Journal of Applied Botany and Green Economy
1(1): 2026

Verdant Legacy


JOURNAL OF
APPLIED BOTANY
AND GREEN ECONOMY

Review Article

Advancing Secondary Metabolite Production: Insights into Hairy Root Systems, Meta-Omics Integration, and Process Intensification in Solanaceae

Fatima Zahid [1], Hafiza Asma Parveen[1], Aysha Arif Chahel[1*], Jaweria Kiran, Kashmala Farooq[1], Mahnoor Khan[1], Manahil Naeem[1], Asfa BiBi [1], Nimra Hazoor Buksh[1]

[1]Department of Botany, The Govt. Sadiq College Women University Bahawalpur, Pakistan;

fatimazahid2911@gmail.com; asmnshf96@gmail.com; aysha.chahel@gscwu.edu.pk; jaweriakiran7@gmail.com; kashmalfarooq@gmail.com; M.K; mahnoorkhan12201@gmail.com; itsmanahilnaeem@gmail.com; A.B; asfasaqi2@gmail.com; N.H.B; balouchnimra1@gmail.com

[*]Corresponding authors: aysha.chahel@gscwu.edu.pk

Abstract

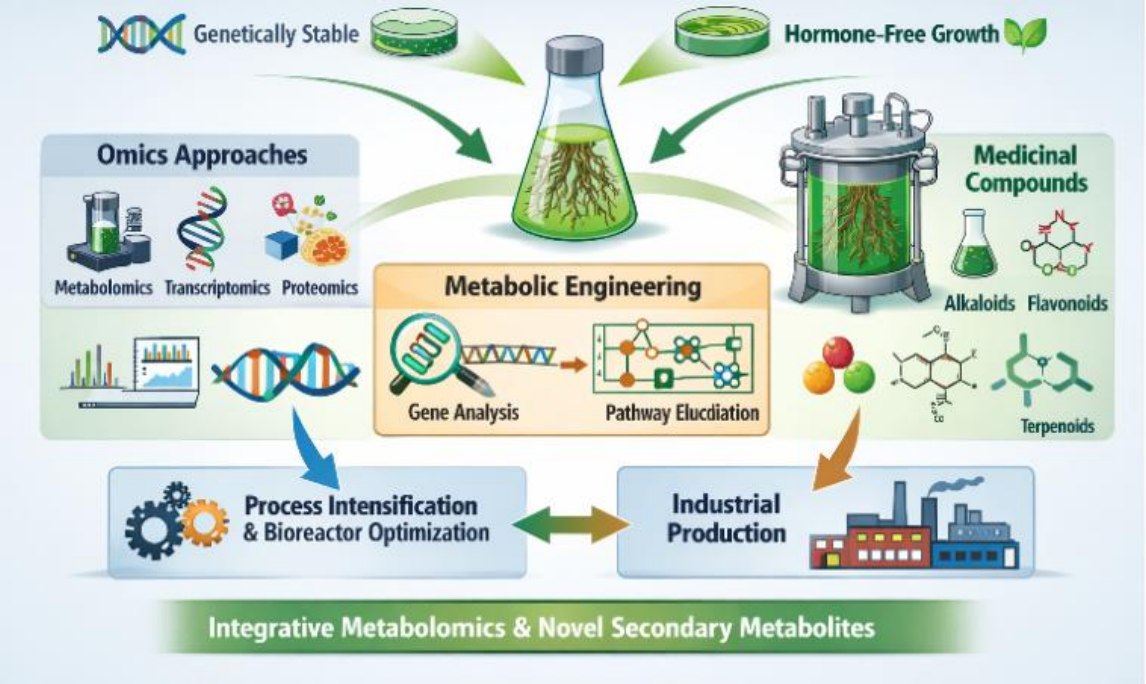
By the mid-1980s, in vitro–produced hairy roots became an effective system for studying plant secondary metabolite biosynthesis. Hairy roots are genetically stable, highly reproducible, and grow rapidly on low-cost media without phytohormones, making them suitable for metabolic studies and bioprocessing. Their application has advanced research on gene function, enzyme regulation, and targeted metabolite production. Understanding complex biosynthetic pathways requires integrated metabolite profiling with multi-omics approaches, particularly transcriptomics (RNA sequencing). Such integrative meta-omics frameworks offer valuable insights into metabolite synthesis in medicinal plants. Species of the Solanaceae family are notable for their nutritional and therapeutic importance and their ability to produce diverse bioactive compounds, including alkaloids, flavonoids, glycosides, and terpenoids. This review highlights recent progress in enhancing secondary metabolite production through improved hairy root induction, process intensification, bioreactor optimization, and integrative metabolomics–transcriptomics strategies for identifying species with industrial potential.

Keywords: Secondary metabolites, meta-omics, transcript profiling, metabolic engineering, bioprocessing;

Received: 31 December 2025 **Accepted**: 20 January 2026 **Published**: 31 January 2026

Citation: Zahid F., H. A. Parveen, A. A. Chahel, J. Kiran, K. Farooq, M. Khan, M. Naeem, A. BiBi, N. H. Buksh (2026).Advancing Secondary Metabolite Production: Insights into Hairy Root Systems, Meta-Omics Integration, and Process Intensification in Solanaceae. Journal of Applied Botany and Green Economy, 1(1): 73-101

Graphical Abstract



1. Introduction

The *Solanaceae* ranked as the third most significant plant family (exceeded barely by grasses and legumes). Nearly 3,000 plant species belong to the *Solanaceae family* (Griffin *et al.*, 2002). It comprises approximately 98 genera and almost 2,700 species for an incredible range of habitats, morphology and ecology. It includes various economically essential crops, such as tomato, potato, petunia, tobacco, pepper and eggplant (Knapp *et al.*, 2004). More than 60% of the species belong to eight genera: *Solanum, Lycianthes, Cestrum, Nolana, Physalis, Lycium, Nicotiana, and Brunfelsia* (Griffin *et al.*, 2002). Most of these plants remain valuable starting materials for plant-derived pharmaceuticals and other fine chemicals. Recently, critical advancements have been made to increase the levels of beneficial secondary metabolites (such as carotenoids and anthocyanins) in *Lycium* fruits by metabolic engineering or breeding (Liu *et al*., 2014). Because of the industrial importance of the family *Solanaceae* and the availability of genetic and genomic resources, various investigations have concentrated on species of *Solanaceae*. In addition, most of the species have a precise propagation time and are best suited for *in vitro* conditions and methods of transformation. In the initial period of tissue culture research, root cultures presented limited activity in this field because of their retarded rate of growth. Although root and shoot organ cultures have been used successfully for alkaloid analysis, coumarins, saponins (Kusakari *et al.*, 2000), phenolic acids (Karam *et al.*, 2003), terpenes, glycosides and steroidal lactones (Ray & Jha, 2001) have been used.

Recently, Solanaceae crops have also been suggested as profitable industrial products for the production of various secondary metabolites and other pharmaceutical proteins. With the development of *in vitro* hairy root systems, this approach has prompted significant improvements to obtain insights into the biological processes of different plant species. Through genetic modification, the engineering of *Solanaceae* food crops has generally been practiced to increase the features of these crops. Furthermore, the hairy root production of various species is being studied. However, from the perspective of increasing concern in the *Solanaceae* family, this article outlines the current advancements in hairy root development, methods of elicitation to increase the yield of secondary metabolites, and the use of multiple omics technologies to highlight the pharmaceutical and industrial importance of food crops belonging to this significant family.

2. Effect of *Agrobacterium rhizogenes* genes and current developments in their understanding:

The term ‘hairy root’ for diseased fruit crops looks like small, thin hair-like extensions of roots usually emerge from infected parts of plants as an aftereffect of microbial infection (Fukuda, 2001). The phrase ‘hairy root syndrome’ was coined by Hildebrandt (Fukuda, 2001) for these discrete disease symptoms. However, Riker *et al.* (1930) were the first to distinguish the causal agent, characterize it, and name it *Agrobacterium rhizogenes*. Currently, *A. rhizogenes* is also called *Rhizobium rhizogenes*. It is a gram-negative, rod-shaped, soil-borne pathogenic bacteria and a determinable member of the genus *Agrobacterium* of *Rhizobiaceae* (Gelvin, 2003; Veena & Taylor, 2007; http://www.Agrobacterium.org). Naturally, the host range of *A. rhizogenes* is limited to a few dicotyledonous plant species. However, under *in vitro* culture conditions, a vast range of plant species can produce hairy roots from monocotyledons to gymnosperms (Chandra, 2012). During plant–pathogen interactions (at the time of infection), the T-DNA of *Agrobacterium* is exchanged with that of host cells. For adequate integration of T-DNA and targeted host cells, few chromosomal genes (*chvA*, *chvB*), *vir* (*virD1*, *virD2*, and *virE1*, *virE2*) and T-DNA genes (present on bacterial pRi) are needed at different times during infection (Chandra, 2012). These *Agrobacterium rhizogenes* mediated transgenic hairy roots can be removed from ex-plants and maintained as axenic cultures. These uncontaminated cultures rapidly grow on growth media without exogenous hormones. Additionally, these roots exhibit plagiotropic growth, profused sidewise branching and fast root tip prolongation (Bonhom *et al*., 2000). Hence, via hairy root technology, transgenic roots can be produced via the introduction of foreign genes via binary vectors (Fukuda, 2001). In *Datura stramonium,* the fastest biomass accumulation period is 1 day, which is comparable to the high biomass aggregation period. However, in transgenic root cultivars, which are not superior, cell cultures are disorganized (Lux *et al.,* 2011). The circumstance of such transformation methodology is that every first root generated at the point of infection is an indication of a single transformation occurrence (Bonhom *et al.,* 2000). Nevertheless, soma-clonal mutations in transgenic root cultivars have also been noted (Sevón & Oksman-Caldentey, 2002; Wilhelmson *et al*., 2005). Transgenic hairy root cultures have been maintained on a significant number of medicinally important plant species (Sevón & Oksman-Caldentey, 2002). Genetically, transgenic clones differ in terms of their morphology (Oksman-Cladentey, 2002), growth, and metabolite production (Batra *et al.*, 2004). This phenomenon is associated with the type of host, infection point, and amount of T-DNA assimilated into the genome of the targeted host. Hence, clone determination might be useful for metabolite profiling through the transgenic hairy root system (Kim *et al.*, 2001). Transgenic hairy root clones of *Datura stramonium* remained genetically and biosynthetically stable for a span of 5 years, with continuous root development and the production of alkaloids (Bulgakov *et al.*, 2002). These roots also presented high levels of cytogenetic stability (Bulgakov *et al.*, 2002). Interestingly, after cryopreservation in *Beta vulgaris* and *Nicotiana rustica* (Dougherty & Morrison, 2004), the production of secondary metabolites, growth rate, and structure of T-DNA in various transgenic root clones remained unaltered.

