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Elicitor-Induced Cellular and Molecular Events Are Responsible for Productivity Enhancement in Hairy Root Cultures: An Insight Study

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Abstract A wide range of external stress stimuli triggers a plant cell to undergo a complex network of reactions that ultimately lead to the synthesis and accumulation of secondary metabolites. These secondary metabolites help the plant to survive under stress challenge. The potential of biotic and abiotic elicitors for the induction and enhancement of secondary metabolite production in various culture systems including hairy root (HR) cultures is well-known. The elicitor-induced defense responses involves signal perception of elicitor by a cell surface receptor followed by its transduction involving some major cellular and molecular events including activation of major secondary message signaling pathways. This result in induction of gene expressions escorting to the synthesis of various proteins mainly associated with plant defense responses and secondary metabolite synthesis and accumulation. The review discusses the elicitor-induced various cellular and molecular events and correlates them with enhanced secondary metabolite synthesis in HR systems. Further, this review also concludes that combining elicitation with in-silico approaches enhances the usefulness of this practice in better understanding and identifying the rate-limiting steps of biosynthetic pathways existing in HRs which in turn can contribute towards better productivity by utilizing metabolic engineering aspects.

Keywords *Agrobacterium rhizogenes* · Elicitor(s) · Elicitation · Hairy roots · Secondary metabolites · Signal transduction

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Abbreviations
HRs Hairy roots
SM Secondary metabolite

Introduction

Elicitor: Inducer of Array of Protection Mechanisms

"Elicitor" is a scientifically described term for stress factors that directly or indirectly triggers the inducible defense changes in a plant system that results in an activation of array of protection mechanisms including induction or expansion of biosynthesis of fine chemicals which do have a major role in the adaptation of plants to the stressful environment. Succinctly, elicitors are the chemical compounds from biotic, abiotic, and physical sources that can stimulate a response in plants leading to the synthesis and accumulation of the same or novel secondary metabolites (SMs) and elicitation is the induced/enhanced biosynthesis of metabolites due to the introduction of elicitors in the system [1]. In this context, where several biotechnological strategies have been hypothesized and applied for the productivity enhancement, elicitation is recognized as the most practically feasible strategy for increasing the production of desirable secondary compounds from cell, organ, and plant systems [2–4]. In general, elicitors can be categorized on the basis of their nature (biotic or abiotic) and origin (endogenous or exogenous). Biotic elicitors are the substances of biological origin that include polysaccharides originated from plant cell walls (pectin, cellulose, etc.), micro-organisms (chitin, glucans, etc.), and G proteins which are bound to the receptors and act by activating or inactivating ion channels or enzymes. Abiotic elicitors comprise of substances that are of nonbiological origin. These include inorganic salts, metal ions, and physical factors such as pH, temperature, etc.

The active responses of plants require detection of signals provided by elicitors. Several classes of molecules derived from pathogens have been shown to be effectual as signals. Some of these act directly, whereas others act indirectly by stimulating any other substance of cell itself. Substances that originate at outer surface of cell comes under the category of exogenous elicitors (polyamines, polysaccharides) whereas those substances which originate at intracellular level like hepta-β-glucosides are known as endogenous elicitors.

Elicitors are also known to be race specific or nonrace specific depending on their interaction with the host plant. General or nonrace-specific elicitors produce resistance response to all cultivars of host and even in nonhost plant species whereas race-specific elicitors induce defense responses according to their specificity to host/cultivars. For example, the viral coat proteins from tobacco mosaic virus formed as structural components but involved in producing hypersensitive response in tomato and tobacco plants specifically. Specific elicitors are encoded by avirulence (*avr*) genes and these peptides are believed to bind with receptor peptide encoded by host resistance genes.

