Biotransformation Through Hairy Roots: Perspectives, Outcomes, and Major Challenges

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Abstract

The *Agrobacterium rhizogenes* mediated hairy root cultures have shown great potential in bringing out structural and/or conformational alteration of a chemical moiety through its enzymatic paraphernalia. The inherent enzymes involved in bioconversions perform various kinds of reactions such as hydroxylation, glycosylation, oxidoreduction, and hydrolysis. Hairy root cultures of variety of plant systems have been explored for the bioconversion of a wide range of substrates into the molecules of improved pharmaceutical properties. Some specific inherent properties of hairy roots like genetic/biochemical stability, hormonal independence, efficient enzyme paraphernalia, and low-cost cultural requirements contribute to the superiority of HRCs over other in vitro production systems. Rational use of hairy root cultures as proficient biotransformation system may result in the synthesis of molecules that have desired physico-chemical properties, adequate solubility, and reduced toxicity, thus more desired in pharmaceutical industries. The present chapter elaborates a comprehensive discussion on hairy root mediated biotransformation, types of reactions, and products formed concomitantly with their commercial importance.

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Keywords

Biosynthetic pathway • Biotransformation • Hairy root cultures • Secondary metabolism • Precursor feeding • Co-culture system

Abbreviations

4-OHBA	4-hydroxybenzalacetone
BG	Betuligenol
DA	Deoxy artemisinin
DHA	Dehydroabietic acid
HBA	*p*-Hydroxy benzyl alcohol
HQ	Hydroquinone or 1–4-benzendiol
HRCs	Hairy root cultures
PCS	Plant cell suspension
PF	Precursor feed or supplementation
RK	Raspberry ketone
SA	Salicylic acid
SM	Secondary metabolism
TBA	3,4,5-trimethoxy benzaldehyde
THPB	Tetrahydroprotoberberines
YE	Yeast extract

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1 Introduction

Biotransformation is defined as structural and/or conformational alteration of a chemical moiety by enzymatic militia of biological system. When referred to the molecules of pharmaceutical and other commercial importance, biotransformation is considered as a flawless methodology aimed to create specific structural changes in rather complex structured bioactive molecules. The transformed products show properties like reduced toxicity, adequate solubility, and improved pharmacokinetics which is more desired in pharmaceutical industries. Thus, this class of molecules with more desired physiochemical and therapeutic properties gain increased commercial demand [1]. During biotransformation of chemical compounds, the biological system functions as suitable biological matrices by offering their inherent enzymatic paraphernalia to catalyze various types of chemical reactions. Majority of these reactions include hydroxylation, glycosylation, oxidoreduction, hydrolysis, etc. Nowadays, biotransformation is increasingly utilized to develop repositories of analogs of variety of naturally occurring phytomolecules with the help of enzymes derived from microbes, plant/animal cells, tissues, and their cultured counterparts [1, 2]. Thus, distinguished from therapeutic protein production, biotransformation not only enables to develop advance version of existing molecules but also to enhance their desired inherent potential. Another phenomenon that sometime comes parallel to this process is fermentation. However, distinguished from fermentation where usually several catalytic steps involve between substrate and product, biotransformation is restricted to one or two. Additionally, the basic backbone structures of the substrate and the product resemble one another in biotransformation but not necessarily in fermentation [3].

The applicability of biotransformation has been envisaged through different biological systems and enzymes thereof, and many studies irrespective of rank and cellular organization have been extensively explored ranging from bacteria (microbe) to higher plants and animals [1]. In animals, this approach is generally confined to adjudge only toxicity or metabolic fate of administered drug (pharmacokinetic studies). On the other hand, the microbial and plant based biotransformation systems are useful for the production of significant molecules which can be of high therapeutic, flavor, or nutritional values. Nevertheless, these two potent candidate systems have their own limitations and advantages. The microbes, though superior in production and processing, have inherent drawback of easy cross-contamination, genetic instability, and comparatively low biotransformation prospects. Plants, on other side, due to complex biological behavior offer broader enzymatic potential for biotransformation and also possess genetic firmness. A comparison of different biotransformation systems has been summarized in Table 1.

The biotransformation procedures carried out by cultured plant cells and organs are independent of seasonal and pathological constraints and offer sustainable use of resources under controlled and defined culture conditions. Plant in vitro systems like plant cell suspensions (PCSs) and hairy root cultures (HRCs) are being utilized for biotransformation of variety of compounds because of their quick response to any variability in culture environment and simple to follow extraction procedures. The

Table 1 Comparative account on plant cell suspension, hairy root, and microbial biotransformation culture

Plant-based biotransformation systems		Microbial biotransformation system
HRCs	Cell suspension	
High growth rate	High growth rate	Very high growth rate
Easy maintenance and relatively low cost	Comparatively high cost	Low cost
Genetically stable	Prone to instability	High mutability (short life-span)
Broader enzyme biosynthetic potential	Broader enzyme biosynthetic potential	Narrow enzyme potential (low organizational status)
Less responsive to the variations in culture conditions	Highly responsive to variation in condition (callus)	Sensitive to culture condition
Capable of virtually infinite growth under defined conditions	Capable of virtually infinite growth under defined conditions	Capable of virtually infinite growth under defined conditions
Not easy downstream processing	Easy downstream processing	Easy downstream processing
Comparatively difficult to upscale	Easy to follow established upscaling methods	Easy to upscale
Susceptible to microbial contamination	Highly susceptible to microbial contamination	High risk of contamination
Free of hazardous chemicals	Free of hazardous chemicals	Free of hazardous chemicals
High prospects of genetic manipulations and its maintenance	High prospects of genetic manipulations but maintenance is not easy	Easy to genetically manipulate
Low yields	Low yields	High yielding

upcoming text elaborates a comprehensive discussion on hairy root mediated biotransformation, types of reactions, and products formed concomitantly with their commercial importance. Additionally, a pictorial presentation of biotransformation procedure is also being given in Fig. 1.

