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Elicitors in Plant Tissue Culture

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Plants or plant cells in vitro, show physiological and morphological response 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. The application of elicitors, which is currently the focus of research, has been considered as one of the most effective methods to improve the synthesis of secondary metabolites in medicinal plants. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavours and other industrial materials. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Commonly tested chemical elicitors are salicylic acid, methyl salicylate, bezoic acid, chitosan and so forth which affect production of phenolic compounds and activation of various defense-related enzymes in plants. Plants are challenged by a variety of biotic stresses like fungal, bacterial or viral infections. This lead to the great loss to a plant yield. Here we discuss the classification of elicitors, mechanism of elicitor, the use of elicitors and the different features of elicitors.

Keyword: Plant tissue culture, elicitors, utilization of elicitors, classification andmechanism of elicitor

1. Introduction

In plant tissue culture studies, the plant tissues are challenged by various stresses and their combinations but the plasticity of plant genome counters remarkably to such stresses under in vitro condition [1].

The stress responses are triggered in the plant tissues for enhanced yield of secondary metabolites using Elicitors, Precussors and Bioinformation, environmental stresses and change in media constituents [1].

Elicitors are compounds which stimulating any type of physiological abnormality of plant [2]. This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitros). Elicitors could be used as enhance of plant secondary-metabolite synthesis and could play an important role in biosynthetic pathways to enhanced production of commercially important compounds.

The secondary metabolites are released due to defense responses which are triggered and activated by elicitors, the signal compound of plant defense responses [2].

2. Classification of Elicitors

According to Radman *et al*. (2003) [2] elicitors are classified as physical or chemical. On the basis of nature elicitors can be devided into two types

Biotic and Abiotic. The biotic elicitors have biological origin, derived from the pathogen or from the plant itself while abiotic elicitors have not a biological origin and are grouped in physical factor and chemical compounds. The first biotic elicitors was discovered in 1968.
Further on the basis of plant elicitor interaction it may be classified into race specific and general elicitors [3,4]

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. This provides an opportunity for intensive research in the field of biosciences for exploitation of plant cells for the production of secondary metabolites. **Table 1** represents the classification of elicitors.

Table 1: Classification of Elicitors

<table>
<tr><th colspan="6">Elicitors</th></tr>
<tr><td>Physical Elicitors</td><td colspan="5">Injury</td></tr>
<tr><td></td><td colspan="2">Abiotic</td><td colspan="3">Metal icons (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 cell 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, elic-Itins, Oligandrin</td></tr>
<tr><td>Lipids</td><td></td><td>Lipopolysaccharides</td></tr>
<tr><td>Glycoproteins</td><td></td><td>Not characterides</td></tr>
<tr><td>Volatiles</td><td></td><td>C_6-C_{10}</td></tr>
</table>

Data source: IJDDHR[5]; Angelova Z.et.al.:2006 [6]

3. Plant hormones as Elicitors

There are various plant hormones which act as a Elicitors. The common plant hormones like Salicyclic acid (SA) and Jasmonic acid (JA) are key signals for defense gene expression , In which SA regulates resistance to pathogens like bacterial, fungal and viral, Whereas JA regulates the production of protiens by the octadecanoid pathway. SA and JA are both synthetic mimics that can be appied externally to induce same metabolic changes that regulates resistance against pathogen. The biochemical pathways of both SA and JA are useful in the plant elicitation process [6].

4. Elicitors from Carbohydrates[5]

In plant tissue culture, there are different carbohydrates useful in the overproduction of secondary metabolites. Albersheim et al. (1977) first isolated to oligosaccharides that regulate variety of plant defense gene. In tobacco cell cultures the carbohydrates elicitors are induce the signal transfer with regard to calcium influx and production of H_2O_2.[17]

- In Rice system, the combination of oligosaccharides and methyl jasmonate has been used to produce phytoalexin.
- When the cultures were treated with a combination of N- acetylchetohexose and methyl jasmonate are produce paclitaxel in taxus Canadensis cell suspension cultures.

The different type of carbohydrate elicitors are shown in table 2. (Angelova z.et. al.:2006).

Table 2: Carbohydrate elicitors and metabolites in plant cell cultures.

