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Review Article

ELICITATION AS A MEANS FOR ENHANCED PLANT SECONDARY METABOLITES THROUGH HAIRY ROOT SYSTEM

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ABSTRACT

Elicitation can regulate a large number of control points and stop the expression of key genes involved in cellular activities at biochemical and molecular levels involving signal compounds. This process not only enhances secondary metabolites but also stimulates defense response. It involves physiological and morphological changes within a range for some physical and chemical factors called elicitors. Elicitors are of much interest to the scientific community due to the difficulty faced in the synthesis of secondary metabolites because of their complicated structures. A large number of synthetic and natural elicitors are being used to enhance the secondary metabolites in plant cell cultures including jasmonic acid, methyl jasmonate, salicylic acid, casein hydrolysate, etc. In addition to natural and synthetic elicitors, researchers have examined the accumulation of bioactive compounds by using biotic elicitors, such as yeast extracts, and abiotic elicitors such as metallic nanoparticles and have reported interesting results. Thus, the present review presents information exclusively on the use of elicitation process for manipulation of secondary metabolites for industry exploitation through hairy root system.

Keywords: Elicitation, biotic and abiotic elicitors, secondary metabolites, hairy root culture.

1. INTRODUCTION

Plants produce various compounds that can be categorized into primary metabolites and secondary metabolites. Primary metabolites include sugar, protein and amino acids that are essential for the plant's survival. In contrast, secondary metabolites are not essential for the survival of plants, but plants suffer in their absence. Some of these metabolites can have beneficial uses, while others can be toxic. Plants offer an extraordinary variety of bioactive small molecule metabolites that have potential applications in pharmaceutical, nutraceutical and agrochemical industries. Production of these metabolites, especially in plant cell cultures, e.g., hairy root metabolism, can be directed through genetic manipulation or environment such as exposure to pathogens.

Researchers studying alkaloid production in transformed hairy root cultures noted that optimization of the medium, specifically mineral composition and sucrose concentration can play either a neutral or positive role in the growth of roots as well as in secondary metabolite production [1].

Secondary metabolites are swiftly gaining renovation in a wide variety of fields such as health care, cosmetics, biomedical, food and feed, drug-gene delivery, environment, health, mechanics, optics, chemical industries, electronics, space industries, energy science, catalysis, light emitters, single electron transistors, nonlinear optical devices and photo-electrochemical applications [2]. Due to secondary metabolites' physical and chemical properties, importance of nanomaterials, and increasing usage in different disciplinary sciences, the scientific communities have focused on their enhancement by different elicitors. However, the exact mechanism of elicitation is yet to be elucidated. Researchers have revealed that metal nanoparticles can cross through the roots or leaf by capillary action [3]. But the uptake of nanoparticles is dependent on size [4] due to the varying nucleation size. A recent study [5] reported that the uptake and distribution of gold nanoparticles depended on the surface charge of the nanoparticles and plant species. Another study demonstrated that exposure of plants to metal nanoparticles had both positive and negative effects, depending on the particle size and shape [6].

The literature shows contradictory reports on the accumulation, biotransformation and toxicity of nanoparticles in various plant species. However, the effects of nanoparticles on different growth parameters mainly depend on plant species [7]. For example, silver nanoparticles influenced the growth of *Brassica juncea*, *Phaseolus vulgaris* and *Zea mays* [8, 9].

Table.1. Effect of various elicitors for Enhancement of Secondary Metabolites through Hairy Roots

