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Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/

ANTI-CANCEROUS SECONDARY METABOLITES FROM PLANT IN-VITRO CULTURE: PRODUCTION AND NEW BIO-ENGINEERING STRATEGIES (REVIEW)

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Abstract

Plant secondary metabolites have great importance in pharmaceutical sector acting as anti-cancerous, anti-inflammatory, anti-oxidant and anti-microbial agents. The secondary metabolites occurring naturally in plants have low availability due to low yield in wild plants, endangered plant and present only under specific conditions. Thus there has been a shift towards in vitro production of secondary metabolites which give higher yield and with no boundaries of time and space. Plants can be grown in cell suspension culture, organ culture and cell culture where a plant develops from an explant. Moreover, the level of production can be enhanced by different strategies like precursor feeding, elicitation, scale-up, and metabolic engineering. Plant cell cultures on large scale has been very promising for large scale production of anti-cancer metabolites which can be used at commercial scale.

Keywords
Secondary metabolites, Elicitation, Anti-cancerous, Secondary metabolites, Bioreactors.

1. Introduction

Plants produce many compounds (phytochemicals) in response to environmental stress (biotic or abiotic) which play role in their adaptation to environment and defense called as secondary metabolites. Many of these compounds have pharmaceutical and medicinal importance used to treat diseases including cancer (Kabera, 2014). The main class of metabolites produced through plant tissue culture include alkaloids, phenylpropanoids, quinones, terpenoids and steroids (Nahata, 2017).

Since, the secondary metabolites produced from whole plants are in low concentration, may be restricted to a specific species, might be produced during a specific developmental stage, under stress, or under certain conditions (Verpoorte *et al*., 2002). Moreover, some plants are difficult to cultivate, or may be at a risk of extinction. For these limitations of getting metabolites from wild, focus has been shifted to produce them

through *in vitro* plant cultures (Buitelaar *et al*., 1992). Thus there is growing importance of producing value added secondary metabolites through *in vitro* plant culture and various strategies are adopted to enhance their production such as precursor feeding, elicitation and manipulating genetic pathways.

Table 1. Examples of some plant species and secondary metabolite obtained from them.

Plant Species	Secondary metabolite	Role	Reference
Camptotheca acuminate	Camptothecin	Anti-tumor activity	(Lorence & Medina-bolivar, 2004)
Podophyllum emodi, Podophyllum peltatum	Epipodophyllotoxins	Anti-tumor agent	(Giri & Lakshmi Narasu, 2000)
Taxus brevifolia	Paclitaxel	Anti- ovarian, breast and non-small cell lung cancer	(Wahab, Sabry, Fatah, & Sayed, 2019)
Tabebuia avellanedae	Beta-lapachone	Anti-inflammatory and anti-neoplastic activity	(Lee, Ban, Chung, Im, & Kim, 2018)
Colchicum autumnale	Colchicine	Acute Pericarditis therapy	(Imazio, 2015)
Zingiber officinale	Gingerol	Anti-inflammatory, anti-cancerous and anti-oxidant	(Mohd Yusof, 2016)
Capsicum annuum	Luteolin	Cardio-protective, anti-inflammatory, anti-cancerous	(Materska et al., 2003)
Gossypium hirsutum	Gossypol	Anti-neoplastic agent	(Stipanovic, Puckhaber, Bell, Percival, & Jacobs, 2005)
Aloe vera	Alexin B	Anti-cancerous activity	(Zadeh & Kor, 2014)

2. In-Vitro Production of Anticancerous Secondary Metabolites

There are various ways for production of secondary metabolites by *in vitro* cultures discussed here.

2.1.Plant Cell Cultures

The plant culture is defined as a culture of cells, tissue, organ or plants, under aseptic condition, under definite chemical and physical environment. The ability to culture ex-plants in laboratory has allowed us to produce secondary metabolites. The in-vitro cultures allows hyper-production of high value secondary metabolites as their synthesis is highly inducible (Isah *et al*., 2018; Kolewe *et al*., 2008).