Elicitors	Culture	Metabolites
ß-linked glucopyranosyl	Glycine max	Phytoalexins
α-1,4-oligogalacturonide	Glycine max	Phytoalexins
Chitosan	N.tobaccum, E.califomaica	Phytoalexins
Hepta- ß-glucoside	Glycine max	Phytoalexins
ß—glucan	Glycine max	H_2O_2

Data source: IJDDHR[5]; Angelova et.al.2006 [6]

Table 3: Classification of Abiotic elicitors

Elicitor	**Plant cell cultures**	**Elicited product**	**References**
Methyl jasmonate	*Taxus* Sp.	Paclitaxel, taxanes,diterpenes	Furmanowa *et al*. (1995) Yukimune *et al*. (1996)
	Glycine max	Vegetable storage proteins	Singh *et al*. (1998), Anderson (1991)
	Oryza sativa leaves	Putrescine	Chen *et al*. (1994)
Salicylic acid	*Daucus carota*	Chitinase	Muller *et al*. (1994)
Calcium chloride	*C. forskohlii*	Forskolin	Prasad babu (2000)
Sodium alginate	*C. forskohlii*	Forskolin	Prasad babu (2000)
Metal ions			
Copper sulphate	*Hyoscymus albus*	Phytoalexins	Lee *et al*. (1998), Mader, (1999
	Lithospermum erythrorhizon	Shikonin	Fujita *et al*. (1982)
Silver nitrate	*Solanum tuberosum*	Free and conjugated polyamines	Mader, (1999)
Vanadium sulphate	*Catharanthus roseus*	Catharanthine, ajmalicine	Smith *et al*. (1987)

Data source: IJDDHR[5]

5. The Use of Elicitors for Protection of Cultured Plants

The method of elicitor-induced resistance to disease in plants is characterized by a number of essential advantages:

(1) ecological safety, because the method is based on induction of the native immune potential of genes;

(2) a systemic and prolonged protective effect:

(3) involvement of multiple defense systems in induced resistance, which makes adaptation of pathogens to protected plants nearly impossible;

(4) induction of non specific resistance to the number of fungi, bacteria, viruses, nematodes, etc.

6. Utilization of elicitation of plant tissue cultures in various areas of research.

(Veersham, 2004) [8]

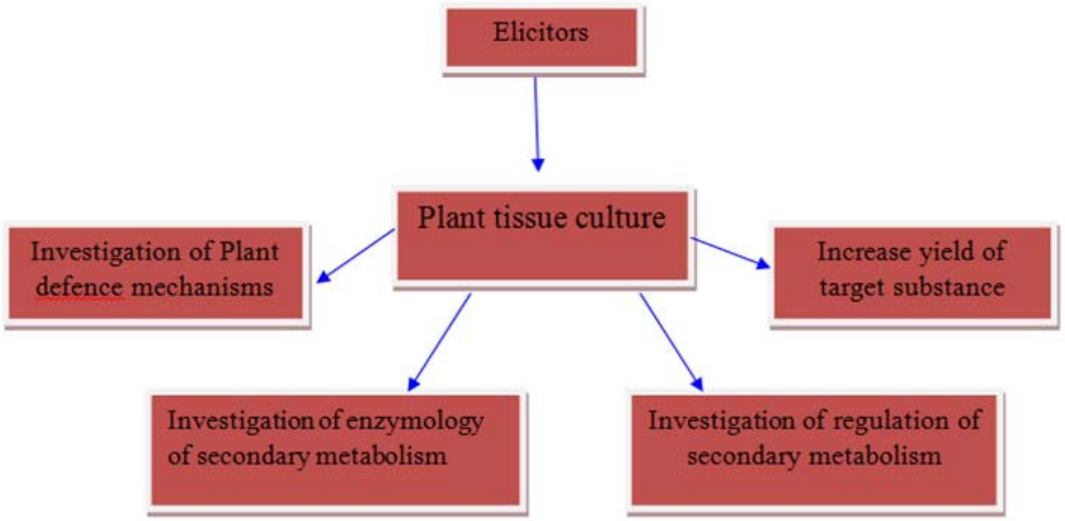

Fig 1: Utilization of elicitation of plant tissue cultures in various areas of research.

7. The Different Features of Elicitors Include [15]

The product which accumulate in plant cell cultures due to elicitation 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.

I). Elicitor concentration

Elicitor concentration plays a very important role in elicitation process. Namdeo *et al*. [11] 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*. [10], Rijhwani and Shanks [11, 13]. High dosage of elicitor has been reported to induce hypersensitive response leading to cell death, whereas, an optimum level was required for induction [14-16].

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* [9] . However, further increasing exposure time

resulted in decrease in ajmalicine content. Similar results were reported by Rijhwani and Shanks [11] Moreno and co-workers [16] and Negeral and Javelle [17].

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 [9]. Highest ajmalicine (166 µg-1 DW) was accumulated in 20-day-old cells elicited with extracts of *T. viride* followed by 90 and 88 µg g-1 DW ajmalicine in cells elicited with *A. niger* and *F. moniliforme* respectively [15]. Similar observations were reported from various workers Rijhwani and Shanks [18] Ganapathi and Kargi [19].

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 production medium as compared to Murashige and Skoog's medium Namdeo *et al*. [9]. Similar observations were reported from various workers Rijhwani and Shanks [18] Ganapathi and Kargi [19] . 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.