Hairy Roots: A Potential System for SM Production

Hairy root (HR) cultures offer a feasible system for the production of biologically active compounds. The HR cultures are generated by exploiting the unique natural ability of a soil-dwelling bacterium *Agrobacterium rhizogenes*. Upon infection, the bacterium transfers its T-DNA which lies between T_R and T_L regions of the Ri plasmid to the infected cell

where it integrates into the nuclear genome of host cell. The genes encoded (*rol* and *aux* genes responsible for HR phenotype and auxin synthesis, respectively) in T-DNA are of bacterial origin but have eukaryotic regulatory sequences enabling their expression in infected host plant cells. This results in the formation of HRs at the sites of infection (Fig. 1). HRs is able to grow without any exogenous hormonal supplementation and show fast growth rate with low doubling time. Besides, they closely mirror their ability with normal and intact root systems by producing a range of SMs at the same time concomitantly with growth. Such uniqueness contributes towards their exploitation for commercial metabolite production utilizing specific bioreactors [5]. Sometimes, HRs produces compounds that are normally not found in nontransformed roots. For example, in *Scutellaria baicalensis* Georgi, the roots obtained after Ri T-DNA-mediated transformation accumulated glucoside conjugates of flavonoids rather than that of glucose conjugates accumulated in nontransformed roots [6]. Owing to the site uncertainty of T-DNA integration into host cell genome and also off-time physiological state of the host cell, the transformed roots generally show different growth and SM accumulation pattern. HRs possesses an exclusive property of genetic and biosynthetic stability and without losing this property these culture systems produce desired SMs over consecutive generations.

A primary cause of the lack of success in commercial production of secondary compounds using HR culture systems is their low yield. The metabolite biosynthesis from HRs under in vitro conditions is highly influenced by its environmental and nutritional ambience. Further, any kind of exogenous supply into the culture medium in the form of growth hormones, precursor supplementation, and elicitation also affect their growth and productivity. In previous researches, enhanced production of important SMs and related precursors from HR systems were achieved by utilizing different strategies such as modification in culture conditions, clone screening, bacterial strain selection, etc. However, during past decade, elicitation is often applied to enhance the productivity of HR culture systems (Table 1). Various physical, chemical, and biological factors elicit the quantitative and qualitative alteration in bioactive SM contents due to the induced enzymatic pathways [2, 3, 7, 8]. Since plant SMs can be defined as compounds that have no recognized role to play in the maintenance of fundamental life processes in the plant that synthesize them, rather they do have a significant role in interaction of plant with its environment, this kind of study also proved to be useful in better understanding of SM production by plants during defense responses against stress challenges.

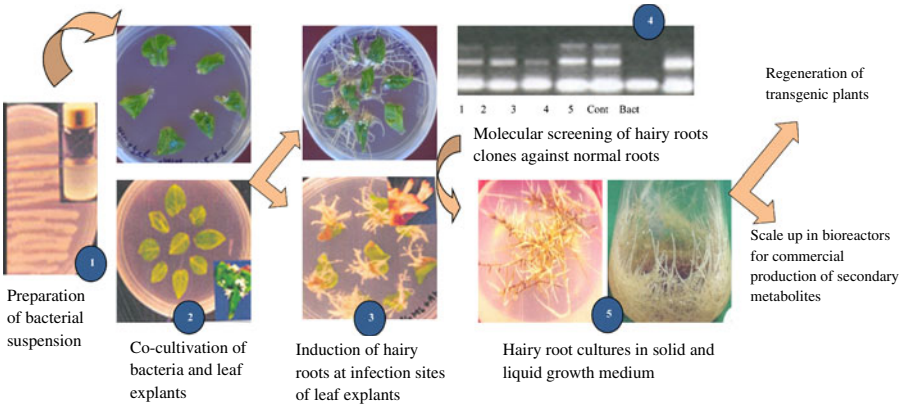

Fig. 1 Hairy root cultures from leaf explants of medicinal plants

Table 1 Plant species in which hairy root cultures showed enhanced secondary metabolite biosynthesis due to elicitation

