted transformed root clones may

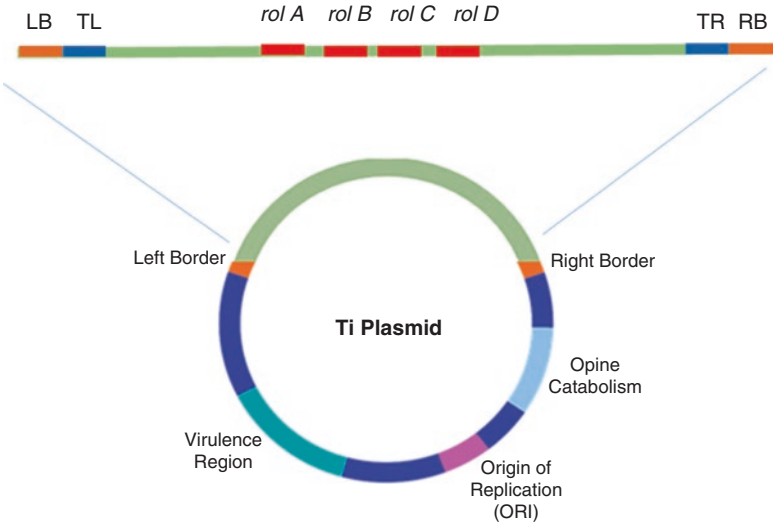

Fig. 7.1 Schematic overview of a Ri plasmid of *A. rhizogenes*

be subcultured indefinitely on basal medium, growing at rates several times quicker than normal roots (Flores and Filner 2012). *Agrobacterium* recognizes various phenolic compounds that have been produced from wounded plant cells, namely, acetosyringone and α-hydroxy acetosyringone.

After microbial colonization, consequently their attachment to plant cells T-DNA will be inserted (Sharma et al. 2013). This T-DNA is a transferable DNA from bacterium to plant cell. The T-DNA has a set of genes that are capable to encode enzymes required for cytokinin biosynthesis, phytohormone auxin control (*iaaM, iaaH, ipt*), and synthesis of sugar and amino acids (unusual amino acids). These segments have eukaryotic regulatory sequences and approximately 10–30 kbp in size and encode for the Ri conjugation, catabolism, opine synthesis, and integration of the T-DNA itself (Pistelli et al. 2010). Genes of T-DNA fragment facilitate the formation of neoplastic crown gall tumor and HR tissues followed by the synthesis of opines (Sharma et al. 2013; Yasin et al. 2016; Meena et al. 2016c; Jaiswal et al. 2016; Jha and Subramanian 2016). Depending on the bacterial strain, these metabolites are used as a carbon and nitrogen source for the bacteria. *A. rhizogenes* strains were categorized into two main classes, namely, Agropine-type and Mann opine-type strains. Among these, agropines are the most often used strains due to their strongest virulence (Sharma et al. 2013). The virulent strains of *A. rhizogenes* contain the Ri plasmids with different gene sequences (Fig. 7.1). Plasmids can be divided in strains producing mannopine and cucumopine with single DNA and strains producing octopine and agropine with two T-DNAs. The two T-DNAs are classified in the T_R-DNA (right DNA) and the T_L-DNA (left DNA). The root-inducing genes (*rol A*, *rol B*, *rol C*, *rol D*) are found in the center of T_L-DNA of the agropine-producing strains. Parts of the T_R-DNA are genes for the biosynthesis of

auxins and the synthesis of mannopine and agropine. After the transfer of the T_L-DNA and T_R-DNA, they are integrated in the genome of the plant cell. The T_L-DNA is vital for the hairy root induction (Chandra 2012).

7.4 Establishment of HR Cultures

Successful HR culture system requires several essential check marks, namely, selection of best *A. rhizogenes* strain, appropriate explants and antibiotic, and a suitable culture medium (Sharma et al. 2013). Strains of *A. rhizogenes* are widely varying in their transforming ability. HRs that are fabricated by using different types of bacterial strains show significantly different morphologies. These observed virulence and morphology differences could be justified by the different strain plasmid harbored (Saha et al. 2016a; Yadav and Sidhu 2016; Nguyen et al. 1992; Meena et al. 2015f). Most plant materials like hypocotyl, stem, cotyledon, leaf, tuber, or storage root may be applied to make HRs (Królicka et al. 2001; Sevón and Oksman-Caldentey 2002; Giri et al. 2001). In order to induce HRs, explants should be infected with strains of *A. rhizogenes* either by cocultivation or direct inoculation (Giri et al. 2001; Ur Rahman et al. 2004). Subsequently, roots are subculture using a medium such as MS or B_5 (Fig. 7.2) (Sevón and Oksman-Caldentey 2002; Le Flem-Bonhomme et al. 2004; Palazón et al. 2003a, b).

7.5 Application of HR Cultures

The HR culture shaves diverse and abundant applications (Fig. 7.3). They traditionally have been used to investigate root physiology in conjunction with biosynthetic pathway elucidation (Ibanez et al. 2016). Nowadays, HR culture technique is now being used for the fabrication of bioactive compounds, secondary metabolites, and phytochemicals. HRs are popular for regeneration of whole plants with desirable phenotypes by infection of ornamental plants with *A. rhizogenes* (Meena et al. 2016d; Saha et al. 2016b; Verma et al. 2015b; Bahadur et al. 2016b; Das and Pradhan 2016; Dominguez-Nunez et al. 2016; Dotaniya et al. 2016).

Moreover, HR cultures have been used for phytoremediation of toxic substances and reactive dye. Presently, several high-value bioactives are fabricated by using HRs from various plant sources which have application in pharmaceutical and cosmetic products (Ono and Tian 2011). Furthermore, recombinant protein production using this system was found to be a sustainable method for producing cytokines as well as protein therapeutics (Talano et al. 2012).

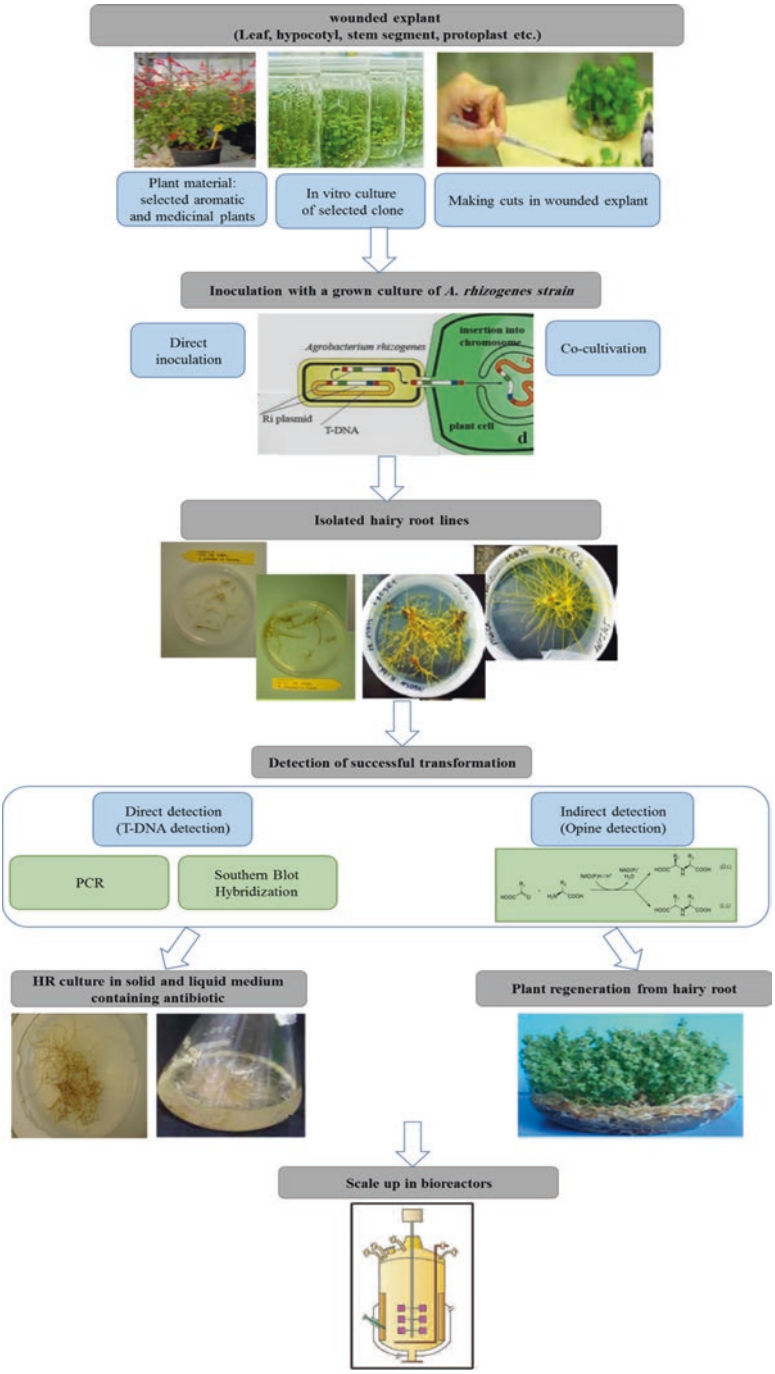

Fig. 7.2 An overview of the HR culture establishment

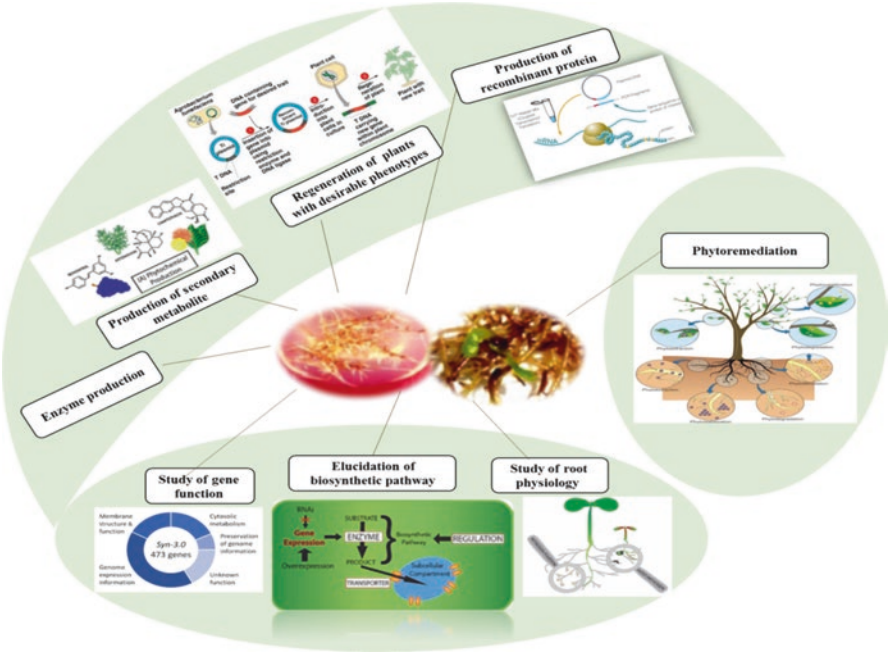

Fig. 7.3 The systematic applications of HR cultures

7.5.1 Application of HR Cultures for Secondary Metabolites Production

Plants are known as chemical factories since they pose the ability to fabricate important phytochemical. However, the major issue is that their growth is dependent to the outside environmental stress. HR cultures are promising source for phytochemical due to sizable biomass production and biosynthetic capacity.

