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Review Article

Effect of elicitation on the production of phyto-constituents through plant tissue culture technique – A review

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Abstract

The evolving commercial importance of secondary metabolites in recent years resulted in a greater interest in study of secondary metabolism; particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology. Different strategies, using an *in-vitro* system, have been extensively studied by various researchers to improve the production of plant active principles. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. By cell culture system only low yields of desired secondary metabolites were obtained, since efforts have been made to improve the productivity of plant tissue cultures by means of elicitation. Therefore this review aims at analyzing the influence of various biotic and abiotic elicitors on production of various phytoconstituents.

Keywords: Plant Tissue Cultures, Effect of Elicitors, Elicitation, Secondary Metabolism

Introduction

Many higher plants are major sources of natural products which are used in pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides. Plant Tissue Cultures are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites, which is an alternatives to production of desirable medicinal compounds from plants.[1]

'Tissue culture is an *in- vitro* propagation technique of a wide range of excised plant parts, through which a mass of cells (callus) is produced from an explant tissue. The callus produced, can be utilized directly to regenerate plantlets or to extract or manipulate some primary and secondary metabolite.'[2]

There are various advantages of a cell culture system over the conventional cultivation of whole plants, Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions, cultured cells would be free of infection from microbes and insects, the cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites, automated control of cell growth and rational regulation of metabolite processes would reduce labor costs and improve productivity also the organic substances are extractable from callus cultures.[3]

Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years. New physiologically active substances of medicinal interest have been found by bioassay. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated. Due to these advances, researches in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.[4] In order to obtain high yields suitable for commercial exploitation, efforts have focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the cultural conditions, selecting high-producing strains, and employing precursor feeding, transformation methods, immobilization techniques and by employing different elicitors.[5, 6]

Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of scientists and microbiologists in several countries during the last decade. In the last few years promising findings have been reported for a variety of medicinally valuable substances, some of which may be produced on an industrial scale in the near future.[7] Thus, the aim of the present review is to focus on the production of plant pharmaceuticals by the effect of different elicitors through tissue culture technology.

Elicitors and Elicitation

Some molecules stimulate the production of secondary metabolites; these are called Elicitors, and the phenomenon is known as Elicitation[8].Elicitors produced within plant cells are termed as endogenous elicitors, while those produced by microorganisms are called exogenous elicitors. Elicitors of plant origin are polysaccharides derived from cell walls, e.g., pectin, pectic acid, cellulose, while those of microorganism origin are cell wall components like chitin, chitosan or glucans. These elicitors are of biological origin; hence they are called biotic elicitors. Low concentrations (50-250 mg/l) of elicitors added to the culture medium dramatically enhance secondary metabolite production by plant cells. Biotic elicitors bind to a receptor protein in plasma membrane, which ultimately leads to the accumulation of secondary metabolites. The response to elicitor polysaccharides seems to be similar to the defense response to micro-organism invasion.

Also, these are the stress agents that induce the accumulation of antibiotically active secondary metabolites (phytoalexins) in plants, as well as in cultivated plant cells. The accumulation of secondary products is regarded as a part of the defense system of the intact plant. Elicitors have also been shown to induce a range of other plant secondary metabolites. The production of these compounds is a dynamic defense response exhibited by plant cells when challenged by an elicitor. The most commonly used biotic elicitors include fungal homogenates from families such as Phytophora, Aspergillus, and Alternaria, and abiotic elicitors,

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e.g., inorganic salts of cadmium, copper, and vanadyl, Jasmonates (plant hormones that have a dual effect on plant growth and development) . The possibility of using chitosan as an elicitor has been intensively studied. Although the use of elicitors does not directly increase secondary metabolite production, they may increase cell permeability. The economic viability of the production process depends in part on the capacity of the cells to secrete the desired metabolite into the surrounding medium.[9, 10.]