Plant species	Secondary metabolite	Type of elicitor		Enhancement in SM yield over the control	Reference (s)
		Abiotic/physical	Biotic		
Ambrosia artemisiifolia	Thiarubrin A	VS	Fungal cell wall elicitor	8-fold	[65]
Ambrosia maritima	Polyacetylenes	MeJA		16.2-fold	[48]
Ammi majus	Coumarine furocoumarine	BION®	*Enterobactor sakazaki*	1.2	[46]
Artemisia annua	Artemisinin	22S & 23S homobrassinolide		57%	[66]
Atropa belladonna	Tropane alkaloids	Glutathione, H_2O_2, Cu^{2+}, Cd^{2+}	Fungal cell wall elicitor (Chitin, chitosan)		[67]
Azadiracta indica	Azadiractin	JA, SA	*Claviceps purpurea*	5- to 9-fold	[68]
Beta vulgaris	Betalines Betaxanthins	pH, temperature, O_2 stress, sonication, MeJA polyamines, Tween 80, CTAB, Triton–X 100, Glutamate, T50C		1.2–1.4-fold	[69, 70]
Brugmansia candida	Scopolamine Hyoscyamine	$CaCl_2$, JA, $AlCl_3$, pH, acetic acid, citric acid	Fungal cell wall elicitor (hemicellulose)	2.5-fold	[44, 55, 56]
Capcicum annum	Sesquiterpene, Phytoalexin	Cellulase		74%	[71]
Catharanthus roseus	Indole alkaloids	pH, $CdCl_2$, loganin, succinic acid, phenobarbitol, verapamil		4–6-fold	[45, 72]
Datura innoxia	Tropane alkaloids	Phenyl alanine, Phenyllactic acid, T-20		55%	[7]

Table 1 (continued)

Plant species	Secondary metabolite	Type of elicitor		Enhancement in SM yield over the control	Reference (s)
		Abiotic/physical	Biotic		
Datura stramonium	Alkaloids	MeJA, GSH, Cu^{++}, Cd^{++}		2.4-fold	[73]
Gloriosa superba	Colchicine	Coumaric acid, tyramine		5–6-fold	[74]
Hyoscyamus albus	Phytoalexin	MeJA, $CuSo_4$			[75]
Hyoscyamus muticus	Hyoscyamine sesquiterpene	MeJA,	Fungal elicitor (Chitosan)	200 times	[52]
Ocimum basilicum	Rosmarinic acid	SA, JA	Fungal elicitor (Chitosan)	2.67-fold	[13]
Oxalis tuberosa	β carbolines	SA, Jasmonate	Fungal elicitor (Chitosan)	7–9-fold	[76]
Panax ginseng	Saponins	VS, Peptone MeJA, Jasmonate	Fungal elicitor (Chitosan)	4-fold	[77–79]
Pharbitis nil	Umbelliferone Scopoletin	$CuSo_4$, MeJA			[80]
Rubia tinctorum	Lucidin anthraquinone	MeJA, SA, ethephon		2-fold	[48]
Salvia militiorrhiza	Diterpenoid tanshinones	Ag^{+}, β amino butyric acid	Carbohydrate fractions from yeast extract	3–9-fold	[81–84]
Scopolia parviflora	Scopolamine		Bacterial elicitors	2.8-times	[85]
Solenum tuberosum	Sesquiterpene, lypoxygenase	MeJA	β cyclodextrin (bacterial origin)		[86]
Swainsona galegifolia	Swainsonine	$CuSO_4$, pipeolic acid, malonic acid		2–3-fold	[87]
Tagetus Patula	Thiophene	Ca^{++}, Ethephon, pH	*A. niger, F. oxysporum, E. coli, B. subtitles*	85%	[88]
Trigonella foenum gracium	Diosgenin	pH	Fungal elicitor (Chitosan),	3 times	[89]
Tropaeolum majus	Glucotropaeolin	MeJA, SA, β amino butyric acid, acetyl SA	Yeast extract	3–5-fold	[90]

The upcoming text discuss various events occur during elicitation at cell and molecular level that leads to the enhancement in metabolite production through HR cultures. It also elucidates the efficacy of elicitation in better understanding and identification of gene expression patterns through transcriptome analysis during stress.

How Elicitors act? Signal Transduction

Signaling System on Cell Surface: Signal Perception

Having an analogy to electronic transducers, the signal perception and its further processing involves a complex interplay of a number of components that transmit information from one source to other leading to different immune responses (Fig. 2). At first instance, these responses begins with the recognition of pathogen-associated or microbe-associated molecular patterns (PAMPs or MAMPs). The PAMPs and MAMPs include cell wall components of pathogens, flagella, membrane lipids, polysaccharides, etc. Theses PAMPs and MAMPs are recognized by the plant through pattern recognition receptors present on its cell surface. Thus, the perception of a stimulus by the cell depends on various receptors with specific properties present on cell surface [9, 10].

