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Plant Cell Elicitation for Production of Secondary Metabolites: A Review

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ABSTRACT

Pharmaceutically significant secondary metabolites or phytopharmaceuticals include alkaloids, glycosides, flavonoids, volatile oils, tannins, resins etc. Currently, most of these secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is either extremely difficult or economically infeasible. Biotechnological production in plant cell cultures is an attractive alternative, but to date this has had only limited commercial success because of a lack of understanding of how these metabolites are synthesized. Plants and/or plant cells *in vitro,* show physiological and morphological responses to microbial, physical or chemical factors which are known as 'elicitors'. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness. Here, we discuss the classification of elicitors, their mechanism of action, and applications for the production of phyto-pharmaceuticals from medicinal plants.

KEY WORDS: Biotic-Abiotic elicitors, Elicitation, Phyto-pharmaceuticals, Plant cell cultures, Secondary metabolites

INTRODUCTION

Plants form an important part of our everyday diet, and plant constituents and their nutritional value have been intensively studied for decades. In addition to essential primary metabolites (e.g. carbohydrates, lipids and amino acids), higher plants are also able to synthesize a wide variety of low molecular weight compounds – the secondary metabolites. Plant secondary metabolites can be defined as compounds that have no recognized role in the maintenance of fundamental life processes in the plants that synthesize them, but they do have an important role in the interaction of the plant with its environment. The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (1, 2).

Higher plants are rich source of bioactive constituents or phyto-pharmaceuticals used in pharmaceutical industry. Some of the plant derived natural products include drugs such as morphine, codeine, cocaine, quinine etc; anti-cancer *Catharanthus* alkaloids, belladonna alkaloids, colchicines, phytostigminine, pilocarpine, reserpine and steroids like diosgenin, digoxin and digitoxin. Many of these pharmaceuticals are still in use today and often no useful synthetic substitutes have been found that possess the same efficacy and pharmacological specificity (3). Currently one-fourth of all prescribed pharmaceuticals in industrialized countries contain compounds that are directly or indirectly, via semi-synthesis, derived from plants. Furthermore, 11% of the 252 drugs considered as basic and essential by WHO are exclusively derived from flowering plants (4). Plant-derived drugs in western countries also represent a huge market value. Prescription drugs containing phytochemicals were valued at more than US$30 billion in 2002 in the USA alone (5). Many plants containing high-value compounds are difficult to cultivate or are becoming endangered because of over-harvesting (4). Furthermore, the chemical synthesis of plant-derived compounds is often not economically feasible because of their highly complex structures and the specific stereochemical requirements of the compounds. The biotechnological production of valuable secondary metabolites in plant cell or organ cultures is an attractive alternative to the extraction of whole plant material. However, the use of plant cell or organ cultures has had only limited commercial success. This is explained by the empirical nature of selecting high-yielding, stable cultures and the lack of understanding of how secondary metabolites are synthesized or how their synthesis is regulated (6, 7). Many biotechnological strategies have been hypothesized and experimented for enhanced production of secondary metabolites from medicinal plants. Some of these include screening of high yielding cell line, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization, biotransformation and others (8-12).

Cell cultures have been established from many plants but often they do not produce sufficient amounts of the required secondary metabolites (11, 13). However, in many cases the production of secondary metabolites can be enhanced by the treatment of the undifferentiated cells with elicitors such as methyljasmonate, salicylic acid, chitosan and heavy metals (14-16). In some cases, secondary metabolites are only produced in organ cultures such as hairy root or shooty teratoma (tumor-like) cultures. For example, hairy roots produce high levels of alkaloids (7), whereas shooty teratomas

produce monoterpenes (17). However, there are a few examples of using plant cells as factories successfully to produce high-value secondary metabolites. These include shikonin production by cell suspension cultures of *Lithospermum erythrorhizon* and berberine production by cell cultures of *Coptis japonica* (18). Rosmarinic acid production by cell cultures of *Coleus blumeii* has also been achieved on a large scale, and sanguinarine, which has market potential in oral hygiene products, has been produced by cell cultures of *Papaver somniferum* (19, 20). An example of a high-value drug produced partially from plant cell cultures is paclitaxel, an anti-cancer drug originally extracted from the bark of 50-60 year old Pacific yew trees (*Taxus brevifolia*). In spite of these few successful cases, the production of secondary metabolites in cell or organ cultures is far from trivial and several technical bottlenecks such as low productivity and process technological issues (e.g. bioreactors and cultivation conditions) need to be solved. This review discusses elicitation as an approach for enhanced production of secondary metabolites from medicinal plants. Definition of elicitors, classification of elicitors, mechanism of elicitation, characteristics of elicitors and their application on medicinal plants have been highlighted.