2.2.Cell Suspension Culture

Nowadays cell suspension culture is mostly used for the production of secondary metabolites through large scale culturing of plant cells (Karam *et al*., 2003). A suspension culture is made by transferring the segment of callus into a liquid medium and hence is sustained under optimum conditions (Murthy *et al*., 2014). In suspension cultures individual cells are distributed evenly throughout the growth medium

because cell suspension facilitates transfer of oxygen and nutrients into each individual cells. The secondary metabolites accumulate in media during the stationary phase of growth. The cell suspension culture develops from callus which should be healthy and growing vigorously (Alvarez, 2014).

2.3.Plant Organ Culture

Some secondary metabolites are dependent on the developmental stage and degree of cell differentiation for which tissue (organ) cultures are used. Two types of organ cultures are there: shoot culture and root culture (Meskaoui, 2015).

Root cultures have the ability to be sub-cultured and propagated for an indefinite period of time. The exogenous auxin in root culture media enhances the root elongation and lateral branching rate. The long-term stability of hairy root cultures have been reported where 500 hairy root cultures of *Datura stramonium* produced alkaloid for over years (Maldonado-Mendoza *et al*., 1993).

The hairy root culture produces secondary metabolites alongside the growth of roots, hence it is possible to obtain secondary metabolites from growing roots. Just like root culture, it is possible to culture shoot for secondary metabolite production. Shoot and root culture are transformed via *Agrobacterium* mediated transformation producing transgenic cultures, which are a potential source of vale added secondary metabolites (Sevón *et al*., 2002).

In one experiment the hairy root and transgenic hairy root cultures of 17 *Hypericum species* were analyzed using liquid chromatography- mass spectrometry (LC-GC) technique. The shoots were induced from root culture by supplementing MS/B5 growth media with shoot promoting hormone thidiazuron. The chemometric analysis revealed presence of secondary metabolites hypericin, pseudohypericin and flavonoid in the species of *Hypericum* (Nigutova et al., 2017).

2.4.Immobilized Plant Tissue Culture

Immobilization technique was initially developed for enzymes to be used in industries. Subsequently, whole cells were immobilized too. The advantage of immobilized plant tissue culture is that it provides high cell density for use in small bioreactors. The secondary metabolites are produced during stationary phase of cells, immobilization provides an excellent environment for production and accumulation of secondary metabolites (Ochoa-Villarreal *et al*., 2015).

To immobilize plant cells, they are entrapped in gel or combination of gels. The examples of matrix include calcium alginate, agar, gelatin, agarose and polyacrylamide. For example, *Th. Minus* cells were immobilized in alginate for the production of anti-cancerous saponin berberine (Sathuluri *et al*., 2002).

2.5. Scale-Up of Cultures (Bioreactors)

Bioreactor cultures are the final step in tissue culture for the production of secondary metabolites at commercial scale. The scale-up is an important phase as growth is modified when cultured in large tanks. Once the conditions and biomass is optimized for large scale production,

plant cultures must undergo well-adapted processes to produce secondary metabolites in good amount (Zhong, 2002). The configuration of bioreactor depends on type of cell culture as no single design can be recommended. The most common bioreactor designs include stirred tank, airlift stirred, bubble column, disc turbine, aerated bioreactor etc. (Valdiani *et al*., 2019).

The process for large scale production depends on the growth stage and secondary metabolite production. Like if the secondary metabolite is produced once the biomass is fully grown then fed-batch reactor may be used. While, if the secondary metabolite is produced concomitantly with biomass batch reactor or continuous fed reactor may be designed (Mishra *et al*., 2008).