Elicitors induce different types of effects in plant cells (figure 1) which is reflected by the influenced cell metabolism –

- Ca^{2+} metabolism.
- Massive variation in membrane integrities, respiration, protein and phosphate metabolism, ethylene production and peroxidase activity.
- Differential gene expression, consequently forming enzymes concerned in the synthesis of polysaccharides as callose, hydroxyproline- rich glucoproteins (HRGP) in cell walls via induction of praline hyroxylase, lignin and polyphenolics (deposited in cell walls) chitinases and protein inhibitors , specific proteins against pathogenic infections, phytoalexins (secondary compounds accumulated in response to microbial attack)

The different features of Elicitors include -

- The products which accumulate in plant cell cultures due to elicitation may be antimicrobial in nature, but they should not be confused with phytoalexins unless there is sufficient proof that the source plant respond to pathogens with rapid accumulation of the same product. Therefore a new term that has been coined for those compounds, which in cell cultures are inducible by way of elicitation, is "Elicitation Product" or "Elicitation Metabolite".
- Elicitors can be regarded as substitute of production media (optimum cultural conditions).
- Optimum employment of elicitors depends upon :
  - Elicitor specificity.
  - Elicitor concentration.
  - Duration of elicitor contact.
  - Elicitor of cell line (clones).
  - Time course of elicitation.
  - Growth stage of culture.
  - Growth regulation and,
  - Nutrient composition.
- Elicitation of cell in suspensions culture may react in the following ways:
  - In a given cell line, different products may show highest level of accumulation, at different times.
  - Product accumulation may be observed in cell lines which area not known to synthesize.
  - Elicitation may not cause an additive effect when applied to cells in production media but may shorten the culture period required for maximum product accumulation.
- Product accumulation due to elicitation has also been observed in growth media. Such occurrence may be excretion or leakage caused by cell breakdown.[11]

Secondary Metabolite Production and Culture Practices

Plants produce biochemicals that are of importance in the healthcare, food, flavour and cosmetics industries. Many pharmaceuticals are produced from the secondary metabolites of plants. Examples are digitalis, L-DOPA, morphine, codeine, reserpine, and the anticancer drugs vincristine, vinblastine and taxol used in treatment of ovarian and breast cancers. Currently, these and many other natural products are produced solely from massive quantities of whole plant parts. Often the source plants are cultivated in tropical or subtropical, geographically remote, areas which are subject to political instability, drought, disease and changing land use patterns and other environmental factors. In addition, the long cultivation periods between planting and harvesting make selection of high-yielding strains difficult, thus resulting in expensive drugs. Cultivation periods may range from several months to decades for instance, the taxol yielding, *Taxus brevifolia* trees[11].Clearly, the development of alternative and complimentary methods to whole plant extraction for the production of clinical medicines is an issue of considerable socioeconomic importance. These factors have generated considerable interest in the use of plant cell culture technologies for the production of pharmaceuticals. Indeed, the plant cell culture technology is now sufficiently advanced to allow for large quantities of relatively homogeneous, undifferentiated cells to be produced. Plant cell and tissue culture systems are complementary and may provide competitive metabolite production systems when compared to whole plant extraction. Several types of high-yielding tissue culture systems have been developed. These include the cultivation of specific organ cultures, suspension cultured cells selected for high productivity on production media and high-density culture. Certain inorganic and organic adjuvants stimulate product synthesis and/or accumulation, but product accumulation is transient. The increased use of plant cell culture systems in recent years is perhaps due to an improved understanding of the biochemistry of secondary metabolic pathways in economically important plants. This trend is expected to increase as advances in plant biotechnology surge on.[12, 13.] Examples of some secondary metabolites and the culture systems are shown in table 1.

Mechanism of Elicitation

Elicitors appear to be recognized by plant cells via interactions with specific receptors on plant plasma membranes. The elicitor-receptor interactions are presumed to generate signals (shown in figure 2) that then activate nuclear genes involved in plant defense reactions, such as the biosynthesis of phytoalexins.[16-18.] The pathway is initiated by the action of local and systemic signal molecules and putative plasma membrane receptors. Wound signal molecules include oligogalacturonic acid, chitosan, physical signals, abscisic acid and systemin. Plasma membrane receptors include a β-glucan-elicitor-binding protein (GEBP), a systemin binding protein of 160 KDa and an unidentified receptor for oligosaccharide elicitors. A lipase translates the wound signal and release linolenic acid from membrane phospholipid, a process stimulatd by ABA, volicitin and β- glucosidase from the oral secretion of insects may also function like linolenic acid i.e be converted to jasmonic acid through the octadecanoid pathway.

Classification of Elicitors

Elicitors are classified (Table 2) as physical or chemical, biotic or abiotic and complex or defined depending on their origin and molecular structure.[19]

Biotic Elicitors

Biotic elicitors are molecules of either pathogen or host origin that can induce defense responses (such as phytoalexin accumulation or hypersensitive response) in plant tissue. Often complex biological preparations have been used as elicitors, where the molecular structure of the active ingredients is unknown.