2 Hairy Root Cultures (HRCs): Suitable Matrix for Biotransformation

Plant in vitro systems in which plant cells, tissues, and organs are cultured under defined sterile conditions offer attractive alternatives for the production of desired metabolites of commercial importance [4–7]. Hairy root cultures (HRCs) have been extensively exploited not only for secondary metabolite production but also to understand the physiology and molecular biology existing behind [8–11]. Under natural conditions, hairy roots are disease manifestations developed by plants that are wounded and subsequently infected by *Agrobacterium rhizogenes* [12]. During

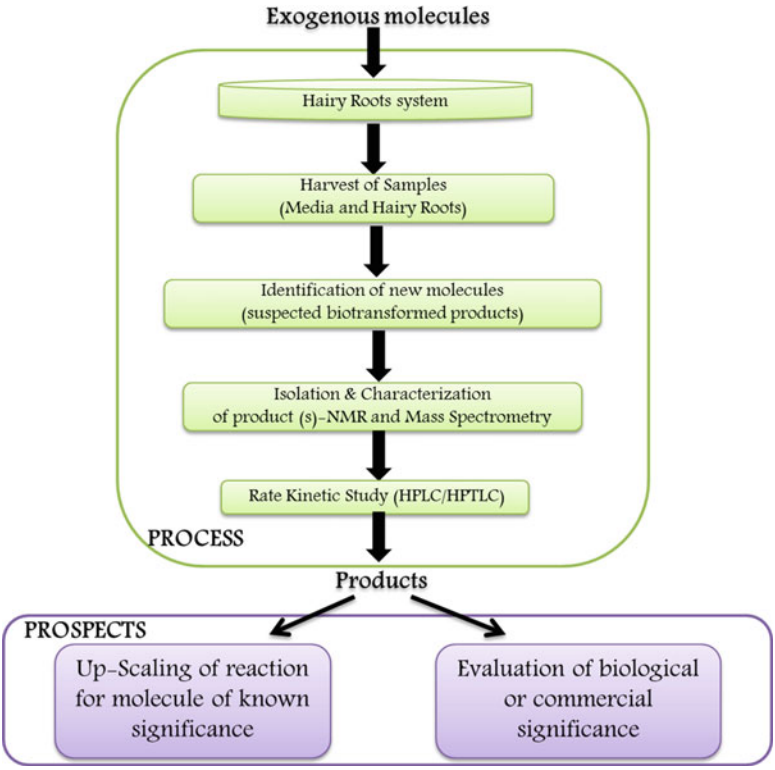


Fig. 1 Process and prospects of hairy roots mediated biotransformation of exogenous molecules

the infection process, root loci (*rol*) genes present in the root inducing (Ri) plasmid of *A. rhizogenes* are incorporated into the nuclear genome of the host plant, eventually causing neoplastic roots and root hair proliferation [12, 13]. The laboratory exploitation of this natural genetic transformation phenomenon and development of HRCs of diverse plant systems offer relatively easy "establish and explore" approach for various purposes including secondary metabolite production [11]. Owing to the characteristic property of fast growth and ability to produce existing and/or novel secondary metabolites in detectable range, the hairy roots have gained attention of global scientific fraternity as a cost effective in vitro production system for desired bioactive compounds [14]. Incessant research on HRCs of various plant species from past three decades have proved their worth as an excellent system as a whole to investigate various aspects of plant secondary metabolism (SM) [8, 11, 14]. Additionally, with time, this system has also emerged as a suitable matrix to explore a broad range of biotechnological applications [11, 14–16].

Hairy root based biotransformation is one such application where structural and/or conformational modification of chemical compounds is achieved by utilizing the enzymatic machinery of root tissue [2, 13]. Because of the defined in vitro conditions, such structural diversifications in parent compounds and subsequent

development of their analogs are independent of seasonal and pathological constraints. In addition to fast growth and secondary metabolite production potential, some specific inherent properties of hairy roots contribute to the superiority of HRCs over other in vitro production systems [11]. These properties include genetic/biochemical stability, hormonal independence, efficient enzyme paraphernalia, and low-cost culture necessities. Additionally, by avoiding hazardous chemicals and reagents, hairy root mediated biotransformation ensures the sustainable utilization of resources under definite culture conditions [2, 11, 17].

3 Properties of Hairy Root Mediated Biotransformation

HRCs mediated biotransformation procedures are recognized with many features, because of which these procedures supersedes over classical method of synthetic chemistry. Moreover, its suitability for context specific metabolite production also occurs due to some novel attributes well exemplified in literature.