In a research, the hairy root cultures of two transgenic *Platycodon grandiflorum* plant was maintained in shake flask and mist bioreactor for the accumulation of secondary metabolite saponin. Saponin is an important pharmaceutical secondary metabolite known to have anti-tumor properties. The two transgenic lines, pl 6 and pl 17 were supplemented with similar growth medium, one in shake flask (250 ml) for 8 weeks while other in mist bioreactor (5L) for 12 weeks, respectively. The results revealed high growth and accumulation of saponin in both reactors compared to field cultivated plants (Urbańska *et al*., 2014).

3. New Bioengineering Strategies

The bioengineering strategies are adopted in order to increase the production levels of secondary metabolites. Various strategies are described here.

3.1.Elicitation

Elicitation is a most widely applied strategy to enhance production of secondary metabolites from cell, tissue or organ cultures. The principle behind elicitation is that when stress factors such as osmotic shock, addition of inorganic salts, heavy metal ions, and microbial factors are applied, the accumulation of secondary metabolites enhances in plant cultures (Radman *et al*., 2003). In *in vitro* cultures plant shows morphological and physiological response to chemical, physical or microbial factors called as "elicitors". There are abiotic and biotic elicitors (Thakur et al., 2019).

3.2.Abiotic elicitation

Abiotic elicitation uses physical and chemical stimuli to enhance phytochemical synthesis in plants. It includes inorganic salts such as Ca, Cu, Cd and physical factors like light, temperature, water stress, pH, salt stress, nanomaterials, heavy metal, heat, cold etc. (Cheynier *et al*., 2013)

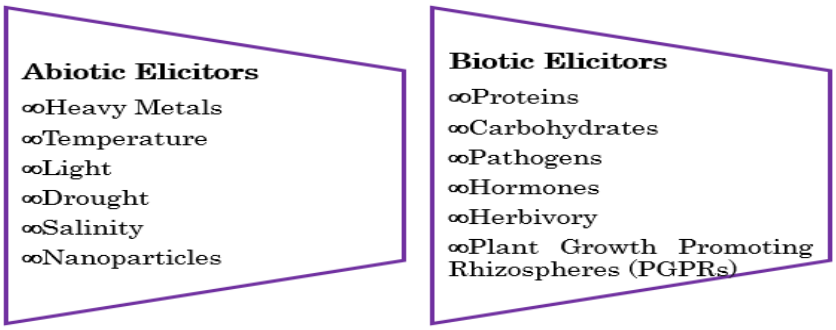

Figure 1. Types of elicitors applied in plant tissue culture

In one research the silver nanoparticles elicitors were applied to Bitter gourd cell suspension culture. The results showed that silver nanoparticles elicited cell suspension culture had significantly enhanced production of total flavonoid and phenolic contents. Due to metabolic changes the pharmacologic properties like anti-cancer, anti-fungal, anti-diabetic, anti-oxidant were also enhanced in AgNP elicited culture (Chung *et al.*, 2018).

3.3.Biotic elicitation

Biotic elicitors are of biological origin which mediate their actions by activating or inactivating ion channels and enzymes. The protein acts as a biotic elicitor which elucidates the ion channels in plant membranes for signal transfer in response to external stimuli. The polysaccharide chitin elicited the production of sanguinarine from *Papaver somniferum* plant culture (Naik *et al*., 2016). Hormones when added in growth culture acts as important elicitors which allows plants to respond physiologically. Most commonly used hormones are jasmonic acid and salicylic acid (Zhou *et al*., 2011).

For example; *Fagonia indica* is a medicinally important plant well known for its anti-cancer phenolics and flavonoids biosynthesis. The synergistic effects of melatonin and light emitting diodes (LED) were investigated on the callus culture of *F. indica*. Both these factors light and melatonin play vital role in biochemical and physiological processes of plant. The results revealed that white LED and melatonin favored the synthesis of phenolics, flavonoid and free radical scavenging activity (97%), in comparison to blue LED or other LED grown plants (Khan *et al*., 2018).