Elicitors from Carbohydrates

From early studies carbohydrates have been implicated in the overproduction of secondary metabolites in plant cell cultures. Albersheim et al., first isolated oligosaccharides that activate a variety of plant defense genes. The signal transfer triggered by carbohydrate elicitors has been studied with regard to calcium influx, pH shifts and production of H_2O_2 in tobacco cell cultures. The different types of carbohydrate elicitors are shown in table 3.

- The combination of oligosaccharides and methyl jasmonate has been employed to induce phytoalexin in rice systems. (Nojiri *et al.* 1996)
- Production of paclitaxel in *Taxus Canadensis* cell suspension cultures was enhanced when the cultures were treated with a combination of N-acetylcheto-hexaose and methyl jasmonate (Linden *et al.* 2000)
- Methyl jasmonate, a lipid-derived elicitor, was also applied as an elicitor in combination with chitopentaose to J. chinensis cell suspension cultures for the enhancement of podophyllotoxin production. (Premjet *et al.*, 2002)[4]

Elicitors from Fungal cell walls

Fungal elicitor is normally one derived from a microorganism (Phytophthora, Botrytis, Verticilium, Alternaria, Fusarium, etc.) pathogenic to the plant species of interest. Although preparations derived from non-pathogenic (Aspergillus, Micromucor, Rhodotorula, etc) microbes have been successfully employed for the elicitation purpose (table 4)

Abiotic Elicitors

All factors which can not be regarded as natural components of the environment of a plant cell are considered as Abiotic Elicitors. Abiotic Elicitors are of non-biological origin mainly the metal ions. Also the abiotic elicitors are of physical or chemical nature working via endogenously formed biotic elicitors.[20-23] The various abiotic elicitors are classified in the table 5.

Table1. Examples of plant cell culture methodologies for the production of Metabolites [12, 14, 15]

S.No.	Products	Production system
1	Vincamine, 3.3 g'L -1	Cell culture of *Vinca minor*
2	Equivincamine,0.9 g.L -1	Cell culture of *Vinca minor*
3	Shikonin, 4 g.L -1	Cell culture of *Lithospermum Erythrohizon*
4	Berberine, 3.5 g.L -1	Cell culture of *Coptis japonica*
5	Ajmaline, 0.4 g.L -1	Cell culture of *Rauwolfia serpentina*
6	Raucaffricine, 1.2 g.L-1	Immobilized strictosidine synthase
7	Strictosidine,	Immobilized strictosidine synthase
8	3',4'-anhydrovinblastine, about 70% yield	Glucose oxidase and peroxidase mediated dimerization
9	3',4'-anhydrovinblastine, about 70% yield,	FeCl3-mediated dimerization
10	vinblastine, about 20% yield	FeCl3-mediated dimerization
11	Methyldigoxin, 505 g produced in 3 months	Biotransformation of methyldigitoxin by *Digitalis lanata* cells

Table2. Classification of elicitors

<table>
<tr><td colspan="6">Elicitors</td></tr>
<tr><td>Physical Elicitors</td><td colspan="5">Injury</td></tr>
<tr><td></td><td colspan="2">Abiotic</td><td colspan="3">Metal ions (lanthanum, europium, calcium, silver, cadmium), oxalate</td></tr>
<tr><td rowspan="8">Chemical Elicitors</td><td rowspan="8">Biotic</td><td>Complex composition</td><td colspan="3">Yeast cell wall, mycelia wall, fungal spores</td></tr>
<tr><td rowspan="7">Defined composition</td><td rowspan="4">Carbohydrates</td><td>Polysaccharides</td><td>Alginate
LBG
Pectin
Chitosan
Guar gum</td></tr>
<tr><td>Oligosaccharides</td><td>Mannuronate
Guluronate
Mannan
Galacturonides</td></tr>
<tr><td>Peptides</td><td>Glutathione</td></tr>
<tr><td>Proteics</td><td>Proteins</td><td>Cellulase,Elicitins, Oligandrin</td></tr>
<tr><td>Lipids</td><td></td><td>Lipopolysaccharides</td></tr>
<tr><td>Glycoproteins</td><td></td><td>Not characterised</td></tr>
<tr><td>Volatiles</td><td></td><td>C_6-C_{10}</td></tr>
</table>

