Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Review Article

PLANT CELL CULTURE TECHNOLOGY AND ITS ENTRÉE INTO THE WORLD OF *Ocimum*

REBECCA MATHEW[1] AND DEEPA SANKAR P[1*]

[1]Plant Biotechnology Division, School of Biosciences and Technology, VIT University, Vellore 632014, Tamil Nadu, India.
Email: pdeepasankar@vit.ac.in

Received: 15 Feb 2013, Revised and Accepted: 25 Mar 2013

ABSTRACT

Higher plants produce various secondary metabolites that are used widely as food additives, food colorants, pharmaceuticals, pesticides and fragrances. '*Ocimum*' of Lamiaceae is famous around the world for its diverse and rich source of pharmaceutical, culinary and aromatic properties. Biotechnology brought in plant cell culture technology and has been considered for long, an attractive alternative source to whole plants for the production of valuable secondary metabolites. This review focuses on the importance and aim of these plant cell cultures, strategies developed over years for the improved production of secondary metabolites; both in quality and quantity, plant cell cultures established successfully on an industrial scale and of how the same could be applied to the genus *Ocimum*. Recent findings in *Ocimum basilicum* L., *Ocimum sanctum* L. and *Ocimum gratissimum* L. cell culture technology have also been taken into consideration for this review.

Keywords: Plant cell cultures, Methyl jasmonate, Chitosan, *Ocimum basilicum* L., *Ocimum sanctum* L., *Ocimum gratissimum* L.

INTRODUCTION

For centuries, mankind has been totally dependent on plants for food and shelter. In addition, plants are also a valuable source of a wide range of secondary metabolites which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides and food additives. Over 80% of the approximately 30,000 known natural products are of plant origin[1] and many of them are unique to the plant kingdom and are not produced by microbes or animals[2]. These secondary metabolites perform no direct metabolic function in plants but fulfill specific ecological functions such as maintaining aesthetics of the plant, attracting insects for pollen transfer, animals for consumption of fruits and as defense mechanism in conditions of stress, wounding or pathogen attack[3]. Despite advancements in synthetic chemistry, biological sources are usually preferred due to lesser side effects and better biodegradability[4,5]. Extraction of these secondary metabolites from naturally grown whole plants on a commercial basis involves large-scale crop cultivation[6]. But with the increase in world population, less availability of cultivable land, fast disappearance of natural habitats for medicinal plants, environmental and geopolitical instabilities make it increasingly difficult for the acquirement of plant derived compounds[7].

Biotechnology offers an opportunity to exploit cell, tissue, organ or the entire organism by *in vitro* studies and to genetically manipulate them to get desired compounds. Plant cell cultures prove an attractive alternative source to whole plant for the production of high-value secondary metabolites[8,9]. The evolving commercial importance of secondary metabolites in recent years has resulted in the possibility of altering the production of bioactive plant metabolites through cell culture technology to a stage where they are yielded more cheaply rather than extracting the whole plant grown under natural conditions or by synthesizing the product[7]. An important requirement for the improvement of secondary metabolite synthesis in plants is the understanding of metabolic pathways and the enzymology of the biosynthesis of products of interest. However, the knowledge of plant metabolic pathways is still very limited.

THE ROYAL PLANT – BASIL

Basil is the popular name given to any aromatic herb belonging to the genus *Ocimum* of Lamiaceae family[10] known widely for their pharmaceutically important plant species[11]. The word "Basil" is derived from the Greek word "Basilica" which means the royal plant[10]. *Ocimum* is an extremely versatile group consisting of about 160 species[12] with a geographic distribution spread over tropical, sub tropical regions of Asia, Africa, South and Central America with the main centre of diversity being in Africa[13]. Plants belonging to this genus are predominantly shrubs, undershrubs and herbs and are perennial, triennial or biennial in habit and rich sources of major secondary metabolites such as phenols, flavonoids, alkaloids, terpenoids and essential oils[10]. *Ocimum* is conventionally propagated through seeds and the seedling progeny show variability due to cross-pollinating nature of the plant[14]. Inter specific hybridisation and polyploidy, common occurrences within this genus have created taxonomic confusion, making it difficult to understand the genetic relationship between basils[15]. The morphological diversity within basil species has been accentuated by centuries of cultivation with great variation in pigmentation, leaf shape and size and pubescence and by the existence of chemotypes or chemical races within species that do not differ significantly in morphology[16].