However, under *in vitro* conditions, the developmental rate of tropane alkaloid generation is progressively reduced in *Duboisia myoporoides* (Kim *et al.*, 2001). Thus, improving a suitable protocol to successfully induce transgenic hairy roots via *Agrobacterium rhizogenes* is critical for establishing an *in vitro* culture system for massive secondary metabolite production. The choice of an efficient strain of *Agrobacterium* for hairy root production is profoundly plant species specific. The successful *A. rhizogenes* strains in the Solanaceae family are provided in Table 1. The effectiveness of various *A. rhizogenes* strains for the production of transgenic hairy root lines was explored. Additionally, the mass accumulation of secondary metabolites is frequently correlated with plant infection with *A. rhizogenes*. This knockout phenomenon has been followed in several Solanaceae plant species. Compelling investigations have been carried out to identify gene loci. Transfer DNA (T-DNA) is important for understanding the term *rol* gene succession. The *rol* genes include the *rolA*, *rolB*, and *rolC* genes. A variety of secondary metabolites can be produced individually by these genes (Palazon *et al*., 1997; Bulgakov *et al.*, 2002). Presumably, *rolB* is a capable inducer for a large part of secondary metabolism accompanied by the *rolC* gene (Bulgakov *et al.*, 2002).

However, a precise system that intermediate secondary metabolite production through *rol* genes is not yet known. Research conducted in the past has demonstrated that the *rol* genes may provide an essential boost to trigger various signal transduction pathways. The *rolB* gene is directly linked to the activity of tyrosine phosphatase and is competent with 14-3-3 proteins (Facchini and Pierre, 2005; Moriuchi *et al*., 2004). Interestingly, these proteins play a paramount role in cell division, growth, differentiation, and death (Dougherty & Morrison, 2004). In plants, tyrosine phosphatases control the major functions of different kinases, transcription factors, and defense signaling molecules. The *rolC* gene has important regulatory effects on different cellular and defense signaling pathways (Laloi *et al*., 2004).

Table 1: Secondary metabolite production and role of different strains of *A. rhizogenes* in the Solanaceae family in hairy root culture

Plant	**Secondary metabolite**	***A. rhizogenes* strain**	**Tissue/organ as an ex-plant**	**Tested genes**	**Reference**
Atropa acuminata	Scopolamine	LBA 9402	Leaves	*rol* genes	Banerjee *et al.* (2008)
Atropa belladonna	Atropine, scopolamine	15834	Stem	*rol* genes	Banerjee *et al.* (2008)
Atropa belladonna	Hyoscyamine, 6-b, hydroxyhyoscyamin,scopolamine, littorine	ATCC 15834, MAFF 03-01724	Leaf segments	*rolA*, *rolB*	Hong, 2006
Atropa belladonna	Scopolamine, hyoscyamine	AR15834	Node, internodes	*rolB*	Chashmi *et al.* (2010)
Brugmansia candida	Scopolamine, hyoscyamine	LBA 9402	Stem	*rol* genes	Spollansky *et al.* (2000)
Brugmansia candida	Cadaverine, putrescine, spermidine,	LBA 9402	Leaves	*rol* genes	Carrizo *et al.* (2001)
Brugmansia suaveolens	Tropine, pseudotropine, scopoline, scopine,hyoscyamine	15834, TR 105	Root tips	*rolC* & *virC*	Zayed & Wink (2004)
Datura stramonium	Hyoscyamine, apohyoscyamine Hyoscyamine, scopolamine Hygrine,	LBA 9402, 1855, AR-10, TR-105,ATCC15834,A4, A41027,15834	Stem and leaf segments	*rolB* & *rolC*	Berkov *et al.* (2003)
Duboisia leichhardtii	Scopolamine	15834, A4	Root segments	*rolB* & *rolC*	Kim *et al.* (2001)
Hyoscyamus albus	Atropine	LBA9402, A4	Leaf	*rolB* & *rolC*	Zahwa *et al.* (2014)
Hyoscyamus muticus	Scopolamine, hyoscyamine	LBA9402, A4	Leaf and nodal segments	*rolB* & *rolC*	Zolala *et al.* (2007)
Nicotiana tabacum cv. Xanthi	Hyoscyamine, scopolamine, nicotine, nornicotine, anabasine,	LBA9402, LBA9402 pLAL21	Leaves	*h6h* gene	Häkkinen *et al.* (2005)
Scopolia parviflora	Scopolamine, hyoscyamine	KCTC 2703 harboring pBEpmt plasmid	Stem tissues	*pmt* gene	Lee *et al.* (2005)
Withania somnifera	Withasteroids	LBA 9402, A4	Shoot segments	*rolB* and *rolC*	Ray and Jha (2001)

2.1: Perspective of transgenic hairy root cultures:

Currently, more than 50 angiospermic plant families with more than 150 genera and various species are being studied for hairy root production (Ono, 2011). The development of the *in vitro* hairy root system begins. Additionally, this approach has led to significant improvements in obtaining insights into the biological processes of different plant species (Figure 1). Unremitting research for decades approximately 1980 and 1990 not only included the best vital subtle fact to *Agrobacterium* biology but also investigated the natural characteristics of genetic transformation on a molecular basis.

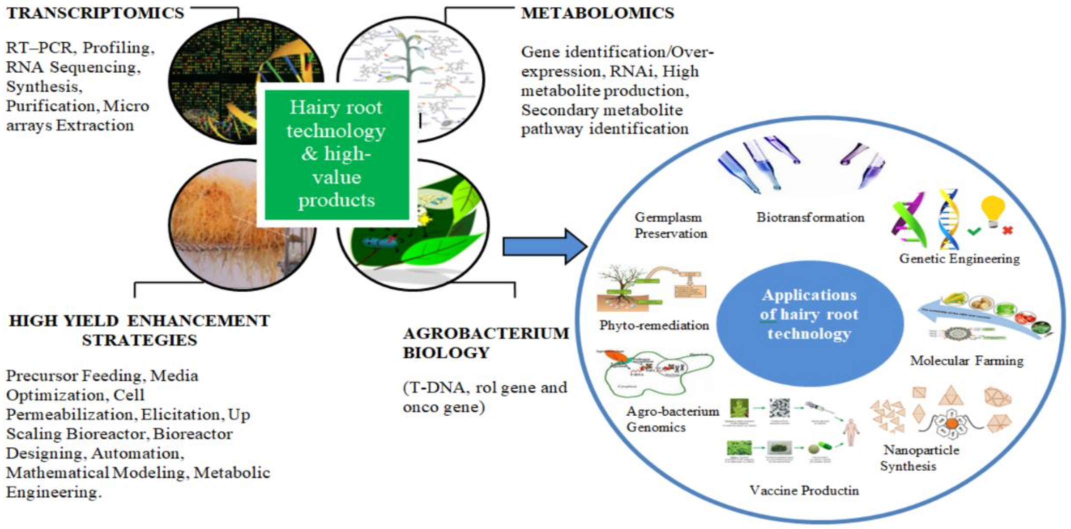


Figure 1: Schematic diagram of hairy root biotechnology and its applications.

2.2: Biosynthesis and localization of secondary metabolites in various tissues or organs:

The production of specific metabolites in plants, according to various studies, is restricted to specific tissues or organs, and their potential is missing in dedifferentiated cells. This may be a direct result of the expression of many genes responsible for particular secondary metabolite pathways. This may be specific to the cell, tissue, or developmental stage (Facchini & St-Pierre, 2005). For *example, withania somnifera*, withanolides are present predominantly in aerial tissues. Therefore, the biosynthetic pathway for withanolides is independent of the roots and shoot organs (Sangwana *et al.*, 2008). The general pathways of secondary metabolite production are represented in Figure 2. Benzylisoquinoline alkaloids in *Papaver somniferum* (codeine, thebaine) accumulate in whole plants but are biosynthesized and translocated by both roots and shoots (Drager B., 2006). Similarly, the biosynthetic pathway for monoterpenoid indole alkaloids (MIAs) in *Catharanthus roseus* involves both the underground and aerial parts of plants, i.e., the roots and leaves (De Luca & St Pierre, 2000). Another kind of terpenoidindole alkaloid, camptothecin, discovered in *Catharanthus acuminata*, *is* produced in each organ of the plant (particularly in newly formed leaves and ripened fruit), although its synthesis is limited to mainly leaves and stems (Valletta *et al*., 2010). *W. somnifera*, *P. somniferum*, and *C. acuminata* have been shown to significantly affect the production of their particular metabolites in their transgenic hairy root cultures (Murthy *et al*., 2008; Lorence *et al.*, 2004). Hairy root cultures of *C. roseus* effectively generated early-pathway MIAs, such as ajmalicine, serpentine, and tabersonine. These results indicate that roots are basic sites of the biosynthesis and accumulation of these secondary metabolites. Alternatively, the locations of the biosynthesis and localization of vindoline in hairy root cultures of *C. roseus* may involve aerial tissues. For example, vinblastin and vincristine are synthesized in highly specific cell types of aerial tissues. For these metabolites, hairy root cultures might not be able to prepare late-pathway MIA (Rothe *et al*., 2003; Murata *et al.*, 2008). Therefore, hairy root cultures have attracted increasing interest, particularly for controlling those metabolites whose accumulation sites are roots but are not formed in calluses or suspension cultures (Mishra & Ranjan, 2008).

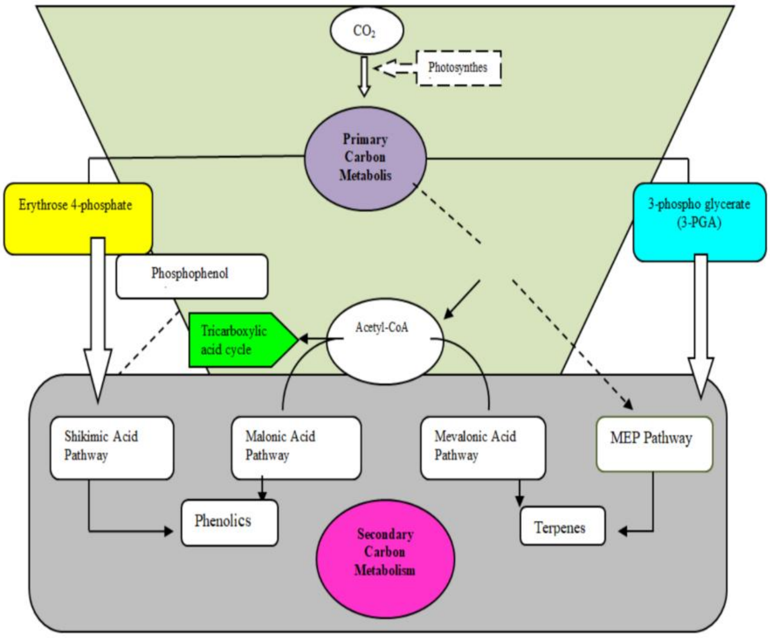


Figure 2: General pathway of secondary metabolite production.