Hairy Roots	Elicitor	Bioactive Compound	Ref.
Brugmansia candida	$AgNO_3$,$CdCl_2$	Hyoscyamine and Scopolamine	[38]
Salvia miltiorrhiza	Ag^+ and YE	Tanshinone	[23]
Datura metel L.	$AlCl_3$ and NaCl	Scopolamine	[22]
Salvia castanea	Methyl Jasmonate	Tanshinone	[54]
Datura metal	Bacillus cereus	Atropine	[55]
	Staphylococcus aureus	Atropine	
Taverniera cuneifolia	Rhizobium leguminosarum	Glycyrrhizic acid	[56]
Camellia sinensis	Maltose	Catechin	[57]
Pamax ginseng	Jasmonic acid	Ginsenoside	[58]
Pamax ginseng	Methyl Jasmonate	Ginsenoside	[59]
Ambrosia artemisiifolia	Vanadyl sulfate	Thiarubrine A	[60]
Ammi majus	*Enterobacter sakazaki*	Umbelliferone	[61]
Tagetes patula	*Fusarium conglutinans*	Total thiophenes	[62]
Cichorium intybus	*Phytopthora parasitica*	Esculin and esculein	[63]
Scopolia parviflora	*S. aureus*	Scopolamine	[64]
Salvia miltiorrhiza	Yeast Elicitor	Rosmarinic acid	[65]
Brugmansia candida	Yeast extract Hemicellulase	Scopolamine and hyoscyamine	[38]
Catharanthus roseus	Methyl jasmonate	Catharanthine	[66]
Catharanthus roseus	*Penicillium jasmonate*	Catharanthine	[67]
Datura stramonium	Wide range of abiotic elicitor	Accumulation of sesquiterpene phytoalexins	[68]
Hyoscyamus muticus	*Rhizoctonia solani*	Phytoalexins	[69]
	Rhizoctonia solani	Solavetivone	[70,71]
	Rhizoctonia solani	Solavetivone and lubimin	[72]
	Lnonotus obliquus	Stimulation of hyoscyamine	[73]
	$CuSO_4$	Stimulation of hyoscyamine	
	Purified chitosan	Stimulation of l-hyoscyamine	[74]
	Jasmonic acid and Methyl Jasmonate	Strong stimulation of polyamines	[75]
	Methyl Jasmonate + Wounding + *R. solani*	Lubimin accumulation	[76]
Nicotiana tabacum	Yeast Extract	Capsidiol and Debneyol	[77]
Panax quinquefolium	trans-anethole	Saponin	[78]

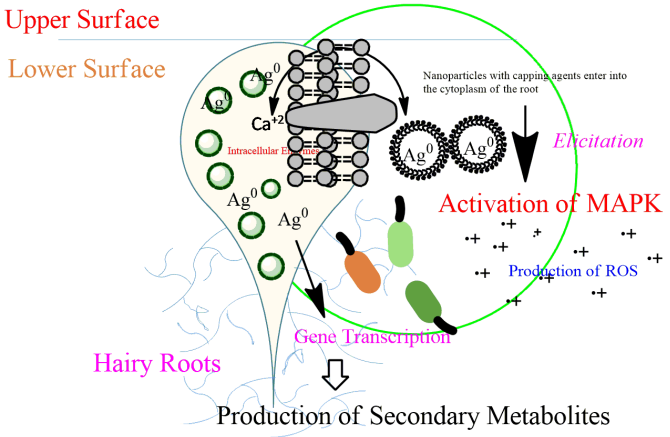
In the present review, we focused on the role of elicitors in terms of increasing secondary metabolites and attempted to understand their mechanism (Schematic Representation 1). Especially elicitation of selective defensive metabolites through biotic and abiotic elicitors has potential applications in the production of high value-added therapeutics (Table. 1) as well as understanding the mechanism of pathogens which have difficulty passing into the cell directly.

2. BACKGROUND

2.1. What is Elicitation?

Elicitation is a process used to enhance secondary metabolites of cell cultures and stimulate defense response [10, 11]. In this process, certain physical and

chemical factors called elicitors facilitate physiological and morphological changes [12]. In 1975, scientist N.T. Keen used the term elicitor when he was working on *Phytophthora megasperma* culture on soybean [13]. Each plant has its own protection scheme against particular pathogens. In case of an attack, plants undergo cell wall reinforcement in which the physiology and structure of the cell wall are changed by deposition of lignin and construction of chitinase and glucanase [14]. This indicates that all alkaloids in a plant are not enhanced during elicitation.



Step- 1. Nanoparticles (abiotic elictor) with capping agents entered into the cytoplasm of the root.

Step - 2. Activating the MAPK and generate ROS which may helps in enhancing the secondary metabolites.

Fig. 1: Schematic Representation of Hypothesized mechanism behind Elicitation and Enhancement of Secondary Metabolites through elicitors in Hairy Roots

Elicitors can trigger physical and morphological responses and phytoalexin accumulation depending on their origin and molecular structure [12, 15]. It may include biotic elicitors from bacteria, yeast extract, fungal cell wall and carbohydrates or abiotic elicitors such as metal ions and inorganic compounds [10]. In some cases, plant cell wall components, as well as chemicals that were released because of physical damage, can act as elicitors [15].