Elicitors and Elicitation

An '*elicitor'* may be defined as a substance which, when introduced in small concentrations to a living cell system, initiates or improves the biosynthesis of specific compounds. *Elicitation* is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors (21).

Classification of Elicitors

Elicitors can be classified on the basis of their '*nature'* like abiotic elicitors or biotic elicitors, or on the basis their '*origin'* like exogenous elicitors and endogenous elicitors. Table 1 represents the classification of elicitors. Abiotic elicitors' are the substances of non-biological origin, predominantly inorganic salts, and physical factors acting as elicitors like Cu and Cd ions, Ca^{2+} and high pH whereas 'biotic elicitors' are substances with biological origin, they include polysaccharides derived from plant cell walls (pectin or cellulose) and micro-organisms (chitin or glucans) and glycoproteins or G-protein or intracellular proteins whose functions are coupled to receptors and act by activating or inactivating a number of enzymes or ion channels (22). 'Exogenous elicitors' are substances originated outside the cell like polysaccharides, polyamines and fatty acids whereas 'endogenous elicitors' are substances originated inside the cell like galacturonide or hepta-ß-glucosides etc. Examples of biotic and abiotic elicitors for secondary metabolite production have been presented in Table 2 and Table 3 respectively.

Mechanism of elicitation in plant cells

'Elicitor' for a plant refers to chemical from various sources that can trigger physiological and morphological responses and phytoalexin accumulation. It may include abiotic elicitors such as metal ions and inorganic compounds and biotic elicitors from fungi, bacteria or herbivores, plant cell wall fragments as well as chemicals that are released at attack site by plants upon pathogen or herbivore attack. It is well known that the treatment of plants with elicitors or attack by incompatible pathogen causes an array of defense reactions, including the accumulation of a range of plant defensive secondary metabolites in intact plants or in plant cell cultures. Even after the intensive research on the effect of biotic and abiotic elicitors on the production of secondary metabolites in plants, the exact mechanism of elicitation is poorly understood. Various mechanisms in this regard have been hypothesized like messenger Ca^{2+}, factors affecting cell membrane integrity, inhibition/ activation of intracellular pathways and changes in osmotic stress etc.

This review summarizes some general mechanism of biochemical responses performed by plant or plant cells when challenged by the elicitor. Some researchers hypothesized the binding of the elicitor to a plasma membrane receptor for elicitation process (63-68). The Ca^{2+} influx to the cytoplasm from the extracellular environment and intracellular Ca^{2+} reservoirs was reported by Gelli et. al. (69). Some groups highlighted the rapid changes in protein phosphorylation patterns and protein kinase activation as mechanism of elicitation (70-72). While others observed mitogen-activated protein kinase (MAPK) stimulation and G-protein activation (73-78). Armero and Tena (79) suggested cytoplasm acidification caused by H^+-ATPase inactivation, whereas the decrease in membrane polarization, extracellular increase of pH has been reported in elicitor treated plant tissues Pugin et. al. (80) and Bolwell et. al. (81). Apostol et. al. (82) explained the production of ROS such as the superoxide anion and H_2O_2 that might have a direct antimicrobial effect as well as contributing to the generation of bioactive fatty acid derivatives. Similar observation of ROS involvement in the cross-linking of cell-wall-bound proline-rich proteins H_2O_2 can act as a secondary messenger and it is involved in the transcriptional activation of defence genes (83). In another hypothesis, accumulation of defence-related proteins pathogenesis related proteins such as chitinases and glucanases, endopolygalacturonases that contribute to the release of signalling pectic oligomers (endogenous elicitors), hydroxyproline-rich glycoproteins, and protease inhibitors (84). Hypersensitive response to cell death at the infection site was observed by some groups (64, 76-78). Transcriptional activation of the corresponding defense response genes for elicitation process has been reported (85-89). The exact mechanism of elicitation is the study of these events and their interconnection and intercorrelation between them is highly complex and is still under investigation. All elicitors do not follow the same sequence of events but varies with their origin, specificity, concentration, physiochemical environment, stage of their growth cycle, nutritional uptake etc.