2.3: Hairy root cultures of various plant species are genetically stable

It is an important perception that differentiated cell cultures are naturally unstable. One of the main reasons for this instability may be modified ploidy. The number of chromosomes in each ploidy level could significantly improve this characteristic. In contrast, hairy root cultures are genetically stable, as their chromosome number and karyotype are generally comparable to those of wild plants. An immediate correlation between the genetic stability of the callus and hairy root lines inferred from the same parent plant was subsequently reported (Sharma *et al.,* 2013). For this study, callus and hairy root cultures were obtained from two model plants, *Datura stramonium* and *Hyoscyamus niger* (Weber *et al., 2008*). Starting with the two types of culture, it was concluded that the callus culture induced the greatest increase in ploidy level because of endoreduplication. However, it was very fascinating that many hairy root cultures underwent endo-reduplication. However, the ploidy levels remained similar among the hairy root clones. These findings provide solid evidence confirming that hairy root cultures are genetically stable. An additional study focused on comparative hairy root cultures of *Beta vulgaris* by a scientist from the same research group likewise concluded that hairy root cultures are genetically stable (Weber *et al.,* 2010). Therefore, the results obtained from hairy root cultures are reproducible, and such findings make hairy root clones advantageous as an experimental system.

2.4: The growth of hairy root cultures is dependent on exogenous phytohormones

Prior investigations related to *Datura stramonium* and *Hyoscyamus niger* also investigated the effects of plant development hormones (2,4-D). It stimulated improvement in calli endo-reduplication (Weber *et al.*, 2008). Previously, in various cases, hairy root cultures were beneficial, as these cultures were developed without exogenous hormones. Plant growth hormones are essential elements that influence the gene expression of secondary metabolite pathways (SMPs). These characteristics of hormone-independent growth become even more advantageous. Auxin (growth hormone) is a notable example that can be examined in this case study. Auxin was added to the callus and cell culture media for plant development. Auxin inhibits the functions of various key enzymes that are responsible for a SMP. For example, the effect of the enzyme tryptophan decarboxylase in *Catharanthus roseus was* suppressed by the addition of auxin to cell suspension cultures. This results in decreased biosynthesis of alkaloids such as ajmalicine (Rothe *et al.,* 2003). Similarly, it stifled the function of putrescine *N*-methyl transferase over calli and cell cultures of *Atropa belladonna*. Eventually, low levels of the alkaloid scopolamine are produced (Rothe *et al.*, 2003).

3. Strategies to increase secondary metabolite production via hairy roots:

Hairy roots have been utilized in a broad range of research fields, such as plant improvement, secondary metabolite synthesis, plant–environment interactions, and the synthesis of monoclonal antibodies. Therefore, scaling up the production of these hairy roots is essential for their industrial application. In this review, we presented various parameters to improve methods of hairy root development that can further improve the production of valuable secondary metabolites.

3.1: Optimization of bioreactor systems:

Streamlining bioreactor frameworks for the development of hairy roots is essential for their scale-up production and industrial applications. A bioreactor framework addresses interesting issues, such as hairy roots being morphologically diverse among various plant species. In terms of aspects such as thickness, length, hirsuteness and root branching. These characteristics are influenced by the plant species and strain of *Agrobacterium* utilized for hairy root induction (Eibl & Eibl, 2008). Bioreactor optimization is a complicated step because hairy roots are nonhomogeneous in terms of cell growth and metabolite production (Huang & McDonald, 2012). The next section of this review provides a description of the benefits and drawbacks of different bioreactor systems. This study provides information for examining the real variables involved in extensive bioreactor cultures. Intensification technologies have been utilized in hairy root bioreactors for the industrial production of secondary metabolites. Furthermore, it is essential to configure scientific models and computer-aided systems that have been used in bioreactor configuration, design and processing.

3.2: Types of bioreactors

Most bioreactors are self-contained. Aseptic conditions that incorporate nutrients and inflow and outpour frameworks for fluid and air are well manufactured to optimize and regulate the conditions of the culture system. A bioreactor has the capacity to monitor microenvironmental parameters such as pH, disintegrated gases, aeration, and temperature (Paek *et al*., 2005). In hairy root culture, bioreactors can be used in various modes and are broadly categorized as either liquid-phase reactors or gas-phase reactors. Other types of bioreactors are further classified as modified reactors. These modified reactors are a combination of both liquid- and gas-phase reactors and disposable reactors. Simplified diagrams for a few of the distinctive types of hairy root bioreactors were studied in a review by Mishra and Ranjan (Mishra & Ranjan, 2008). For various forms of bioreactors that are effectively produced for growing hairy roots, there are significant advantages and disadvantages. Therefore, various variables need to be considered when selecting the best bioreactor plan. A review of the distinctive types of bioreactors is included in Table 2.

Table 2: Convenience and drawbacks of the main forms of bioreactors.

Types of bioreactor	Convenience	Drawback
	Reactors having liquid phase	
1. Stirred tank	Preventing cell aggregation by mixing and removing air bubbles.	Direct force of shear, complex design, unsterilized conditions.
2. Airlift and Bubble column	Shear stress is low, easy design and configuration, less contamination.	Growth is not uniformed, due to air.
	Reactors having gas Phase	
1. Nutrient mist	Oxygen supply is abundant, concentration of sugar is low	Construction is complicated, consumes high energy.
2.Trickle bed	Sufficient oxygen supply and less consumption of energy.	It creates a high mass transfer barrier due to a viscous liquid film production on the hairy roots.

3.3: Parameters of the bioreactors:

Hairy root cultures showed genetic stability and increased biosynthesis of secondary metabolites. However, it is also affected by nutritional and environmental factors. Moreover, the biological characteristics of hairy root clones differ among different species, even within the same species (Mishra & Ranjan, 2008). Different factors must be acknowledged to develop efficient scale-up bioreactor systems, together with the physiology, morphology, and stress affectability of hairy root cultures. Submerged hairy root establishment contributes to the formation of thick aggregates because of the inclination of hairy roots to structure parallel extensions and the hirsuteness of the roots (Asplund & Curtis, 2001; Kim *et al*., 2002).

In liquid-phase reactors, these thick clumps oppose the liquid stream and nutrient supplements to the tissues, making them stand out among those major problems in reactor scale-up. The principle bottleneck in scale-up is the conveyance of enough available oxygen. However, other factors, such as light, different gases, temperatures, nutrients and inoculum conditions, likewise control the growth and yield benefits of hairy root cultures (Choi *et al.,* 2006).

3.3.1: Levels of oxygen and supplementary gases

The oxygen dissolved in the bioreactor microenvironment is assumed to play a paramount role. However, the demand for oxygen may differ among species. Hairy roots cultured in bioreactors need a propensity to structure thick root aggregates that minimize oxygen transfer. Therefore, explaining oxygen exchange and supply has been exceptionally important for scrutinizing the bioreactor configuration for hairy root development. Generally, it may be important that the level of dissolved oxygen remains at a discriminating level at all times for ideal cell growth (Bordonaro & Curtis, 2000). Shiao and Doran reported that as root bristliness increased, the oxygen impostor exchange limit layer expanded, restricting oxygen utilization in hairy root cultures (Shiao & Doran, 2000).

Numerous protocols have been used to improve the oxygen accessibility of hairy roots over that of bioreactors. In reactors with a liquid phase, extra tubing is adjusted. This tubing can increase oxygen availability. For example, in a bioreactor containing *Atropa belladonna* hairy root cultures, micro porous polypropylene tubing was included in the air pocket of the bioreactor. This decreases the oxygen constraint and enhances both biomass and secondary metabolite production.

3.3.2: Effects of temperature and light

Light is assumed to play a part in both the growth and biosynthesis of secondary metabolites. The morphological tenet of hairy root cultures hindered the attainment of consistent brightening. The stimulatory impact of light on secondary metabolite production has been shown for the plant species *Perilla fruitescnens* and *Artemisia annua* (Abbasi *et al*., 2007; Wang *et al*., 2001). Additionally, *Ipomea aquatica* hairy roots develop twice the amount of biomass generated in light and four times the rate of peroxidase activity compared with hairy roots established and developed in the dark (Sudo *et al.,* 2002).

In an investigation of the impact of temperature on *Solanum aviculare* hairy roots, 25 °C was recognized as the ideal temperature

(Yu *et al*., 2005). Similarly, Hilton and Rhodes (Fukushima *et al*., 2009) reported that *Datura stramonium* hairy roots generated at 30 °C produced approximately 4 times greater biomass, and the level of hyoscyamine production might be as high as 7 times greater. In hairy root cultures of *Panax ginseng*, a correlation between temperature shifts resulted in significant biomass production of the hairy roots at 20 °C or 13 °C for 16/8 h day cycles; moreover, the net ginsenoside production level might be ideal for cultures incubated at 25 °C (Yu *et al*., 2005).

4. Composition of the nutrient medium

Sivakumar *et al*. (2005) analyzed *Panax ginseng* hairy roots. He reported that frequently used MS media resulted in less hairy root growth, biomass and ginsenoside production. Therefore, the nutrient media should be optimized. They distinguished supplement accessibility as the significant chemical factor for scaling up the production of metabolites. These findings suggest that mineral components are paramount factors that regulate hairy root development and biomass processing. An investigation of tropane alkaloid production by *Datura stramonium* L. hairy roots was performed to assess the combined effects of nutrients and elicitation (Amdoun *et al.,* 2009). These authors inferred that the level of elicitation is associated with the response of nitrate and calcium levels. A total of 101 root cultures were tested in this study.

These findings indicated a decreased elicitation effect of jasmonic acid. Another investigation investigated the impact of diluted MS media on the biomass and biosynthesis of phenols and flavonoids in *Hypericum perforatum.* Maximum root development was observed during four weeks of growth when half-strength MS was used, and the total phenols and flavonoids were also increased when quarter- or half-strength MS was used (Cui *et al.*, 2010).

5: Approaches for intensification

Various approaches are being utilized for system intensification to improve biomass generation and the production of secondary metabolites in plant cell and tissue cultures. In the process of elicitation, certain chemical compounds that initiate the increased production of metabolites are applied. A system used to increase mass exchange over plant cell suspension cultures has been applied in bioreactors with hairy root cultures.

4.1: Method of elicitation:

The advancement of plant tissue culture (especially for counting organs and cells) for the processing of secondary metabolites has progressed for more than three decades. However, well-established systems for plant tissue culture are available. The implications of these methods for extensive-scale bioprocessing are now restricted to several methods (Abbasi *et al*., 2007; Amdoun *et al*., 2009; Ding *et al*., 2010).