Table3. Carbohydrate elicitors and metabolites in plant cell cultures[19]

Elicitor	Culture	Metabolites / Products
β-linked glucopyranosyl	Glycine max	Phytoalexins
α-1,4-oligogalacturonide	Glycine max	Phytoalexins
Chitosan	N.tobaccum, E.californica	Phytoalexins
Hepta-β-glucoside	Glycine max	Phytoalexins
Pectic oligomers	Citrus limon	Phytoalexins
β-1,6-1,3 glucans	Glycine max	Isoflavonoids
β- glucan	Glycine max	H_2O_2
β- D-glucans	N.tobaccum	Desease resistance
Chitin	Papaver somniferum	Sanguinarine
Chitosan	Polygonum tinctorium	Indirubin
Chitosan	Lupinus albus	Isoflavonoids
Chito- oligosaccharides	Juniperus chinensis	Podophyllotoxin
Oligo galaturonides	N.tobaccum	H_2O_2
Chitosan	Lupinus albus	Genistein
Chitin and Chitosan oligosaccharides	Taxus canadensis	Taxol
Rhamsan, xanthan	Morinda citrifolia	Anthraquinones
Laminarin	N.tobaccum	H_2O_2
Mannan	Hypericum perforatum	Hypercins
N-Acetylchitohexose	Taxus canadensis	Taxol
Chitosan	Rheum palmatum	Anthracene
N-Acetylchito-oligosaccharides	Avana sativa	Anthranilate

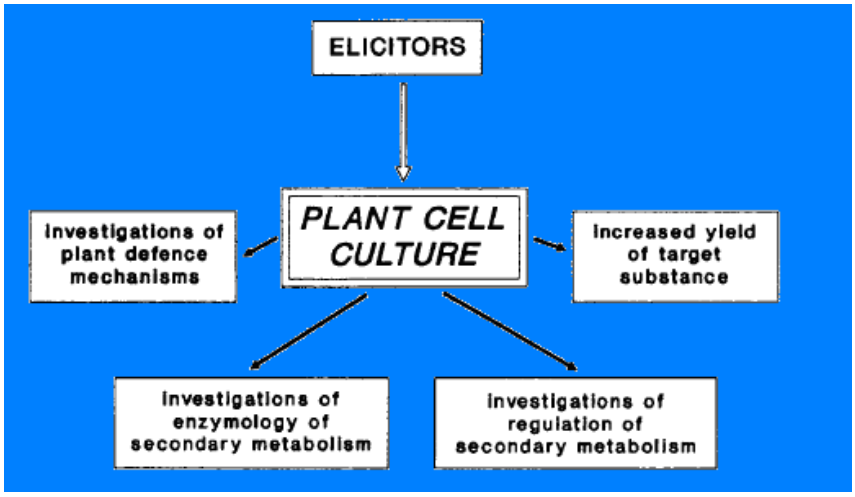
Table4. Fungal elicitors

S.N.	Elicitor Studied	Plant Cell Cultures	Elicitated Product	References
1.	Ustilaginodia verens (mycelial homogenate)	Catharanthus roseus	Indole alkaloid	Zhao et al., 2000
2.	Rhodoturula glutinis, R. rubra, Panus conchatus, coriolus versicolor	Xanthophyllomyces dendrorhous	Carotenoids, asthaxanthin	Wang et al., 2005
3.	Pseudomonas lachrymons	Tobacco cell cultures	Caspidiol, lubimin, phytuberin, phytuberol, rishitin	Cappell et al., 1987
4.	Fungus isolated from bark of T.Chinensis	Taxus chinensis	Taxol	Jiang et al., 2001
5.	Pythium aphanidermatum, Phytopthora parasitica	Witloof chicory	Coumarins	Bias et al., 2000
6.	Mycelia of Tuber aestivum vittad	Ganoderma lucidum	Ganoderic acid	Zhu et al., 2008
7.	Fungal elicitor	Glycine max seedlings	Increase in enzymatic activity(lepoxegenase)	Kondo et al., 1993
8.	Conidia from Alternaria alternate	Citrus limon	Umbelliferone, scoparone	Castaneda et al., 1995
9.	Colliotrichum lindermuthianum	Phaseolus vulgaris	Increase in protein concentration	Hughes & Dickerson,1988
10	Ectomycorrhizal fungas	Pine cell cultures	Phenolics	Campbell & elist, 1991
11.	Yeast cell wall	Alfa cell cultures	Increase in enzymatic activity (S-adenosyl –L- methionine)	Robert Edwards, 1996
12.	Phytopthora megasperma	Soyabean cells	Glyceollin	Stab & Ebel, 1987
13.	Chitosan	Oryza sativa	Increase in octadecanoid pathway	Rakwal et al., 2002
14.	Aspergillus niger, Rhizopus oryzae	Arnebia euchroma	Shikonin	Quing Fu & Lu, 1999
15.	Phytopthora species	Rose cell cutures	Increase in ion transport & H_2O_2 synthesis	Arnot & Murphy, 1990
16	Chitosan	N.Tobaccum, Eschscholtzia californica	Alkaloids	Funk et al., 1989
17.	Aspergillus Fumigatus, A.Flavus, A.Ochraseus	Carmathus tinctorius	Kinobeon A. formation	Hanagata et al., 1994
18.	Botrytis species	Papaver somniferum	Dihydro-sanguinarine	Eilert et al., 1986
19.	Callulase	C.annum	Capsidiol	Patrica et al., 1996
20.	Verticillium dahliae	Caphalotaxus harringtonia	Alkaloid	Heinstein, 1985

