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Effect of elicitors on plant cell suspension culture for the enhancement of secondary metabolite production

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Abstract
In present era, different tissue culture techniques are applied to enhance secondary metabolites production by triggering stress response. These stresses include application of elicitors, biotransformation, change in environmental conditions, change in medium constituents, precursors, etc. Among them, elicitors are substances that induce defense responses in plants. Elicitors are introduced into various organ cultures and in cell suspension cultures to increase the stress tolerance. The present review focuses on secondary metabolite production in plant cell suspension culture. This will help in conservation of rare and endangered medicinally important plants and it will also provide increased secondary metabolite production with less time and low cost.

Keywords: Cell suspension culture, elicitor, secondary metabolite production.

Introduction
The extensive research work in the modern biological and chemical sciences have described the role of primary metabolites in vital life functions like cell division and growth, respiration, storage, and reproduction. The concept of secondary metabolite was first explained by Kossel [1]. He defined that secondary metabolites are opposed to primary ones. Later on, Czapek [2] have taken an important step and devoted an entire volume named "end product" to his 'plant biochemistry' series. As per Czapek, the secondary modification i.e. de-amination process in nitrogen metabolism gives rise to these end products. The secondary metabolites are often located at specific cell or organs and less than 1% of total carbon as compared to other main molecules. In the middle of 20th Century, advancement in different analytical techniques resulted in to identification and purification of several molecules which formed the basis of phyto-chemistry discipline. The paper chromatography revealed that some of the molecules are pigments. However, the possible role of these secondary metabolites in life cycle of plant is still mysterious as mentioned by Czapek as end product. Plant fitness is largely depending on production of secondary metabolites under environmental influence. Most of the secondary metabolites possesses bioactivities like antifungal, antibiotic, antiviral etc. Hence, they protect plant against pathogens and shows allelopathic effect which prevents germination of other species present in their vicinity. In addition, they possess UV absorbing compounds and prevents leaf damage from UV rays [3].
The classification of plant secondary metabolites is usually based on their biosynthetic pathways [4]. The secondary metabolites are grouped in to four families such as alkaloids, phenolics, steroids and terpenes. Among these phenolic family is widespread as its compounds are involved lignin synthesis and ubiquitous in higher plants. The alkaloids are sparsely distributed in plants and are specific to genus and species. Such narrow distribution of secondary metabolites forms basis of chemotaxonomy and chemical ecology. Because of the numerous biological activities of secondary metabolites, they have been used in traditional medicines for centuries.
In recent days, secondary metabolites correspond to value added products like cosmetics, fine chemicals or currently neutraceutics. Recent studies have well established that in pharmaceutical industries chemistry is said to be backbone, however about 25% molecule used in this industry have natural plant origin [5]. The secondary metabolite production requires large scale cultivation of medicinal plants. However, it is difficult to cultivate specific biotype away from their natural ecosystem. Sometime the common plants with pathogen sensitivity are unable to grow in large fields, for example anthracnose on *Arnica montana* and *Hypericum perforatum.* Due to this, plant cell, tissue and organ cultures become popular choice for production of secondary metabolites.

Different tissue culture techniques are being used for increase content of secondary metabolites by application of elicitors, alternation in environmental conditions and medium constituents etc.

Elicitors

An elicitor is a substance which, when introduced in small concentrations to a living cell system, it initiates or triggers the biosynthesis of specific compounds [6].

Classification of elicitors

Generally, elicitors are classified as physical or chemical, biotic or abiotic and complex or defined depending on their origin and molecular structure [6].

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. Biotic elicitors can be classified on the basis of their exact molecular structure as follows: [7, 8].

A. Proteins and glycoproteins as elicitors

Proteins and enzymes are class of elicitors that trigger defense response e.g. in plant cell cultures. Cellulase causes rapid accumulation of phytoalexins, enhanced production of debneyol and capsidol in cell cultures of Nicotiana tabacum [9]. The glycoproteins are also involved in triggering of different phytoalexins in plant cell cultures. In cell cultures of *Plantanus acerifolia*, the coumarin concentration was increased due to application of glycoprotein in its native conformation extracted from a fungus *Ceratocystis fimbriata* cell suspension culture [10].