Different incitement and process methodologies have been used to increase the production of secondary metabolites in plant tissue culture systems. The basic aim of elicitation is to measure the ability of biotic and biotic elicitors to increase secondary metabolite production both in hairy roots and in cell suspension cultures (Woodard *et al*., 2009). This technique meets expectations on the basis of the plant–pathogen infection process. During pathogen attack, plants exhibit various defense mechanisms not only against pathogen infection but also during periods of environmental stress. This mechanism results in the significant production of various secondary metabolites. The causal agents for defensive responses in plants are generally termed elicitors. Table 3 provides a review of different elicitors and general protocols of elicitor treatment to increase the production of secondary metabolites in growing hairy root cultures.

5.1: Biotic and A-biotic elicitors

Plant–microbe interactions in hairy root systems provide a convenient experimental system. These interactions can be further used in hairy root cultures to increase the production of secondary metabolites. As sources of fungal elicitors, different kinds of fungi have been utilized, including pathogenic and endophytic fungi as well as fungi that are nonspecific to plants. Mostly, raw extracts of culture filtrates or mycelial biomass are applied to hairy root cultures as fungal elicitors. *Fusarium conglutinans* were cultured on potato dextrose media for 15 days. The fungal elicitor was biologically prepared for rapidly improving the production of thiophene in hairy roots of *Tayetes patula* (Ramakrishnan & Curtis, 2004).

In concentrated silver thio-sulfate ($Ag_2S_2O_3$), the silver ion Ag^+ was formulated at a 1:4 molar ratio by combining $AgNO_3$ and $Na_2S_2O_3$. Silver ion elicitation in the culture medium also promoted the release of valuable natural products. In hairy root cultures of *Brugmansia candida*, after 24 h, treatment with 1.0 mM $AgNO_3$ lowered the scopolamine and hyoscyamine accumulation levels and markedly increased the scopolamine accumulation in the medium (Asplund PT & Curtis WR, 2001).

However, cadmium (Cd) is a substantial poisonous metal that has adverse effects on plant growth and development. Similarly, Cd^{2+} has been utilized as an intense elicitor for the processing of secondary metabolites in hairy root and plant cell cultures. These include ajmalicine (Merchuk *et al.*, 2007), podophyllotoxiand tanshinones (Kim *et al.*, 2001). To stimulate secondary metabolite production in hairy root cultures, hyperosmotic stress is another viable abiotic elicitor.

Two common agents, NaCl and sorbitol, are utilized to induce hyperosmotic stress in plant tissue cultures. NaCl at 1–5 g/L did not have a critical effect on the hairy root biomass of *D. stramonium*, but with 1 and 2 g/L NaCl treatment, the root hyoscyamine level was threefold-fold (3.54 and 4.53 mg/g DW, respectively) greater than that of the control (Ramakrishnan & Curtis, 2004).

In *Datura stramonium* hairy root culture, 1 mM Cd^{2+} elicited sesquiterpenoid lubimin production and 3-hydroxylubimin production, which were not detected in cultures without Cd2+ elicitation. Cd^{2+} treatment did not result in a detectable increase in the level of alkaloids in the roots, but after 40–60 h, there was a remarkable increase (up to 50–75% from net alkaloids) in alkaloid production in the medium from the roots (Curtis WR, 2000). At 1.0–2.0 mM $CdCl_2$, in hairy root cultures of *B. candida* (Asplund PT & Curtis WR, 2001), extremely retarded the growth of hairy roots but essentially triggered the accumulation of two tropane alkaloids, hyoscyamine and scopolamine (up to 24-fold), which were released into the liquid medium. However, Cd is not a redox-active metal that directly produces ROS. In general, plants collect ROS and experience oxidative stress (Dhaouadi *et al.*, 2008). Cd-induced oxidative outburst might be associated with the production of secondary metabolites in the culture medium (Kim *et al.*, 2001; Liu *et al.*, 2011).

5.2: Transgenic hairy root culture and multiple-omics technology:

Approaches based on meta-mics have served as methods of natural product discovery. At the organism level, natural product discovery began with system-level disruption. Elicitation methods use certain chemicals, such as jasmonic acid or other elicitors, to stimulate the synthesis of natural products. Without previous knowledge of biosynthetic genes or enzymes (Nak-abayashi *et al.*, 2015). Notably, metabolomics has been the primary contributor to our present information about metabolite diversity and bioactive compounds enhanced in distinctive medicinal plant species. Metabolomics has also served as a standard tool for the identification of key bioactive compounds to explore the targeted pharmaceutical properties of plants (Rai *et al.*, 2017). Metabolite profiling to suppress or overexpress a target gene in transgenic lines and its correlation with wild-type/background control often provides information on particular metabolites specific to the target gene. Moreover, it serves as a good approach to elucidate its biosynthetic pathway and, further, theory, as determined by experimental test acceptance (Tohge and Fer-nie, 2009).

For example, virus-induced gene silencing (VIGS) and RNAi-based approaches, along with metabolome profiling, have been effectively utilized for identifying benzylisoquinoline alkaloids (BIAs). Such approaches might evaluate the functional characterization of key enzymes from their metabolic pathways (Desgagne-Penix & Facchini, 2012; Farrow and Facchini, 2013). Similar techniques have been used for the recognition and functional characterization of genes correlated with the biosynthesis of other specific metabolites. In tomato and potato plants, five genes *(CYP97A29, DFR, FLS, NIK and PMEI)* were selected to examine their roles in plant–nematode interactions. Using bioinformatics tools, the promoters of selected genes were isolated, and potential cis-regulatory elements in their sequences were characterized. *β-glucuronidase* gene fusions with promoters were constructed and integrated into the tomato and potato genomes via transformation with *Agrobacterium rhizogenes* to produce hairy roots. The analyzed promoters presented distinct activity patterns in nematode-infected and uninfected transgenic hairy roots (Wis´niewska *et al.*, 2013). Hence, integrative meta-omics provides insights into the phenotypic transformation of plants and the interactions between diverse organization levels and provides conclusive and functional information.

Brugmansia candida (Solanaceae) is a native tree dispersed across South America. It biosynthesizes a medicinally significant group of tropane alkaloids containing scopolamine. *The hyoscyamine 6-β hydroxylase (H6H, EC 1.14.11.11)* at the end of the tropane alkaloid pathway is responsible for the synthesis of this biocompound from hyoscyamine. This study reported the tissue- and organ-specific expression of *h6h* mRNA from *B. candida* anthers and hairy root transgenic cultures. In

Table 3: Impact of various elicitors on hairy roots of Solanaceous plants

Hairy Roots	Elicitor	Effects	Reference
Brugmansia Candida	Hemicelluloses,Theophylline $CaCl_2$	Stimulation of hyosyamine and scopolamine	Pitta *et al.*, 2000
Brugmansia Candida	Salicylic Acid, Yeast extract, $CaCl_2$, $AgNO_3$, $CdCl_2$	Stimulation of hyosyamine and scopolamine	Pitta *et al.*, 2000
Datura Stramonium	Wide range of abiotic elicitors (metal ions)	sesquiterpene phystoalexins (lubimin, 3 hydroxylubimin, rishtine) accumulation	Furze *et al.*, 1991
Hyoscyamus mutics	*Rhizoctonia Solani*	sesquiterpene phytoalexin accumulation	Signs MW & Flore He, 1989
Hyoscyamus muticus	*Rhizoctonia Solani*	solavetivone accumulation	Dunlop DS & Curtis WR, 1989;
Hyoscyamus muticus	*Rhizoctonia Solani*	Solavetivone & alubimin accumulation	Ramakrishna G & Signs MW, 1993
Hyoscyamus muticus	*Inonotus obliquus*	Stimulation of hyoscyamine	Sevon N, 1997
Hyoscyamus muticus	$CuSO_4$	Stimulation of hyoscyamine	Sevon N, 1997
Hyoscyamus muticus	Purifield Chitosan	Stimulation of hyoscyamine	Sevon *et al.*, 1992
Hyoscyamus muticus	Jasmonic acid Metyl Jasmonate	Slight stimulation of hyoscyamine. Strong stimulation of polyamines	Biondi *et al*., 2000
Hyoscyamus muticus	Methyl Jasmonate +wounding+Rhizoctonia Solani	Solavetivone and lubimin accumulation	Singh *et al*., 1998
Nicotiana tabacum	Yeast extract, Botrytis Fabae extract	Accumulation of sesquiterpene phytoalexins	Wibberely *et al.*, 1994

this report, cDNA isolation, cloning and sequencing were performed via RT–PCR analyses (Cardillo, *et al.,* 2006). In another study, tropane alkaloids were produced via hairy roots of *Hyoscyamus niger* at high levels (Rai *et al.,* 2017). Similarly, two putative solavetivone biosynthetic genes, *terpene synthase 1 (hatps1)* and *hyoscyamus premnaspirodiene oxygenase 1 (hahpo1*), were cloned from *Hyoscyamus albus*. The WRKY transcription factor DNA-binding element, the W-box, was recognized in the *hatps1* and *hahpo1* promoters. The WRKY transcription factor *H. albus wrky transcription factor 1 (hawrky1)* was cloned under solavetivone production conditions. From the phylogenetic analysis, *hawrky1* was categorized into group IIa. The expression pattern of *hawrky1* was comparable to that of *hahpo1*. Therefore, *hawrky1* may be a significant gene that stimulates the production of solavetivone in *H. albus* (Kawauchi *et al.,* 2016). This integrative approach is usually exploited for two purposes: gene function identification and characterization of the precise interactions of biosynthetic pathways. In the next section of this review, we focused on the current opportunities and challenges of integrative studies with multiple omics data in members of the *Solanaceae* family.