Paclitaxel is an important chemotherapeutic agent having biological effects against range of cancers. Paclitaxel can be produced through *in vitro* cell suspension culture of *Corylus avellana.* The two elicitors derived from endophytic fungi *Chaetomium globosum* and *Paraconiothyrium brasiliense* were shown to increase paclitaxel production (Salehi *et al*., 2019).

4. Metabolic and Genetic Engineering

4.1.Homologous Overexpression of Secondary Metabolites Key Genes

Another strategy could be the over-expression of single or multiple genes. As biosynthesis of secondary metabolites is strictly controlled by the enzymes, so regulation of the genes encoding

enzymes could enhance production (De Geyter, Gholami, Goormachtig, & Goossens, 2012). For example, the enzyme chalcone isomerase (CHI) is a rate limiting enzyme in flavonoids pathways. The gene *AaCHI* was isolated from *Artemisia annua* and overexpressed in same plant (transgenic) which significantly improved the production of artemisinin. The gene is involved in artemisinin production in *A. annua* and linked with terpenoid and flavonoid production (Ma *et al*., 2019).

The overexpression of multiple metabolic genes could also enhance the production level. In one research the transgenic plants were generated by agrobacterium mediated transformation of four major artemisinin biosynthetic pathway genes. The genes were amorpha-4,11-diene synthase gene (*ADS*), cytochrome P450 reductase gene (*CPR*), amorpha-4,11-diene 12-monooxygenase gene (*CYP71AV1*), and aldehyde dehydrogenase 1 gene (*ALDH1*). All the transformants had high expression level and production of artemisinin (Shi *et al*., 2017).

4.2.Suppression of Competitive Pathways

Another strategy to enhance the production of anti-tumor secondary metabolites is by suppressing the expression of competitive pathways. The artemisinin is a potential anti-cancer metabolite synthesized by *A. annua*. Sterol pathways acts as a competitive pathway for artemisinin synthesis. Down-regulation of key sterol pathway gene *SQS* by RNA interference showed significant increase in artemisinin content (Lu *et al*., 2016).

4.3.Regulating Primary Metabolism

To improve the production levels of secondary metabolites can be achieved by regulating relevant primary metabolic pathways. For example; carbohydrates are important primary metabolites. The experiments showed that carbohydrates played important role in influencing the yield of geraniol in cell suspension culture of transgenic tobacco. Moreover, light is also important for photosynthesis and carbohydrate synthesis which also had potent effect on geraniol synthesis (Ochoa-Villarreal *et al*., 2015).

4.4.Precursor Feeding

The concept of precursor feeding is based upon the idea that a compound which is an intermediary during the secondary metabolite biosynthesis pathway if fed in can increase the yield of final product. For example; anti-cancerous secondary metabolites phenolics, flavonoids, and wedelolactone in *Sphagneticola calendulacea (L.)* were augmented by precursor feeding with phenylalanine (Kundu *et al*., 2018).

4.5.Regulating Level Of Endogenous Plant Hormones

By regulating the level of phyto-hormones involved in biosynthetic pathways of secondary metabolites their level can be enhanced. The phytohormone abscisic acid plays role in the development of plant and stress response. Abscisic acid also regulates the biosynthesis of pharmaceutical terpenoids. Moreover, terpenoids production may be regulated through jasmonate biosynthetic pathway (Ahmad *et al*., 2016).

4.6.Synthetic Biology

Synthetic biology is a new approach to gain precise control over heterologous expression of phyto-chemicals and modification of central metabolism to enhance the supply of precursor compounds into biosynthetic pathway (Kotopka *et al*., 2018).

5. Conclusion

Secondary metabolites from plants are of great industrial and medical importance. Plant cell cultures on large scale has been very promising for large scale production of anti-cancer metabolites. In order to enhance the production levels metabolic engineering is a very helpful tool. Biotechnology plays an important role in high yield production high value secondary metabolites.

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