Furthermore, HR cultures frequently accumulate phytochemical at much higher levels than cell or callus cultures (McCoy and O'Connor 2008; Ono and Tian 2011). Secondary metabolites as summarized in Table 7.1 are naturally more complex as compared to primary metabolites. These compounds have been categorized into terpenoids, phenolics, and alkaloids (Chinou 2008).

Several HR cultures have attracted significant amount of attention due to their potential in production of valuable phytochemical including *Artemisia annua*, *Catharanthus roseus*, *Arachis hypogaea*, and *Camptotheca acuminata/Ophiorrhiza pumila* (McCoy and O'Connor 2008). List of secondary metabolites produced by wild HR cultures is summarized in Table 7.2. Although, non-transgenic HR cultures continue to serve as a good source for phytochemicals and secondary metabolites (Ono and Tian 2011).

However, application of metabolic engineering methods requires acritical understanding about the regulation of secondary metabolite pathways and the metabolic

Table 7.1 Classification of secondary metabolites (Rao and Ravishankar 2002)

Terpenes (composed of C and H)	Phenols (composed of sugars, benzene ring, H and O)	Alkaloids	Steroids
Monoterpenes	Phenolic acids	Acridines	Cardiac glycoside
Sesquoiterpenes	Coumarins	Glucosinolates	Pregnenolone derivatives
Diterpenes	Lignins	Betalains	
Triterpenes	Flavonoids	Quinolizidines	
Tetraterpenoids	Tannins	Furonoquinones	
	Anthocyanins	Harringtonines	
	Hydroxycinnamoyl derivatives	Isoquinolines	
	Phenalenones	Indoles	
	Proanthocyanidins	Purines	
	Stilbenes	Pyridines	
	Tanins	Tropane alkaloids	

phenotype of the HR culture to regulate the dynamic distribution of metabolites between different biochemical pathways (Kruger and Ratcliffe 2009; Talano et al. 2012). Table 7.3 tabulated the common secondary metabolites that are produced by transgenic HRs.

7.5.2 Application of HR Cultures in Phytoremediation

Environmental remediation is a method that deals with the removal of toxins in order to support the environment (Macek et al. 2008). Remediation processes can be expensive; therefore, efficient and inexpensive technologies are still developing to address the needs in the field. Bioremediation includes the environmental treatment with the use of microorganisms and plants (Novakova et al. 2007; Najmanova et al. 2007). In case of using plants, this is so-called phytoremediation (Garbisu and Alkorta 2001; Korde et al. 2016). The use of plants to absorb and accumulate organic and inorganic pollutants or to transform toxic molecules to harmless once has attracted attention (Eapen et al. 2007; Doty 2008; Macek et al. 2008; Ibanez et al. 2016).

Phytoremediation process usually occurs through several complex interactions between the key involved sources (Krystofova et al. 2009; Guillon et al. 2006). Roots are normally the main contact point between contaminants and the plant tissues they are the key point of assessment of the phytoremediation potential (Verma et al. 2014; Meena et al. 2014a, 2015e; Teotia et al. 2016).

However, in this sense HR culture has been found suitable in order to study the xenobiotic detoxification without the soil matrix or microbes interaction (Talano et al. 2012). Figure 7.4 summarizes how a plant deposits the toxin efficiently. Plants are able to chemically modify toxic substances through their direct metabolism (Krystofova et al. 2009). Greater genotypic and phenotypic stability are the

Table 7.2 List of the secondary metabolites that are produced by wild HR cultures

Secondary metabolite	Function	HR	References
Ajmalicine, ajmaline	Antihypertensive	*Rauvolfia micrantha*	Sudha et al. (2003)
Artemisinin	Antimalarial	*Artemisia annua*	Weathers et al. (2005)
Azadirachtin	Biopesticide	*Azadirachta indica*	Srivastava and Srivastava (2012c)
Benzylisoquinoline alkaloids (morphinan, codeine, and sanguinarine)	Analgesic, antibiotic	*Papaver somniferum*	Park and Facchini (2000) and Le Bonhomme et al. (2004)
Betalain	Red pigments for food industry, strong aphrodisiac, laxative	*Beta vulgaris*	Rudrappa et al. (2004) and Pavlov et al. (2003)
Camptothecin	Antitumor, AIDS, falciparum malaria, colorectal and ovarian cancers treatment	*Ophiorrhiza alata* Craib, *Ophiorrhiza pumila*	Ya-ut et al. (2011) and Sato et al. (2001)
3,4-Dihydroxyl-L-phenylalanine	Therapeutic agent against Parkinson's disease	*Stizolobium hassjoo*	Sung and Huang (2006)
Dopa and dopamine	Neurotransmitters	*Beta vulgaris*	Rudrappa et al. (2004)
Flavone glycosides	Anti-inflammatory action	*Catharanthus roseus*	Talano et al. (2012)
Flavonoids	Meant for the treatment of gastric ulcers, anti-inflammatory, and antitussive	*Glycyrrhiza pallidiflora*	Li et al. (2002)
Flavonoids	Antimutagenic, antiulcer, antitumor, antimicrobial	*Glycyrrhiza uralensis*	Zhang et al. (2009)
Glycyrrhizin	Artificial sweetener and pharmaceutical products (peptic ulcers treatment)	*Glycyrrhiza inflate*	Wongwicha et al. (2011)
Glycyrrhizin	Diuretic, tonic, alexiteric, antifertility	*Abrus precatorius*	Dixit and Vaidya (2010)
Hyoscyamine	Narcotic and antispasmodic activity, used against Parkinson's disease	*Datura stramonium*	Pavlov et al. (2009)
Indole alkaloids (vinblastine, vincristine)	Anticancer	*Catharanthus roseus*	Ayora-Talavera et al. (2002)
Iridoid glycosides	Anti-inflammatory, analgesic, antidiabetic	*Harpagophytum procumbens*	Georgiev et al. (2006)

(continued)

Table 7.2 (continued)

Secondary metabolite	Function	HR	References
6-Methoxy-podophyllotoxin	Anticancer	*Linum album*, *Linum persicum*	Wink et al. (2005)
Physalins	Diuretic, febrifuge, vermifuge	*Physalis minima*	Azlan et al. (2002)
Plumbagin	Diuretic, antibacterial and used against leprosy	*Plumbago zeylanica*	Sivanesan and Jeong (2009)
Resveratrol	Anti-inflammatory, antioxidant, anti-infective, anticancer	*Arachis hypogaea*	Kim et al. (2008)
Rosmarinic acid	Astringent, antioxidant, anti-inflammatory, antimutagenic, antimicrobial, antiviral	*Nepeta cataria*	Yang (2010)
Rutin	Antioxidant, anticarcinogenic, antithrombotic, cytoprotective, vasoprotective	*Fagopyrum esculentum*	Kim et al. (2010)
Rutin, hispidulin, and syringin	Anti-inflammatory; antifungal	*Saussurea involucrata*	Fu et al. (2005)
Serpentine	Diabetes treatment	*Catharanthus roseus*	Datta et al. (2010)
Sesquiterpenes	Phytoalexins	*Hyoscyamus albus*	Kawauchi et al. (2010)
Shikonin	Dye for silk and food industry, anti-inflammatory, anti-allergic, and antineoplasic activities	*Arnebia*	Talano et al. (2012)
Stilbenoids (resveratrol, pinosylvin, and derivatives)	Antioxidant, anticancer, antiatherosclerosis, neuroprotective, and estrogenic activities	*Arachis hypogaea*	Medina-Bolivar et al. (2010)
Tropane alkaloids	Narcotic, anticholinergic and antispasmodic activity	*Datura metel*, *Hyoscyamus muticus*	Moyano et al. (2003)
Tropane alkaloids	Narcotic, anticholinergic and antispasmodic activity	*Hyoscyamus niger*	Zhang et al. (2004)
Tropane alkaloids (scopolamine and hyoscyamine)	Narcotic, anticholinergic and antispasmodic activity	*Datura innoxia*	Dechaux and Boitel-Conti (2005)
Tropane alkaloid (hyoscyamine, atropine, and hyoscine)	Used against Parkinson's disease	*Atropa belladonna*	Richter et al. (2005)

(continued)

Table 7.2 (continued)

Secondary metabolite	Function	HR	References
Tropane alkaloids (scopolamine and hyoscyamine)	Parasympatholytic	*Przewalskia tangutica*	Lan and Quan (2010)
Withanolide A	Brain regenerative properties	*Withania somnifera*	Praveen and Murthy (2012)
Xanthotoxin (furocoumarin)	Leucoderma	*Ammi majus*	Krolicka et al. (2001)

important advantages of HR cultures which provide a more promising system over time for phytoremediation (Doran 2009). Additionally, the organized nature of HR cultures offers an added advantage that makes them more useful for cultivation in bioreactors at large scale (Angelini et al. 2011; Bahadur et al. 2017; Verma et al. 2017b; Kumar et al. 2017b). HRs that have been functionalized by genetic engineering are expected to become a new solution for environmental treatment in near future (Guillon et al. 2006).

HRs produced by hyper-accumulators are capable to uptake nickel, uranium, or cadmium from polluted environment (Boominathan and Doran 2003; Boominathan et al. 2004; Eapen et al. 2003; Agostini et al. 2003; Suresh et al. 2005; Gujarathi et al. 2005). Phytoremediation of several environmental pollutants by wild-type and transgenic HR cultures is shown in Table 7.4.

7.6 Recent Advances in HR Cultures Scale-Up

Root tissues are not identical to microbial cultures in many ways. Therefore, bioreactors for HR cultures are more challenging to be controlled, operated, and scaled up. Development of innovative bioreactors and process intensification will allow to optimize cell growth and large-scale production (Sharma et al. 2016; Meena et al. 2013c, 2016e; Verma et al. 2015a; Bahadur et al. 2016a; Masood and Bano 2016).

7.6.1 Development of Groundbreaking Bioreactors in HR Cultures

Design and optimization of bioreactors have been the great advance in HRs for industrial-scale production of metabolites (Huang and McDonald 2012). Production of HRs in bioreactors helps to have a better control on operating conditions and consequently optimize the growth and biosynthesis of the secondary metabolite (Eibl and Eibl 2008). Bioreactor optimization for fabrication of HRs is of critical importance for scale-up strategies.