5.3: Integrative metabolomic and transcriptomic analysis

Presently, comparative transcriptomics is a strategy to unfold the biosynthetic pathways of secondary metabolites in nonmodel plant species (especially medicinal plants). In a transcriptome-wide system, the total genome sequences and annotations for these plants are not yet accessible. In the *Solanaceae* family, *de novo* transcriptome sequencing and characterization have been executed successfully for *Lycium chinense* (Zhao *et al.,* 2013). Transcriptome profiling originated with next-generation sequencing (NGS)-based RNA sequencing (RNA-seq). Unlike microarray analysis, RNA-seq-based transcriptome profiling does not require former genomic information. Hence, a fundamental strategy to facilitate transcriptome profiling and characterization is promoted (Wang *et al.,* 2009). RNA-seq reads might be collected *de novo* without mapping the reference genomic sequences. This quality makes RNA sequencing valuable for expression analysis and gene identification in nonmodel medicinal plants (Rai *et al.,* 2017). In recent years, RNA-seq-based transcriptome profiling, *de novo* transcriptome assembly, coexpression and similar transcriptome examination of different plant species have led to considerable efforts to provide genetic assets for biologically important plants (Martin & Wang 2011; Jain, 2012). Therefore, RNA-seq-based transcriptome profiling and coexpression analysis have been utilized in various investigations. These analyses are used to identify biosynthetic enzymes that affect the production of pharmaceutically significant phytochemicals. As in the three varieties of *Opium poppy,* RNA-seq-based transcriptome profiling, along with coexpression analysis, resulted in large amounts of morphine, thebaine and noscapine. The function of metabolic enzymes can be determined by testing gene overexpression or RNA interference vectors. As in *Nicotiana glauca, the function* of an enzyme involved in pyridine alkaloid biosynthesis has been elucidated (DeBoer *et al.,* 2009). Similarly, *Agrobacterium rhizogenes* was used to identify genes responsible for lowering the programmed cell death triggered by fumonisin

B1 in plants. In this study, transformed tomato roots (*Solanum* spp.) were analyzed for resistance to fumonisin 1 (Harvey *et al.,* 2008). In addition, *A. rhizogenes*-mediated transformation of tomato roots expressing the *baculovirus p35* gene was utilized to determine the presence of proteases. With substrate site specificity, proteases are functionally identical to animal caspases (Lincoln *et al.*, 2002). In another study, *A. rhizogenes-mediated gene transformation was used as a measure* for rapid and effective gene expression analysis. This study provided cell-level information for genes expressed in the roots of *Solanum lycopersicum* and *Solanum pennellii*. Hence, this study serves as a resource for producing root cell-specific promoters in tomato. Hairy root-based transformation techniques include the INTACT method to capture nuclei and the TRAP method to immunopurify polyribosomes (polysomes) in tomato roots. Finally, this study revealed that a transgenic hairy root establishment system could be used as a tool to identify gene function through the application of certain new technologies, such as CRISPR-mediated gene mutation (Ron *et al.,* 2014).

5.4: Planned alternations in metabolomics: Identifying the role of novel genes and enzymes to elucidate secondary metabolite pathways

The stable integration and expression of foreign genes in medicinally important plants suggest incredible promise for genetic manipulation of plant secondary metabolism. The *Agrobacterium*-Ti or Ri plasmid-based technique is usually utilized for transferring genes into dicotyledonous plants (Zhang *et al.*, 2004). In particular, the Ri-*Agrobacterium* system can be helpful for plants for which regeneration methods have been well established, e.g., those involving *tobacco, potato, and Arabidopsis* (Rothe *et al.,* 2003). The *Agrobacterium*-Ri plasmid-based binary vector system can be utilized to maintain transgenic hairy roots incorporating the T-DNAs of the helper Ri plasmid (Fukuda, 2001). This protocol provides an easy and understandable approach to obtain transformed tissues/organs with selected foreign genes. Therefore, manipulation of genes can increase biosynthetic capacity and produce abundant plant-based secondary metabolites (Li *et al*., 2006). Similarly, this approach serves as a heterologous system for the production of significant natural compounds, including foreign proteins (Huang & McDonald, 2012). Once a genetically transformed line is established, a single hairy root from the ex-plant tissue is treated as a clone (Fukuda, 2001). Upon proper establishment, these clones proliferate unlimitedly from the site of infection (Rothe *et al*., 2003). Hence, hairy root cultures have been used to identify unknown genes required in biosynthetic pathways in *plant* cell cultures (Chandra & Chandra, 2011). In contrast to ordinary root cultures, hairy roots present great characteristics. These roots are genetically and biochemically stable, grow rapidly, are easy to maintain, and grow independently of plant growth hormones. Moreover, these materials could serve as promising future transgenic systems. Transformed roots of various plants have been maintained *in vitro* for the processing of natural secondary metabolites (Zhang *et al., 2004*).

One of the most critical research areas of expertise is metabolic engineering (ME) of metabolite pathways. Principles of genetic engineering used to increase product output after planned alternations in metabolomics. In addition to elicitation, product enhancement strategies, the regulation of culture conditions and the composition of the medium are planned and altered to increase the production of secondary metabolites. Through the use of hairy root cultures, such amendments might be achieved either by the overexpression of an individual gene or through the expression of more than one gene. An individual gene or more than one gene enhances targeted catalyst activity, resulting in increased production of the target compound through single-step engineering or multistep engineering, respectively (Mehrotra *et al*., 2010, 2013; Zhou *et al*., 2011). As in *Atropa belladonna,* the overexpression of the *pmt* (putrescine N methyltransferase) gene individually did not influence the production of tropane alkaloids in transgenic hairy roots (Sato *et al.,*2001). Compared with control products such as scopolamine, hyoscyamine, tropine, pseudotropine and tropinone remained unchanged or, to some extent, decreased in *pmt*-overexpressing lines. Moreover, the expression level of *pmt* alone may not limit tropane alkaloid formation in *A. belladonna* (Rothe *et al*., 2003). Moyano *et al.* (2002) integrated the *pmt* gene from tobacco (*Nicotiana tabacum* L.) into the hairy roots of *Duboisia hybrids,* and the levels of N-methylputrescine increased (two-fourfold) in the resulting engineered hairy roots compared with those in wild-type roots. Another study revealed that, in two related plant species, similar biosynthetic pathways can be differentially regulated and that the overexpression of a selected gene does not result in the same pattern of secondary metabolite accumulation (Moyano *et al.,* 2003). A binary vector system was developed in an attempt to increase the production of hyoscyamine and scopolamine in hairy root cultures. The T-DNA of the Ri plasmid was introduced with the tobacco *pmt* gene into the genomes of *Datura metel* and *Hyoscyamus muticus* under the CaMV 35S promoter. This gene encodes putrescine N-methyltransferase (PMT), which catalyzes the first dedicated step in the tropane alkaloid pathway. In this investigation, it was concluded that both hyoscyamine and scopolamine production in hairy root cultures of *D. metel* improved, whereas in *H. muticus,* only the hyoscyamine content increased with *pmt* gene overexpression (Moyano *et al.,* 2003). The results showed that the overexpression of a selected gene might not always lead to the same accumulation pattern of secondary metabolites; even in two related plant species, the same biosynthetic pathway could be differentially regulated in different systems.

Even when a rate-limiting enzyme is targeted, a considerable increase in productivity is feasible in most biosynthetic pathways. During secondary metabolite production, there might not be more than one rate-limiting enzyme. Therefore, systems should strengthen multiple steps by overexpressing multiple biosynthetic genes. Regulatory genes that regulate the expression of multiple pathway enzymes, genes or both (Zhang *et al.,* 2005). Here, the concurrent integration and overexpression of genes encoding the rate-limiting upstream enzyme putrescine N-methyltransferase (PMT) and the downstream key enzyme hyoscyamine 6 β hydroxylase (H6H) of scopolamine biosynthesis in transgenic henbane (*Hyoscyamus niger*) hairy root cultures were explored (Zhang *et al.,* 2004). Compared with the wild-type and transgenic lines carrying a single gene (*pmt or* *h6h*), the transgenic hairy root lines expressing both pmt and h6h produced promisingly higher levels of scopolamine. These activities resulted in overcoming rate-limiting steps in the pathway, excluding focused pathways, or lessening the catabolism of the targeted product. Rather, designing the expression level of regulatory genes that control the biosynthesis of numerous genes is also supportive of regulating product biosynthesis (Peebles *et al.*, 2009; Chandra & Chandra 2011). Nevertheless, when a variety of root-based metabolic systems are used, it is challenging to control the entire pathway to adjust the functions of only a single gene or collectively as a set of genes.

5.5: Production of metallo-organic semiconductor quantum dots:

Plants utilize various protection components as a defensive system to protect against the poisonous impacts of heavy metals. The formation of complexes with these natural particles reduces the cytotoxicity of metal ions, permits the transport of metals and enhances the capacity of vacuoles. Phytochelatin peptides are composed of three amino acids: glucose, cysteine and glycine. These amino acids are organized as (Glu-Cys)n-Gly (‘n’ ranging between 2 and 11), which is common in the plant kingdom (Al-Shalabi Z, 2010). In the presence of metal ions such as Cd, the synthesis of phytochelatin in plants is strongly increased (Al-Shalabi Z, 2010). The metal-binding ability of phytochelatins and related peptides has been misused to improve the chemical synthesis and photophysical properties of semiconductor quantum dots (Bailey *et al*., 2004).

For the biological synthesis of CdS quantum dots in *Solanum lycopersicum* (tomato), hairy roots have been investigated (Al-Shalabi Z, 2010). The concentrations of Cd are frequently higher in roots than in other parts of entire plants (Conn & Gilliham, 2010; lux *et al*., 2011). This phenomenon suggested that the root system may be a viable form of plant culture for the production of nanoparticles.

The decision to choose tomato as a model plant is based on the ability of tomato plants to aggregate Cd metal naturally as phytochelatin-capped CdS crystallites (Ray and Jha, 2001). Tomato cv. Grosse Lisse hairy roots were generated and treated with 100 lM Cd. For high-level preparation of CdS quantum dots, different dosages of Cd, cultures and harvesting times were tested. The ideal consolidation of these factors might have been characterized with respect to root growth, accumulated levels of Cd in the net biomass, and the proportion of inorganic sulfide to Cd in crude root extracts. UV absorbance of root extracts and their radiance properties inside the spectral ranges linked with CdS quantum dots were also inspected. Using gel filtration, the method of size fractionation allowed the recovery of phytochelatin-capped Cd- and inorganic sulfide-containing nanoparticles displaying the size- and size-dependent optical/electronic properties of CdS quantum dots. At 4–10 nm in diameter, these particles fluoresced at wavelengths corresponding to blue–violet light in the color spectrum and exhibited a high level of photo stability with prolonged excitation.

Whereas 69% of the Cd extracted from the roots was associated with phytochelatin peptides, the maximum yield of CdS nanocrystals was 1.4% of the total Cd taken up into the biomass with quantum dot properties (Al-Shalabi *et al.,* 2014). An attainable biological system that can be exercised precisely to produce metal-based nanoparticles is 'Hairy roots'. A major benefit of utilizing plant cells for the production of quantum dots is their ability to passivate the surface of nanocrystals for regular plant-based natural capping layers to produce phytochelatin peptides.

5.6: As an alternative for phytoremediation

Transgenic hairy root (HR) systems are promising substitutes for enhancing the effectiveness of phytoremediation. *Peroxidases (Pxs)* are associated with the removal of phenolic compounds. The transformed tobacco hairy roots, which expressed basic *Px* genes from tomato (*tpx1* and *tpx2*), were maintained and analyzed for phenol elimination. In view of the development index, 10 tobacco HR clones were selected for assaying the existence of the rol C sequence, *tpx1* or *tpx2* genes and the encoded proteins, as well as for *Px* activity determination for phenol removal. The removal efficiency was high for all HRs. However, a few transgenic HRs presented essentially higher removal efficiencies than did the controls.