This genus has a long history of being used as pharmaceutical agents[17,18], as flavoring agents in soups, salads, confectionaries, cheese, meat, dental and oral products and as fragrances in perfumery[19], herbal toiletries and aromatherapy treatment[20]. The economically important parts are leaves and tender shoots[10].

Ocimum basilicum L.

O. basilicum also called as the "Common Basil" or the "Sweet Basil" is a native of tropical Asia and grown in several regions of the world[21]. It is herbaceous and 20 - 60cm in length[22]. Sweet basil is cultivated worldwide as a garden ornamental, culinary herb and as a source of essential oils to be used in foods, flavors and fragrances. The presence of essential oils and their composition determine the specific aroma of plants and the flavor of the condiments[23,24,25]. Historically, due to its pleasant aroma, basil has been widely used in religious rituals in various cultures and times[26,27,28]. The essential oils extracted from leaves and flowers are used as flavoring in liquors and for fragrance in perfumes and soaps[29,30].

Variable uses of sweet basil depend on variable varieties. There are several types of ornamental basils. Basil with all its varieties is a popular herb known for its flavorful foliage. The popularity of basil has led to the introduction of many products into the market place[31,32]. As a pot herb, *O. basilicum* has a trade value of 15 million USD per year[13]. Scented basils are used fresh or dried in potpourri, jellies, honeys, vinegars and baked foods[33]. Fresh and dried sweet basil is used widely in the Mediterranean kitchen such as tomato products, vegetables, salads, pizza, meat products, soups and marine foods[16,23,34,35,36].

Sweet basil is also a well known as a plant of folk medicinal value and has been accepted officially in a number of countries[34,37]. Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts,

worms, and kidney malfunction. Major aroma compounds from volatile extracts of basil present antioxidative activity[38]. The plant is stomatic, antihelminthic, antipyretic, diaphoretic, expectorant, carminative, stimulant and pectoral[39,40]. Basil tea when taken hot is good for treating nausea, dysentery and flatulence. The oil of sweet basil is also used for the alleviation of mental fatigue, colds, spasms and as a first aid treatment of wasp stings and snake bites[41].

***Ocimum sanctum* L.**

O. sanctum called as the "Holy Basil" in English and "Tulsi" in Hindi is an erect, soft, aromatic herb or undershrub and is commonly cultivated in gardens[17]. It is a 30-75cm high erect herb which is grown practically in every part of India. Its odor and taste are aromatic and sharp. Two types of *O. sanctum* are met within cultivation in India namely, the ones with green leaves called as "Sri Tulsi" and the ones with purple leaves called as "Krishna Tulsi"[42]. Holy basil is held sacred by Hindus all over the world due to its manifold curative uses[43].

Several medicinal properties have been attributed to Tulsi. Different parts of Tulsi plant such as leaves, flowers, stem, root, seeds are known to possess therapeutic potentials and have been used by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic and antistress agents. Tulsi has also been used in the treatment of fever, bronchitis, arthritis, convulsions, gastric and hepatic disorders[42,44]. Herbal preparations containing Tulsi have been suggested to shorten the course of illness, clinical symptoms and biochemical parameters in patients suffering from viral hepatitis[44]. The leaf juice along with Triphala is used in Ayurvedic eye drop preparations recommended for glucoma, cataract, chronic conjunctivitis and other painful eye diseases. The juice of fresh leaves is used to treat chronic fever, dysentery, hemorrhage and dyspepsia. A decoction of Tulsi leaves is a popular remedy for cold[42,44]. Tulsi leaves also check vomiting and act as antihelmintic[45]. As a prophylactic against malaria, fresh Tulsi leaves are taken with black pepper in the morning[42]. Ayurvedic preparation containing *O. sanctum, Allium sativum, Piper nigram* and *Curcuma longa* has been shown to possess anti-malarial activity. Tulsi extracts and essential oil have also been found to possess insecticidal and larvicidal activities against mosquitoes. Aqueous extract of Tulsi is found effective against viral encephalitis[44]. Aqueous decoction of whole plant has been said to control diabetes mellitus[46]. Paste of Tulsi leaves are found effective in the treatment of ringworm and other skin diseases. Tulsi has also been recommended as an antidote for dog bite, scorpion bite and insect bite in traditional systems of medicine[42,44,45]. The fresh leaves and flower tops have been used as antispasmodic agent[42,44]. The seeds are mucilaginous and demulcent and are used to treat disorders of the genitourinary system. The leaves of Tulsi plant have also been shown to possess good antistress, analgesic, antihyperlipidemic, antioxidant potentials in experimental animals[44,47,48,49,50]. Leaves and seeds of Tulsi plants have been reported to reduce blood and urinary uric acid level in albino rabbits and possess diuretic property[51]. Gastric ulceration and secretion are reported to be inhibited by Tulsi in albino rats[52]. *O. sanctum* has also got anti fertility effect[53].