Characteristics of Elicitors

The enhanced production of the secondary metabolites from plant cell cultures through elicitation has opened up a new area of research which could have important economical benefits for pharmaceutical industry. Several parameters such as elicitor concentration and selectivity, duration of elicitor exposure, age of culture, cell line, growth regulation, nutrient composition, quality of cell wall materials,

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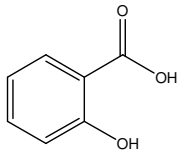
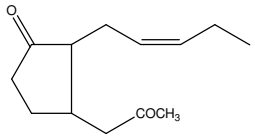
Table 1. Classification of elicitor for the production of secondary metabolites

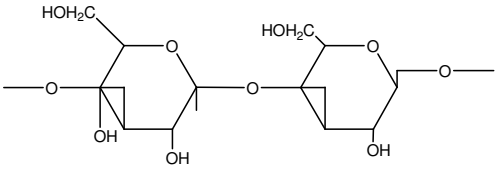
A) Nature of Elicitor	
Biotic elicitors	**Abiotic elicitors**
- Directly released by microorganisms and recognized by the plant cell (enzymes, cell wall fragments) - Formed by action of microorganisms on plant cell wall (fragments of pectins etc.) - Formed by the action of plant enzymes on microbial cell walls (chitosan, glucans) - Compounds, endogenous and constitutive in nature, formed or released by the plant cell in response to various stimuli.	- Of physical or chemical nature working via endogenously formed biotic elicitors - UV light - Windfall - Denatured proteins (RNase) - Freezing and thawing cycles - Non essential components of media (agarose, tin, etc.) - Heavy metals - Chemicals - With high affinity to DNA - With membrane-destroying activities like detergents: xenobiochemicals - Fungicides (Maneb, Butylamine, Benomyl) - Herbicides (Acifluorofen)
B) Origin of Elicitor	
Exogenous elicitors	**Endogenous elicitors**
- originated outside the cell, including the reaction immediately or via endogenous mediators - Polysaccharides: Glucomannose, Glucans, Chitosan - Peptides as poly cations: Monilicolin, Poly-L-lysine, Polyamines, Glycoproteins - As enzymes: Polygalacturonase, Endo-polygalacturonic acid lyase, Cellulase - Fatty acids: Arachidonic acid, Eicosapentanoic acid	- formed via secondary reactions induced by a signal of biotic or abiotic nature in the cell - dodeca-β-1,4-D-galacturonide - hepta-β-glucosides - alginate oligomers



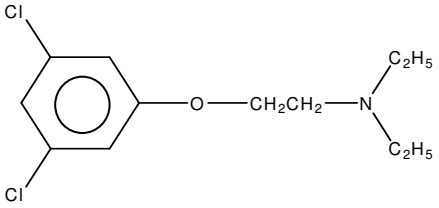
Salicylic acid



Methyl jasmonate



Curdlan



Diethyl amino ethyl dichloro phenyl ether

Arachidonic acid

Figure 1. Chemical structures of some compounds commonly used as elicitors for the elicitation of secondary metabolites from plant cells.