Among the different classes of elicitor receptors known, the major groups comprises of plant R-proteins and transmembrane receptor like protein kinases (RLKs). The plant R-proteins are generally responsible for the identification of race-specific elicitors encoded by *avr* genes expression of which produces hypersensitive response in plants against pathogenic stress [11].

Following the "gene-for-gene interaction" concept, this kind of response is a result of expression of disease resistance genes which allows the plant to detect and resist pathogenic strains that express genetically complementary genes called *avr* genes. An *avr* gene promotes the production of a unique signal molecule by the pathogen which functions as a specific elicitor. Only those plants which carry the complementary resistance gene respond to this elicitor and show hypersensitive response. That is why all plants do not respond to all types of elicitors (elicitor specificity). This specificity of elicitors suggests that plants have the ability to sense a number of structurally different molecules as signals as apparent by the existence of specific receptor for each type of elicitor and accordingly respond to them through their altered physiological and biochemical changes [12]. Also, the elicitor specificity in conjunction with the type of host tissue challenged influence the production of

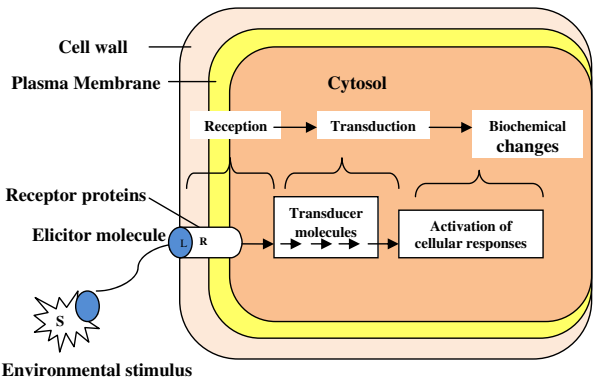

Fig. 2 Elicitor-induced cellular events in a cell

different stress-related compounds. This was evident from the case of *Oxalis tuberosa* hairy root exudates in response to cell wall extracts of two fungal species that differentially altered the quantitative production of compounds in the challenged root tissue [13] and this in vitro root exudation was very much comparable to the exudation in rhizosphere from *Oxalis* and other plant systems in response to various stresses including microbial infection and mechanical injury [14].

Occurrence of variable length leucine-rich repeat domains and thus enabled for frequent protein–protein interactions, the RLKs class of elicitor receptors perceive a wide array of stimuli and is likely to be present in various plant species ([9] and references there in). The identification of a LysM type receptor kinase gene in *Lotus japonicas* roots is a well-known example of RLKs class of receptors on the root cell surface. It is supposed to be involved in legume perception of rhizobial signals. In the presence of this Nod factor receptor kinase (*nfr* gene product), the molecular communication initiates the interaction and signals the secretion of legume flavones that stimulate the bacteria to produce a lipochitin-oligosaccharide signal molecule (Nod-factor), which in turn triggers the plant response towards the microbe [15, 16]. In a recent study, *L. japonicas* HR cultures were used for the in-planta functional analysis of nod factor receptor 1 and 5 (*nfr*) and also for receptor-mediated signaling. These two receptors are the major candidates for bacterial nod factors and required by the plant at initial phase of response to bacteria. The work concluded that *nfr* mutated root lines upon infection with *Mesorhizobium loti* could not be able to produce bacteria-filled nodules as compared to nonmutant root lines. This was very much similar to *nfr* mutant *L. japonicus* plants where nodules are not formed due to nonfunctioning of receptors at early stages of signaling [17].

These kinds of evidences suggest that HR in vitro systems closely mimic the biochemical and physiological responses occur in intact plant during stress conditions. Further, the *L. japonicas* HR cultures were also utilized Weerasinghe et al. [16] as a model system to visualize the changes in cytoskeletal dynamics and the morphology of microtubule and actin filaments in root hair cells for signaling during host–pathogen interaction. For this purpose, the HR cultures were generated having green fluorescent protein coding sequence fused with microtubule-associated protein, MAP4, and the actin-binding protein, talin.