The slightly hairy, pale green leaves of holy basil are also used as a flavoring agent in Southeast Asian cuisine, especially in Thai stir-fries. Holy basil leaves are spicy and have lemony notes[54], and many Indians consume small quantities of the young leaves either as an offering after divine worship in temples or as a food additive[55].

***Ocimum gratissimum* L.**

O. gratissimum is otherwise called as the "Camphor Basil" in English and "Ram Tulsi" in Hindi. The plant grows to a height of one to two feet. The leaves taste like cloves and hence used for flavoring of vegetables. It is a valuable multi-purpose medicinal plant. The whole plant and the essential oil are used in traditional medicine especially in Africa and India[45,56].

Extracts of the plant have been used in the treatment of treatment of upper respiratory tract infections, diarrhea, headache, fever, ophthalmic and skin diseases and pneumonia[57,58]. Extracts contain antimicrobial activity[59], antibacterial activity[60], antifungal activity[61], antimalarial activity[62] and antiprotozoal activity[63]. *O. gratissimum* is associated with chemopreventive, anticarcinogenic, free radical scavenging, radio protective and numerous other pharmacological uses[56]. Earlier reports have shown the smooth muscle contracting lipid soluble principles and antimutagenic activity in organic solvent extracts of *O. gratissimum* leaves[57,64]. This medicinal plant also acts as an antibacterial, antifungal, antimicrobial, antihelmintic agent[60,65,66,67]. The aqueous leaf extract and seed oil have shown antiproliferative and chemopreventive activity on HeLa cells[68]. Aqueous extract of *O. gratissimum* leaves have been reported to inhibit tumor growth and angiogenesis[69].

Eugenol, thymol, citral, geraniol and linalool have been extracted from the essential oil of *O. gratissimum*[70]. Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties[71]. *O. gratissimum* is germicidal[60,63] and widely used in toothpastes and mouth washes as well as some topical ointments. It is used as an excellent gargle for sore throats and tonsillitis. The plant extract is used against gastrointestinal helminths of animals and man[72]. In addition, its carminative properties make it a good choice for stomach upsets. The plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhea and mental illness. In addition, the plant is also used as a spice and condiment in the southern part of Nigeria[70].

PLANT TISSUE CULTURE AND ITS AID TO THE WORLD OF PLANT SCIENCE

There is an increasing concern through out the world about the uncontrolled exploitation and depletion of earth's natural resources. Current progress in genetics and biotechnology is highly adopted upon the use of *in vitro* culture[73]. Hence, the application of plant tissue culture has gained major industrial importance in three main areas. One, in breeding and genetics, two, used as model systems for the study of plant cell genetics, physiology, biochemistry and pathology and three, for the production of secondary metabolites from organ or liquid cell cultures[74].

It is unlikely that tissue culture would compete economically with the agricultural methods for the production of food substances from plants. But, they can do so for secondary metabolites as extraction of these from whole plants is extremely expensive[75,76] and hence much exploitation has been made in recent years on plant cell cultures.

COMMENCEMENT OF CELL CULTURE TECHNOLOGY

At the beginning of the century, Haberlandt attempted cultivation of isolated plant cells, but cell division was never observed in these cultures[77]. In the 1930s, the first *in vitro* cultures were established[78,79] and this was followed by a period of development of culture media and cultivation methods[80]. However, low yields of secondary metabolites in these suspension cultures were clearly a bottleneck for commercialization and the prospect of the use of plant cells for chemicals production was unimaginable. In those early efforts, plant cells in culture were treated in direct analogy to microbial systems, with little knowledge of plant cell physiology and biochemistry or the influence of bioreactor operation on the physiologic state of such systems. Strategies to optimize growth and product formation were developed separately during the period between 1975 and 1985 and in the year 1982, at least 30 compounds were found to be accumulated in plant culture systems in concentrations equal to or higher than that of the plant[81]. Considerable progress has been made since then to stimulate formation and accumulation of secondary metabolites using plant cell cultures.