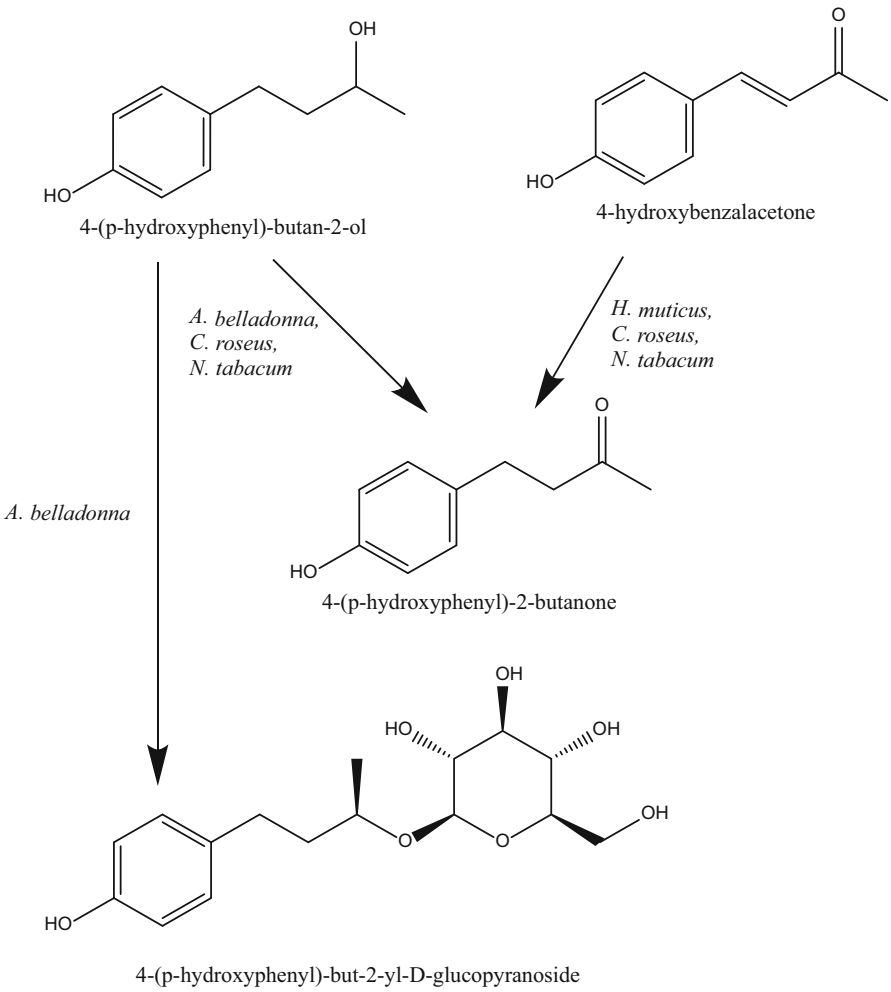
3.1 Regio-Selectivity

Regio-selectivity is the specificity of enzymes that refers to the preference of one location of catalyzing a reaction out of over all other possible locations. Regio-specific glycosylation of different position isomers of hydroxyl benzoic acid by *Panax ginseng* HRCs is one of the relevant examples of this category [18]. This study showed glycosyl esterification of *para*- and *meta*-hydroxy benzoic acid but not of *ortho*-hydroxy benzoic acid. Likewise, HRCs of *Polygonum multiflorum* depicted efficient regio-selective glucosylation of phenolic hydroxyl groups of compounds into its derivatives [19]. In the same way, *Atropa belladonna* hairy roots also show regio-specific glucosylation during biotransformation of betuligenol (BG) into 4-(*p*-hydroxyphenyl) but-2-yl-*β*-D-glucopyranoside or betuloside [20] (Scheme 1).

Further, in a recent study, HRCs of *P. ginseng* exhibited high degree of regio-selective glycosylation for tetrahydroprotoberberine (THPBs) substrates [21]. These HRCs showed greater catalytic efficiency on THPBs with phenolic hydroxyl at C-9 as compared to the positions other than C-9. In this way, HRCs provide an easy way by avoiding the tedious methods of chemistry involving protection of functional equivalent/groups to attain desired derivatization to get specific products with desired properties.

3.2 Reaction Flexibility

Biotransformation reactions are flexible in nature in which one group is prone to be transformed into any of its possible derivatives. With this feature, exogenous



Scheme 1 Biotransformation of 4-(*p*-hydroxyphenyl)-butan-2-ol (BG) and 4-hydroxy benzalacetone(4-OHBA) utilizing HRCs (Biotransformation of 4-OHBA has not been investigated in *A. belladonna* HRCs) [20, 50]

molecule can undergo any of the possible transformation events. In an example of reaction flexibility, both oxidation and reduction of formyl group of 3, 4, 5-trimethoxy benzaldehyde (TBA) was observed by *A. belladonna* hairy roots [22]. Same hairy roots also performed oxidation and glucosylation at secondary alcohol during biotransformation [20]. Besides, this kind of reaction flexibility have also been well presented in studies with *Pharbitis nil* and *P. multiflorum* HRCs mediated biotransformation reactions [19, 23, 24].

3.3 Stereoselectivity

The stereoselectivity in a reaction refers to the preferential stereoisomer formation over another, as a result of inherent reaction specificity, substrate, enzymes, etc. More specifically, according to the type of reactions and products the terms enantioselectivity and diastereoselectivity are referred. In context of HRCs mediated biotransformation, steric nature of substrate and products remains tightly maintained and only one of the isomer in racemic mixtures of substrate is attacked by respective enzymes. Moreover, in enantioselective reactions, where numbers of enantiomers are possible, production of only one enantiomer prevails. *Daucas carota* HRCs based stereoselective reduction of aromatic ketones, keto esters, and a simple aliphatic ketone is one such example where all these substrate reduced to corresponding derivatives with excellent stereoselectivity [25]. In another example, HRCs of *P. multiflorum* depicted efficient regio and stereoselective glucosylation of phenolic hydroxyl groups of compounds into corresponding compounds [26]. Similarly, enantioselective biotransformation of prochiral heteroaryl and prochiral alkylaryl ketones was achieved through HRCs of *Brassica napus* and *Raphanus sativus*, respectively, to their corresponding chiral alcohols [27, 28]. This advocates the property of stereoselectivity in HRCs based bioconversion of different substrates. However, on contrary, in some examples such as bioconversion of tetrahydroprotoberberins (THPBs) through *P. ginseng* HRCs, the conversion reactions exhibit nonstereoselectivity towards their substrates [21].

3.4 Activation of Molecules

There are molecules which are chemically inactive because of lack of functional groups in its backbone. The biotransformation processes have the ability to decorate the back bone of any molecule through the addition of chemically reactive groups (like hydroxylation etc.). This kind of property can be observed in cell suspension cultures of *Catharanthus roseus*, where Shimoda et al. [29] had demonstrated hydroxylation of 2-hydroxybenzoic acid into 2, 5-dihydroxybenzoic acid and subsequent glucosylation of this newly formed phenolic hydroxyl group into 5-*O*-(*β*-D-glucopyranosyl)-2,5-dihydroxybenzoic acid. Though also possible in HRCs based biotransformation of compounds yet, such modifications are not much explored in hairy root systems. However, the example of glycosylation of primary alcohol derived after aldehyde reduction in *Pharbitis nil* HRCs based biotransformation of benzaldehyde- and acetophenone-type derivatives would be relevant to state [30].