B. Oligosaccharides as elicitors

The oligosaccharides have shown to induce defense responses in plant cells. Like proteins, carbohydrates also act as chemical trigger i.e. elicitor to enhance the secondary metabolite production in cell suspension culture of plants. In Soybean cell cultures, the role of carbohydrates elicitors in accumulation of phytoalexin was observed [12]. The partial acid hydrolysis of mycelium of *Phytophthora gasperma* revealed involvement of eight oligosaccharides in phytoalexin production [12]. The oligosaccharides showing enhancement of secondary metabolites in cell cultures of plants are enlisted in Table 1.

C. Plant hormones as elicitors

The hormones play an important role in plant defense mechanism against different environmental stresses. The plat hormones, Jasmonic acid (JA), salicylic acid (SA), derivatives and analogs of SA, Brassinosteroids act as elicitor and accumulates specific metabolites to trigger defense mechanism. They upregulate expression of plethora of defense genes in plants. The plant resistance to bacterial, fungal and viral pathogens is regulated by SA [14, 15]. while the resistance against insects is regulated by JA [16, 17]. JA derived elicitation results in to enhanced synthesis of various proteins via octadecanoic pathway [16, 17].

Abiotic elicitors

The abiotic elicitors have acquired less attention as compared to biotic elicitors [6]. They include different stress causing physical and chemical factors of abiotic origin like salt, metal etc. Treatment of rare earth metal lanthanum to *Taxus sp.* cell cultures resulted in tremendous increase in taxol content (280%) [18]. The other abiotic factors like salinity, heat, drought, mechanical wounding, UV irradiation also shown increased content of secondary metabolites in plants. For example, exposure of cell cultures of *Vaccinium corymbosum* enhanced production of non-volatile phenolic compounds [19].

Cell suspension cultures

Initially, it was stated that the undifferentiated cells like callus or cell suspension cultures will not be able to synthesize secondary products [20]. However, Zenk and co-workers demonstrated that the cell cultures of *Morinda citrifolia* yields about 2.5 g of anthraquinones [21]. This finding opened the door to a large community of *vitro* culturists who extensively studied the possible use of plant cell cultures for the production of secondary compounds of industrial interest (mainly pharmaceutics and dyes). For the establishment of *in vitro* cell lines promising individual plants are selected and callus culture established for them. Once calli are established then they can undergo somaclonal variation during subculture cycles. After a period of time (from several weeks to several years) genetic stability occurs and each callus can be considered as homogeneous cell aggregate, just as if it was derived from single cell cloning.

When genetic stability is reached, it is necessary to screen the different callus lines according to their aptitudes to provide an efficient metabolite production. Hence, each callus must be assessed separately for its growth speed as well as intracellular and extracellular metabolite concentrations i.e. growth and production kinetics. This allows an evaluation of the productivity of each cell line (mg of products g^{-1} of cell day^{-1} or mg of products l^{-1} day^{-1}) so that only the best ones will be taken to cell suspensions and reactor studies. The cell growth kinetics is usually in exponential curve; however, the secondary metabolites are produced during stationary or plateau phase. This meager production in initial stages is a result of allocation of more carbon for cell structure building and respiration i.e. primary metabolism when cells growth is very active. On the contrary, when cell growth reaches to stationary phase, the carbon flow is diverted from primary metabolism to secondary metabolism and enhances the process of secondary metabolites synthesis. During lag or log phases, the enzyme activities are generally absent or less but in plateau phase they start appearing. These observations lead researchers to predict possible biochemical differentiation of cell in plateau phase [5]. But, some secondary products like betalains and carotenoids are associated with growth of undifferentiated cells.