The results revealed that *TPX1* is involved in phenol removal not only when it might be overexpressed in tomato but also when it is expressed in different plants, e.g., tobacco. The greater effectiveness of *TPX2* transgenic HR indicated that this *Px* additionally participates in the transformation process. The contributions of other mechanisms (adsorption, H_2O_2-free enzymatic processes) could be recognized as depreciable, which *strongly suggests that Px is involved* in phenol removal (Lucas *et al.,* 2009).

5.7: Tomato and potato hairy roots for plant protection against nematodes

Conventional strategies (fallow periods, inundation, crop rotation, and nematode-repelling soil treatments) for plant protection against nematodes need expensive aid and are not sufficiently powerful. On the other hand, ant nematode chemicals might cause natural harm. However, biological strategies for pathogen control (e.g., nematode-pathogenic fungi) are still under development (Yan *et al.,* 2011). Similarly, biotechnological systems focused on transgenic plants and classical breeding methods for the selection of natural host resistance genes have yet to be developed. *Solanum tuberosum* is closely related to *Solanum lycopersicum* (Hoffmann *et al.,* 2006), and both are good hosts for *Globodera rostochiensis* (nematode).

Hence, the comparable action patterns of the investigated promoters over syncytia prompted in tomato and potato roots suggest that their mechanism of regulation is the same in both solanaceous species. Furthermore, these promoters should be used to control the expression of antinematode products in related plants. Only in the case of the Ro1 pathotype of *Globodera rostochiensis* is the resistance mediated by tomato *H1* (Bakker *et al.,* 2004) effective, and the resistance provided by the *Gpa2* gene against *Globodera pallida* (van der Vossen *et al.,* 2000) has been overcome by this nematode (Fududa, 2001). For all pathotypes of *G. rostochiensis and G. pallida,* the tomato *Hero* gene presented distinctive levels of resistance (Ernst *et al.,* 2002). The introduction of *Hero* into a susceptible tomato cultivar resulted in a visibly diminishing effect on the number of developing nematode females, but transgenic potato plants harboring the *Hero* gene presented no resistance (Sobczak *et al.*, 2005).

5.8: Anti-feeding properties of tomato hairy root extract against Chewing Insects:

Many previous reports have suggested that various flavonoids in living systems are lethal to insects/pests. Leaf extracts of *Nothofagus dombeyi* and *Nothofagus pumilio*, which are composed of triterpenes and flavonoids, are effective against the larvae of *Ctenopsteustis obliquana* and have shown antifeeding activity (Thoison *et al.*, 2004). A high amount of rutin accumulation is the cause of the insect-resistant genotype of soybean (*Glycine max* PI 227687) (Hoffmann *et al.*, 2006). The phenolic compounds extracted (PCEs) from hairy roots of *Helicoverpa armigera* and *Spodoptera litura* transgenic plants presented a noticeable mortality rate against both insects. However, the growth of the surviving larvae was retarded, with a very low average weight. The HPLC data clearly demonstrated the accumulation of various flavonoids and phenolic acids in the PCE. This could be a reason for insect toxicity. The mortality rate of insects additionally demonstrated that extracted phenolic compounds were concentrated in their biologically active forms (Singh *et al.*, 2014).

6. Conclusion leading to future prospects:

By producing high levels of plant secondary metabolites, solanaceous species are promising for pharmaceutical and industrial applications. In particular, alkaloids, flavonoids, phenolics, terpenoids and glycosides constitute the largest groups of natural products involved in plant secondary metabolism. This review provides an effective approach for the use of hairy root cultures as a genetic engineering technique to improve the end products of secondary metabolite pathways. A suitable combination of genetic engineering and elicitor treatment is ideal in this regard. One of the next main directions of the metabolic system is filling the knowledge gap between gene function and pathway regulation. Therefore, functional characterization of enzymatic genes in specific biosynthetic pathways is very important.

In this context, integrated approaches with multiple omic data provide valuable information for the identification of targeted key genes and descriptions of pathway interactions in various processes. However, for the investigation of plant behavior, incorporating new concepts and cutting-edge technologies is mandatory. Programmed investigations of hairy root research have revealed traditional research domains. Integrative investigations on hairy root induction, planned alterations in metabolomics and genetic engineering will make hairy root technology more commercially attainable.

However, dissection of hairy root cultures through the integration of metabolomic studies with transcriptomic analysis (e.g., RNA sequencing) based on overexpression and RNAi methods is also needed. In the future, considerable priority will be given to exploration, which will provide a comprehensive picture in light of this natural gene transfer phenomenon in economically important members of the family *Solanaceae*. However, more scientific and specialized solutions for the utilization of hairy root cultures in industrial and pharmaceutical applications are needed.

Acknowledgment

This work was supported by grants from the National Natural Science Foundation of China (31470391, 31770334), the Youth Innovation Promotion Association CAS (2015286) and the Scientific Project of Ningxia Agriculture Comprehensive Development (znnfkj2015).

Authors Contribution: Conceptualization and supervision, FZ and AAC; writing original draft preparation, FZ., HAP and JK writing review and editing. KF and MK; validation, MN., AB and NHB; assisted with literature screening and revision of manuscript

Conflict of Interest: The authors declare no conflicts of interest

Declaration: it is to declare that AI is used to generate figures

Funding: This research received no external funding

References

Abbasi, B.H., Tian, C.L., Murch, S.J., Saxena, P.K., and Liu, C.Z. (2007). Light-enhanced caffeic acid derivatives biosynthesis in hairy root cultures of *Echinacea purpurea*. *Plant Cell Rep.* **26**, 1367–1372.

Al-Shalabi, Z. (2010). Production of cadmium sulphide quantum dots in tomato hairy root cultures. Dissertation: University of New South Wales, Australia

Ambros, P.F., Matzke, A.J.M., and Matzke, M.A. (1986a). Localization of *Agrobacterium rhizogenes* T-DNA in plant chromosomes by *in situ* hybridization. *Eur Mol Biol Organ J.* **5,** 2073–2077.

Amdoun, R., Khelifi, L., Khelifi-Slaoui, M., Amroune, S., Benyoussef, E.H., Thi, D.V., AssafDucrocq, C., and Gontier, E. (2009). Influence of minerals and elicitation on *Datura stramonium* L. tropane alkaloid production: Modelization of the *in vitro* biochemical response. *PlantSci*. **177,** 81–87.

Asplund, P.T., and Curtis, W.R. (2001). Intrinsic oxygen use kinetics of transformed plant root culture. *Biotechnol Prog.* **17**(3), 481–489.

Bailey, R.E., Smith, A.M., and Nie, S. (2004). Quantum dots in biology and medicine. *Physica E.* **25**, 1–12.

Bakker, E., Achenbach, U., Bakker, J., van Vliet, J., Peleman, J., Segers, B., van der Heijden, S., van der Linde, P., Graveland, R., Hutten, R., van Eck, H., Coppoolse, E., van der Vossen, E., Bakker, J., and Goverse, A. (2004). A high-resolution map of the H1 locus harboring resistance to the potato cyst nematode *Globodera rostochiensis*. *Theor Appl Genet*. **109,**146–152.

Banerjee, S., Madhusudanan, K.P., Chattopadhyay, S.K., Rahman, L.U., and Khanuja, S.P.S. (2008). Expression of tropane alkaloids in the hairy root culture of Atropa acuminata substantiated by DART mass spectrometric technique. *Biomed Chromatogr*. **22**, 830–834.

Batra, J., Dutta, A., Singh, D., Kumar, S., and Sen, J. (2004). Growth and terpenoid indole alkaloid production in Catharanthus roseus hairy root clones in relation to left- and rightterminilinked Ri T-DNA gene integration. *Plant Cell Rep*. **23**,148–154.

Berkov, S., Pavlov, A., Kovatcheva, P., Stanimirova, P., and Philipov, S. (2003). Alkaloid spectrum in diploid and tetraploid hairy root cultures of *Datura stramonium*. *Z Naturforsch* **58c**, 42–46.

Bonhom, V., Laurain-Mattar, D., Lacoux, J., and Fliniaux, M. (2000). Tropane alkaloid production by hairy roots of *Atropa belladonna* obtained after transformation with *Agrobacterium rhizogenes* 15834 and *Agrobacterium tumefaciens* containing *rol* A, B, C genes only. *J. Biotechnol*. **81**, 151–158.

Bordonaro, J.L., and Curtis, W.R. (2000). Inhibitory role of root hairs on transport within root culture bioreactors. *Biotechnol Bioeng.* **70**, 176–186.

Bulgakov, V. P., Kusaykin, M., Tchernoded, G. K., and Zvyagintseva, T. N. (2002). Carbohydrase activities of the *rolC* gene transformed and nontransformed ginseng cultures. *Fitoterapia*. **73**, 638–643.

Cardillo, A. B., Giulietti, A. M., and Marconi, P. L. (2006). Analysis and sequencing of h6hmRNA, last enzyme in the tropane alkaloids pathway from anthers and hairy root cultures of *Brugmansia candida* (Solanaceae). *Elect J of Biotech*. 9, 3.

Carrizo, C.N., Pitta-Alvarez, S.I., Kogan, M.J., Giulietti, A.M., and Tomaro, M.L. (2001) Occurrence of cadaverine in hairy roots of *Brugmansia candida*. *Phytochemistry*. **57**,759–763.

Chandra, S. (2012). Natural plant genetic engineer *Agrobacterium rhizogenes*: role of T-DNA in plant secondary metabolism. *Biotechnol Lett*. **34**, 407–415.

Chandra, S., and Chandra, R. (2011). Engineering secondary metabolite production in hairy roots. *Phytochem Rev*. **10**, 371–395.

Chashmi, N.A., Sharifi, M., Karimi, F., and Rahnama, H. (2010) Differential production of tropanealkaloids in hairy roots and *in vitro* cultured two accessions of Atropa belladonna L. under nitrate treatments. *Z Naturforsch.* **65**c, 373–379.

Choi, Y., Kim, Y., and Paek, K. (2006). Types and designs of bioreactors for hairy culture. In: Duttagupta S, Ibaraki Y (eds) Plant tissue culture engineering. Springer, Dordrecht, pp 161–172.

Conn, S., and Gilliham, M. (2010). Comparative physiology of elemental distributions in plants. *Ann Bot*. **105**, 1081–1102.

Cui, X.H., Chakrabarty, D., Lee, E.J., and Paek, K.Y. (2010). Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L in a bioreactor. *Bioresour Technol* **101**, 4708–4716.

Curtis, W.R. (2000). Bioreactor growth of hairy roots. In: Spier R (ed) Encyclopedia of Cell Technology. John Wiley and Sons, New York, pp 827–841.