3.5 Nonextreme Conditions and No Hazardous Chemicals Required

The in vitro culture procedures, including HRCs, necessitate the maintenance of controlled, aseptic, and defined conditions. All kinds of HRCs based bioconversion

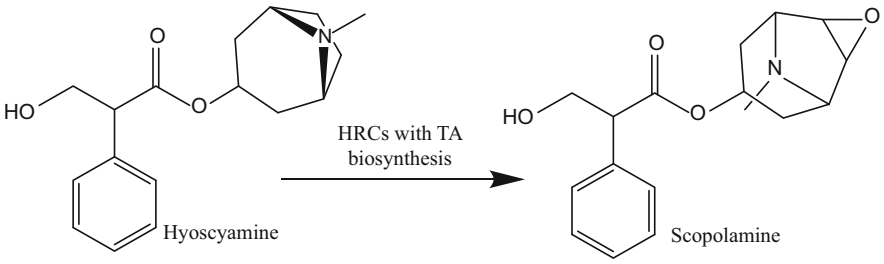
reactions takes place under very nominal in vitro conditions including optimum pH, temperature, etc. Thus, a thermolabile compound can be converted into product of interest using low energy / effort consumption, without undesired decomposition or isomerisation of substrate. Additionally, the biotransformation procedures do not require any hazardous solvents/reagents except the organic solvent(s), which are generally used to solubilize the substrate or making fraction/extract, which are volatile in nature and can easily separate out from reaction products. Therefore, with reduced usage of toxic chemicals the molecules thus produced are safer to use and does not require much toxicity analysis [1, 2]. In this way, biotransformation procedures carried out utilizing inherent enzymatic potential of plant cell and/or organ cultures are gaining recognition as environment friendly, sustainable, and safe methods to produce valuable compounds.

4 HRCs Mediated Various Biotransformation Strategies

4.1 Biotransformation Through Pathway Precursor Feeding

Hairy roots, due to their easy maintenance, offer a suitable tissue system for the elucidation of various metabolic pathways and investigation of various intermediates and precursor compounds in a pathway [11, 15]. Till date various SM biosynthetic pathways in different related or unrelated plant species have been partially or completely explicated. Tropane alkaloid (TA), terpenoid indole alkaloid (TIA), and benzyl isoquinoline alkaloid (BIA) pathways are such examples which are well understood not only in terms of their reaction steps but also for precursor, intermediates, and enzyme/genes required for various steps [8, 15]. Further, this has also paved the way for enhancement in product flux via precursor feeding (PF) [31, 32]. Sometimes, in spite of presence of adequate enzymatic activity and chemical ambience, the production of metabolite of interest is not satisfactory due to scarcity of its near or distant precursors. To address the problem, advantage of feeding precursor to the immediate environment of the system has been explored in many plants to get the metabolite of interest through biotransformation. The earlier studies, where addition of hyoscyamine to the growth medium containing HRCs of TA bearing plants individually, resulted improved production of scopolamine are the classical examples of precursor feeding (Scheme 2) [33]. In these studies, hyoscyamine was biotransformed into scopolamine utilizing enzymatic epoxidation (through H6H of TA pathway) reaction.

In a relatively recent study, silymarin accumulation was enhanced due to feeding of precursor compound in *Silybum marianum* HRCs. After feeding of *L*-phenylalanine as precursor in these HRCs, the accumulation of silymarin and naringenin was found to be significantly affected [34]. At present, PF is also being utilized as an integrative approach along with other enhancement methods like elicitation, metabolic engineering, and upscaling for desired metabolite production [35–37].



Scheme 2 Biotransformation of hyoscyamine to scopolamine in hairy root cultures of TA pathway bearing plants [33]

4.2 Biotransformation Using Co-culture Approach

Fundamentally, a co-culture system is a defined cultivation setup where two or more different populations of cells/tissues are grown together with distinct degree of contact. In recent years, in plant tissue culture with particular reference to secondary metabolite production through in vitro systems, the concept of co-culture systems have gained noteworthy interest [38]. Utilizing in vitro systems, the production of desired bioactive molecule is accomplished by acquiring the simplest form of any co-culture scheme by growing two complementary systems in one matrix (culture medium). The two systems complement each other in a way that one produces the substrate for the other to metabolize [39]. Here, the medium in which two systems grow behaves as a "mean of translocation." Apart from being beneficial for studying natural interaction between two or more related on unrelated cells/tissues/populations, this co-culture system has shown its suitability in secondary metabolite production through hairy roots co-cultured with in vitro growing shoots and/or cell suspensions of intra and/or inter species [39, 40]. For example, in vitro shoot and hairy root cultures of *Genista tinctoria* were co-cultured to produce detectable amounts of two pharmaceutically important secondary metabolites, viz. daidzin and daidzein. In this co-culture system, hairy roots produced isoliquiritigenin, a daidzein precursor absent in intact plant. The external supplementation of ABA in the medium resulted in release of isoliquiritigenin from G.tinctoria HRCs in the medium from which it was used by the shoots to convert it into significant amounts of daidzin and daidzein [40]. Further, successful production of podophyllotoxin from co-culture of *Linum flavum* hairy roots and *Podophyllum hexandrum* cell suspensions is also a relevant example [41]. In an earlier study *P. hexandrum* cell suspension cultures were found able to convert only coniferin out of seven precursors from the phenylpropanoid routing into podophyllotoxin, an important therapeutic compound widely used in drugs meant for the treatment of cancers [42]. Keeping this in mind, the co-culture system of *L. flavum* hairy roots and *P. hexandrum* cell suspension was developed [41]. Hairy roots of *L. flavum* naturally produce coniferin which is released in growth medium and utilized by the cells of *P. hexandrum* to produce podophyllotoxin. Similarly, the co-culture of *Ammi majus* hairy roots along with *Ruta graveolens* shoots for the synthesis of furanno coumarin