2.2. Biotic Elicitors

Biotic elicitors are directly released by microorganisms and recognized by the plant cell wall fragments and enzymes [16]. In 1995, a research group [17] found that peroxidase activity reduced during the exponential growth of hairy roots due to the depletion of phenolics in the medium. However, the peroxidase levels increased by three and a half folds when they used yeast extract as elicitors. A few researchers [18] worked on hairy root culture of *Beta vulgaris* for elicitation of betalain synthesis with dry cell powders of various yeasts, bacteria and fungi like *Aspergillus niger*, *Penicillium notatum*, and *Rhizopus oligosporus*. Similar results were also obtained in another study [19], where polysaccharide fraction of yeast extract was used as a potent elicitator for tanshinonc accumulation in hairy roots of *Salvia miltiorrhiza* with control. Biotic elicitors (hormonal) such as methyl jasmonate and jasmonic acid have shown effective stimulation of various secondary metabolites in various plant species [20, 21]. In *Datura metel* L., the addition of yeast extract in the media resulted in increased growth index of hyoscyamine and scopolamine in root culture. However, the incorporation of very high concentration of yeast extract resulted in the decline of alkaloid content [22].

2.3. Abiotic Elicitors

Research on the use of abiotic elicitors in hairy root culture is still underway. However, in recent years, various strategies have been reported for enhancement of bioactive compounds. In *S. miltiorrhiza*, the addition of Ag^+ (30 µM) to the medium stimulated the hormone responsible for tanshinone production [23]. In general, biotic elicitors increase the alkaloid content but fail to stimulate the hormone responsible for alkaloid pathway. Recent reports have suggested that treatment with $AlCl_3$ increased hyoscyamine and scopolamine production in datura root culture and stimulated the production of alkaloids [22].

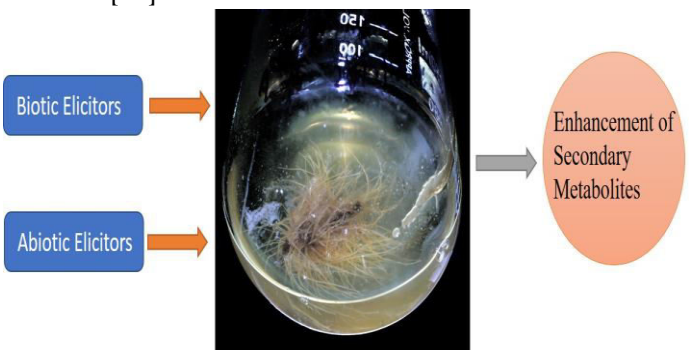


Fig. 2: Schematic representation of enhancing metabolites by using biotic and abiotic elicitors.

2.4. Nanoparticles for elicitation of secondary metabolites

Among the various strategies available to increase the level of metabolites, nanoparticles can be effectively used as elicitors in plant biotechnology [24]. This may be

because of their unique properties such as number of atoms on surface, increase in total free energy, etc.

2.5. Synthesis and Importance of Nanoparticles

Phytosynthesis of nanoparticles increased massively when different macro-microscopic organisms such as plant, bacteria, fungi, seaweeds and microalgae [25, 26] were used. Their structures exhibited significantly novel and improved physical, chemical and biological properties. However, microbe-mediated phytosynthesis is more expensive and disadvantageous [27]. Thus, plant extract-derived nanoparticles have shown superior antioxidant activity due to their greater biocompatibility, scalability and applicability [27]. Another reason favoring plant-mediated synthesis is the readily available reducing agents such as enzymes (hydrogenases and reductases). Reports from FTIR studies concluded that extracts containing terpenoids, flavonoids, phenols, etc. [28-31] help as precursors for generating nanoparticles.

Nanoparticles are associated with potential toxicity that affects their applicability. However, it should be noted that the synthesis of nanoparticles involve zero oxidation state, and thus, they cannot react directly with any other living organism. In previous studies, the synthesis method [32] included different organic and inorganic reducing agents such as sodium borohydride, sodium citrate, ascorbate, elemental hydrogen, Tollen's reagent, N,N-dimethyl formamide and (poly ethylene glycol) [33,34]. It also included separate capping agents. Thus, plant extract-mediated synthesis played a major role as the compounds present in the extract acted as capping agents.