The cell suspension is the best biological material to study biosynthetic and metabolic pathways. As compared to callus, the recovery of individual cells in large quantities for isolation of enzymes is easier [22]. The studies on biosynthetic pathways helps to restrict enzyme activities (or expression of associated genes) in synthesis of potent metabolites. Such restriction steps can be altered by feeding the cells suspension cultures with a precursor compound of desired product. This phenomenon also has high risk of activation of feedback inhibition at other places on the pathway [23]. Among multiple traditional strategies employed for increased production of secondary metabolites, the elicitation is one of the most successful technique. The

elicitor compounds impose physical or chemical stresses on cell suspension cultures which triggers production of stress induced secondary metabolites which are not produced under normal conditions. This elicitation of cell suspension cultures involves both biotic and abiotic factors. The fungal and bacterial attacks are most efficient to trigger elicitation process and increase in content of secondary metabolites. Immobilization is also used in liquid culture system for enhancing metabolite production [24]. In this technique, plant cells are encapsulated with polymers like alginate, carrageenan's etc. which improves yield of desired metabolites [25]. This may be due to possible effect of polymers around the cell which may mimic tissue organization between cells. This is supposed to give rise to the so-called biochemical differentiation which favors the synthesis of secondary products [26]. The present review aimed to summarize information of different elicitors used for enhancement of secondary metabolites in cell suspension cultures.

Table 1: List of plants and their elicitors used to enhance secondary metabolites

No.	Plant name	Product	Elicitors	Reference
1	*Abrus precatorius*	Glycyrrhizin	*Aspergillus niger* and *Rhizopus stolonifer*	[27]
2	*Agrostis tenuis*	Jasmonic acid	Fungal Elicitor	[28]
3	*Ammi majus*	Scopoletin	*Enterobacter sakazaki*	[29]
4	*Ammi majus*	Coumarin Umbelliferon	*Phytophthora megasperma*, *Alternaria carthami*	[6]
5	*Apium graveolens*	Furocaumarin	*Sclerotinia sclerotiorum*	[30]
6	*Apium graveolens*	Furocaumarin	*Erwinia carotovora*	[31]
7	*Apium graveolens*	Furocaumarin	UV, CuSO4	[32]
8	*Arabidopsis*	Indoleglucosinolates, Camalexin	Thaliana fungal elicitor, Methyl Jasmonate, Salicylic acid	[33]
9	*Arabidopsis thaliana*	Indole-glucosinolates, camalexin	*Erwinia carotovora*	[33]
10	*Arabidopsis thaliana*	Anthocyanin	Jasmonic acid	[34]
11	*Arabidopsis thaliana*	Indole-glucosinolates and anthocyanins	Jasmonic acid	[35]