Elicitor Signaling System Inside the Cell

Elicitor signal transduction is a multiple component/transducer network comprises of various chronological reactions to establish a competent defense. These diverse components cover some parallel or cross-linked signaling pathways leading to different target responses. An elicitor signaling pathway may vary with perception of different elicitor signals or with target defense responses. Subsequent to signal perception, the sequentially occurring cellular changes can be organized as follows: receiving of elicitor by a receptor, reversible phosphorylation, and dephosphorylation of plasma membrane and cytosolic proteins, fluxes in Ca^{2+} and other ions, extracellular alkalization and cytoplasmic acidification, activation of major secondary messaging pathways of phospholipases (PLC)/1,4,5-triphosphate (IP_3)-diacylglycerol (DAG)/PKC, cAMP and mitogen-activated protein kinases (MAPK) and reactive oxygen species production ([18] and reference within). Some signaling pathways related to jasmonate, salicylic acid and ethylene metabolism are also known to be involved in this phenomenon.

The association of highly conserved heterotrimeric guanine nucleotide binding proteins (G proteins) to cell signaling is an important aspect to cover. The existence of plant G

protein coupled signaling in *Arabidopsis* and tobacco is well documented where the GTP binding proteins comprises one α, one β subunit, and two γ subunits [19, 20]. The rice dwarf variety mutated at α G protein subunit gene level show concealed H_2O_2 production and pathogenic-related gene expression induced by elicitors [21]. Biochemical and genetic evidences obtained from studies on G protein activators or inhibitors shows that heteromeric and monomeric subunits of G proteins have undisputable contribution in transmitting signals to molecules, including GTPase, ion channels, phospholipases and several signaling pathways that are effectors in the responses to various biotic and abiotic stress conditions including pathogen elicitation, O_3 treatment, and drought etc. [22–24]. Supplementation of G protein inhibitors such as suramin in the ambience of cell inhibits the activity of receptor-coupled G proteins and consequently the phytoalexin production suggesting the possible involvement of G protein is signal communication within the cell [25].

Elicitor-induced Ca^{2+} fluctuation is thought to be one of the early events that occur as a key herald for almost all downstream reactions (Fig. 3). However, K^+/H^+ exchange, Cl^- effluxes are also known to be the early responses of plant cell induced by avirulent pathogen or elicitors. Several techniques that are used to detect intracellular Ca^{2+} showed that elicitor-induced calcium influx within the cell generally results in changes of calcium level ranging between 50–100 nM and 1–5 μM within few minutes of elicitor treatment [26]. Elicitor-induced Ca^{2+} is imperative for elicitor-induced accumulation of plant SMs. This essential event triggers many intracellular processes through Ca^{2+} sensors such as calmodulin which after binding with Ca^{2+} get activated and further stimulate calmodulin-dependent protein kinases, membrane-bound enzymes, and protein phosphatase. For example, NAD(P)H oxidase, a major cause of generation of reactive oxygen species (H_2O_2 and O_2^-) and Ca^{2+}-dependent kinases are regulated by Ca^{2+} binding induced by elicitors and acquire key roles in plant defense responses ([26] and references within). Ca^{2+} is also found to be responsible for administering the role of extracellular ATP. In *Salvia miltiorrhiza* hairy roots, yeast elicitor induced as well as exogenously supplied ATP

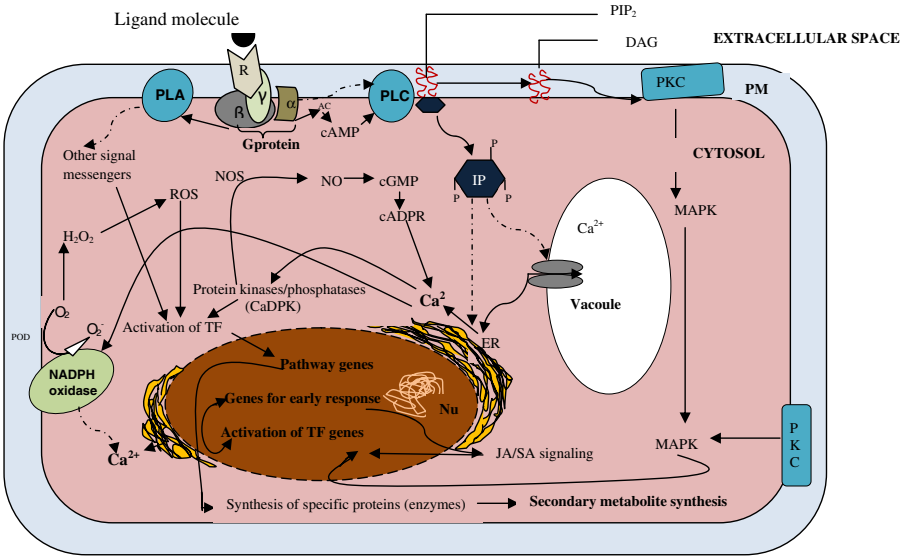

Fig. 3 Schematic presentation of involvement of various signaling molecules in a stress-challenged system