HR bioreactors can be in general divided into gas or liquid phase. In liquid-phase bioreactors, roots are always placed in the medium; as a result they are called submerged reactors. On the other hand, in gas-phase reactors, the roots are almost exposed

Table 7.3 List of secondary metabolites produced through transgenic HR cultures (Talano et al. 2012)

Secondary metabolite	Function	Transgenic HR	Foreign genes
Solanoside	Antineoplastic agent	*Solanum khasianum*	Gene encoding a specific antibody that binds solanoside
Indole	Beneficial effects on cancer, sedative and hypotensive action	*Catharanthus roseus*	Modified anthranilate synthase (AS) alpha subunit (*trp5*) and tryptophan decarboxylase gene (TDC)
Ginseng	Traditional Chinese medicine, tonic, antiaging, anticancer, and anti-diabetes properties	*Panax ginseng*	*cs* gene for cycloartenol synthase enzyme
Scopolamine		*Hyoscyamus niger*	Putrescine *N*-methyltransferase *pmt*) and hyoscyamine 6ß-hydroxylase (*h6h)* genes
Catharanthine	Anticholinergic agents that act on parasympathetic nervous system	*Catharanthus roseus*	*Geraniol 10-hydroxylase (G10H) and a jasmonate-responsive transcript factor (ORCA3)*
Hyoscyamine, scopolamine		*Scopolia parviflora*	Putrescine *N*-methyltransferase (*pmt*) and hyoscyamine 6ß- hydroxylase (*h6h)*
Anisodamine, anisodine, hyoscyamine, scopolamine		*Anisodus acutangulus*	Putrescine *N*-methyltransferase (*pmt*) and gene codifying tropinone reductase I (TRI)
Glycyrrhizin	Medicine, healthcare products, food (sweetener), and cosmetics	*Glycyrrhiza uralensis*	Chalcone synthase
Flavones: baicalin, baicalein, wogonin	Diuretic, anti-inflammatory, antiseptic, antispasmodic, and anticancer	*Scutellaria baicalensis*	Chalcone isomerase
Vitamin C	Antioxidant properties	*Solanum lycopersicon*	*gal* UR gene
Total sterols	Hypocholesterolemic, anticarcinogenic properties	*Centella asiatica*	Farnesyl diphosphate synthase from *Panax ginseng*

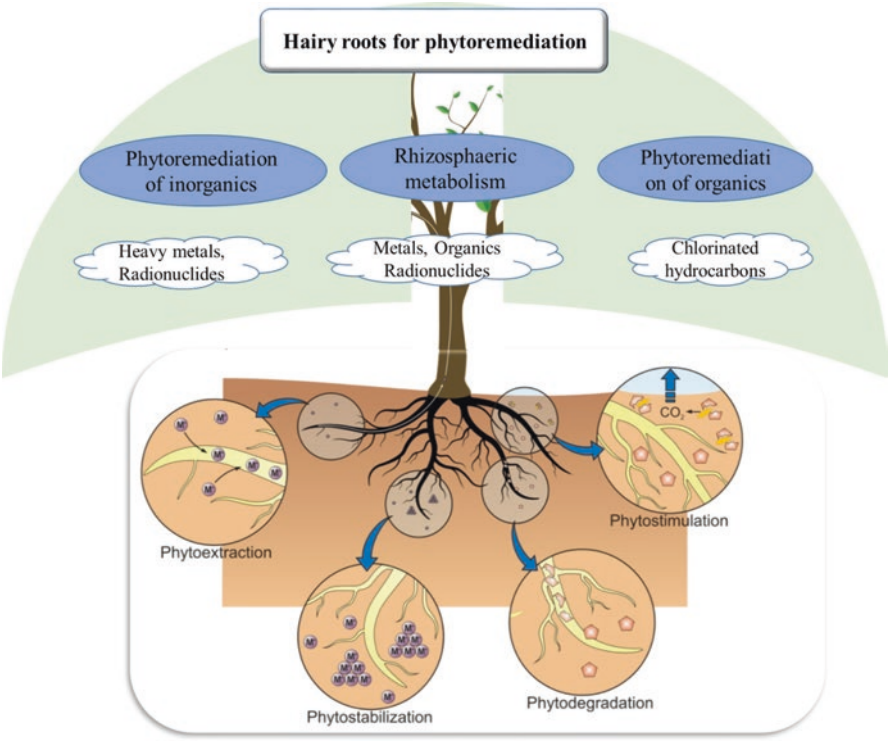

Fig. 7.4 Types of phytoremediation. Plant metabolizes the pollutant via organic and inorganic phytoremediation or rhizospheric metabolism. Phytoremediation includes several approaches, namely, phytostabilization, phytoextraction, phytodegradation, and phytostimulation

to air or another gas mixture (Kim et al. 2002a, b; Stiles and Liu 2013). The design of the reactor also depends on the product location, which is either intracellular or extracellular (Meena et al. 2013b, 2015d; Shrivastava et al. 2016; Singh et al. 2015).

HR cultivation is usually associated with clumps formation that are naturally composed of primary roots and their bridged lateral roots. It is very difficult to find appropriate bioreactor for HR cultures because the rheological properties of HR cultures vary from one species to another and even within clones of a single species. Several bioreactor designs have been reported for HRs (Mishra and Ranjan 2008). Schematic diagrams of promising bioreactor types that have been successfully tested are depicted in Fig. 7.5.

7.6.1.1 Liquid-Phase Bioreactors

In liquid-phase reactors, the culture space is filled up with liquid medium, and several techniques are used to provide the required aeration to the media. Since the roots are submerged, therefore, mixing and mass transfer are the main issues in scaling up the process (Eibland Eibl 2008; Curtis 2000; Nath et al. 2017; Sarkar et al. 2017; Verma et al. 2017a).

Table 7.4 Phytoremediation of several environmental pollutants by wild-type and transgenic HR cultures

Chemical nature of pollutant	Nature of HR	Pollutant	Plant species	References
Inorganic	Wild type	Arsenic	*Nicotiana tabacum*	Talano et al. (2014)
		Cadmium	*Thlaspi caerulescens*	Nedelkoska and Doran (2000)
		Cadmium and lead	*Brassica juncea*	Eapen et al. (2007)
		Chromium	*Brassica napus* and *Pantoea* sp. FC1	Ontañon et al. (2014)
		Nickel	*Alyssum murale*	Vinterhalter et al. (2008)
		Nickel	*Alyssum bertolonii*	Boominathan et al. (2004)
		Uranium	*Armoracia rusticana*	Soudek et al. (2011)
		Uranium	*Daucus carota*	Straczek et al. (2009)
		Zinc and nickel	*Brassica juncea*	Ismail and Theodor (2012)
	Transgenic	Copper	*Nicotiana tabacum*	Ibanez et al. (2016)
Organic	Wild type			
		Explosives (DNT, TNT; ADNTs; DANTs)	*Armoracia rusticana*	Nepovim et al. (2004)
		N-acetyl-4-aminophenol	*Armoracia rusticana*	Huber et al. (2009)
		PCBs	*Solanum nigrum*	Rezek et al. (2007), (2012)
		Phenol and chloro derivatives	*Brassica juncea*	Singh et al. (2006), Coniglio et al. (2008), and Gonza'lez et al. (2012)
			Daucus carota, *Ipomoea batatas* L.	De Araujo et al. (2006)
			Nicotiana tabacum	Talano et al. (2010)
			Helianthus annuus	Jha et al. (2013)
			Nicotiana tabacum	Talano et al. (2010)
			Solanum lycopersicon	Khoudi et al. (2012)
			Solanum lycopersicum	Gonzalez et al. (2006); (2008)

(continued)

Table 7.4 (continued)

Chemical nature of pollutant	Nature of HR	Pollutant	Plant species	References
		Reactive red 198 dye	*Tagetes patula* L.	Patil et al. (2009)
		Tetracycline, oxytetracycline	*Helianthus annuus*	Gujarathi et al. (2005)
		Textile dye: Methyl orange	*Brassica juncea.*	Telke et al. (2011)
		Textile dye: reactive green 19A- HE4BD	*Sesuvium portulacastrum* L.	Lokhande et al. (2015)
	Transgenic	Phenol	*Nicotiana tabacum*	Alderete et al. (2009)
			Brassica juncea inoculated with two rhizobacteria	Gonzalez et al. (2013)
			Solanum lycopersicum	Oller et al. (2005)
			Brassica juncea inoculated with *Pantoea* sp. FC1	Ontañon et al. (2014)
			Nicotiana tabacum expressing *tpx1* and/or *tpx2* genes	Sosa Alderete et al. (2009), (2012)
			Nicotiana tabacum expressing *tpx1* gene + AMF	Ibanez et al. (2011)
		TCE	*Atropa belladonna*	Banerjee et al. (2002)

DNT 2,4-dinitrotoluene, *PCB* polychlorinated biphenyls, *TNT* 2,4,6-trinitrotoluene, *DANTs* diaminonitrotoluenes, *ADNTs* aminodinitrotoluenes, *AMF* Arbuscular Mycorrhizal Fungi, *TCE* trichloroethylene

Design considerations comprise of mechanisms to provide adequate nutrient to the roots. Mixing and aeration strategy represents the main design differences between the various types of liquid-phase bioreactors. The most commonly used liquid-phase bioreactors are, namely, pneumatic reactors and stirred tanks. Methods for immobilizing HRs include cages, meshes, and polyurethane foam (Eibl and Eibl 2008).

Past studies evaluated the use of stirred tank reactors (STR) for HRs cultivation. In order to supply required amount of oxygen, compressed air is always spared into the bioreactor from a placed device in the impeller region. STRs are normally not useful for HR cultures despite their wide range of application in biotechnology. This is mainly because of the callus formation and wound response which are usually a response to the impeller rotation shear stress (Taya et al. 1989; Mishra and Ranjan 2008). Pneumatic bioreactors are the ones that include both airlift and bubble column reactors.