Cell cultures have a higher rate of metabolism than intact differentiated plants because the initiation of cell growth in culture leads to fast proliferation of cell mass and to a condensed

biosynthetic cycle. This is the most important advantage of plant cell cultures as secondary metabolite formation can take place within a short cultivation time[82]. Plant cells are biosynthetically totipotent, retaining complete genetic information in each cell of the culture and hence be able to produce the range of chemicals found in the parent plant. The advantages of this technology over the conventional agricultural production are as follows - Independent of geographical and seasonal variations and various environmental factors, a defined production system ensuring continuous supply of products of uniform quality and yield, possibility of production of novel compounds that are not found in the parent plant, independent of political interference, efficient downstream recovery and rapidity of production[2]. A number of plant cell cultures have been established successfully such as production of vanillin from *Vanilla planifolia*[83], mint oil from *Mentha piperata*[84] and some at an industrial scale eg., rosmarinic acid (yield 36% DW) from *Salvia officinalis*[85] and shikonin (yield 12.4 % DW) from *Lithospermum erythrorhizon*[86]. However, there are still problems in the production of metabolites by cell cultures due to instability of cell lines, low yields, slow growth and scale-up problems[87].

PLANT CELL CULTURES SO FAR

Certain metabolites in certain plant cell cultures have already been proved to show a higher accumulation than those obtained from parent plants[74]. The possibilities achieved so far through plant cell culture technology include food additives such as anthocyanins from *Vitis vinifera*[89], vanillin from *Vanilla planifolia*[83], garlic from *Allium sativum*[90], sweetener stevioside from *Stevia rebaudiana* [91], aromas and fragrances such as aromas of cocoa and coffee produced from the cell cultures of *Tlaeobroma cacao* and *Coffea Arabica* [92], pigments such as Shikonin from *Lithospermum erythrorhizon* cell cultures[88] and pharmaceuticals such as the high-value anti-cancer drug paclitaxel produced from *Taxus brevifolia* cell cultures[93] .

STRATEGIES FOR IMPROVED PRODUCTION OF SECONDARY METABOLITES

The strategies adopted in plant cell culture technology for enhancement of secondary metabolite content in less amount of time and in increased product volumes include screening and selection of highly productive cell lines[94,95], manipulation of nutrients[96] which may include sugar[97], nitrate[98], growth regulators[99] and precursor feeding[100]. Optimizing the culture environment such as light, temperature, medium pH and oxygen have also been examined for their effect upon secondary metabolite accumulation in many types of cultures[101]. Permeabilization[102,103] and *in situ* product removal[104,105,106] have also been reported for the successful recovery of secondary metabolites.

Elicitation has been one of the most effective strategies for improving the productivity of bioactive secondary metabolites[107]. Secondary metabolites serve as defensive, protective, or offensive chemicals against environmental stress, microorganisms, insects, and higher herbivorous predators. When infected by pathogenic microorganism, plants respond with rapid activation of various spatially and temporally regulated defense reactions and thus the induction of synthesis of secondary metabolites. Elicitors may be formed inside or outside plant cells. Elicitors have been used to stimulate secondary metabolite product formation in plant cell cultures by reducing the process time for increased culture volumes and high product concentrations[7]. This has opened a new area of research having important economical benefits for bio industry[108]. Various elicitors such as chitosan, *ß*-glucan, and yeast extracts, as well as plant hormonal chemicals such as jasmonic acid (JA) and MeJA have been used to induce biotic and abiotic stresses upon plants[109]. In many cases, elicitors used in cell culture are MeJA, salicylic acid, chitosan, and heavy metals[110].

Depending on their origin, elicitors are classified as biotic and abiotic. Biotic stress can be caused by bacterial, viral, or fungal attack, as well as by biotic elicitors[111]. Abiotic elicitors are substances of non-biological origin such as chemicals like inorganic salts, heavy metals, chemicals that disturb membrane integrity, physical factors like mechanical wounding, ultraviolet irradiation, high salinity, high or low osmolarity, extreme temperature (freezing, thawing) and high pressure. 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, and substantial enhancement of product accumulation have been reported[110].