Table5. Classification of Abiotic elicitors

S.No.	Elicitor Studied	Plant Cell Cultures	Elicitated Product	References
1.	Methyl jasmonate	Lithospermum erythrorhizon	Rasmarinic acid	Mizukami et al., (1993)
		Taxus sp.	Paclitaxel,	Furmanowa et al., (1995)
			caphalomannines,	Yukimune et al., (1996)
			taxanes, diterpenes	Yukimune et al., (1996)
		Vaccinium pahlae	Anthocyanins	Fang et al., (1999)
		Glycine max	Vegetative storage proteins	Singh et al., (1998), Anderson (1991)
		Oryza sativa leaves	Putrescine	Chen et al., (1994)
		Coleus blumei	Rosmarinic acid	Szabo et al., (1999)
		C. forskohlii	forskolin	Prasad babu (2000)
2.	Salicylic acid	Daucus carota,	chitinase	Muller at al., (1994)
		C. forskohlii	forskolin	Prasad babu (2000)
3.	Calcium chloride	C. forskohlii	forskolin	Prasad babu (2000)
4.	Sodium alginate	Glycyrrhiza echinata	Echinatin	Ayabe et al., (1986)
5.	Colchicine	Valeriana wallichi	Valepotriates	Becker and chavadej (1985)
6.	Activated carbon	Lithospermum erythrorhizon	Benzoquinone	Fukui et al., (1983)
7.	Curdlan, xanthan	Capsicum frutiscens	Capsaicin	Johnson et al., (1991)
8.	Metal ions			
	Copper sulphate	Hyoscymus albus	Phytoalexins with vetispyrane skeleton	Lee at al., (1998), Mader, (1999)
		Lithospermum erythrorhizon	Shikonin	Fujita et al., (1982)
9.	Silver nitrate	Solanum tuberosum	Free and conjugated polyamines	Mader, (1999)
10.	Vanadium sulphate	Catharanthus roseus	Catharanthine, ajmalicine	Smith et al., (1987)
11.	Cu^{2+}, Cd^{2+}	Atropa belladonna	Tropane alkaloids	Lee et al., (1998)
12.	Cu^{2+}, Mn^{2+}, Hg^{2+}	Cicer arientinum	Pterocarpenes	Threfall and whitehead (1998)
13.	Al^{3+}, Cr^{3+}, Co^{2+}. Ni^{2+}	Datura stramonium	Sesquiterpenoids	Threfall and whitehead (1998)
14.	Arachidonic acid	Capsicum annum	Capsidiol, Rishitin	Hoshino et al., (1994)

Fig. 1 Utilization of elicitation of plant cell cultures in various areas of research.