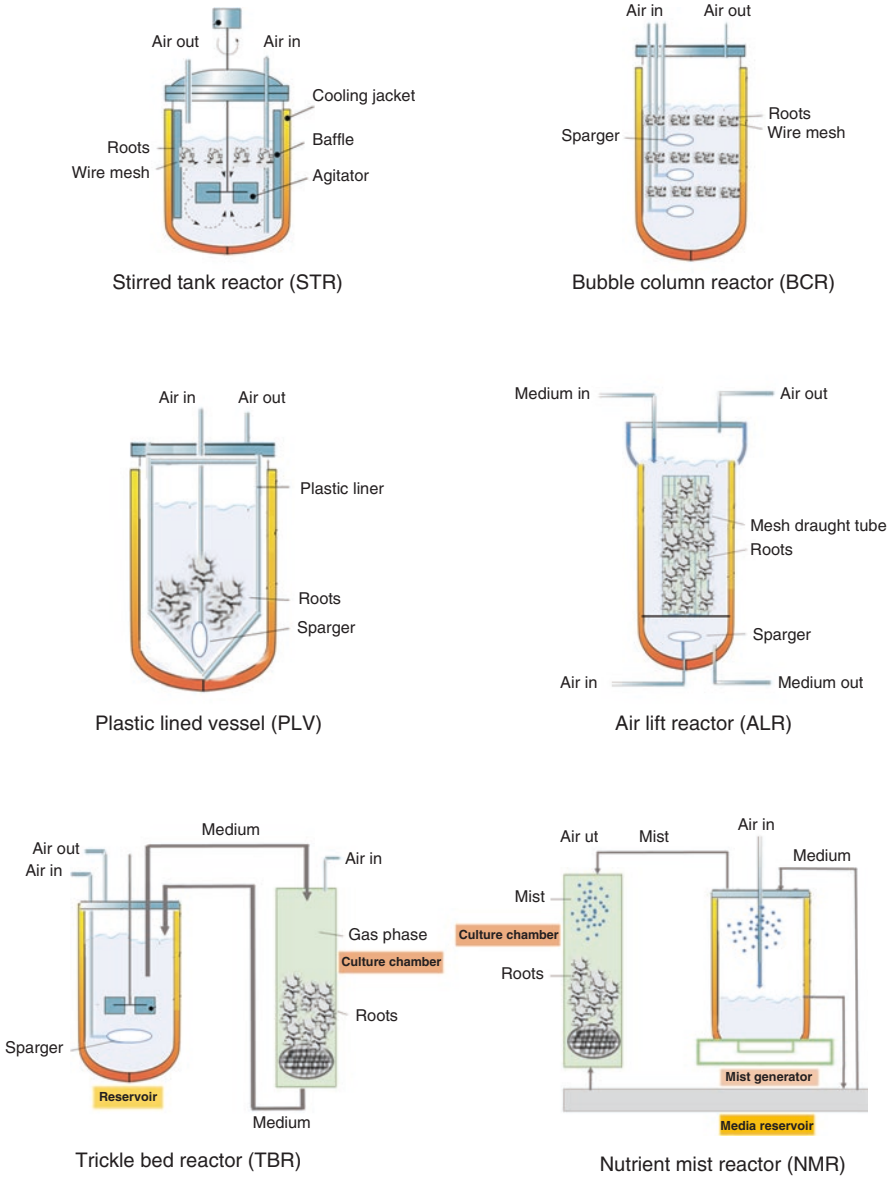

Fig. 7.5 Schematic diagrams of the bioreactors used for HR culture

Bubble column reactors (BCR) are among the simplest bioreactors that are easy to scale up. The use of bubbles instead of mechanical mixers minimizes the shear stress on the cultures (Choi et al. 2008; Huang and McDonald 2012). However, the major drawback with BCRs are the undefined flow pattern of the liquid (Choi et al. 2008) and the reduced growth performance (Kwok and Doran 1995). In the

presence of high biomass, the bubbles may coalesce resulting in the reduction of gas-liquid interface area (Huang and McDonald 2012). BCRs are liquid-phase bioreactors in which the roots are submerged in the medium. Liquid mixing is obtained by the upflow of air bubbles generated from an air distributor situated at the bottom of the column. In contrast to BCRs, airlift reactors (ALRs) contain a draft tube (either internal or external) to avoid coalescing bubbles. ALRs distribute shear stress more evenly, reduce shear stress, consume little energy, and promote a cylindrical mixing of the medium (Stiles and Liu 2013).

The draft tube in ALRs prevents bubble coalescence by forcing the bubbles to move in one direction. It also distributes shear stresses equally throughout the reactor. As a result, cells are able to grow in a more stable physical environment than those growing under high shear, a condition causing cell damage and lower productivity in STRs. It has also been shown experimentally that shear stress rates generated in ALRs are lower than those generated in BCRs.

ALRs have been extensively used for HRs since the initiation of HR bioreactor studies for species including *Panax ginseng* (Yoshikawa and Furuya 1987), *Armoracia rusticana* (Taya et al. 1989), *Trigonella foenum-graceum* (Rodriguez-Mendiola et al. 1991), *Lippia dulcis* (Sauerwein et al. 1991), *Lithospermum erythrorhizon* (Shimomura et al. 1991), *Ophiorrhiza pumila* (Sudo et al. 2002), and *Echinacea purpurea* (Abbasi et al. 2009).

Conventional ALRs have been extensively used for scale-up cultures of HR lines; however, they are generally not appropriate for high-density cultures due to inadequate mixing and oxygen mass transfer (Choi et al. 2008). This phenomenon is mainly based on uneven distribution of root tissue at certain regions as well as excessive gas-phase channeling (Taya et al. 1989).

7.6.1.2 Gas-Phase Bioreactors

In gas-phase reactors, roots are exposed to a mixture of air or gas mixture. The liquid nutrient is usually sprayed onto the top of the root bed (Kim et al. 2002a, b). These reactors have been widely used in plant tissue and HR cultures due to their abundant oxygen supply (Stiles and Liu 2013; McKelvey et al. 1993; Katuri et al. 2011; Wyslouzil et al. 1997). However, gas-phase reactors yet require a matrix for anchoring the HRs. These can be mesh trays or mesh cylinders. In addition, these reactors are labor intensive as their requirement for uniform loading (Eibl and Eibl 2008; Choi et al. 2008; Srivastava and Srivastava 2007; Velazquez et al. 2016; Meena et al. 2014b, 2015c; Sindhu et al. 2016; Singh et al. 2016; Ramakrishnan and Curtis 2004).

Nutrient mist reactors (NMRs) are another one of gas-phase-type reactors. In these systems plant organ is usually dispersed in the air phase with the help of a mesh support. NMRs have definite advantages, such as easy operation, high dissolved oxygen tension present in the mist, lack of shear, and ease of scaling up. Whitney (1992) investigated the performance of different types of bioreactors for *D. stramonium* and *Nicotiana tabacum* cultivation. Authors stressed that the growth rate and yield of tobacco HRs were greater in NMRs than in STRs, TBRs, and ALRs. Recently, HRs of *Stizolobium hassjoo* (velvet bean) were cultivated in 3 and

9 l NMRs by Huang et al. (2004) to evaluate the oxygen uptake rate, effects of intermittent medium supply, and other growth-related parameters.

Several other comparative studies also have been evaluated the optimal bioreactor type for a particular species of HRs. The production of *Artemisia annua* HRs was compared both in bubble and mist bioreactors. Based on the results, authors suggested that the overall biomass was higher in the BCR (Kim et al. 2002a, b). However, the mist reactor usually accumulates lower amount of biomass as compared to the BCR. This behavior could be due to insufficient nutrient availability (Choi et al. 2008; Srivastava and Srivastava 2012a, b).

7.6.1.3 Novel Bioreactors

Hybrid reactor can be seen as a method to address this issue, a reactor which allows the roots to attach uniformly to the anchoring system (Stiles and Liu 2013). Disposable bioreactors could also be seen as another alternative to the traditional protocols. These reactors can significantly reduce the operation costs by eliminating the need for cleaning or sterilization though out the process (Eibl and Eibl 2006). Disposable wave bioreactor systems could also be another advancement in the bioreactor design area (Mishra and Ranjan 2008). These systems work on the basis of using wave for agitation purpose which in turn reduces the stress levels (Palazón et al. 2003a, b). Large-scale wave bioreactors having the capacities of up to 600 L are now commercially applicable (Mishra and Ranjan 2008; Eibl and Eibl 2006).

7.6.2 Process Intensification

Process intensification methods could also be utilized in plant and tissue culture works (Stiles and Liu 2013). The ability to exploit HR cultures as a source of bioactive chemicals depends on the development of a suitable bioreactor system where several physical and chemical parameters must be taken into consideration. Selection of highly productive cell lines, manipulation of nutrients, optimizing the culture environment, elicitation, metabolic engineering, in situ product removal, and ultrasound have been applied for process intensification in HR bioreactor cultures (Mishra and Ranjan 2008; Stiles and Liu 2013).

7.6.2.1 Optimization of Bioreactor Parameters

Development of an appropriate bioreactor depends on several physical and chemical parameters, such as optimum pH, sufficient substrate, controlled temperature, salts for nutrition, product and by-product removal, oxygen, inoculation size and density, and product recovery. The agro-bacterial concentration has an important role in the production of transformed roots (Mishra and Ranjan 2008). Dissolved oxygen is another important factor in the bioreactor microenvironment. HRs cultured in bioreactors have the affinity to form clumps which critically inhibits the oxygen transfer (Bordonaro and Curtis 2000). Nutrient availability is also a major point for scale-up, and minerals are an important regulatory factor for HR growth (Sivakumar et al. 2005). Furthermore, periodic measurement of nutrients

concentration during periods in bioreactors would provide key information regarding metabolic production (Wilhelmson et al. 2006; Sivakumar et al. 2005).

Light also plays a key role for both growth and production of secondary metabolites. The stimulatory role of light on the production of secondary compounds has been demonstrated using plant species "*Perilla frutescens* and *Artemisia annua*" (Zhong et al. 1991; Wang et al. 2001; Abbasi et al. 2007; Taya et al. 1994; Jacob and Malpathak 2004).

7.6.2.2 Elicitation

Elicitation is the effective technique which is currently used for improving the production of secondary metabolites (Zhao et al. 2005; Baenas et al. 2014). Overall, based on origin, elicitors are classified biotic and abiotic. Basically, biotic elicitors are either physical factors or chemical factors such as ultraviolet light heavy metals and salts (Stiles and Liu 2013). Salts including $AlCl_3$, $AgNO_3$, $CdCl_2$, $CaCl_2$, $CuCl_2$, $CoCl_2$, KCl, $HgCl_2$, $MgSO_4$, $VOSO_4NiSO_4$, and Zn ions have been used to increase the secondary metabolite production in a variety of plant (Ramirez-Estrada et al. 2016; Li et al. 2006; Vasconsuelo and Boland 2007). Abiotic elicitors are usually cheaper than biotic; however, they are not as efficient for the cultivation of the target microorganism (Georgiev et al. 2007). Specificity of the elicitor, culture growth stage, treatment interval, the concentration, medium composition, and light are the main factors that affect the effectiveness of elicitation (Sharma et al. 2013).

7.6.2.3 Metabolic Engineering

Metabolic engineering of biosynthetic pathways has been established recently to enhance the fabrication of secondary metabolites. In this case some general issues have to be taken into account, namely, competing pathways, cofactors for the reaction, and rate-limiting enzymatic steps which are among the major metabolic engineering issues (Ludwig-Müller et al. 2014; Georgiev et al. 2010; Chandra and Chandra 2011).