8. Conclusion

Above all these, Elicitation of the tissue culture has been found to be more economical beneficial [1]. In *in vitro* cultures, few decades many strategies such as media manipulation phytohormone regulation, biotransformation. Phytochemistry and Biochemistry of elicitors are useful to exploit the potential of plant cells for the production of plant secondary metabolites like alkaloids, flavonoids volatile oils, tannins, resins etc. These secondary metabolites to ensure their survival, persistence and competitiveness. [15]

9. References

1. Manorma Sharma, Archana Sharma, Ashwani Kumar and Saikat Kumar Basu. Enhancement of secondary Metabolites in Cultured Plant Cells through Stress Stimulus. American Journal of Plant Physiology. 2011, 6 (2): 50-71.
2. Radman R, Sacz T, Bucke C Keshvartz T. Biotehnol. Appl. Biochem., 2003; 37,91-10.
3. Staskawicz B.J., F.M. Ausubel, B.J. Baker, J.G. Ellis , J.D.G Jones. Molecular genetics of plant disease resistance Science., 1991;268:661-667.
4. Vasconsuelo, A. and R. Boland., Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci., 2007, 172:861-875.
5. Interenational journal of Drug discovery and Herbal reaserch (IJDDHR)., April-june:2011;1(2):84-90.
6. Angelova Z., Georgiev S, Roos W. Elicitation of plants, Biotechnol. & Biotechnol; 2006;72-83.
7. Alami I., Maris S , Clerivet A. Phytochemistry.,1988; 48, 771-776.
8. C.Veersham. In Elicitation: Madicinal Plant Biotechnology, CBS Publisher, India.,2004; 270-293.
9. A.G. Namdeo, S. Patil, D.P. Fulzele. Influence of fungal elicitors on production of ajmilicine by cell culture of *Catharanthus roseus*. Biotechnol Prog.,2002, 18:159-162.
10. C. Nef-Campa, M.F. Trousot, P. Trouslot, H. Chrestin. Long-term effect of a Phythium elicitor treatment on the growth and alkaloid production of *Catharanthus roseus* cell suspensions. Planta Med.,1994;60(2): 149-152.
11. S.K. Rijhwan, J.V. Shanks. Effect of elicitor dosage and exposure time on biosynthesis of indole alkaloids by *Catharanthus roseus* hairy root cultures. Biotechnol Prog.,1998a; 14 (3): 442-449.
12. D.B. Collinge , A.J. Susarenka. Plant gene expression in response to pathogens. Plant Mol. Biol.,1987; 9: 389-410.
13. U. Mukandan, M.A Hjorosto. Effect of fungal elicitor on thiophene production in hairy root cultures of Tagetes patula. Appl. Microb Biotechnol.,1990; 33: 145-147.
14. I. A. Roewer, N. Cloutier and R. Van der Heijden. Transient induction of tryptophan decarboxylase (TDC) and strictosidine synthase, (SS) genes in cell suspension cultures of *Catharanthus roseus*. Plant Cell Rep.,1992;11(2): 86-89.
15. A. G. Namdeo. 'Investigation on pilot scale bioreactor with reference to the synthesis of bioactive compounds from cell suspension cultures of *Catharanthus roseus* Linn. Ph.D.

Thesis, Devi Ahilya Vishwavidyalaya, Indore, M.P. India.,2004.
16. P.R.H. Moreno, R. Van der Heijden, R. Verpoorte of terpenoid precursor feeding and elicitation on formation of indole alkaloids in cell suspension cultures of *Catharanthus roseus*. Plant Cell Rep.,1993;12: 702-705.
17. J. Negeral, F. Javelle. Induction of phenyl propanoid and tyramine metabolism in pectinase or pronase elicited cell suspension culture of tobacco. Physiol Plant.,1995; 95:569-574.
18. S.K. Rijhwani, J.V.Shanks. Effect of subculture cycle growth and indole alkaloid production by *Catharanthus roseus* hairyroot cultures. Enz Microb Technol., 1998b; 22 : 606-611.
19. G. Ganapathi , F. Kargi. Recent advances in indole alkaloid production by *Catharanthus roseus* (Periwinkle). J Exptl Bot.,1990; 41: 259-267.
20. M.H. Zenk, H. El Shagi, H. Arens, J. Stockigst, E.W. Weiler, D. Deus. Formation of indole alkaloids serpentine and ajmalicine in cell suspension cultures of *Catharanthus roseus*. In Plant Tissue culture and its Biotechnological Application. Barz, W., Reinhard, E. and Zenk, M.H. (eds.), Springer-Verlag, Berlin. Pp., 1977; 27-44.
21. T. Murashige , F. Skoog. A revised medium for rapid growth and bioassays with tobacco J. 12: 113-120, (tissue cultures. Physiol. Plant).,1962; 15, 473-479..