12	*Arabidopsis thaliana*	Anthocyanins	Jasmonic acid	[36]
13	*Arabidopsis thaliana*	Aliphatic glucosinolates	Jasmonic acid	[37]
14	*Arabidopsis thaliana*	Camalexin	Jasmonic acid	[38]
16	*Artemisia annua*	Artemisinin	Jasmonic acid	[39]
17	*Azadirachta indica*	Azadirachtin	*Anabaena* sp. and *Nosto carneum*	[40]
18	*Azadirachta indica*	Azadirachtin	Jasmonic acid, salicylic acid	[41]
19	*Azadirachta indica*	Azadirachtin	Cyanobacterial elicitor	[42]
20	*Boswellia serrata*	boswellic acid	PVP. *Fusarium*, Penicillin, *Mucor sp*. and yeast extract, NaCl, NaSO4, UV-C, light intensity	[43]
21	*Brugmansiasu aveolens*	Tropane alkaloids	*Spodoptera frugiperda*, Methyl jasmonate	[44]
22	*Calendula officinalis*	Oleanolic acid	*Trichoderma viride*, Pectin, Chitosen	[45]
23	*Camptotheca acuminate*	Indol alkaloid	Methyl Jasmonate	[46]
24	*Carthamus tinctorius*	A-tocopherol and pigment	*Trametes varsicolor*, *Mucor sp*., *Penicillium notatum*, *Rhizopus stolonifer*, and *Fusarium oxysporum* and abiotic (NaCl, $MgSO_4$, $FeSO_4$,and $FeCL_3$)	[47]
25	*Catharanthus roseus*	5'diesterase	*Alteromonas acleodii*, Alginate oligomers	[48]
26	*Catharanthus roseus*	Indol alkaloid	Methyl Jasmonate	[49]
27	*Citrus aurantium*	Chitin oligomers	Chitin	[50]
28	*Citrus grandis*	Limonene, Linalool	Chitosan	[51]
29	*Coleus blumei*	Rosmarinic acid	Yeast Elicitor	[52]
30	*Coleus forskolin*	Methyl jasmonate	Forskolin	[53]
31	*Cupressus lusitanica*	B-thujaplicin	Oligosaccharide, Methyl Jasmonate	[54]
32	*Dacus carota*	Methoxymellein, 4-hydroxybenzoic acid	Fungal elicitor	[55]
33	*Datura stramonium*	Alkaloids (tropane)	*Phytopthora megasperma*	[56]
34	*Daucus carota*	*Pythium aphanidermatum*	6-Methoxymellein; 4-hydroxybenzoic acid	[56]
35	*Daucus carota*	Furocaumarin	Unknown	[57]
36	*Digitalis lanata*	Flavonoids	Copper sulphate	[58]
37	*Dioscorea zingiberensis*	Diosgenin	*Fusarium*	[59]
38	*Drosera burmanii*	Plumbagin	Methyl jasmonate, yeast Extract and chitosan	[60]
39	*Eschscholzia californica*	Benzophenanthridines, sanguinarine	Methyl Jasmonate, Fungal Elicitor Puccinia	[61]
40	*Eschscholzia californica*	Benzo-phenanthridine	Fungal elicitor	[62]
41	*Eschscholzia californica*	Alkaloids	Yeast extract	[63]
42	*Euphorbia pekinensis*	Terpenoids	*Fusarium*	[64]
43	*Eurycoma longifolia*	Alkaloids	Chitosan, polyvinylpyrrolidone	[65]
44	*Ganoderma lucidum*	Ganoderic acid and Ganoderma polysaccharides	Fungal	[66]
45	*Ginkgo biloba*	Bilobalide and ginkgolides	Biotic	[67]
46	*Glehnia littoralis*	Furanocoumerin	Yeast extract	[68]
47	*Glehnia littoralis*	Furocaumarin	*Pseudomonas cichorii*, UV	[69]
48	*Glycine max*	Glyceollins, Apigenin, Genistein, luteolin	Glucan, Methyl Jasmonate	[70]
49	*Glycine max*	Lignin	Fungal Elicitor	[71]