7.7 Concluding Remark and Future Developments of HR Cultures

To date, significant progresses have been made in the genetic transformation and tissue culture in order to amplify the key pathways for the biosynthesis of targeted metabolites. Commercial production of HRs has attracted much attention recently as compared to the other plant cells. HR cultures are unique due to their much higher genetic and biosynthetic stability. However, exploration into inexpensive novel elicitors and bioreactors are required in order to warrant their industrial implementation. Further, generated knowledge from plant metabolic pathways and advancements in genetic engineering will help HRs to become a promising and sustainable fabrication system in the near future.

Acknowledgments The authors would like to thank Food Research Institute of Sahand University of Technology for cooperation in preparation of this review.

References

Abbasi BH, Tian CL, Murch SJ, Saxena PK, Liu CZ (2007) Light-enhanced caffeic acid derivatives biosynthesis in hairy root cultures of *Echinacea purpurea*. Plant Cell Rep 26:1367–1372

Abbasi BH, Liu R, Saxena PK, Liu CZ (2009) Cichoric acid production from hairy root cultures of *Echinacea purpurea* grown in a modified airlift bioreactor. J Chem Technol Biotechnol 84:1697–1701

Agostini E, Coniglio MS, Milrad SR, Tigier HA, Giulietti AM (2003) Phytoremediation of 2,4-dichlorophenol by *Brassica napus* hairy root cultures. Biotechnol Appl Biochem 37:139–144

Ahmad M, Nadeem SM, Naveed M, Zahir ZA (2016) Potassium-solubilizing bacteria and their application in agriculture. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 293–313. doi:10.1007/978-81-322-2776-2_21

Alderete LG, Talano MA, Ibáñez SG, Purro S, Agostini E, Milrad SR, Medina MI (2009) Establishment of transgenic tobacco hairy roots expressing basic peroxidases and its application for phenol removal. J Biotechnol 139:273–279

Angelini VA, Orejas J, Medina MI, Agostini E (2011) Scale up of 2, 4-dichlorophenol removal from aqueous solutions using *Brassica napus* hairy roots. J Hazard Mater 185:269–274

Ayora-Talavera T, Chappell J, Lozoya-Gloria E, Loyola-Vargas VM (2002) Overexpression in *Catharanthus roseus* hairy roots of a truncated hamster 3-hydroxy-3-methylglutaryl-CoA reductase gene. Appl Biochem Biotechnol 97:135–145

Azlan GJ, Marziah M, Radzali M, Johari R (2002) Establishment of *Physalis minima* hairy roots culture for the production of physalins. Plant Cell Tissue Organ Cult 69:271–278

Baenas N, García-Viguera C, Moreno DA (2014) Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19:13541–13563

Bahadur I, Meena VS, Kumar S (2014) Importance and application of potassic biofertilizer in Indian agriculture. Int Res J Biol Sci 3:80–85

Bahadur I, Maurya BR, Kumar A, Meena VS, Raghuwanshi R (2016a) Towards the soil sustainability and potassium-solubilizing microorganisms. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 225–266. doi:10.1007/978-81-322-2776-2_18

Bahadur I, Maurya BR, Meena VS, Saha M, Kumar A, Aeron A (2016b) Mineral release dynamics of tricalcium phosphate and waste muscovite by mineral-solubilizing rhizobacteria isolated from indo-gangetic plain of India. Geomicrobiol J. doi:10.1080/01490451.2016.1219431

Bahadur I, Maurya BR, Meena VS, Saha M, Kumar A, Aeron A (2017) Mineral release dynamics of tricalcium phosphate and waste muscovite by mineral-solubilizing rhizobacteria isolated from indo-gangetic plain of India. Geomicrobiol J. doi:10.1080/01490451.2016.1219431

Banerjee S, Shang TQ, Wilson AM, Moore AL, Strand SE, Gordon MP, Lafferty Doty S (2002) Expression of functional mammalian P450 2E1 in hairy root cultures. Biotechnol Bioeng 77:462–466

Boominathan R, Doran PM (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*. Biotechnol Bioeng 83:158–167

Boominathan R, Saha-Chaudhury NM, Sahajwalla V, Doran PM (2004) Production of nickel bio-ore from hyperaccumulator plant biomass: applications in phytomining. Biotechnol Bioeng 86:243–250

Bordonaro JL, Curtis WR (2000) Inhibitory role of root hairs on transport within root culture bioreactors. Biotechnol Bioeng 70:176–186

Chandra S (2012) Natural plant genetic engineer *Agrobacterium rhizogenes*: role of T-DNA in plant secondary metabolism. Biotechnol Lett 34:407–415
Chandra S, Chandra R (2011) Engineering secondary metabolite production in hairy roots. Phytochem Rev 10:371–395
Chinou I (2008) Primary and secondary metabolites and their biological activity. In: Waksmundzka-Hajnos M, Sherma J, Kowalska T (eds) Thin layer chromatography in photochemistry. CRC Press, Boca Raton, pp 59–76
Choi YE, Kim YS, Paek KY (2008) Types and designs of bioreactors for hairy root culture. In: Gupta SD, Ibaraki Y (eds) Plant tissue culture engineering. Springer, Netherlands, pp 161–172
Coniglio MS, Busto VD, González PS, Medina MI, Milrad S, Agostini E (2008) Application of *Brassica napus* hairy root cultures for phenol removal from aqueous solutions. Chemosphere 72:1035–1042
Curtis WR (2000) Bioreactor growth of hairy roots. In: Spier R (ed) Encyclopedia of cell technology. Wiley, New York, pp 827–841
Das I, Pradhan M (2016) Potassium-solubilizing microorganisms and their role in enhancing soil fertility and health. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 281–291. doi:10.1007/978-81-322-2776-2_20
Datta A, Bhattacharya S, Pal CB, Sen J, Dasgupta S, Biswas A, Batra J (2010) Process for production of anti-diabetic compound in root culture of Catharanthus roseus.US Patent WO2010004584 Aug 2010
De Araujo BS, Dec J, Bollag JM, Pletsch M (2006) Uptake and transformation of phenol and chlorophenols by hairy root cultures of *Daucus carota*, *Ipomoea batatas* and *Solanum aviculare*. Chemosphere 63:642–651
Dechaux CE, Boitel-Conti MI (2005) A strategy for overaccumulation of scopolamine in Datura innoxia hairy root cultures. Acta Biol Cracov Ser Bot 47:101–107
Dixit AK, Vaidya S (2010) *Agrobacterium rhizogenes* induced hairy root development and its effect on production of glycyrrhizin in *Abrus precatorius* (L). Int J Curr Res 6:33–38
Dominguez-Nunez JA, Benito B, Berrocal-Lobo M, Albanesi A (2016) Mycorrhizal fungi: role in the solubilization of potassium. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 77–98. doi:10.1007/978-81-322-2776-2_6
Doran PM (2009) Application of plant tissue cultures in phytoremediation research: incentives and limitations. Biotechnol Bioeng 103:60–76
Dotaniya ML, Meena VD, Basak BB, Meena RS (2016) Potassium uptake by crops as well as microorganisms. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 267–280. doi:10.1007/978-81-322-2776-2_19
Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
Eapen S, Suseelan KN, Tivarekar S, Kotwal SA, Mitra R (2003) Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Environ Res 91:127–133
Eapen S, Singh S, D'souza SF (2007) Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol Adv 25:442–451
Eibl R, Eibl D (2006) Design and use of the wave bioreactor for plant cell culture. In: Gupta SD, Ibaraki Y (eds) Plant tissue culture engineering. Springer, The Netherlands, pp 203–227
Eibl R, Eibl D (2008) Design of bioreactors suitable for plant cell and tissue cultures. Phytochem Rev 7:593–598
Ferdosi A, Kashefi B (2014) A review hairy roots and secondary metabolite production in salvia. Intl J Farm Allied Sci 3:940–945
Flores HE, Filner P (2012) Metabolic relationships of putrescine, GABA and alkaloids in cell and root cultures of Solanaceae. In: Neumann KH, Barz W, Reinhard E (eds) Primary and secondary metabolism of plant cell cultures. Springer Science and Business Media, p 174–185

Fu CX, Zhao DX, Xue XF, Jin ZP, Ma FS (2005) Transformation of *Saussurea involucrata* by *Agrobacterium rhizogenes*: hairy root induction and syringin production. Process Biochem 40:3789–3794

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236

Georgiev M, Heinrich M, Kerns G, Pavlov A, Bley T (2006) Production of iridoids and phenolics by transformed *Harpagophytum procumbens* root cultures. Eng Life Sci 6:593–596

Georgiev MI, Pavlov AI, Bley T (2007) Hairy root type plant in vitro systems as sources of bioactive substances. Appl Microbiol Biotechnol 74:1175–1185

Georgiev MI, Ludwig-Müller J, Bley T (2010) Hairy root culture: copying nature in new bioprocesses. In: Arora R (ed) Medicinal plant biotechnology. CAB International, Oxon, pp 156–175

Georgiev MI, Ludwig-Müller J, Alipieva K, Lippert A (2011) Sonication-assisted *Agrobacterium rhizogenes*-mediated transformation of *Verbascum xanthophoeniceum* Griseb for bioactive metabolite accumulation. Plant Cell Rep 30:859–866

Giri A, Narasu ML (2000) Transgenic hairy roots: recent trends and applications. Biotechnol Adv 18:1–22

Giri A, Ravindra ST, Dhingra V, Narasu ML (2001) Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and artemisinin production in *Artemisia annua*. Curr Sci 81:378–382

Gonzalez PS, Capozucca C, Tigier H, Milrad S, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzym Microb Technol 39:647–653

Gonzalez PS, Agostini E, Milrad SR (2008) Comparison of the removal of 2,4-dichlorophenol and phenol from polluted water, by peroxidases from tomato hairy roots, and protective effect of polyethylene glycol. Chemosphere 70:982–989

Gonzalez PS, Ontanon OM, Armendariz AL, Talano MA, Paisio CE, Agostini E (2013) *Brassica napus* hairy roots and rhizobacteria for phenolic compounds removal. Environ Sci Pollut Res 20:1310–1317

Guillon S, Trémouillaux-Guiller J, Pati PK, Rideau M, Gantet P (2006) Hairy root research: recent scenario and exciting prospects. Curr Opin Plant Biol 9:341–346

Gujarathi NP, Haney BJ, Park HJ, Wickramasinghe SR, Linden JC (2005) Hairy roots of *Helianthus annuus*: a model system to study phytoremediation of tetracycline and oxytetracycline. Biotechnol Prog 21:775–780

Huang TK, McDonald KA (2012) Bioreactor systems for in vitro production of foreign proteins using plant cell cultures. Biotechnol Adv 30:398–409

Huang SY, Hung CH, Chou SN (2004) Innovative strategies for operation of mist trickling reactors for enhanced hairy root proliferation and secondary metabolite productivity. Enzym Microb Technol 35:22–32