De Luca, V., and St Pierre, B., (2000). The cell and developmental biology of alkaloid biosynthesis. *Trends Plant Sci.* 5, 168–173.

Deboer, K.D., Lye, J.C., Aitken, C.D., Su, A.K.K., and Hamill, J.D. (2009). The A622 gene in *Nicotiana glauca* (tree tobacco):

evidence for a functional role in pyridine alkaloid synthesis. *Plant Mol Biol*. **69**, 299–312.

Desgagné-Penix, I., Facchini, J. (2012). Systematic silencing of benzylisoquinoline alkaloid biosynthetic genes reveals the major route to papaverine in opium poppy. *The Plant J.* **72,** 331–344.

Dhaouadi, H., Poncin, S., Hornut, J.M., and Midoux, N. (2008). Gas–liquid mass transfer in bubble column reactor: analytical solution and experimental confirmation. *Chem Eng Proc.*. **47**, 548–556.

Ding, J., Wang, X., Zhou, X.F., Ren, N.Q., and Guo, W.Q. (2010). CFD optimization of continuous stirred-tank (CSTR) reactor for biohydrogen production. *Bioresour Technol* **101**, 7005–7013.

Dougherty, M. K., and Morrison, D. K., (2004). Unlocking the code of 14-3-3. *J. Cell Sci.* **117,** 1875–1884.

Drager, B., (2006). Tropinone reductases, enzymes at the branch point of tropane alkaloid metabolism. *Phytochemistry*. **67**, 327–337.

Ernst, K., Kumar, A., Kriseleit, D., Kloos, D.U., Phillips, M.S., and Ganal, M.W. (2002). The broad-spectrum potato cyst nematode resistance gene (Hero) from tomato is the only member of a large gene family of NBS–LRR genes with an unusual amino acid repeat in the LRR region. *Plant J.* **31**, 127–136.

Facchini, P. J., and St-Pierre, B. (2005). Synthesis and trafficking of alkaloid biosynthetic enzymes. *Curr. Opin. Plant Biol.* **8**, 657–666.

Farrow, S.C., Facchini, P.J. (2013). Dioxygenases catalyze o-demethylation and o,o-demethylenation with widespread roles in benzylisoquinoline alkaloid metabolism in Opium poppy. J. Bio Chem. **288**, 28997-29012.

Fukuda, T. (2001). Phylogeny & biogeography of genus *Lycium* (solanaceae): Inferences from chloroplast DNA sequences. *Mol. Phylogene & evol.* **19**(2), 246-58.

Fukushima, A., Kusano, M., Redestig, H., Arita, M., and Saito, K. (2009). Integrated omics approaches in plant systems biology. *Curr Opin in Chem Bio.* **13**, 532-538.

Gelvin, S.B. (2003). Agrobacterium-mediated plant transformation: the biology behind the “gene-jockeying” tool. *Microbiol Mol Biol Rev*. **67,** 16–37.

Häkkinen, S.T., Moyano, E., Cusidó, R.M., Palazón, J., Piñol, M.T., and Oksman-Caldentey, K.M. (2005). Enhanced secretion of tropane alkaloids in *Nicotiana tabacum* hairy roots expressing heterologous hyoscyamine-6b-hydroxylase. *J Exp Bot*. **56**, 2611–2618.

Harvey, J.J., Lincoln, J.E., and Gilchrist, D.G. (2008). Programmed cell death suppression in transformed plant tissue by tomato cDNAs identified from an *Agrobacterium rhizogenes*-based functional screen. *Mol Genet Genomics*. **279**, 509–521.

Hoffmann-Campo, C. B., Neto, J. A. R., de Oliveira, M. C. N., and Oliveira, L. J. (2006). Detrimental effect of rutin on *Anticarsia gemmatalis*. *Pesqui. Agropecu. Bras.* **41**, 1453-1459.

Hong, S., Peebles, C., Shanks, J.V., and San, K. (2006). Terpenoidindole alkaloid production by *Catharanthus roseus* hairy roots induced by *Agrobacterium tumefaciens* harboring *rol ABC* genes. *Biotechnol. Bioeng*. ***93,*** 386–390.

Huang, T.K., and McDonald, K.A. (2012). Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures. *Biotechnol Adv*. **30**, 398–409.

Jain, M. (2012). Next-generation sequencing technologies for gene expression profiling in plants. *Brief Funct Genomics.* **11**, 63–70.

Karam, N.S., Jawad, F.M., Arikat, N.A., and Shib, R.A. (2003). Growth and rosmarinic acid accumulation in callus, cell suspension, and root cultures of wild *Salvia fruiticosa*. *Plant Cell Tiss Org* **73**, 117–121.

Kawauchi, M., Arima, T-H., and Kuroyanagi, M. (2016). Molecular cloning and transcriptional analysis of *WRKY* and solavetivone biosynthetic genes in the hairy roots of *Hyoscyamus albus*. *Plant gene*. **5**, 78-86.

Kim, Y., Wyslouzil, B.E., and Weathers, P.J. (2001). A comparative study of mist and bubble column reactors in the *in vitro* production of Artemisinin. *Plant Cell Rep*. **20**, 451–455.

Kim, Y., Wyslouzil, B.E., and Weathers, P.J. (2002). Invited review: secondary metabolism of hairy root cultures in bioreactors. *In vitro Cell Dev Biol Plant*. **38**, 1–10.

Knapp, S., Bohs, L., Nee, M., and Spooner, D.M. (2004). *Solanaceae*—A model for linking genomics with biodiversity. *Comp. Funct. Genom.*, **5**, 285–291.

Kusakari, K., Yokoyama, M., and Inomata, S. (2000). Enhanced production of saikosaponins by root culture of *Bupleurum falcatum* L. using two-step control of sugar concentration. *Plant Cell Rep*. **19**, 1115–1120.

Laloi, C., Apel, K., and Danon, A. (2004). Reactive oxygen signaling: the latest news. *Curr. Opin. Plant Biol.* **7,** 323–328.

Lee, O.S., Kang, Y.M., Jung, H.Y., Min, J.Y., Kang, S.M., Karigar, C.S., Prasad, D.T., Bahk, J.D., and Choi, M.S. (2005). Enhanced production of tropane alkaloids in *Scopolia parviflora* by introducing the pmt (putrescine n-methyltransferase) gene. *In Vitro Cell Dev Biol Pant*. **41**,167–172.

Li, F.X., Jin, Z.P., Zhao, D.X., Cheng, L.Q., Fu, C.X., and Ma, F.S. (2006). Overexpression of the *Saussurea medusa* chalcone isomerase gene in *Saussurea involucrata* hairy root cultures enhances their biosynthesis of apigenin. *Phytochemistry***. 67,** 553–560.

Lincoln, J.E., Richael, C., Overduin, B., Smith, K., Bostock, R., and Gilchrist, D.G. (2002). Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. *Proc Natl Acad Sci USA*.99, 15217–15221.

Liu, R., Sun, W., and Liu, C.Z. (2011) Computational fluid dynamics modeling of mass-transfer behavior in a bioreactor for hairy root culture. II. Analysis of ultrasound-intensified process. *Biotechnol Prog*. **27,** 1672–1679.

Liu, Y., Sun, W., Zeng, S., Hu, W., Liu, D., Hu, W., Shen, X., and Wang, Y. (2014). Virus-induced gene silencing in two novel functional plants, *Lycium barbarum* L. and *Lycium ruthenicum* Murr. Sci. Hortic. **170**, 267–274.

Lorence, A., Medina-Bolivar, F., and Nessler, C.L. (2004). Camptothecin and 10-hydroxycamptothecin from *Camptotheca acuminate* hairy roots. *Plant Cell Rep*. **22**, 437–441.

Lucas, G., Sosa, A., Melina, A.T., Sabrina, G.I., Silvia, P., Elizabeth, A., Silvia, R., and María, I. M., (2009). Establishment of transgenic tobacco hairy roots expressing basic peroxidases and its application for phenol removal. *J. Biotech*. **139**, 273–27.

Lux, A., Martinka, M., Vaculík, M., and White, P.J. (2011). Root responses to cadmium in the rhizosphere. *J Exp Bot*. **62**, 21–37.

Martin, J.A., and Wang, Z. (2011). Next-generation transcriptome assembly. *Nat Rev Genet*. **12**, 671–682.

Mehrotra, S., Rahman, L.U., and Kukreja, A.K. (2010). An extensive case study of hairy-root cultures for enhanced secondary-metabolite production through metabolic-pathway engineering. *Biotechnol Appl Biochem*. **56,** 161–172.

Mehrotra, S., Srivastava, V., Rahman, L.U., and Kukreja, A.K. (2013). Overexpression of a Catharanthus tryptophan decarboxylase (tdc) gene leads to enhanced terpenoid indole alkaloid (TIA) production in transgenic hairy root lines of *Rauwolfia serpentina*. *Plant Cell Tiss Org cult*. **11,** 377–384.

Merchuk, J., Garcia-Camacho, F., and Molina-Grima, E. (2007). Photobioreactor design and fluid dynamics. *Chem Biochem Eng Quarterly*. **21,** 345–355.

Mishra, B.N., and Ranjan, R. (2008). Growth of hairy-root cultures in various bioreactors for the production of secondary metabolites. *Biotechnol Appl Biochem*. **49**, 1–10.

Moriuchi, H., Okamoto, C., Nishihama, R., and Yamashita, I. (2004). Nuclear localization and interaction of *rolB* with plant 14-3-3 proteins correlates with induction of adventitious roots by the oncogene *rolB*. *Plant J. 38*, 260–275.

Moyano, E., Fornale,´ S., Palazo,´n. J., Cusido,´ R.M., Bagni, N., and Pinˇol, M.T. (2002). Alkaloid production in *Duboisia hybrid* hairy root cultures overexpressing the pmt gene. *Phytochemistry*. **59**, 697–702.

Moyano, E., Jouhikainen, K., Tammela, P., Palazo, J., Cusido, R.M., Pinˇol, M.T., Teeri ,T. H., and Oksman-Caldentey, K.M. (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.

Murata, J., Roepke, J., Gordon, H., and De Luca, V. (2008). The leaf epidermome of *Catharanthus roseus* reveals its biochemical specialization. *Plant Cell*. ***20,*** 524–542.

Murthy, H. N., Dijkstra, C., Anthony, P., and White, D. A. (2008). Establishment of *Withania somnifera* hairy root cultures for the production of withanolide A. *J. Integr. Plant Biol*. **50**, 975–981.

Nakabayashi, R., Yang, Z., Nishizawa, T., Mori, T., and Saito, K. (2015). Top-down targeted metabolomics reveals a sulfur-Containing metabolite with inhibitory activity against Angiotensin-Converting Enzyme in *Asparagus officinalis. J. Nat. Prod.* **78**, 1179–1183.