accumulation resulted in induction of H_2O_2 and nitric oxide [27, 28]. Both the events require the membrane Ca^{2+} influx. Both H_2O_2 and nitric oxide are well-known signal transducers and these studies showed that in elicitor-challenged hairy root system ATP dependant on Ca^{2+} plays an important role in cell signaling. Ca^{2+} binding may sometimes results in activation of PLC that ultimately leads to the activation of some other secondary messengers such as IP_3 and DAG [29] (Fig. 3). Elicitor-induced breakdown of phosphoinositide by specific PLC which is activated by Ca^{2+} fluctuations, produces IP_3 and DAG [30]. An increased PLC activity or IP_3 level in cytosol of plant cells in response to elicitor treatment is required for SM accumulation [30]. As far as involvement of phospholipases, IP_3, and DAG during defense is concerned, there are reports that suggest that this cascade plays a crucial role in response of plants to a number of elicitors. In *Arabidopsis*, gene coding for PLC was cloned that showed stumpy expression at normal conditions whereas under abiotic stress, it showed noteworthy expression level [31]. Similar studies have been done in *Vigna radiata* and *Solanum tuberosum* where isoforms of PLC have been identified which are greatly affected under stress conditions [32, 33]. Likewise, the involvement of IP_3 signaling in biotic elicitor-induced accumulation of anthraquinones in *Rubia tinctorum*, furanocoumarines in parsley, and pisatin in pea at cellular level was observed in cell cultures of these plants [34, 35]. In pea, elicitor-induced Ca^{2+} fluctuations cause increment in DAG level in host cell cytosol which is found to be mandatory for phytoalexin accumulation since inhibition of DAG production by limiting PLC activity can limit the pisatin production.

Among other important components that are involved in elicitor-induced signaling inside cell is MAPKs. These MAPK cascades are involved in downstream of receptors of cell surface that transduce external signal into intracellular responses. This cascade can turn on by a variety of biotic and abiotic elicitors which ultimately leads to proceeding pathways and specific genes. Several signals triggered by different elicitors can be transmitted by MAPK pathways. Further, a single elicitor not only activates one but several MAPKs and different stimuli can activate the same pathway [36]. In *Catharanthus roseus* hairy roots, the mitogen-activated protein (MAP) kinase-like activity was determined in protein isolated from the extracts obtained from transformed roots. The study evidenced that this 56 kDa polypeptide with MBP kinase activity may be involved in signaling the response to cold and osmotic stress to the hairy roots [37]. Further, the isolation of another 51 kDa protein of MAP kinase family from beet roots grown in hyperosmotic ambience is reported that supposedly participate during osmotic stress tolerance [38]. Though, the exact phenomenon of MAP activation and results is still holds some unresolved aspects, it is widely accepted that their activation leads specific gene expression encoding for those enzymes, which play an important role in biosynthetic pathway of SMs. Sometimes this activation involves induction at transcriptional, translational, or post-translational levels. In *R. tinctorum* cell cultures, chitosan provided by fungal wall elicitor signals stimulated MAPKs that results in the activation of transcription factors, which leads to the overexpression of isochorismate synthase enzyme encoding gene that gives an overall outcome of increased anthraquinone level [10, 39].