Huber C, Bartha B, Harpaintner R, Schröder P (2009) Metabolism of acetaminophen (paracetamol) in plants-two independent pathways result in the formation of a glutathione and a glucose conjugate. Environ Sci Pollut Res 16:206–213

Ibanez SG, Medina MI, Agostini E (2011) Phenol tolerance, changes of antioxidative enzymes and cellular damage in transgenic tobacco hairy roots colonized by *Arbuscular mycorrhizal* fungi. Chemosphere 83:700–705

Ibanez S, Talano M, Ontañon O, Suman J, Medina MI, Macek T, Agostini E (2016) Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. New Biotechnol 33:625–635

Ismail AM, Theodor PA (2012) The effect of heavy metals Zn and Ni on growth of in vitro hairy root cultures of Indian mustard *Brassica juncea* L. Int J Adv Biotechnol Res 3:688–697

Jacob A, Malpathak N (2004) Green hairy root cultures of *Solanum khasianum* Clarke-a new route to in vitro Solasodine production. Curr Sci 87:1442–1447

Jaiswal DK, Verma JP, Prakash S, Meena VS, Meena RS (2016) Potassium as an important plant nutrient in sustainable agriculture: a state of the art. In: Meena VS, Maurya BR, Verma JP,

Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 21–29. doi:10.1007/978-81-322-2776-2_2

Jat LK, Singh YV, Meena SK, Meena SK, Parihar M, Jatav HS, Meena RK, Meena VS (2015) Does integrated nutrient management enhance agricultural productivity? J Pure Appl Microbiol 9(2):1211–1221

Jha Y, Subramanian RB (2016) Regulation of plant physiology and antioxidant enzymes for alleviating salinity stress by potassium-mobilizing bacteria. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 149–162. doi:10.1007/978-81-322-2776-2_11

Jha P, Jobby R, Kudale S, Modi N, Dhaneshwar A, Desai N (2013) Biodegradation of phenol using hairy roots of *Helianthusannuus* L. Int Biodeterior Biodegrad 77:106–113

Katuri SR, Ranjan R, Khanna R (2011) Mathematical modeling of mist bioreactor for the growth of hairy roots In: National workshop-cum-conference on recent trends in Mathematics and Computing RTMC (13)

Kawauchi M, Arima T, Shirota O, Sekita S, Nakane T, Takase Y, Kuroyanagi M (2010) Production of sesquiterpene-type phytoalexins by hairy roots of *Hyoscyamus albus* co-treated with copper sulfate and methyl jasmonate. Chem Pharm Bull 58:934–938

Khoudi H, Maatar Y, Gouiaa S, Masmoudi K (2012) Transgenic tobacco plants expressing ectopically wheat H+-pyrophosphatase (H+-PPase) gene *TaVP1* show enhanced accumulation and tolerance to cadmium. J Plant Physiol 169:98–103

Kim YJ, Weathers PJ, Wyslouzil BE (2002a) Growth of *Artemisia annua* hairy roots in liquidand gas-phase reactors. Biotechnol Bioeng 80:454–464

Kim Y, Wyslouzil BE, Weathers PJ (2002b) Invited review: secondary metabolism of hairy root cultures in bioreactors. In Vitro Cell Dev Biol Plant 38:1–10

Kim JS, Lee SY, Park SU (2008) Resveratrol production in hairy root culture of peanut, *Arachis hypogaea* L. transformed with different *Agrobacterium rhizogenes* strains. Afr J Biotechnol 7:3788–3790

Kim YK, Xu H, Park WT, Park NI, Lee SY, Park SU (2010) Genetic transformation of buckwheat (*Fagopyrum esculentum* M.) with *Agrobacterium rhizogenes* and production of rutin in transformed root cultures. Aust J Crop Sci 4:485–490

Korde VV, Dhas SS, Gurave NA (2016) Hairy root culture: a promising approach in biotransformation. Asian J Plant Sci 6:6–11

Królicka A, Staniszewska I, Bielawski K, Maliński E, Szafranek J, Łojkowska E (2001) Establishment of hairy root cultures of *Ammi majus*. Plant Sci 160:259–264

Kruger NJ, Ratcliffe RG (2009) Insights into plant metabolic networks from steady-state metabolic flux analysis. Biochimie 91:697–702

Krystofova O, Shestivska V, Galiova M, Novotny K, Kaiser J, Zehnalek J, Babula P, Opatrilova R, Adam V, Kizek R (2009) Sunflower plants as bioindicators of environmental pollution with lead (II) ions. Sensors 9:5040–5058

Kumar A, Bahadur I, Maurya BR, Raghuwanshi R, Meena VS, Singh DK, Dixit J (2015) Does a plant growth-promoting rhizobacteria enhance agricultural sustainability? J Pure Appl Microbiol 9:715–724

Kumar A, Meena R, Meena VS, Bisht JK, Pattanayak A (2016a) Towards the stress management and environmental sustainability. J Clean Prod 137:821–822

Kumar A, Patel JS, Bahadur I, Meena VS (2016b) The molecular mechanisms of KSMs for enhancement of crop production under organic farming. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 61–75. doi:10.1007/978-81-322-2776-2_5

Kumar A, Maurya BR, Raghuwanshi R, Meena VS, Islam MT (2017a) Co-inoculation with Enterobacter and Rhizobacteria on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the alluvial soil under indo-gangetic plain of India. J Plant Growth Regul. doi:10.1007/s00344-016-9663-5

Kumar A, Meena VS, Maurya BR, Raghuwanshi R, Bisht JK, Pattanayak A (2017b) Towards the biological nitrogen fixation and nitrogen management in legume under sustainable agriculture. Appl Soil Ecol. doi:10.1016/j.apsoil.2017.05.013

Kwok KH, Doran PM (1995) Kinetic and stoichiometric analysis of hairy roots in a segmented bubble-column reactor. Biotechnol Prog 11:429–435

Lan X, Quan H (2010) Hairy root culture of *Przewalskia tangutica* for enhanced production of pharmaceutical tropane alkaloids. J Med Plant Res 4:1477–1481

Le Flem-Bonhomme V, Laurain-Mattar D, Fliniaux MA (2004) Hairy root induction of *Papaver somniferum* var. album, a difficult-to-transform plant, by *A. rhizogenes* LBA 9402. Planta 218:890–893

Li W, Koike K, Asada Y, Hirotani M, Rui H, Yoshikawa T, Nikaido T (2002) Flavonoids from *Glycyrrhiza pallidiflora* hairy root cultures. Phytochemistry 60:351–355

Li FX, Jin ZP, Zhao DX, Cheng LQ, Fu CX, Ma FS (2006) Overexpression of the *Saussurea medusa* chalcone isomerase gene in *Saussurea involucrata* hairy root cultures enhances their biosynthesis of apigenin. Phytochemistry 67:553–560

Lokhande VH, Kudale S, Nikalje G, Desai N, Suprasanna P (2015) Hairy root induction and phytoremediation of textile dye, reactive green 19A-HE4BD, in a halo-phyte, *Sesuvium portulacastrum* L. Biotechnol Rep 8:56–63

Ludwig-Müller J, Jahn L, Lippert A, Püschel J, Walter A (2014) Improvement of hairy root cultures and plants by changing biosynthetic pathways leading to pharmaceutical metabolites: strategies and applications. Biotechnol Adv 32:1168–1179

Macek T, Kotrba P, Svatos A, Novakova M, Demnerova K, Mackova M (2008) Novel roles for genetically modified plants in environmental protection. Trends Biotechnol 26:146–152

Masood S, Bano A (2016) Mechanism of potassium solubilization in the agricultural soils by the help of soil microorganisms. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 137–147. doi:10.1007/978-81-322-2776-2_10

Maurya BR, Meena VS, Meena OP (2014) Influence of Inceptisol and Alfisol's potassium solubilizing bacteria (KSB) isolates on release of K from waste mica. Vegetos 27:181–187

McCoy E, O'Connor SE (2008) Natural products from plant cell cultures. In: Petersen F, Amstutz R (eds) Natural compounds as drugs, vol 1. Birkhäuser, Basel, pp 329–370

McKelvey SA, Gehrig JA, Hollar KA, Curtis WR (1993) Growth of plant root cultures in liquid- and gas-dispersed reactor environments. Biotechnol Prog 9:317–322

Medina-Bolivar LF, Dolan M, Bennett S, Condori JM, Hubstenberger JF, inventors; Condori Jose M, Hubstenberger John F, assignee (2010) Production of stilbenes in plant hairy root cultures. US patent 7,666,677, 23 Feb 2010

Meena VS, Maurya BR, Bohra JS, Verma R, Meena MD (2013a) Effect of concentrate manure and nutrient levels on enzymatic activities and microbial population under submerged rice in alluvium soil of Varanasi. Crop Res 45(1, 2 & 3):6–12

Meena OP, Maurya BR, Meena VS (2013b) Influence of K-solubilizing bacteria on release of potassium from waste mica. Agric Sust Dev 1:53–56

Meena VS, Maurya BR, Verma R, Meena RS, Jatav GK, Meena SK, Meena SK (2013c) Soil microbial population and selected enzyme activities as influenced by concentrate manure and inorganic fertilizer in alluvium soil of Varanasi. Bioscan 8(3):931–935

Meena VS, Maurya BR, Bahadur I (2014a) Potassium solubilization by bacterial strain in waste mica. Bang J Bot 43:235–237

Meena VS, Maurya BR, Verma JP (2014b) Does a rhizospheric microorganism enhance K+ availability in agricultural soils? Microbiol Res 169:337–347

Meena VS, Maurya BR, Meena RS (2015a) Residual impact of wellgrow formulation and NPK on growth and yield of wheat (*Triticum aestivum* L.). Bangladesh. Aust J Bot 44(1):143–146

Meena VS, Maurya BR, Verma JP, Aeron A, Kumar A, Kim K, Bajpai VK (2015b) Potassium solubilizing rhizobacteria (KSR): isolation, identification, and K-release dynamics from waste mica. Ecol Eng 81:340–347