has been reported [43]. Sometimes hairy roots are also co-cultured in association with microbes and plant nematodes. For example, enhanced tanshinone production from *Salvia militiorrhiza* hairy roots was observed in a root-bacteria (*Bacillus cereus*) co-culture [44]. The results suggested the stimulation of tanshinone production by elicitors of bacterial origin in the root cultures. In a co-culture of *Solanum tuberosum* and root knot nematode *Meloidogyne chitwoodi* enhanced production of volatiles is reported [45]. Nevertheless, the latter two examples show perspectives of co-culture system involving HRCs in association with other organisms. These systems need thoughtful attention for their utilization in HRCs based biotransformation. Further, though such examples present a coordinated way of production and/or transformation of substrate molecule through two partners, yet, there are certain points that are needed to be scientifically attended. For example co-culturing requires extensive optimization and stabilization of growth of culture partners for incessant production. Additionally, deep insight of physiological behavior of partners and degree of their contact/separation also require attention for culture stability. Further, extending towards upscaling, the optimization of culture densities, volume of medium, the interaction of partners at different time scale, etc. are the points of consideration.

4.3 Biotransformation Using Nonspecific/Exogenous Molecules

It has been seen that many compounds are present in immensity in source plant system, but those are not biologically very active and valuable from commerce point of view. On the other hand, small structural diversification in these molecules can add or enhance their commercial significance [2]. Hairy roots have the ability to biochemically transform exogenously added foreign substrates into their corresponding derivatives (Table 2) [1, 2].

This system has been widely reported to utilize the desired regio-, stereo-, and enantioselectivity of the reaction and also substrate specificity. The kind of reaction explored depends upon the functional groups in the substrate and the type of enzyme present in host system. HRCs of various plant species have been utilized for the bioconversion of a variety of exogenous compounds supplemented in their growth medium through distinguished reaction types [2, 17]. A comprehensive account on HRCs of different plant species, transformed products, and reaction types has been earlier reviewed by Banerjee et al. [2]. Bioconversion of exogenous molecules is most widely applied in HRCs based biotransformation processes and subject of choice for generation/derivatization of potential molecules. The substrate for this purpose ranges from synthetic to natural origin. In a recent example of HRCs based biotransformation of nonspecific exogenous molecule, bioconversion of artemisinin into its derivative compounds is reported [46]. These derivative compounds reveal significant antimalarial activity and tumor necrosis factor (TNF) lowering potential.

The HRCs of representative genera and species from various plant families like Asteraceae, Solanaceae, Campanulaceae, Brassicaceae, Apiaceae, Lamiaceae, etc. have been exploited for biotransformation of exogenous molecules with almost no

Table 2 Biotransformation of exogenous molecules utilizing hairy root cultures

Plant	Reaction explored	Substrate	Product	Reference
Atropa belladonna	Oxidation and reduction	3,4,5-trimethoxy benzaldehyde	3,4,5-trimethoxy benzoic acid and 3,4,5-trimethoxy benzyl alcohol	[22]
	Reduction	3,4,5-trimethoxy acetophenone	1-(3,4,5-trimethoxy phenyl)-ethanol	[22]
Atropa belladonna	Oxidation and glycosylation	Betuligenol	Raspberry ketone and betuloside	[20]
Atropa belladonna; Hyoscyamus muticus; Ocimum basilicum	–	Artemisinin	3-α-hydroxy-1-deoxyartemisinin; 4-hydroxy-9,10-dimethyloctahydrofuro-(3,2-i) - isochromen-11(4H)-one	[46]
Nicotiana tabacum (expressing 35S-*Ri*ZS1- 4-OHBA reductase)	Reduction	4-hydroxybenzalacetone (4-OHBA)	Raspberry ketone	[50]
Catharanthus roseus	Reduction and oxidation	4-hydroxybenzalacetone and betuligenol	Raspberry ketone	[50]
N. tabacum SR1	Reduction and oxidation	4-hydroxybenzalacetone and betuligenol	Raspberry ketone	[50]
Hyoscyamus muticus	Reduction	4-hydroxybenzalacetone	Raspberry ketone	[50]
Rhodiola kirilowii	Glycosylation	Cinnamyl alcohol	Rosarin and rosavins	[66]
Polygonum multiflorum	Glycosylation	Esculetin and eight synthetic hydroxyl coumarins	Corresponding glycosides	[62]
Datura tatula L.	Glucosylation	p-hydroxybenzyl alcohol	Gastrodin	[57]
Polygonum multiflorum	Glycosylation	7-hydroxy-4-phenylcoumarin and 5,7-dihydroxy-4-phenylcoumarin	4-phenylcoumarin-7-*O*-*β*-D-glucopyranoside and 7-hydroxy-4-phenylcoumarin-5-*O*-*β*-D-glucopyranoside	[61]