Transcription Factors Involved in Elicitation and Activation of Biosynthetic Pathway Genes: Role of Jasmonic Acid and Allied Compounds

The transcription factors (TF) are sequence-specific DNA binding proteins that interact with target gene at its promoter region and regulate the rate of initiation of mRNA synthesis mediated by RNA polymerase II. These TF proteins regulate transcription depending upon

tissue type as well as in response to the internal signals produced by physical and chemical challenging factors. It is supposed that all signal transduction pathways finally congregate on TFs and almost all genes for SM biosynthesis are regulated by specific TFs. These TFs can be activated either directly by elicitor molecule or by end results of activation of any of the existing signaling pathways. TFs may be constitutively synthesized or activated by some other transcription factors through protein–protein interaction, phosphorylation/dephosphorylation, etc. Sensing of external and/or internal signals followed by their transduction supposedly modulate the levels and activities of these regulators (TF), leading to appropriate response to any kind of stress or elicitor. One of the best-studied examples of elicitor-induced SM accumulation in hairy root cultures is that of stress hormone jasmonic acid (JA)-mediated biosynthesis of terpenoid indole alkaloids (TIA) [40]. In *C. roseus*, addition of methyl jasmonate (MeJA) to culture medium induces TIA production through cells. The regulation of JA responsive activation of several TIA biosynthetic genes is carried out by octadecanoid-responsive *Catharanthus* AP2/ethylene-responsive factors (ERF) domain (ORCA) class of transcriptional factors. The possible role of octadecanoid pathway in TIA production was assessed and reported by Peebles et al. [41]. The study included a comparison of increased TIA accumulation due to exogenous addition of JA to *C. roseus* HR cultures with that of endogenous production of JA through analyzing octadecanoid pathway genes. The promoter element having the affiliation with ORCA class of transcription factors and their involvement in jasmonate and elicitor-responsive gene expression has been identified in *strictosidine synthase* gene responsible for the conversion of secologanin into strictosidine during TIA biosynthesis [42]. Other transcriptional factors ORCA2 and ORCA3 of AP2/ERF domain class (APETALA2/ethylene responsive factor) have also been identified to be involved in elicitor especially MeJA responsive gene expression [42]. The effect of overexpression of octadecanoid-responsive *Catharanthus* AP2/ERF domain and MeJA on transcriptional activation of catharanthin biosynthetic pathway in *C. roseus* HR cultures was explored by Zhou et al. [43]. However, the study revealed that ORCA3 overexpression causes slight decrease in catharanthine accumulation while MeJA treatment caused an increment in transcript levels of pathway genes as well as catharanthin concentration. Thus, this transcriptional repressor response may explain the antagonistic effects of MeJA on alkaloid biosynthesis.

Apart from their involvement in transcriptional gene regulation, the JA and allied compounds also act as transducers of elicitor signals for the production and/or promotion and accumulation of various groups of SMs including alkaloids [44, 45], Coumarines and furocoumarines [46], antraquinones and saponins [47], etc. in HRs and cell suspension systems. The exposure of *Brugmansia candida* and *Ambrosia maritima* HR cultures to jasmonic acid promoted hyoscyamine and polyacetylenes production respectively [44, 48]. The exogenous supply of JA and its volatile methyl ester MeJA sets off various morphological and physiological changes by inducing gene expression escorting to the synthesis of different proteins mainly associated with plant defense responses as well as elevated SM accumulation in *R. tinctorum* HR cultures [47, 49]. Many elicitors stimulate endogenous JA biosynthesis mediated through octadecanoid pathway in plants which provide the evidence that JA signaling pathway can be regarded as a transducer or mediator for elicitor signaling ([50, 51] and references within). This endogenous biosynthesis of JA in turn activates defensive genes eventually yielding a variety of JA-induced proteins. Therefore, a transcriptome analysis of a JA or MeJA-challenged tissue can be helpful in discovering and exploring those genes which actively take part in biosynthetic pathways. However, in some cases as in *Hyoscyamus muticus* HR cultures, these molecules alone cannot produce the desired sesquiterpenes in the culture system suggesting the need of

some advance knowledge with reference to their mode of action [50, 52, 53]. Though SA is also a well-known elicitor signal transducer but it is not the universal one. The exogenous supplementation or fluctuation in endogenous level of SA by any means induces gene expression related to biosynthesis and production of some classes of SMs [54]. In HR cultures of *B. candida* instability in endogenous SA level significantly stimulates the release of tropane alkaloids. Further, flux in SA level is also found to be accountable for the stimulation of related biosynthetic gene expression in *Scopolia parviflora* HR cultures [55–57].