Meena RS, Meena VS, Meena SK, Verma JP (2015c) The needs of healthy soils for a healthy world. J Clean Prod 102:560–561
Meena RS, Meena VS, Meena SK, Verma JP (2015d) Towards the plant stress mitigate the agricultural productivity: a book review. J Clean Prod 102:552–553
Meena VS, Meena SK, Verma JP, Meena RS, Ghosh BN (2015e) The needs of nutrient use efficiency for sustainable agriculture. J Clean Prod 102:562–563. doi:10.1016/j.jclepro.2015.04.044
Meena VS, Verma JP, Meena SK (2015f) Towards the current scenario of nutrient use efficiency in crop species. J Clean Prod 102:556–557. doi:10.1016/j.jclepro.2015.04.030
Meena VS, Bahadur I, Maurya BR, Kumar A, Meena RK, Meena SK, Verma JP (2016a) Potassium-solubilizing microorganism in evergreen agriculture: an overview. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 1–20. doi:10.1007/978-81-322-2776-2_1
Meena RS, Bohra JS, Singh SP, Meena VS, Verma JP, Verma SK, Sihag SK (2016b) Towards the prime response of manure to enhance nutrient use efficiency and soil sustainability a current need: a book review. J Clean Prod 112(1):1258–1260
Meena VS, Meena SK, Bisht JK, Pattanayak A (2016c) Conservation agricultural practices in sustainable food production. J Clean Prod 137:690–691
Meena SK, Rakshit A, Meena VS (2016d) Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions. Biocatal Agric Biotechnol 6:68–75
Meena RK, Singh RK, Singh NP, Meena SK, Meena VS (2016e) Isolation of low temperature surviving plant growth-promoting rhizobacteria (PGPR) from pea (*Pisum sativum* L.) and documentation of their plant growth promoting traits. Biocatalysis Agric Biotechnol 4:806–811
Mishra BN, Ranjan R (2008) Growth of hairy-root cultures in various bioreactors for the production of secondary metabolites. Biotechnol Appl Biochem 49:1–10
Moyano E, Jouhikainen K, Tammela P, Palazón J, Cusidó RM, Piñol MT, Teeri TH, Oksman-Caldentey KM (2003) Effect of *pmt* gene overexpression on tropane alkaloid production in transformed root cultures of *Datura metel* and *Hyoscyamus muticus*. J Exp Bot 54:203–211
Najmanova J, Mackova M, Macek T, Kotrba P (2007) Preparation of transgenic flax with enhanced metal tolerance. J Biotechnol 131:S38–S39
Namdeo AG (2007) Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn Rev 1:69–79
Nath D, Maurya BR, Meena VS (2017) Documentation of five potassium- and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. Biocatal Agric Biotechnol 10:174–181
Nedelkoska TV, Doran PM (2000) Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Miner Eng 13:549–561
Nepovim A, Podlipná R, Soudek P, Schröder P, Vanek T (2004) Effects of heavy metals and nitroaromatic compounds on horseradish glutathione S-transferase and peroxidase. Chemosphere 57:1007–1015
Nguyen C, Bourgaud F, Forlot P, Guckert A (1992) Establishment of hairy root cultures of *Psoralea* species. Plant Cell Rep 11:424–427
Novakova M, Mackova M, Sylvestre M, Macek T (2007) Preparation of genetically modified plants containing bacterial dioxygenase – tool for preferable phytoremediation. J Biotechnol 131:S36
Oller AL, Agostini E, Talano MA, Capozucca C, Milrad SR, Tigier HA, Medina MI (2005) Overexpression of a basic peroxidase in transgenic tomato (*Lycopersicon esculentum* Mill. cv. Pera) hairy roots increases phytoremediation of phenol. Plant Sci 169:1102–1111
Ono NN, Tian L (2011) The multiplicity of hairy root cultures: prolific possibilities. Plant Sci 180:439–446
Ontañon OM, González PS, Ambrosio LF, Paisio CE, Agostini E (2014) Rhizoremediation of phenol and chromium by the synergistic combination of a native bacterial strain and *Brassica napus* hairy roots. Int Biodeterior Biodegrad 88:192–198

Palazón J, Cusidó RM, Bonfill M, Mallol A, Moyano E, Morales C, Piñol MT (2003a) Elicitation of different *Panax ginseng* transformed root phenotypes for an improved ginsenoside production. Plant Physiol Biochem 41:1019–1025

Palazón J, Mallol A, Eibl R, Lettenbauer C, Cusidó RM, Piñol MT (2003b) Growth and ginsenoside production in hairy root cultures of *Panax ginseng* using a novel bioreactor. Planta Med 69:344–349

Parewa HP, Yadav J, Rakshit A, Meena VS, Karthikeyan N (2014) Plant growth promoting rhizobacteria enhance growth and nutrient uptake of crops. Agric Sustain Dev 2(2):101–116

Park SU, Facchini PJ (2000) *Agrobacterium rhizogenes*-mediated transformation of opium poppy, *Papaver somniferum* L., and California poppy, *Eschscholzia californica* Cham, root cultures. J Exp Bot 51:1005–1016

Patil P, Desai N, Govindwar S, Jadhav JP, Bapat V (2009) Degradation analysis of reactive Red198 by hairy roots of *Tagetes patula* L. (Marigold). Planta 230:725–735

Pavlov A, Georgiev V, Kovatcheva P (2003) Relationship between type and age of the inoculum cultures and betalains biosynthesis by *Beta vulgaris* hairy root culture. Biotechnol Lett 25:307–309

Pavlov A, Berkov S, Weber J, Bley T (2009) Hyoscyamine biosynthesis in *Datura stramonium* hairy root in vitro systems with different ploidy levels. Appl Biochem Biotechnol 157:210–225

Pistelli L, Giovannini A, Ruffoni B, Bertoli A, Pistelli L (2010) Hairy root cultures for secondary metabolites production. In: Teresa M, Giuseppina G, Berra R, Berra B (eds) Bio-farms for nutraceuticals. Springer, US, pp 167–184

Prakash S, Verma JP (2016) Global perspective of potash for fertilizer production. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 327–331. doi:10.1007/978-81-322-2776-2_23

Praveen N, Murthy HN (2012) Synthesis of with anolide A depends on carbon source and medium pH in hairy root cultures of *Withania somnifera*. Ind Crop Prod 35:241–243

Priyadharsini P, Muthukumar T (2016) Interactions between arbuscular mycorrhizal fungi and potassium-solubilizing microorganisms on agricultural productivity. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 111–125. doi:10.1007/978-81-322-2776-2_8

Qaderi A, Akbari Z, Kalateh-jari S, Fatehi F, Tolyat M, Jalali Moghadam M, Naghdi Badi H (2016) Improving trigonelline production in hairy root culture of fenugreek (*Trigonella foenum-graecum*). J Med Plants 59:73–80

Raghavendra MP, Nayaka NC, Nuthan BR (2016) Role of rhizosphere microflora in potassium solubilization. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 43–59. doi:10.1007/978-81-322-2776-2_4

Ramakrishnan D, Curtis WR (2004) Trickle-bed root culture bioreactor design and scale-up: growth, fluid-dynamics, and oxygen mass transfer. Biotechnol Bioeng 88:248–260

Ramirez-Estrada K, Vidal-Limon H, Hidalgo D, Moyano E, Golenioswki M, Cusidó RM, Palazon J (2016) Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules 21:182–205

Rao SR, Ravishankar GA (2002) Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv 20:101–153

Rates SM (2001) Plants as source of drugs. Toxicon 39:603–613

Rawat J, Sanwal P, Saxena J (2016) Potassium and its role in sustainable agriculture. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 235–253. doi:10.1007/978-81-322-2776-2_17

Rezek J, Macek T, Mackova M, Triska J (2007) Plant metabolites of polychlorinated biphenyls in hairy root culture of black nightshade *Solanum nigrum* SNC-90. Chemosphere 69:1221–1227

Rezek J, Macek T, Doubsky J, Mackova M (2012) Metabolites of 2,2′-dichlorobiphenyl and 2,6-dichlorobiphenyl in hairy root culture of black nightshade *Solanum nigrum* SNC-90. Chemosphere 89:383–838

Richter U, Rothe G, Fabian AK, Rahfeld B, Dräger B (2005) Overexpression of tropinone reductases alters alkaloid composition in *Atropa belladonna* root cultures. J Exp Bot 56:645–652
Rodriguez-Mendiola MA, Stafford A, Cresswell R, Ariascastro C (1991) Bioreactors for growth of plant-roots. Enzym Microb Technol 13:697–702
Rudrappa T, Neelwarne B, Aswathanarayana RG (2004) In situ and ex situ adsorption and recovery of betalains from hairy root cultures of *Beta vulgaris*. Biotechnol Prog 20:777–785
Saha M, Maurya BR, Bahadur I, Kumar A, Meena VS (2016a) Can potassium-solubilising bacteria mitigate the potassium problems in India? In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 127–136. doi:10.1007/978-81-322-2776-2_9
Saha M, Maurya BR, Meena VS, Bahadur I, Kumar A (2016b) Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic Plains of India. Biocatal Agric Biotechnol 7:202–209
Sarkar D, Meena VS, Haldar A, Rakshit R (2017) Site-specific nutrient management (SSNM): a unique approach towards maintaining soil health. In: The adaptive soil management: from theory to practices. p 69–88 doi. 10.1007/978-981-10-3638-5_3
Sato F, Hashimoto T, Hachiya A, Tamura KI, Choi KB, Morishige T, Fujimoto H, Yamada Y (2001) Metabolic engineering of plant alkaloid biosynthesis. Proc Natl Acad Sci 98:367–372
Sauerwein M, Yamazaki T, Shimomura K (1991) Hernandulcin in hairy root cultures of *Lippia dulcis*. Plant Cell Rep 9:579–581
Sevón N, Oksman-Caldentey KM (2002) *Agrobacterium rhizogenes*-mediated transformation: root cultures as a source of alkaloids. Planta Med 68:859–868
Sharma S, Shahzad A, Sahai A (2013) Hairy root culture: an efficient system for secondary metabolite production. In: Shahid M, Shahzad A, Malik A, Sahai A (eds) Recent trends in biotechnology and therapeutic applications of medicinal plants. Springer, The Netherlands, pp 51–78
Sharma A, Shankhdhar D, Shankhdhar SC (2016) Potassium-solubilizing microorganisms: mechanism and their role in potassium solubilization and uptake. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 203–219. doi:10.1007/978-81-322-2776-2_15
Shimomura K, Sudo H, Saga H, Kamada H (1991) Shikonin production and secretion by hairy root cultures of *Lithospermum erythrorhizon*. Plant Cell Rep 10:282–285
Shrivastava M, Srivastava PC, D'Souza SF (2016) KSM soil diversity and mineral solubilization, in relation to crop production and molecular mechanism. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 221–234. doi:10.1007/978-81-322-2776-2_16
Sindhu SS, Parmar P, Phour M, Sehrawat A (2016) Potassium-solubilizing microorganisms (KSMs) and its effect on plant growth improvement. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 171–185. doi:10.1007/978-81-322-2776-2_13
Singh S, Melo JS, Eapen S, D'souza SF (2006) Phenol removal using *Brassica juncea* hairy roots: role of inherent peroxidase and H_2O_2. J Biotechnol 123:43–49
Singh NP, Singh RK, Meena VS, Meena RK (2015) Can we use maize (Zea mays) rhizobacteria as plant growth promoter? Vegetos 28(1):86–99. doi:10.5958/2229-4473.2015.00012.9
Singh M, Dotaniya ML, Mishra A, Dotaniya CK, Regar KL, Lata M (2016) Role of biofertilizers in conservation agriculture. In: Bisht JK, Meena VS, Mishra PK, Pattanayak A (eds) Conservation agriculture: an approach to combat climate change in Indian Himalaya. Springer, Singapore, pp 113–134. doi:10.1007/978-981-10-2558-7_4
Sivakumar G, Yu KW, Hahn EJ, Paek KY (2005) Optimization of organic nutrients for ginseng hairy roots production in large-scale bioreactors. Curr Sci 89:641–649
Sivanesan I, Jeong BR (2009) Induction and establishment of adventitious and hairy root cultures of *Plumbago zeylanica* L. Afr J Biotechnol 8:5294–5300
Sosa Alderete LG, Talano MA, Ibáñez SG, Purro S, Agostini E, Milrad SR et al (2009) Establishment of transgenic tobacco hairy roots expressing basic peroxidases and its application for phenol removal. J Biotechnol 139:273–279