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Table 2. Biotic elicitors and production of secondary metabolites

Sl. No.	Plant species	Product	Biotic Elicitor	Reference
1.	*Arabidopsis*	Camalexin, indole glucosinolates	*Erwinia carotovora.*	(23)
2.	*Bidens pilosa*	Phenylheptaryn	Fungal culture filtrate	(14)
3.	*Brugmansia candida*	Hyosujamine	*Hemicellulase*	(24)
4.	*Capsicum annuum*	Capsidol	Cellulase	(25)
5.	*Catharanthus roseus*	Indole alkaloids	Fungal elicitor	(26)
6.	*Catharanthus roseus*	N-acetyl-tryptamine	*Pythium aphanidermatum*	(27)
7.	*Catharanthus roseus*	Ajmalicine	*Trichoderma viride*	(10)
8.	*Carthamus tinctorius*	Polyacetylenes	Fungal polysaccharide	(28)
9.	*Cicer arientium*	Medicarpin, Maackiain	*Ascochyta rabiei*	(29)
10.	*Cupressus lusitanica*	Beta-thujaplicin	Fungal elicitor	(30)
11.	*Datura stramonium*	Lubimin	*Fungal spores*	(31)
12.	*Dioscorea deltoidea*	Steroid (Diosgenin)	Fungal mycelia	(32)
13.	*Glycine max*	Glyceollin	Fungal glucan	(33)
14.	*L. erythrorhizon*	Shikonin	Agaropectin	(34)
15.	*Medicago sativa*	Isoflavonoid	*C. indemuthianum*	(35)
16.	*Medicago sativa*	Phytoalexins	Fungal elicitor	(36)
17.	*Medicago truncatula*	Beta-amyrin	Yeast elicitor	(37)
18.	*Papaver somniferum*	Morphine, codeine	*Vertcillium dahliae*	(38)
19.	*Petroselinum crispum*	Enzymes	Fungal Elicitor	(39)
20.	*Phaseolus vulgaris*	Krevitone	Fungal polysaccharide	(40)
21.	*Ruta graveolens*	Rutacridone epoxide	Chitosan	(41)
22.	*Salvia miltiorrhiza*	Diterpenoid tanshinones	Yeast elicitor	(42)
23.	*Silybum marianum*	Silymarin,	Yeast extract	(43)
24.	Various plant cells	Enzymes and sec. metabolites	*Erwinia carotovora*	(44)

Table 3. Abiotic elicitors and production of secondary metabolites

Sl. No.	Plant species	Abiotic Elicitor	Product	Reference
1.	*Arabidopsis*	Oxidative stress, amino acid starvation	Camalexin	(45)
2.	*Atropa belladona*	Cu^{2+}, Cd^{2+}	Tropane alkaloids	(46)
3.	*Catharanthus roseus*	Diethyl amino ethyl dichloro phenyl ether	Indole alkaloids	(46)
4.	*Catharanthus roseus*	Vanadium sulphate	Catharanthine	(47)
5.	*Capsicum annuum*	Arachidonic acid	Capsidiol, Rishitin	(48)
6.	*Capsicum frutescens*	Curdlan, Xanthan	Capsaicin	(49)
7.	*Coleus blumei*	Methyl jasmonate (MeJA)	Rosmarinic acid	(50)
8.	*Coleus forskolin*	Methyl jasmonate (MeJA)	Forskolin	(51)
9.	*Cupressus lusitanica*	Methyl jasmonate (MeJA)	β-thujaplicin	(30)
10.	*Datura stramonium*	Metal ions: Al^{3+}, Cr^{3+}, Co^{2+} Ni^{2+} ,Cu^{2+}, Zn^{2+}	Sesquiterpenoids	(52)
11.	*Daucus carota*	Salicylic acid	Chitinase	(53)
12.	*Glycyrrhiza echinata*	Na-alginate	Echinatin	(54)
13.	*Hyoscyamus albus*	Copper sulphate	Phytoalexin	(55)

14.	*Hyoscyamus albus*	Methyl jasmonate (MeJA)	Phytoalexins	(56)
15.	*L. erythrorhizon*	Activated carbon	Benzoquinone	(34)
16.	*Medicago truncatula*	Methyl jasmonate (MeJA), UV light	Triterpene β-amyrin	(32)
17.	*Panax ginseng*	Low-energy ultrasound	Saponins	(57)
18.	*Rauvolfia canescens*	Jasmonic acid	Secondary metabolites	(58)
19.	*Silybum marianum*	Methyl jasmonate (MeJA)	Silymarin	(43)
20.	*T. canadensis, T. cuspidata*	Methyl jasmonate (MeJA)	Taxoids	(59)
21.	*T. wallichiana*	Methyl jasmonate (MeJA)	Taxanes	(51)
22.	*Taxus chinensis*	Trifluoroethyl salicylate (TFESA)	Taxuyunnanine C (Tc)	(60)
23.	*Taxus spp.*	Arachidonic acid	Taxol	(61)
24.	*Valeriana wallichii*	Colchicine	Valepotriates	(62)