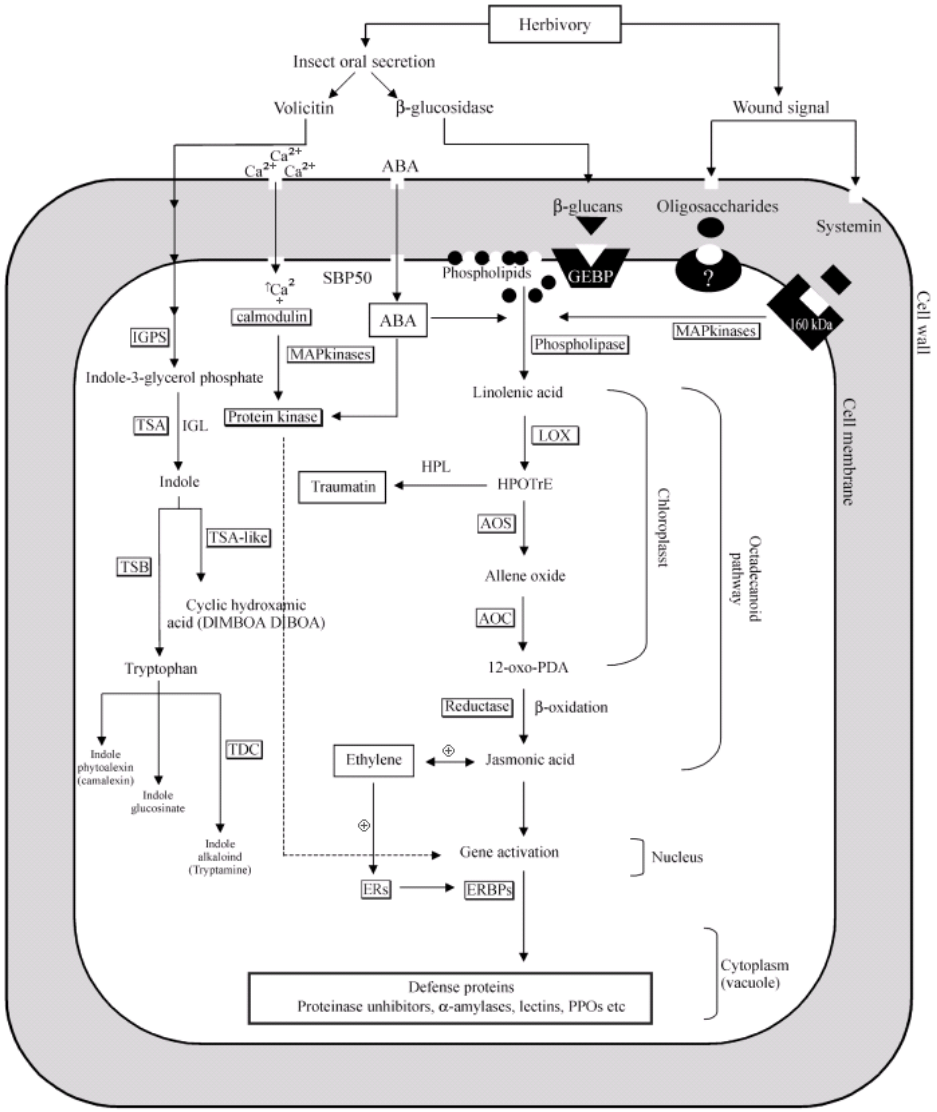


Fig 2. Putative intracellular wound signal transduction pathway leading to the induction of defense protein gene expression

→ : illustrate portion of pathway proposed from direct evidence.
--->: illustrates inferred pathways or interaction.
Boxes indicate orthologous present in sugarcane.

LOX: Lipoxygenase
HPL: Hydroxy peroxyde lyase
AOS: Allene oxide synthase
AOC: Allene oxide cyclase
SA: Salicyclic acid
HPOTrE: 13(s)-Hydroperoxylenolenic acid
12-oxo-PDA: 12-Oxy phytodienoic acid
IGP: Indole-3-glycerol phosphate
IGPS: Indole-3-glycerol phosphate synthase
TSA: Tryptophan synthase-subunit-α (BX1 in maize) (a constitutive enzyme which catalyses the conversion of IGP

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Conclusion

At present relatively few plants secondary metabolites can be produced economically by cell cultures. With increasing knowledge of the biochemistry of secondary product synthesis and accumulation, and how these processes are regulated, it seems likely that this situation will however gradually change. Knowledge of biosynthetic pathways of desired compounds in plants as well as in cultures is often still in its infancy, and consequently, strategies are needed to develop an information base on a cellular and molecular level. An optimum production process cannot be found by sequentially optimizing each step of the process individually, because strategies leading to improved product synthesis seem to be interactive. Thus, we believe that only continuation and increase of efforts in this field will lead to controllable and successful biotechnologic production of specific, valuable, and as yet unknown plant biochemicals.[24, 25]

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