Sosa Alderete LG, Ibăne˜z SG, Agostini E, Medina MI (2012) Phytoremediation of phenol at pilot scale by tobacco hairy roots. Int J Environ Sci 3:398–407
Soudek P, Petrová S, Benesova D, Vanek T (2011) Uranium uptake and stress responses of in vitro cultivated hairy root culture of *Armoracia rusticana*. Agrochimica 55:15–28
Srivastava S, Srivastava AK (2007) Hairy root culture for mass-production of high-value secondary metabolites. Crit Rev Biotechnol 27:29–43
Srivastava S, Srivastava AK (2012a) Azadirachtin production by hairy root cultivation of *Azadirachta indica* in a modified stirred tank reactor. Bioprocess Biosyst Eng 35:1549–1553
Srivastava S, Srivastava AK (2012b) In vitro Azadirachtin production by hairy root cultivation of *Azadirachta indica* in nutrient mist bioreactor. Appl Biochem Biotechnol 166:365–378
Srivastava S, Srivastava AK (2012c) Statistical medium optimization for enhanced azadirachtin production in hairy root culture of *Azadirachta indica*. In Vitro Cell Dev Biol Plant 48:73–84
Stiles AR, Liu CZ (2013) Hairy root culture: bioreactor design and process intensification. In: Doran PM (ed) Biotechnology of hairy root systems. Springer, Berlin, pp 91–114
Straczek A, Wannijn J, Van Hees M, Thijs H, Thiry Y (2009) Tolerance of hairy roots of carrots to U chronic exposure in a standardized in vitro device. Environ Exp Bot 65:82–89
Sudha CG, Reddy BO, Ravishankar GA, Seeni S (2003) Production of ajmalicine and ajmaline in hairy root cultures of *Rauvolfia micrantha* Hook f., a rare and endemic medicinal plant. Biotechnol Lett 25:631–636
Sudo H, Yamakawa T, Yamazaki M, Aimi N, Saito K (2002) Bioreactor production of camptothecin by hairy root cultures of *Ophiorrhiza pumila*. Biotechnol Lett 24:359–363
Sung LS, Huang SY (2006) Lateral root bridging as a strategy to enhance L-DOPA production in *Stizolobium hassjoo* hairy root cultures by using a mesh hindrance mist trickling bioreactor. Biotechnol Bioeng 94:441–447
Suresh B, Sherkhane PD, Kale S, Eapen S, Ravishankar GA (2005) Uptake and degradation of DDT by hairy root cultures of *Cichorium intybus* and *Brassica juncea*. Chemosphere 61:1288–1292
Talano MA, Frontera S, González P, Medina MI, Agostini E (2010) Removal of 2,4- diclorophenol from aqueous solutions using tobacco hairy root cultures. J Hazard Mater 176:784–791
Talano MA, Oller AL, Gonzalez PS, Agostini E (2012) Hairy roots, their multiple applications and recent patents. Recent Patents Biotechnol 6:115–133
Talano MA, Oller AL, González P, González SO, Agostini E (2014) Effects of arsenate on tobacco hairy root and seedling growth, and its removal. In Vitro Cell Dev Biol Plant 50:217–225
Taya M, Yoyama A, Kondo O, Kobayashi T, Matsui C (1989) Growth characteristics of plant hairy roots and their cultures in bioreactors. J Chem Eng Jpn 22:84–89
Taya M, Sato H, Kinooka M, Tone S (1994) Characterization of Pak-bung green hairy roots cultivated under light irradiation. J Ferment Bioeng 78:42–48
Telke AA, Kagalkar AN, Jagtap UB, Desai NS, Bapat VA, Govindwar SP (2011) Bio-chemical characterization of laccase from hairy root culture of *Brassica juncea* L. and role of redox mediators to enhance its potential for the decolorization of textile dyes. Planta 234:1137–1149
Teotia P, Kumar V, Kumar M, Shrivastava N, Varma A (2016) Rhizosphere microbes: potassium solubilization and crop productivity-present and future aspects. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 315–325. doi:10.1007/978-81-322-2776-2_22
Thwe A, Arasu MV, Li X, Park CH, Kim SJ, Al-Dhabi NA, Park SU (2016) Effect of different agrobacterium rhizogenes strains on hairy root induction and phenylpropanoid biosynthesis in tartary buckwheat (*Fagopyrum tataricum gaertn*). Front Microbiol 7:318–328
Ur Rahman L, Ikenaga T, Kitamura Y (2004) Penicillin derivatives induce chemical structure-dependent root development, and application for plant transformation. Plant Cell Rep 22:668–677
Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY, Tsay HS (2004) Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. Bot Bull Acad Sin 45:1–22
Vasconsuelo A, Boland R (2007) Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci 172:861–875

Velazquez E, Silva LR, Ramírez-Bahena MH, Peix A (2016) Diversity of potassium-solubilizing microorganisms and their interactions with plants. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 99–110. doi:10.1007/978-81-322-2776-2_7

Verma R, Maurya BR, Meena VS (2014) Integrated effect of bio-organics with chemical fertilizer on growth, yield and quality of cabbage (*Brassica oleracea* var capitata). Indian J Agric Sci 84(8):914–919

Verma JP, Jaiswa DK, Meena VS, Meena RS (2015a) Current need of organic farming for enhancing sustainable agriculture. J Clean Prod 102:545–547

Verma JP, Jaiswal DK, Meena VS, Kumar A, Meena RS (2015b) Issues and challenges about sustainable agriculture production for management of natural resources to sustain soil fertility and health. J Clean Prod 107:793–794

Verma R, Maurya BR, Meena VS, Dotaniya ML, Deewan P (2017a) Microbial dynamics as influenced by bio-organics and mineral fertilizer in alluvium soil of Varanasi. India Int J Curr Microbiol App Sci 6(2):1516–1524

Verma R, Maurya BR, Meena VS, Dotaniya ML, Deewan P, Jajoria M (2017b) Enhancing production potential of cabbage and improves soil fertility status of Indo-Gangetic Plain through application of bio-organics and mineral fertilizer. Int J Curr Microbiol App Sci 6(3):301–309

Vinterhalter B, Savić J, Platiša J, Raspor M, Ninković S, Mitić N, Vinterhalter D (2008) Nickel tolerance and hyper accumulation in shoot cultures regenerated from hairy root cultures of *Alyssum murale* Waldst et Kit. Plant Cell Tissue Organ Cult 94:299–303

Wang YC, Zhang HX, Zhao B, Yuan XF (2001) Improved growth of *Artemisia annua* L hairy roots and artemisinin production under red light conditions. Biotechnol Lett 23:1971–1973

Weathers PJ, Bunk G, McCoy MC (2005) The effect of phytohormones on growth and artemisinin production in *Artemisia annua* hairy roots. In Vitro Cell Dev Biol Plant 41:47–53

Whitney PJ (1992) Novel bioreactors for the growth of roots transformed by *Agrobacterium rhizogenes*. Enzym Microb Technol 14:13–17

Wilhelmson A, Hakkinen ST, Kallio PT, Oksman-Caldentey KM, Nuutila AM (2006) Heterologous expression of *Vitreoscilla* hemoglobin (VHb) and cultivation conditions affect the alkaloid profile of *Hyoscyamus muticus* hairy roots. Biotechnol Prog 22:350–358

Wink M, Alfermann AW, Franke R, Wetterauer B, Distl M, Windhövel J, Krohn O, Fuss E, Garden H, Mohagheghzadeh A, Wildi E (2005) Sustainable bioproduction of phytochemicals by plant *in vitro* cultures: anticancer agents. Plant Genet Resour C 3:90–100

Wongwicha W, Tanaka H, Shoyama Y, Putalun W (2011) Methyl jasmonate elicitation enhances glycyrrhizin production in *Glycyrrhiza inflata* hairy roots cultures. Z Naturforsch 66:423–428

Wyslouzil BE, Whipple M, Chatterjee C, Walcerz DB, Weathers PJ, Hart DP (1997) Mist deposition onto hairy root cultures: aerosol modeling and experiments. Biotechnol Prog 13:185–194

Yadav BK, Sidhu AS (2016) Dynamics of potassium and their bioavailability for plant nutrition. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 187–201. doi:10.1007/978-81-322-2776-2_14

Yang YK (2010) Exogenous auxins and polyamines enhance growth and rosmarinic acid production in hairy root cultures of Nepeta cataria L. Plant Omics 3(6):190–193

Yasin M, Munir I, Faisal M (2016) Can Bacillus spp. enhance K+ uptake in crop species. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp 163–170. doi:10.1007/978-81-322-2776-2_12

Ya-ut P, Chareonsap P, Sukrong S (2011) Micropropagation and hairy root culture of *Ophiorrhiza alata* Craib for camptothecin production. Biotechnol Lett 33:2519–2526

Yazaki K, Sugiyama A, Morita M, Shitan N (2008) Secondary transport as an efficient membrane transport mechanism for plant secondary metabolites. Phytochem Rev 7:513–524

Yoshikawa T, Furuya T (1987) Saponin production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogenes*. Plant Cell Rep 6:449–453

Zahedi H (2016) Growth-promoting effect of potassium-solubilizing microorganisms on some crop species. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium

solubilizing microorganisms for sustainable agriculture. Springer, India, pp 31–42. doi:10.1007/978-81-322-2776-2_3

Zhang L, Ding R, Chai Y, Bonfill M, Moyano E, Oksman-Caldentey KM, Xu T, Pi Y, Wang Z, Zhang H, Kai G (2004) Engineering tropane biosynthetic pathway in *Hyoscyamus niger* hairy root cultures. Proc Natl Acad Sci USA 101:6786–6791

Zhang HC, Liu JM, Lu HY, Gao SL (2009) Enhanced flavonoid production in hairy root cultures of *Glycyrrhiza uralensis* Fisch by combining the over-expression of chalcone isomerase gene with the elicitation treatment. Plant Cell Rep 28:1205–1213

Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 23:283–333

Zhong JJ, Seki T, Kinoshita S, Yoshida T (1991) Effect of light irradiation on anthocyanin production by suspended culture of *Perilla frutescens*. Biotechnol Bioeng 38:653–658