The studies related to the role of jasmonates in inducing biosynthesis of many SMs clearly indicate the involvement of sequence-specific DNA binding proteins as a regulator of several genes. Such information repositories may shed some light on similar mechanism that is occurring in HR cells at present, when HR cultures of several TIA-producing plants are being employed for active alkaloid production and several methodologies are being exploited for their productivity enhancement. In further extension, if assistance could be taken from the information gained by transcriptome, proteome, and metabolome analysis, collected in global "omic" banks will certainly resolve related molecular mysteries.

Among other members of AP2/ERF domain class of transcription factors, are ethylene-responsive element-binding proteins (EREBPs) and ERFs. These have been isolated from tobacco and *Arabidopsis* [58]. These proteins specifically bind with ethylene-sensitive guanine cytosine-rich *cis* element (GCC box) found commonly in several genes responsive for resistance mechanism under stress conditions. Such genes have structural and functional analogy with pathogen-responsive (PR) genes. Some times in the presence of ethylene these EREBPs and ERFs are regulated by ethylene insensitive3 proteins which are crucial for their activation. This kind of ethylene-sensitive transcriptional regulation is present in phenyl propanoid pathway that leads to the biosynthesis of flavonoids, lignin, and phenolic compounds upon elicitor challenge. Here, the rate-limiting enzyme chalcone synthase (CHS), phenylalanine ammonium lyase (PAL), and 4-coumariate Co A ligase (4CL) comprises of highly conserved@ *cis* elements in their promoter region of their corresponding gene *CHS*, *PAL*, and *4CL*. These *cis* element can bind by TF_S such as Myb and bHLH (basic helix loop helix) in response to wounding, light fluctuation, UV irradiation, and microbial challenge etc. Successful genetic manipulation of Myb and bHLH genes leads to the increased pigmentation and accumulation of flavonoids and anthocyanins [59, 60].

Sometimes, the TF gene expression can be autoregulated [61]. Their structure and arrangement of components of functional sequence becomes necessary and sufficient for quick elicitor-responsive activation. For example, in case of parsley WRKY1 protein which is associated with expression of parsley *PR1* genes (specific pathogen-responsive gene) has its unique functionally defined TGAC-containing promoter sequence responsible for its own functions in response to elicitation [62].

Conclusion

In the present scenario, hairy root-type plant in vitro systems are being used worldwide not only to explore plant-based secondary metabolite production but also to evaluate related metabolic processes both on biochemical and molecular level. Besides, HR cultures are also utilized to explore the root-specific responses during root pathogen interactions [14, 63]. This kind of analysis can help to understand early molecular responses in terms of recognition, reception, and further processing of signals within the tissue. Hairy root cultures are preferred for such kind of studies as they are easy to handle and closely mimic

the natural activities in controlled physical and nutritional environment irrespective of any limitation. Elucidation of the signaling network during elicitor challenge will help to discover the interaction between signal transducers and activation of SM biosynthetic genes and regulatory factors. A combination of this information with transcriptomic and metabolomic approach may open new and exciting perspectives in the area of developing links between identification of rate limiting steps, transcriptional, and post-transcriptional regulation of metabolite synthesis and product level. Transcriptome analysis of HR culture under different elicitor treatments could display differential expression patterns and regulation of plant SM-related genes and stress-resistant genes in an individual system and can also facilitate their isolation and further exploitation through engineering approaches. Microarrays are providing a powerful approach for this purpose. For example, micro array-based screening of differentially expressed genes of fungus-infected and drought-stressed peanut (legume) [64] provides an information bank that can be utilized in exploiting fungus-challenged or dehydrated HR cultures of related legume species to study the expression of common genes that positively influences the synthesis and/or accumulation of commercially important metabolites. Besides, expressed sequence tags are also contributing in the same way to explore the genes involved in various types of stress tolerance. Such resources of information can facilitate in utilizing the specific and efficient metabolic engineering approaches and in developing new pathways for enhanced production of the target molecule in a homologous or heterologous system. As the regulation is mainly controlled at enzyme and gene expression levels, a quantitative and real-time metabolome scrutiny of metabolic intermediates will provide useful information about exact flux shifts due to stresses. In fact, a systemic biology approach, whereas many parameters as possible are considered, quantified, and combined with various computational tools for identifying correlations, similarities, and differences, should be the next evolving insight in the complex and dynamic processes of the elicitation and secondary metabolism of hairy root cultures.

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