Table 4. Secondary metabolite production by cell cultures of medicinal plants

S. No.	Secondary metabolite	Plant	Elicitor	Reference
1.	5'-phosphodiesterase (PDase)	*Catharanthus roseus*	*Alteromonas macleodii,* Alginate oligomers	(105)
2.	Acridone expoxide	*Ruta gravelones*	Fungal poly saccharide	(41)
3.	Ajmalicine	*Catharanthus roseus*	a) *Pythium* sp.	(106)
			b)Yeast elicitor, MeJA	(107,108)
			c) *Trichoderma viride*	(10, 91)
			d)Pythium aphanidermatum	(96)
			e) Jasmonic acid	(26)
4.	Alkaloids (tropane)	*Datura stramonium*	*Phytopthora megasperma*	(109)
5.	Anthraquinones	*Morinda citrifolia*	Chitin 50	(110)
6.	azadirachtin	*Azadirachta indica*	Jasmonic acid, salicylic acid	(111)
7.	Berberine	*Thalictrum rugosum*	*Sacharomyces cerevisiae*	(149)
8.	Capsidiol	*Capsicum annum*	*Trichoderma viride*	(112)
9.	Capsidiol, debneyol, scopoletin, nicotine	*Nicotiana tabacum*	*Phytopthora cryptogea*	(113)
			Yeast extract	(114)
			Cryptogein	(115)
			Cellulase, MeJA	(116)
10.	Codeine, morphine	*Papaver somniferum*	Fungal spores	(38)
11.	Diosgenin	*Dioscorea deltoida*	*Rhizopus arrhizus*	(32)
12.	Diterpenoid tanshinones	*Salvia miltiorrhiza*	Yeast elicitor	(117)
13.	Glyceollins, apigenin, genistein, luteolin	*Glycine max*	β-glucan, MeJA	(118, 119)
14.	Hyoscyamine, scopolamine	*Hyoscyamus niger, H. muticus*	Fungal elicitor, MeJA	(120)
15.	Indole glucosinolates, Camalexin	*Arabidopsis thaliana*	Fungal, MeJA	(23)
16.	Isoflavonoids	*Lotus corniculatus*	Glutathione	(121)
17.	Kinobeon A	*Carthamus tinctorius*	Blue green algae	(122)
18.	Methoxymellein, 4-hydroxybenzoic acid	*Dacus carota*	Fungal elicitor	(123, 124, 125)
19.	Raucaffrincine	*Rauwolfia canescens*	Yeast elicitor, MeJA	(58, 126)