Panax ginseng	Glycosylation and hydrolysis	Tetrahydroprotoberberines (THPBs)	Corresponding glycosides	[21]
Polygonum multiflorum	Glycosylation	7-Hydroxy-2,3-dihydrocycl openta [c]chromen-4-one, 7,9-dihydroxy-2,3-dihydrocyclopenta[c]chromen-4-one and 6,7-dihydroxy-2,3-dihydrocyclopenta[c]chromen-4-one	7-*O*-β-D-glucopyranosyl-2,3-dihydrocyclopenta[c]chromen-4-one, 9-*O*-β-D-glucopyranosyl-7-hydroxy-2,3-dihydrocyclop enta[c]chromen-4-one and 6-*O*-β-D-glucopyranosyl-7-hydroxy-2,3-dihydrocyclop enta[c]chromen-4-one	[60]
Nicotiana tabacum	Glycosylation	Dehydroabietic acid (DHA)	DHA-18-*O*-glucoside	[50]
Catharanthus roseus	Hydroxylation and glycosylation	DHA	17-hydroxy-DHA, DHA-17-*O*-α- glucoside and DHA-17-*O*-β-glucoside	[50]

dependency on additional biosynthetic pathway (secondary metabolic pathway) [2]. Among the plant systems explored, the maximum utilization of HRCs of *P. multiflorum* [24, 26], *P. ginseng* [18, 21, 47], *P. nil* [23, 30], and *A. belladonna* [17, 20, 22, 46] for the bioconversion of variety of substrate has been reported. These reports advocate the wide potential of hairy roots to perform variety of reactions on structurally unrelated exogenous molecules to produce corresponding compounds with more desired chemical properties (Table 2).

5 Reactions Carried Out by Hairy Roots

5.1 Reduction

A variety of carbonyl substrates undergo hairy root based enzymatic reduction and produce primary or secondary alcohols. The HRCs of *A. belladonna* [22], *P. multiflorum* [26], and *P. nil* [30] clearly exhibit aldehyde reduction. Besides, HRCs of *A. belladonna* [17, 21] also shares the property of acetophenone/ketone reduction with HRCs of *Daucas carota* [25], *Brassica napus* [27], and *Raphanus sativa* [27, 28]. Additionally, HRCs of few plant species have demonstrated biotransformation of artemisinin to its reduced derivative, i.e., deoxy artemisinin (e.g., *Cyanotis arachnoidea* and *Rheum palmatum*) [48, 49]. Recently, the biotransformation of 4-OHBA (p-hydroxybenzalacetone) into RK (raspberry ketone) utilizing cell cultures of plants of different families have also demonstrated reduction reactions [50].

5.2 Oxidation

The oxidation reactions in biotransformation procedures are confined mainly to aldehyde and alcohol substrate. The oxidation of aldehyde, a reaction mostly favored by microbes, is normally less observed in hairy roots based conversions. Despite, *A. belladonna* HRCs are known to perform this reaction against 3,4,5-trimethoxy benzaldehyde (TBA) leading to production of 3,4,5-trimethoxy benzoic acid [22]. Additionally, the same HRCs have also exhibited oxidation of betuligenol (BG; secondary alcohol) to raspberry ketone (RK) [20]. Furthermore, HRCs of *C. roseus* and *N. tabacum* also demonstrated oxidation of BG into RK (Scheme 1) [50]. The other significant example of oxidation of substrate was depicted by HRCs of *Levisticum officinale* [51]. This report describes that exogenous supplementation of geraniol in growth medium as a substrate induced production of six new volatiles out of which some were oxidized products. Likewise, hairy roots of *Anisodus tanguiticus* exhibited conversion of dehydroepiandrosterone (DHEA), as a substrate for oxidation into its corresponding compounds [52].

5.3 Esterification

The esterification reactions involve formation of ester bond in substrate. HRCs of *P. ginseng* have demonstrated esterification of digitoxigenin into different esters such as digitoxigenin stearate, digitoxigenin palmitate, digitoxigenin laurate, and digitoxigenin myristate [53].

5.4 Acetylation

Acetylation of exogenously supplemented monoterpenes (menthol and geraniol) was observed in HRCs of *Anethum graveolens* [54]. In this case, menthyl acetate was formed as biotransformation product of menthol, whereas geraniol was transformed into ten new products which were alcohols (linalool, alpha-terpineol, and citronellol), aldehydes (neral and geranial), esters (citronellyl, neryl) and geranyl acetates etc. Substrate specificity in biotransformation was also observed with *L. officinale* HRCs [51]. In comparison to hairy roots of *A. graveolens* [54] which acetylate both monoterpenes (menthol and geraniol) in *L. officinale* acetylation was restricted to geraniol only [51].

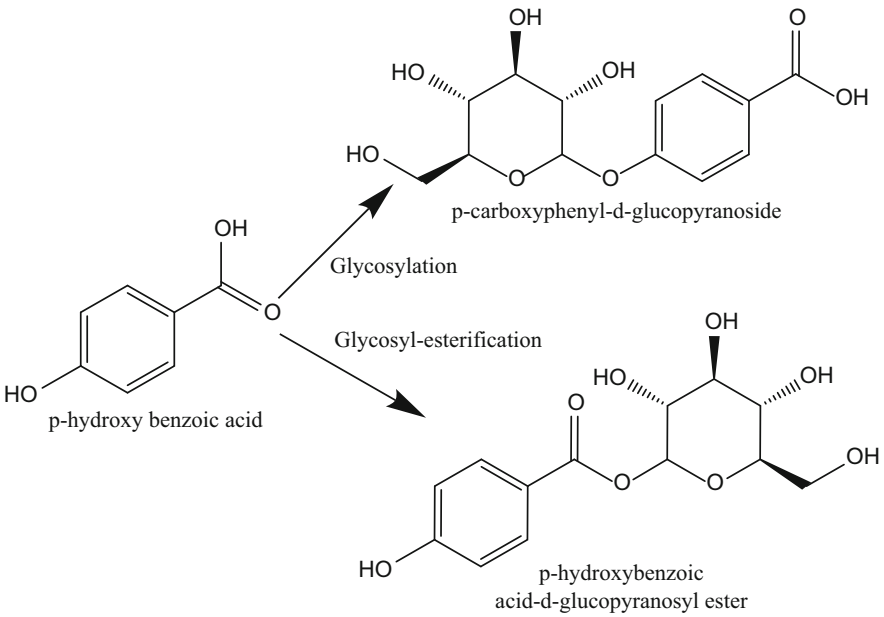
5.5 Hydroxylation

The bioconversions utilizing hydroxylation reactions are well explored in plant cell suspension cultures [1, 55]. However, HRCs has also been documented to acquire biotransformation utilizing hydroxylation reactions. Derivatization of trans-cinnamic acid into *p*-coumaric acid has been achieved by hydroxylation utilizing HRCs of *Lobelia sessilifolia, L. cardinalis, Campanula medium,* and *Fragaria xananassa* [56].