Ono, N.N., and Li, T. (2011). The multiplicity of hairy root cultures: prolific possibilities. *Plant Sci*. **180**, 439–446.

Paek, K.Y., Chakrabarty, D., and Hahn, E.J. (2005). Application of bioreactor systems for large scale production of horticultural and medicinal plants. *Plant Cell, Tissue Organ Cult.* **81,** 287–300.

Palazon, J., Cusid,´ O. R. M., Roi, C., and Pi´ nol, M. T. (1997). Effect of˜ *rol*genes from *Agrobacterium rhizogenes* TL-DNA on nicotine production in tobacco root cultures. *Plant Physiol. Biochem*. **35**, 155–162.

Peebles, C.A.M., Hughes, E.H., Shanks, J.V., and San, K.Y. (2009). Transcriptiona response of the terpenoid indole alkaloid pathway to the overexpression of ORCA3 along with jasmonic acid elicitation of *Catharanthus roseus* hairy roots over time. *Metab Eng*. **11,** 76–86.

Rai, A., Saito, K., and Yamazaki, M. (2017). Integrated omics analysis of specialized metabolism in medicinal plants. *The Plant J.* **90**, 764-787.

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

Ray, S., and Jha, S. (2001). Production of withaferin A in shoot cultures of *Withania somnifera*. *Planta Med*. **67**, 432–436.

Riker, A.J., Banfield, W.M., Wright, W.H., Keitt, G.W., and Sagen, H.E. (1930). Studies on infectious hairy root of nursery apple trees. *J Agri Res*. **41**, 507–540.

Ron, M., Kajala, K., Pauluzzi, G., Wang, D., Reynoso, M.A., Zumstein, K., Garcha, J., Winte, S., Masson, H., Inagaki, S., Federici, F., Sinha, N., Deal, R.B., Bailey-Serres, J., and Brady, S.M. (2014). Hairy root transformation using Agrobacterium rhizogenes as a tool for exploring cell type-specific gene expression and function using tomato as a model. *Plant Physiol*. **166**, 455–469.

Rothe, G., Hachiya, A., Yamada, Y., and Hashimoto, T., and Dra¨ger, B. (2003). Alkaloids in plants and root cultures of *Atropa belladonna* overexpressing putrescine *N*-methyltransferase. *J Exp Bot.* **390**, 2065–2070.

Sangwana, R. S., Chaurasiyaa, N. D., Lala, P., and Misraa, L. (2008). Withanolide A is inherently *de novo* biosynthesized in roots of the medicinal plant Ashwagandha (*Withania somnifera*). *Physiol. Plant.* **133**, 278–287.

Sato, F., Hashimoto, T., Hachiya, A., Tamura, K., Choi, K.B., Morishige, T., Fujimoto, H., and Yamada, Y. (2001). Metabolic engineering of plant alkaloid biosynthesis. *Proc Nat Aca Sci USA*. **98,** 367–372.

Sevón, N., and Oksman-Caldentey, K.M. (2002). Agrobacterium rhizogenes-mediated transformation: root cultures as a source of alkaloids. *Planta Med*. **68**, 859–868.

Sharma, P., Padh, H., and Shrivastava, N. (2013). Hairy root cultures: A suitable biological system for studying secondary metabolic pathways in plants. *Eng Life Sci*. **1**, 62-75.

Shiao, T.L., and Doran, P.M. (2000). Root hairiness: effect on fluid flow and oxygen transfer in hairy root cultures. *J Biotechnol***. 83,** 199–210.

Singh, H., Sameer, D., Verma P.C., and Singh, P.K. (2014). Evaluation of total phenolic compounds and insecticidal and antioxidant properties of tomato hairy root extract. *J Agric Food chem*. **62**, 2588-2594.

Sivakumar, G., Yu, K.W., Hahn, E.J., and Paek, K.Y. (2005). Optimization of organic nutrients for ginseng hairy roots production in large-scale bioreactors. *Curr Sci*. **89**, 641–649.

Sobczak, M., Avrova, A., Jupowicz, J., Phillips, M.S., Ernst, K., and Kumar, A. (2005). Characterization of susceptibility and resistance responses to potato cyst nematode (*Globodera* spp.) infection of tomato lines in the absence and presence of the broad-spectrum nematode resistance *Hero* gene. *Mol Plant Microbe Interact*. **18**, 158–168.

Spollansky, T.C., Pitta-Alvarez, S.I., and Giulietti, A.M. (2000). Effect of jasmonic acid and aluminum on production of tropane alkaloids in hairy root cultures of *Brugmansia candida*. *Electron J Biotechnol*. **3,** 72–75.

Sudo, H., Yamakawa, T., Yamazaki, M., Aimi, N., and Saito, K. (2002). Bioreactor production of camptothecin by hairy root cultures of *Ophiorrhiza pumila*. *Biotechnol Lett*. **24,** 359–363.

Thoison, O., Sevenet, T., Niemeyer, H. M., and Russell, G. B. (2004). Insect antifeedant compounds from Nothofagus dombeyi and N. pumilio. *Phytochemistry*. **65**, 2173–2176.

Tohge, T., and Fernie, A.R. (2009). Web-based resources for mass-spectrometry-based metabolomics: a user's guide. *Phytochem*. **70**, 450-456.

Valletta, A., Trainotti, L., Santamaria, A. R., and Pasqua, G. (2010). Cell-specific expression of tryptophan decarboxylase and 10-hydroxygeraniol oxidoreductase, key genes involved in camptothecinbiosynthesisin *Camptotheca acuminata*Decne (Nyssaceae). *BMC Plant Biol*. **10**, 69.

Van der Vossen, E.A., van der Voort, J.N., Kanyuka, K., Bendahmane, A., Sandbrink, H., Baulcombe, D.C., Bakker, J., Stiekema, W.J., and Klein-Lankhorst, R.M. (2000). Homologs of a single resistance–gene cluster in potato confer resistance to distinct pathogens: a virus and a nematode. *Plant J*. **23**, 567–576.

Veena, V., and Taylor, C.G. (2007). *Agrobacterium rhizogenes*: recent developments and promising applications. *In Vitro Cell Dev Biol – Plant*. **43**, 383–403.

Wang, Y.C., Zhang, H.X., Zhao, B., and Yuan, X.F. (2001). Improved growth of *Artemisia annua* L. hairy roots and artemisinin production under red light conditions. *Biotechnol Lett.* **23**, 1971–1973.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. **10**, 57–63.

Weber, J., Georgiev, V., Haas, C., and Bley, T. (2010). Ploidy levels in *Beta vulgaris* (red beet) plant organs and *in vitro* systems. *Eng. Life Sci*. 10, 139–147.

Weber, J., Georgiev, V., Pavlov, A., and Bley, T. (2008). Flow cytometric investigations of diploid and tetraploid plants and *invitro* cultures of *Datura stramonium* and *Hyoscyamus niger*. *Cytometry A.* **73**, 931–939.

Wilhelmson, A., Kallio, P.T., Oksman-Caldentey, K.M., and Nuutila, A.M. (2005). Expression of Vitreoscilla hemoglobin enhances growth of *Hyoscyamus muticus* hairy root cultures. *Planta Med.* **71**, 48–53.

Wis´niewska, A., Da˛browska-Bronk, J., Szafran´ski, K., Fudali, S., S´wie˛cicka, M., Czarny, M., Wilkowska, A., Morgiewicz, K., Matusiak, J., Sobczak, M., and Filipecki, M. (2013). Analysis of tomato gene promoters activated in syncytia induced in tomato and potato hairy roots by *Globodera rostochiensis*. *Transgenic Res*. **22**, 557–569.

Woodard, S.L., Wilken, L.R., Barros, G.O.F., White, S.G., and Nikolov, Z.L. (2009). Evaluation of monoclonal antibody and phenolic extraction from transgenic Lemna for purification process development. *Biotechnol Bioeng*. **104,** 562–571.

Yamazaki,Y., Kitajima, M., Arita, M., Takayama, H., Sudo, H., Yamazaki, M., Aimi, N., and Saito, K. (2004). Biosynthesis of camptothecin: in silico and *in vivo* tracer study from [1-13C] glucose. *Plant Physiol*. **134**, 161–170.

Yan, X.N., Sikora, R.A., and Zheng, J.W. (2011). Potential use of cucumber (Cucumis sativus L.) endophytic fungi as seed treatment agents against root-knot nematode Meloidogyne incognita. *J Zhejiang Uni Sci B.* **12,** 219–225.

Yu, K.W., Murthy, H.N., Hahn, E.J., and Paek, K.Y. (2005). Ginsenoside production by hairy root cultures of Panax ginseng: influence of temperature and light quality. *Biochem Eng J.* **23,** 53–56.

Zahwa Al-Shalabi, Marion, A., Stevens-Kalceff, and Pauline M.D. (2014). Application of *Solanum lycopersicum* (tomato) hairy roots for production of passivated CdS nanocrystals with quantum dot properties. *Biochem. Eng. J.* **84**, 36–44.

Zayed, R., and Wink, M. (2004). Induction of tropane alkaloid formation in transformed root cultures of *Brugmansia suaveolens* (Solanaceae). *Z Naturforsch*. **59c**, 863–867.

Zhang, L., Ding, R.X., Chai, Y.R., BonWll, M., Moyano, E., OksmanCaldentey, K.M., Xu, T.F., Pi, Y., Wang, Z.N., Zhang, H.M., Kai, G.Y., Liao, Z.H., Sun, X.F., and Tang, K. (2004). Engineering tropane biosynthetic pathway in *Hyoscyamus niger* hairy root cultures. *Proc Natl Acad Sci USA*. **101**, 6786–6791.

Zhang, L., Kai, G.Y., Lu, B.B., Zhang, H.M., Tang, K.X., Jiang, J.H., and Chen, W.S. (2005). Metabolic engineering of tropane alkaloid biosynthesis in plants. *J Integ Plant Biol*. **47**, 136–143.

Zhao, S., Tuan, P. A., Li, X., Kim, Y. B., Kim, H. R., and Park, C. G. (2013). Identification of phenylpropanoid biosynthetic genes and phenylpropanoid accumulation by transcriptome analysis of *Lycium chinense*. *BMC Genomics*. **14**, 802.

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

Zhou, M.L., Zhu, X.M., Shao, J.R., Tang, J.R., and Wu, Y.M. (2011). Production and metabolic engineering of bioactive substances in plant hairy root culture. *Appl Microbiol Biotechnol.* **90,** 1229–1239.

Zolala, J., Farsi, M., Gordan, H.R., and Mahmoodnia, M. (2007). Producing a high scopolamine hairy root clone in *Hyoscyamus muticus* through transformation by *Agrobacterium rhizogenes*. *J Agric Sci Technol*. **9**, 327–339.