20.	Rishitin	*Jimson* weed	Copper sulphate	(127)
21.	Rosmarinic acid	*Coleus blumei*	Yeast elicitor	(50)
22.	Rosmarinic acid	*Ocimum basilicum*	*Aspergillus niger*	(128)
23.	Salidroside	*Rhodiola sachalinensis*	*Aspergillus niger, Coriolus versicolor, Ganoderma lucidum*	(129)
24.	Sanguinarine	*Eschscholtzia californica*	Yeast extract	(130)
25.	Sanguinarine	*Papaver bracteatum*	Dendryphion	(131)
26.	Sanguinarine	*Sanguinaria canadensis*	*Verticillum dahliae*	(132)
27.	Saponin	*Panax ginseng*	Oligogalacturonic acid Low energy ultra sound	(52, 133, 134)
28.	Scopoletin	*Lycopersicon esculentum*	Fungal elicitor, MeJA	(135)
29.	Scopoletin	*Ammi majus*	*Enterobacter sakazaki*	(136)
30.	Sesquiterpenes	*Hyoscyamus muticus*	*Rhizoctonia solani*	(120)
31.	Shikonin	*Lithospermum erythrorhizon*	Endogenous source	(137)
32.	Soyasaponin 5-deoxyflavonoid	*Glycyrrhiza glabra*	MeJA	(138)
33.	Stilbene, resveratrol, anthocyanins	*Vitis vinifera*	MeJA, ethylene	(139, 140)
34.	tanshinone	*Salvia miltiorrhiza*	hyperosmotic stress, yeast elicitor	(141)
35.	Taxol	*Taxus chinensis*	fungal elicitation	(142)
36.	Taxol, baccatin III	*Taxus brevifolia,T. cuspidate*	Fungal elicitor	(143, 144, 145)
37.	Taxuyunnamine C Tc	*Taxus Chinensis*	Trifluoroethyl salicylate (TFESA)	(60)
39.	Tcibulin, tcibulin2	*Allium cepa*	Ca^{+}, cAMP	(146)
40.	Thiophene	*Tagetes patula*	*Furasium conglutanis A. niger*	(94, 147)
41.	Tropane alkaloids	*Brugmansia suaveolens*	*Spodoptera frugiperda* Methyl jasmonate	(148)
42.	β-Thujaplicin	*Cupressus lusitanica*	Fungal and MeJA	(30)

Table 5. Comparison of production of secondary metabolite after elicitation

S. No.	Species	Elicitor	Products	Product concentration in control	Product concentration after elicitation	Reference
1.	*Capsicum annum* (cells)	*Trichoderma viride* (crude)	Capsidiol	0	1mg per flask	(112)
2.	*Carthamus tinctorius*	*Blue green algae (crude)*	Kinobeon A	0.6mg/L	5.78 mg/L	(130)
3.	*Catharanthus roseus* (hairy roots)	*Penicillium* sp. (crude)	Alkaloids (indole)	3mg/gm dry weight	9mg/gm dry weight	(140)
4.	*Catharanthus roseus* (Cell culture)	*Alteromonas macleodii,* alginate oligomers	5'-phosphodiesterase (PDase)	0.022 U/ml.	to 0.235 U/ml.	(105)
5.	*Catharanthus roseus* (cells)	*Pythium* sp. (crude)	Ajmalicine	0	400mcg/L	(106)

6.	*Catharanthus roseus* (cells)	*Trichoderma viride*	Ajmalicine	79 (µg g^{-1} DW)	166 (µg g^{-1} DW)	(10)
7.	*Catharanthus roseus* (cells)	*Pythium aphanidermatum* (crude)	Alkaloids (indole)	50µmol/L	75µmol/L	(96)
8.	*Cupressus lusitanica* suspension cultures	fungal elicitor	Beta-thujaplicin	0	187 mg/g dry weight	(30)
9.	*Datura stramonium* (cells)	*Phytopthora megasperma* (crude)	Alkaloids (tropane)	0.85mg/gm dry weight	4.27mg/gm dry weight	(139)
10.	*Dioscorea deltoida* (cells)	*Rhizopus arrhizus* (crude)	Diosgenin	134mg/L	230mg/L	(32)
11.	*Eschscholtzia californica* (cells)	Yeast extract (crude)	Sanguinarine	20mg/L	60mg/L	(130)
12.	*Hyoscyamus muticus* (hairy roots)	*Rhizoctonia solani*	Sesquiterpenes	0	1mg per 10gm fresh weight	(120)
13.	Jimson weed (hairy roots)	Copper sulphate	Rishitin	0	traces	(127)
14.	*Lithospermum erythrorhizon* (cells)	Endogenous (crude)	Shikonin	0	28µgm/ 10ml	(149)
15.	*Lotus corniculatus* (hairy roots)	Glutathione (abiotic)	Isoflavonoids	0	160 µg /gm fresh weight	(121)
16.	*Morinda citrifolia* (cells)	Chitin50 (pure)	Anthraquinones	3µg/gm fresh weight	7mcg/gm fresh weight	(110)
17.	*Nicotiana tabacum* (hairy roots)	Yeast extract	Sesquiterpenes	1µgm/gm fresh weight	87µgm/gm fresh weight	(114)
18.	*Nicotiana tabacum* (cells)	*Phytopthora cryptogea* (crude)	Capsidiol	0	25µg/ml	(113)
19.	Panax ginseng (hairy roots)	selenium	ginseng saponin	control levels	1.33 times control levels	(150)
20.	*Papaver bracteatum* (cells)	*Dendryphion* (crude)	Sanguinarine	50µg/gm fresh weight	450µg/gm fresh weight	(131)
21.	*Sanguinaria canadensis* (cells)	*Verticillium dahliae* (cells)	Dopamine	3mg/gm fresh weight	15 mg/gm fresh weight	(132)
22.	*Sanguinaria canadensis* (cells)	*Verticillum dahliae* (crude)	Sanguinarine	3µg/gm fresh weight	12µg/gm fresh weight	(132)
23.	*Tagetes patula* (hairy roots)	*Furasium conglutanis* (crude)	Thiophene	0.2gm per 100gm dry wt	0.55gm per 100gm dry wt	(94)
24.	*Tagetes patula* (hairy roots)	*Aspergillus niger* (crude)	Thiophene	1.5µmol/gm dry weight	3.5µmol/gm dry weight	(147)
25.	*Taxus chinensis* (Cell culture)	Trifluoroethyl salicylate (TFESA)	Taxuyunnanine C (Tc),	14.0 mg/gm dry weight	21.9 mg/gm dry weight	(60)
26.	*Thalictrum rugosum* (cells)	*Sacharomyces cerevisiae* (crude)	Berberine	0.5% of dry weight	2% of dry weight	(149)
27.	Thorn apple (hairy roots)	Cadmium chloride	Sesquiterpenes	0	140nmol/gm	(127)