5.6 Glycosyl Conjugation

The biochemical reactions in which a carbohydrate moiety (saccharide unit) conjugates with any acceptor molecule thereby making it hydrophilic (water soluble) comes under glycosyl conjugation. The acceptor molecule can be protein, lipid, or any other organic molecule. It is later most category of acceptor which is normally targeted in biotransformation. Two kinds of glycosyl conjugation have been observed in plant hairy root systems. First is glycosyl esterification, which occurs as a consequence of esterification of carboxylic acids and sugar moieties, and second is glycosylation, which occurs due to ether formation of alcohols and sugar moieties. The glycosylation and glycosyl esterification of *p*-hydroxy benzoic acid has been noticed in *P. ginseng* HRCs (Scheme 3) [18].

Besides, glycosylation has also been observed in HRCs of various plants utilizing diverse substrate backbone [2]. It is the most explored biochemical reaction through



Scheme 3 Biotransformation of *p*-hydroxy benzoic acid utilizing *Panax ginseng* HRCs showing glycosylation and glycosyl esterification [18]

HRCs mediated biotransformation [2, 17]. The production of gastrodin as a consequence of glycosylation of 4-hydroxybenzyl alcohol was obtained by HRCs of *Datura tatula* [57]. Glucosylation of thymol into glucoside-5-methyl-2-(1-methylethyl) phenyl-*β*-D-glucopyranoside has been observed in HRCs of *P. multiflorum* [58].

6 Transgenic Hairy Roots and Biotransformation

Approaches like clonal selection, physical elicitation, permeabilization, alteration of chemical conditions, etc. are normally used to optimize the production potential of an in vitro system. Transfer and overexpression of candidate genes of required reactions in a pathway is one such approach that has gained much interest in hairy root mediated research. Being unique in their genetic and long lasting biosynthetic stability, HRCs have been sought as a competent means of biomass production concomitant with their use for transforming various substrates into their analogs. Besides, its recognition as a proficient system to study transfer and expression of gene segment relevant to metabolic pathways has also paved the way for transgene functional characterization. Additionally, in the context of biotransformation, use of well established HRCs appears more expedient as most of the studies do not require transgenic plant development. To be precise, with reference to the biotransformation

of exogenous/inherent compound utilizing enzymatic machinery of HRCs, identification and characterization of enzymes and related genes involved in biotransformation will lead to their isolation and further utilization through engineering approaches. Enhancement of biotransformation potential of selected HRCs can be done either by modulating expression of gene already existing in the system or through heterologous overexpression. Vanillin production in transgenic HRCs of *Beta vulagaris* through heterologous expression of *Pseudomonas fluorescens* HCHL gene is a relevant example to discuss [59]. The study demonstrated bioconversion of inherently available ferulic acid into vanillin through bacterial HCHL overexpression. Besides, this study also showed enhancement of vanillin production after ferulic acid PF. Similarly, in a recent study, raspberry ketone, a natural flavor compound of high commercial value, was produced through transgenic HRCs of *Nicotiana tabacum* [50]. The study revealed that overexpression of *Rubus idaeus* ketone/zingerone synthase1 (*Ri*ZS1), a NADPH-dependent reductase, which is found to be responsible for the production of raspberry ketone from *p*-hydroxybenzalacetone (4-OHBA), resulted in accumulation of ketone. On the whole, these results persuade the use of infinite opportunities to develop and explore transgenic HRCs for biotransformation of a wide range of chemical compounds through activation or suppression of functional genes [11, 14].

7 Recent Reports on HRCs Based Biotransformation and Production of High Value Compounds

The research area of HRCs mediated biotransformation is still in its infancy as hairy roots of only few plant systems have been explored for this purpose [2]. The progress in this field can be measured in terms of establishment of new candidate HRCs systems solely for bioconversions as well as through the widening of substrate range. Out of many successful biotransformation reports, the significance of some of the biotransformed products has also been observed as listed in Table 3.

A detailed account on different hairy root systems, biotransformation reactions carried out there with, and transformed products has been reviewed by Banerjee et al. 2012 [2]. The upcoming text, therefore, discusses recent reports illustrating different hairy root systems used for biotransformation of various substrates and thereby production of commercially more valuable corresponding compounds (Tables 2 and 3).

HRCs of Atropa belladonna: The biotransformation capability of *A. belladonna* HRCs has been investigated utilizing three carbonyl substrates [22]. Out of three substrate utilized, TBA and 3,4,5-trimethoxy acetophenone got biotransformed; however, no biotransformation product was detected for 3,4,5-trimethoxy benzoic acid. The TBA was transformed into 3,4,5-trimethoxy benzoic acid and 3,4,5-trimethoxy benzyl alcohol after oxidation and reduction of substrate, respectively. The reduction of 3,4,5-trimethoxy acetophenone was also achieved resulting into 1-(3,4,5-trimethoxyphenyl)-ethanol. Utilizing this HRCs biotransformation of other substrates of different backbone was also investigated [20, 46]. The

Table 3 Production of value added molecules through HRCs mediated biotransformation