2.6. Importance of Hairy Root Synthesis

Considering the importance of secondary metabolites in pharmaceutical, cosmetics and various fields, it is important to study the enhancement of secondary metabolites. This is because cell cultures are genetically unstable, tend to produce low yields of secondary metabolites and are time-consuming [35,36], except for a few metabolites such as berberine and shikonin. Since synthesis is linked to root differentiation [37], undifferentiated cultures do not produce effectively. In this respect, hairy or transformed roots have several advantages over normal ones; some of these advantages include genetic and biochemical stability and requirement of only normal free media [38]. The secondary metabolites formed by hairy roots are the same as those usually synthesized in mother plants with higher yields [39].

2.7. Possible Mechanism behind Hairy Root Synthesis

Hairy roots are produced by infecting explants with *Agrobacterium rhizogenes*—a gram-negative soil bacterium that induces Ri plasmid, which is commonly known as virulence plasmid that integrates into the genome of a plant [40, 41]. In flora, *Agrobacterium* attacks mainly at the wounded site [42] a wounded site helps in the entry of bacteria by the secretion of phenolics via the transferred DNA (T-DNA) process. During the infection process, T-DNA from the bacteria integrates into a plant cell genome [43]. Even if the particular mechanism of transformation remains unknown, DNA homologous sequence to Ri plasmid are found in transformed DNA but not in normal plant DNA [41]. This is similar to that found in *Agrobacterium tumefaciens*, which causes crown gall tumors, whereas *Agrobacterium rhizogenes* abuses root formation. Wide literature is available regarding Ti plasmids; therefore, readers can refer to some recent review articles for more information [44].

Plant signals induce the bacteria for the expression of a range of virulence proteins. At the wounding site, T-DNA and Vir proteins are imported into the host cell through the bacteria. The expression of T-DNA encoded bacterial genes in the plant system (host) results in the production of enzymes that catalyze the synthesis of plant hormones responsible for inducing hairy roots [45]. The T-DNA that integrates into plant genome is located on the Ri plasmids and are approximately 10 to 30 kilo base pairs in size and mediated by another segment on the plasmid known as the virulence (Vir) region [40] that do not enter the plant cell but helps in the transfer of T-DNA. The signals received by the Vir region encodes monosaccharides, low pH and activates VirA/VirG system at much lower phenol concentration [46]. It also leads to the formation of hairy roots at the wounded site. Several decades have passed since the establishment of hairy root culture; however, this system has not yet been utilized globally in the commercial scale.

To date, the most studied *Agrobacterium* strains belong to agropine-type strains which synthesize agropine and mannopine. Agropinic acid and mannopinic acid contain two T-DNA regions on their roots-inducing plasmid called TL-DNA and TR-DNA. However, the strains that produce all agropine opines, except agropine, are known as mannopine strains [47, 48]. TL-DNA encodes four important genes named as *rol A, rol B*, *rol C* and *rol D* [49-52] that have been reported in different plant species for the induction of hairy roots. Their presence was

confirmed through molecular techniques, although the functions of the genes are unknown. The root inducing right DNA carries the genes responsible for opine synthesis. To date, several plant species have been transformed by integrating T-DNA into the plant genome.

3. CONCLUSION AND FUTURE PROSPECTS

In the present scenario, bioactive compounds are mainly obtained from medicinal plants as synthetic bioactive compounds have many negative effects. Research is being carried out to enhance secondary metabolites; however, alternative methods should be developed to enhance bioactive compounds. Undoubtedly, the use of biosynthesized nanoparticles for enhancing secondary metabolites has drawn great attention in the field of nanotechnology in recent times. The affect of various biotic and abiotic elicitors for enhancement of secondary metabolite production in hairy root culture are dependent on the specific secondary metabolites However, the type, dose, and treatment schedule for elicitors are major factors determining their effects on secondary metabolite production [53]. There is a tremendous scope for the large- scale production or enhancement of secondary metabolites in hairy root system by using elicitor as an agent. Hence, hairy root cultures have shown promising biosynthetic capability towards production of secondary metabolites. At the same time they have provide many challenges for large scale culture by using elicitors. Therefore, more studies on improvement of secondary metabolite production in hairy root cultures are needed to meet industrial demand.

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Conflict of Interest

Authors declared there is no conflict of interest.

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