substantial enhancement of product accumulation etc. has been reported (90). Some of these parameters were highlighted on elicitation of *Catharanthus roseus* cell suspension cultures for the production of active compounds.

i) Elicitor concentration

Elicitor concentration plays a very important role in elicitation process. Namdeo et. al. (10) reported higher accumulation of ajmalicine in *C. roseus* cultures when treated with different concentrations of elicitor extracts of *T. viride, A. niger* and *F. moniliforme*. Ajmalicine accumulation was higher in cells elicited with higher concentration (5.0 %) of elicitor extracts as compared to lower concentration (0.5%). However, increasing the concentration further upto 10.0 % adversely affected the accumulation of ajmalicine. These results are also supported by the findings of Nef-Campa et al. (92), Rijhwani and Shanks (26). High dosage of elicitor has been reported to induce hypersensitive response leading to cell death, whereas, an optimum level was required for induction (93-95).

ii) Duration of elicitor exposure

In a study, cells of *C. roseus* exposed with elicitor extracts of *T. viride, A. niger* and *F. moniliforme* for 24h, 48h, 72h and 96h. About 3-fold increase in ajmalicine production by *C. roseus* cells elicited with extracts of *T. viride* for 48 h, whereas, about two-fold increase was observed in cells elicited with *A. niger* and *F. moniliforme* (10, 91). However, further increasing exposure time resulted in decrease in ajmalicine content. Similar results were reported by Rijhwani and Shanks (26) Moreno and co-workers (96) and Negeral and Javelle (97).

iii) Age of culture

Age of subculture plays is an important parameter in production of bioactive compounds by elicitation. In a study, *C. roseus* cells of 20-day-old cultures showed higher yields of ajmalicine on elicitation. Highest ajmalicine (166 μg^{-1} DW) was accumulated in 20-day-old cells elicited with extracts of *T. viride* followed by 90 and 88 $\mu g\ g^{-1}$ DW ajmalicine in cells elicited with *A. niger* and *F. moniliforme* respectively (10, 91). Similar observations were reported from various workers Rijhwani and Shanks (98) Ganapathi and Kargi (90).

iv) Nutrient composition

Composition of medium or selection of medium also played a vital role for elicitation process. Ajmalicine accumulation was observed more in Zenk's (99) production medium as compared to Murashige and Skoog's (100) medium Namdeo et. al. (10, 91). Similar observations were reported from various workers Rijhwani and Shanks (98) Ganapathi and Kargi (90).