Transformed products	Therapeutic significance	Parent molecule	Hairy roots system	Reference
Gastrodin	Anti-inflammatory, anticonvulsion, analgesic, and antianoxemia	p-hydroxybenzyl alcohol (HBA)	*Datura tatula*	[57]
		4-hydroxybenzaldehyde and 4-hydroxybenzyl alcohol	*Polygonum multiflorum*	[26]
Raspberry ketone	Flavor molecule, antiobese, antibacterial, anticancer, and depigmenting activities	Betuligenol	*Atropa belladonna*	[20]
		Betuligenol and 4-OHBA	*Nicotiana tabacum; Catharanthus roseus*	[50]
		4-OHBA	*Hyoscyamus muticus*	[50]
Betuloside	Anti-inflammatory and hepatoprotective	Betuligenol	*Atropa belladonna*	[20]
Arbutin	Skin-lightening agent	Hydroquinone	*Brugmansia candida,*	[67]
			Physalis ixocarpa	[68]
		1–4-benzendiol	*Polygonum multiflorum*	[26]
3-α-hydroxy-1-deoxyartemsinin	Antiplasmodial and TNF lowering potential	Artemisinin	*Atropa belladonna; Hyoscyamus muticus; Ocimum basilicum*	[46]
Rosavin	Antidepressant and anxiolytic	Cinnamyl alcohol	*Rhodiola kirilowii*	[66]

biotransformation of 4-(*p*-hydroxyphenyl)-butan-2-ol or BG exhibited oxidation and glucosylation of substrate into raspberry ketone and betuloside, respectively (Scheme 1). Both of the derivatives were found to have significant pharmaceutical interest (Table 3).

HRCs of A. belladonna, *Hyoscyamus muticus*, and *Ocimum basilicum:* Pandey et al. (2015) have reported the biotransformation of antimalarial molecule artemisinin utilizing HRCs of *A. belladonna, H. muticus*, and *O. basilicum* [46]. The biotransformation reaction resulted into 3-α-hydroxy-1-deoxyartemisinin and 4-hydroxy-9,10-dimethyloctahydrofuro-(3,2-i)-isochromen-11(4H)-one. The HRCs of *H. muticus* and *A. belladonna* were found superior, and 3-α-hydroxy-1-deoxyartemisinin exhibit antiplasmodial activity with notable TNF lowering potential.

HRCs of Polygonum multiflorum: The glycosylation of 3,4-cyclocondensed coumarins (7-Hydroxy-2,3-dihydrocyclopenta[c]chromen-4-one,7,9-dihydroxy-2,3-dihydrocyclopenta[c]chromen-4-one, 6,7-dihydroxy-2,3-dihydrocyclopenta[c]chromen-4-one) have been evaluated [60]. This led to the formation of its corresponding glycosides. Moreover, this study also highlights the use of elicitation with salicylic acid (SA), yeast extract (YE), and $HgCl_2$ for enhancement of biotransformation reactions. Out of tested elicitors, the biotransformation reaction was enhanced by SA (11%) and YE (12%).

The glycosylation of two 4-phenylcoumarins (7-hydroxy-4-phenylcoumarin and 5,7-dihydroxy-4-phenylcoumarin) was also achieved utilizing *P. multiflorum* HRCs [61]. Additionally, other substrates were also analyzed for biotransformation utilizing *P. multiflorum* HRCs [62–65].

HRCs of P. ginseng: The biotransformation of tetrahydroprotoberberines (THPBs) was investigated utilizing *P. ginseng* HRCs. This substrate though alkaloid structurally also bear phenolic hydroxyl groups. The study demonstrates efficient glycosylation of THPBs having single phenolic hydroxyl and has presented no stereoselectivity. Furthermore, the culture also demonstrated hydrolysis of other substrate followed by glycosylation [21].

HRCs of Rhodiola kirilowii: The biotransformation of cinnamyl alcohol into corresponding glycosides (rosarin and rosavin) was achieved utilizing HRCs of *R. kirilowii* [66].

8 Conclusions

The hairy root research till now has made its significant contribution in plant biotechnology, and HRCs are being globally explored to investigate diverse aspects of hairy root biotechnology right from root initiation to translational prospects [8, 9, 11, 14]. This is the reason behind continuous inflow of reports involving hairy root establishment in diverse plant species of which number even crossed 0.5 K as per recent reviews [11, 14]. With such immense interest, the curiosity related to the application of HRCs also transited towards metabolism, elicitation, phytoremediation, metabolic engineering, and biotransformation from hairy root based traditional SM production. Despite, the HRCs are also proving worthwhile to study elemental research related to root biology [11, 14].

The biotransformation though an old practice since the beginning of civilization simultaneously recognized as an efficient step towards green chemistry. Biotransformation exists in diverse life forms under natural conditions, and exploitation of this ability of living entities into the production process of wider utility compounds has been judiciously focussed. In context of biotransformation mediated by different plant in vitro systems, the indubitable superiority of HRCs in terms of cost, maintenance, stability, and enzymatic potential has been well established [2, 17]. The HRCs based biotransformation procedures involve different strategies which are precursor feeding, co-culture system, and exogenous molecule supplementation. Whichever the strategy involved, the rational is either to enhance inherent potential

of machinery or to get rid of substrate (precursor) limitation. The reaction potential here provides the additional advantage of regiospecificity, enantioselectivity, and almost nominal culture conditions. The literature suggested that diverse substrates which include phenolics, carbonyl compounds, coumarins, terpenes, etc. have been explored for biotransformation utilizing HRCs. Besides, the production of significant molecules has also been achieved which validates the concept of "value addition" through biotransformation (Table 3).

Altogether, many successful hairy root systems have been established that can be considered for having potential prospects for commercial scale setups. However, the incorporation of integrative approaches like elicitation and gene overexpression is also needed to explore broader utility of HRCs based transformation. Further, optimization of various other factors like selection of suitable hairy root system with desired enzymatic pool, time scale management for different reactions to deal with a reaction flexible system, optimization of growth medium composition, and consumption require special attention to develop a successful HRCs based biotransformation system.

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