50	*Glycyrrhiza glabra*	Soyasaponin, 5-deoxyflavonoid	Methyl Jasmonate	[72]
51	*Gossypium arboretum*	Gossypol	Jasmonic acid	[73]
52	*Gymnema sylvestre*	Gymnemic acid	*Aspergillus niger*	[74]
53	*Helianthus tuberosa*	Insulin	*Aspergillu sniger* and *Saccharomyces cerevisiae;*	
54	*Hyoscyamus muticus*	Solavetivone, rishitin, lubimin, Scopolamine	Salicylic acid	[76]
55	*Linum album*	Podophyllotoxin	Ag, Pb, Cd, Yeast	[77]
56	*Lithospermum erythrorthizon*	Shikonin, Rosmarinic acid	Polysaccharides, fungal elicitor, Methyl Jasmonate	[78, 79]
57	*Lycopersicon esculentum*	Scopoletin	Fungal elicitor, Methyl Jasmonate	[61]
58	*Medicago truncatula*	Beta-amyrin	Yeast elicitor	[80]
59	*Morinda citrifolia*	Anthraquinone	Chitin, Pectine	[81]
60	*Nicotiana attenuata*	Volatile terpenes	Jasmonic acid	[82]
61	*Nicotiana benthamiana*	Nicotine	Jasmonic acid	[83]
62	*Nicotiana plumbaginifolia*	Nicotine	Cellulase, Methyl Jasmonate	[84]
63	*Nicotiana tabacum*	Capsidiol, Debneyol, Scopolatin, Nicotine	Cryptogein	[85]
64	*Nicotiana tabacum*	Sesquiterpenecyclise, Chitinase, Proteinase inhibitor	Algal Elicitor	[86]
65	*Nicotiana tabacum*	Nicotine	Jasmonic acid	[87]
66	*Nicotiana tabacum*	Phenylpropanoids	Jasmonic acid	[88]
67	*Nicotiana tabacum*	Capsidol	Fungal elicitor	[89]
68	*Oryza sativa*	Momilactones, Sakuranetin, Phytocassans	N-acetylachitoheptaose, Methyl Jasmonate	[90, 91]
69	*Panax ginseng*	Saponins	Low energy ultrasound	[92]
70	*Panax ginseng*	Saponin	Oligogalacturonic acid Low energy ultra sound	[93, 94]
71	*Panax ginseng*	Ginsenosides	Methyl Jasmonate	[95]
72	*Panax ginseng*	Saponin	Chitosan	[94]
73	*Papaver somniferum*	Sanguinarine	Methyl jasmonate, Phenidone, Fungal elicitor	[96]
74	*Pastinaca sativa*	Furocaumarin	*Ceratocystis fimbriata*	[97]
75	*Pastinaca sativa*	Furocaumarin	*Phomacompanata*	[98]
76	*Phaseolus vulgaris*	Phaseolin	Fungal elicitor	[99]
77	*Pinelli aternata*	Alkaloids	*Pseudomonas sp., Enterobacter sp*.	[100]
78	*Pinustaeda*	Flavonoids and isoprenoids	Jasmonic acid	[101]
79	*Psoralea cinerea*	Furocaumarin	CuSO4	[102]
80	*Rhodiola sachalinensis*	Salidroside	*Aspergillus niger*	[103]
81	*Rubia tinctorum*	Anthraquinone	Fungal polysaccharides, Salicylic acid, Gibbrelic acid	[104]
82	*Ruta graveolens*	Furocaumarin	UV, *Rhodotula rubra*	[105]
83	*Ruta graveolens*	Furocaumarin	NaCl	[106]
84	*Salvia miltiorrhiza*	Ditepenoidtanshinones	Yeast elicitor	[107]
85	*Silybum marianum*	Silymarin	Yeast extract	[108]
86	*Solanum tuberosum*	Hydroxy-cinnamoyltyramins	*Phytophthora infestans*	[109]
87	*Taxus baccata*	Taxol	Vanadyl sulphate	[110]
88	*Taxus chinensis*	Trifluoroethyl salicylate	Taxuyunnanine C (Tc)	[111]
89	*Taxus chinensis*	Taxol	Fungal elicitation	[112]
90	*Taxus chinensis*	Taxol	Nitric oxide	[113]
91	*Taxus chinensis*	Taxane	Methyl jasmonate, Salicylic acid	[114]
92	*Taxus unnanensis*	Taxane	Oligogalacturonides	[115]
93	*Vaccinium corymbosum*	Nonvolatile phenolic compounds	UV B radiation	[19]
94	*Vanilla planifolia*	Vanillin	Acetone dried red (Beetperoxidase)	[116]
95	*Vitis vinifera*	Stilbene, resveratrol, Anthocyanins	Methyl Jasmonate, Ethylene	[117]
96	*Vitis vinifera*	Anthocyanin	Salicylic acid, Abscisic acid, Jasmonic acid, Manitol	[118]
97	*Eschscholzia Californica*	Macarpine	Salicylic Acid	[119]
98	*Lithospermum erythrorhizon*	Rosmarinic acid	Yeast extract, Methyl jasmonate	[120]
99	*Taxus chinensis*	Paclitaxel	Chitosan	[121]

Conclusion

Plants are well known source of pharmaceuticals but due to low yield, they are not economically feasible. In biodiversity conservation point of view, it is necessary to avoid destruction of medicinally important plants. To overcome these obstacles, cell suspension culture is one of the potential *in vitro* tissue culture techniques to get these metabolites in less time and high amount. In addition, it is proving that applications of biotic and abiotic elicitors can enhance production of these metabolites. Elicitation in plants has opened up opportunities for better understanding of the pathways leading to the production of novel plant metabolite and overproduction of defense compounds useful in the protection against plant pathogens. However, the mechanism involved in elicitation process is not yet fully understood. To unravel the potential elicitation process, the future efforts must consider all the aspects of biochemistry, molecular biology, microbiology, phytochemistry, pharmacognosy, and fermentation technology associated with secondary metabolite production.

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