Apart from these characteristics, the efficiency of elicitation also depends on elicitor specificity, cell line or clones of microbial elicitor used, presence of growth regulators, nutrient composition of the medium, and the environmental conditions.

Elicitation and production of secondary metabolite by plant cell cultures -

The living plants may be considered as a biochemical factory not only for the production of primary metabolites like sugars, amino acids and fatty acids but also for the production of secondary products of pharmaceutical significance such as alkaloids, glycosides, flavonoids, volatile oils, tannins, resins etc. Secondary metabolites may represent chemical adaptations to environmental stress, or they may serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators. They are some times considered to be waste or secretary products of plant metabolism.

In spite of the progress made in organic synthesis or semi-synthesis of a wide range of compounds similar to those produced by the plants, extraction of secondary metabolites from plants is still of considerable commercial importance. A large number of these metabolites are difficult or virtually impossible to synthesize at economic values. In several cases, the natural product is more easily accepted by consumers than an artificially produced one. Both reasons, objective and subjective, explain that natural extraction still applies to a large number of aromas or fragrances which are the result of a mixture of hundreds of different compounds as is the case of jasmine and strawberry, or to biochemicals that have complex molecular structures (e.g. some alkaloids and glycosides).

There is great interest in developing alternatives to the intact plant for the production of plant secondary metabolites. This originally had centered on the use of tissue and cell cultures though the most recent approaches involve applying molecular biology techniques to enhance the metabolic pathways leading to specific compounds.

When infected by pathogenic microorganism plants, respond with rapid activation of various spatially and temporally regulated defense reactions. These responses include oxidative cross linking of cell wall proteins, production of phytoalexins, hydrolytic enzymes, incrustation of cell wall proteins with phenolics and finally hypersensitive death of plant cell. Microbial invasion of plants induce the synthesis of anti-microbial secondary metabolites in the same way as stress factors like UV-irradiation, osmotic shock, fatty acids, inorganic salts and heavy metal ions induce the synthesis of secondary metabolites in plants. The molecules that stimulate the production of secondary metabolites are termed as elicitors. Both biotic and abiotic elicitors induce product accumulation not only in intact plants or plant organs but also in plant cell cultures as a result of their defensive, protective or offensive reactions (26-27, 30, 90, 98, 101-104). Table 4 illustrates different plant species producing various secondary metabolites on elicitation with different elicitors.

Most of the strategies employing fungal elicitors utilize fairly undefined mixtures such as autoclaved fungal homogenate (151) or fungal culture filtrates (61, 152). With the consideration of several parameters such as elicitor specificity and concentration, duration of contact and quality of cell wall materials, substantial enhancement of product accumulation has been reported (90). The enhancement of production of secondary metabolites after elicitation is compared with that of control is exhibited in Table 5.

CONCLUSION

Secondary metabolites or bioactive constituents or phyto-pharmaceuticals occurring in intact plants are employed

either directly or indirectly by a large number of pharmaceutical industries. The supply of these secondary metabolites is however, often having many limitations. It is widely recognized that cultured plant cells represents a potential source of phyto-pharmaceuticals but very few cell cultures synthesize secondary metabolites in comparison to those produced in intact plants. Over few decades many strategies like media manipulation, phyto-hormone regulation, precursor feeding, plant cell immobilization, biotransformation and bioconversion, hairy root cultures and genetically modified cells etc. have been tried but failed to synthesize the desired products in appreciable quantity and at competitive economic value. This reflects the poor understanding of basic secondary metabolic regulation in plant cell cultures. Elicitation of plant cell culture system may be promising as it showed favorable results in fermentation of antibiotics and many other fermented products. Though, elicitation enhances secondary metabolism in plants or plant cells *in vitro* but the exact mechanism of elicitation is not exactly understood. This provides an opportunity for intensive research in the field of biosciences for exploitation of plant cells for the production of secondary metabolites. Combined efforts of experts of Plant science, Pharmacognosy, Microbiology, Phyto-chemistry, Biochemistry, Molecular biology, and Fermentation technology can exploit the potential of plant cells for the production of plant secondary metabolites.

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