THE ELICITORS – MeJA AND CHITOSAN

Shikimate pathway is often referred to as the common aromatic biosynthetic pathway, even though all aromatic compounds are synthesized by this route. The pathway converts the primary metabolites phosphoenol pyruvate (PEP) and erythrose-4-phosphate to chorismate, the last common precursor for the three aromatic amino acids phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). These aromatic amino acids are central molecules in plant metabolism. Besides functioning as building blocks of proteins, the three amino acids also serve as precursors for a variety of plant hormones, such as auxin and salicylate and for a very wide range of aromatic secondary metabolites with multiple biological functions and biotechnological value in the health promoting, medical and food industries[112,113,114].

Transcriptional regulation of the shikimate pathway and aromatic amino acid metabolism in plants has so far not been studied extensively. However, the expression of 3-deoxy-d-arabino-heptulonosate-7-phosphate synthase (DAHPS*)* gene encoding the first enzyme of the shikimate pathway was reported to be induced by MeJA[115,116], Endogenous jasmonic acid (JA) and its volatile component MeJA are plant hormonal signals derived principally from linolenic acid through the octadecanoid pathway and controllers of secondary metabolites as well as defense system[117,118]. Under stress conditions such as wounding and pathogen attack, volatile MeJA could be released into the air from wounded plants and the wounding signal transferred to other healthy plants. In turn, the healthy plants that receive the signal often respond through their defensive systems with increasing secondary metabolites. This could explain for the trigger of secondary metabolite production on elicitation with exogenous MeJA[119].

In addition, endogenous JA and MeJA have also been found to get accumulated after treatment of cell cultures with fungal elicitors such as chitosan[120,121] triggering the start of shikimate pathway and thereby the production of secondary metabolites.

MeJA

Addition of MeJA to a broad range of plant-cell suspensions has resulted in higher accumulation or *de novo* synthesis of secondary metabolites[120]. The effect of MeJA on the production of bioactive chemicals and gene expression in sweet basil was investigated[122]. The total amount of phenolic compounds was found to be significantly increased in sweet basil after 0.5 mM MeJA treatment. Among the phenolic compounds, rosmarinic acid (RA) and caffeic acid (CA) were identified, and their amounts were increased by 55 and 300%, respectively. An increase in the total terpenoid content was also observed respectively.

The effect of MeJA vapors on content of phenolic compounds such as free phenolic acids, total quercetin, and total phenolics in etiolated buckwheat seedlings was studied. Low concentration of MeJA (10^{-8} M) was observed to stimulate the accumulation of chlorogenic acid in hypocotyls and cotyledons and a moderate dose of MeJA (10^{-6} M) for the sysnthesis of cinnamic acid (CA) synthesis in cotyledons. MeJA had stimulatory effect on caffeic acid forming, but inhibited synthesis of vanillic acid in hypocotyls and cotyledons[123].

MeJA was found to enhance capsaicin production in cell suspension cultures of *Capsicum frutescens* Mill[124]. MS media supplemented with 3 mg/L picloram and JA in dark conditions was found useful for the production of silymarin (an isomeric mixture of flavonolignans) and growth in cell suspension cultures of *Silybum marianum*[125].

In bioreactor cultures of *Eleutherococcus senticosus, e*leutheroside content increased significantly by elicitation of MeJA. The highest

total eleutheroside (7.3 fold increment) and chlorogenic acid (3.9 fold increment) yield was obtained with 200 µM MeJA treatment[126]. Two and five fold increases in serpentine and ajmalicine were obtained when MeJA was added to *Catharanthus roseus* cell cultures on day 6 of growth at a concentration of 10µM and 100 µM[127].

Cell suspension cultures of *Taxus canadensis* and *Taxus cuspidata* rapidly produced paclitaxel (Taxol) and other taxoids in response to elicitation with MeJA. The greatest accumulation of paclitaxel occurred when MeJA was added to cultures at a final concentration of 200 µM on day 7 of the culture cycle. The concentration of paclitaxel increased in the extracellular (cell free) medium to 117 mg/day within 5 days following elicitation, equivalent to a rate of 23.4 mg/L per day[128].

Ginsenoside accumulation in *Panax ginseng* was enhanced by elicitation with MeJA (range 50 - 400 µM). However, fresh weight, dry weight and growth ratio of the cells was strongly inhibited by increasing MeJA concentration. The highest ginsenoside yield was obtained at 200 µM of MeJA[129].

When investigated for induced resistance in eggplant with respect to cell wall strengthening and defense enzyme activation affected by MeJA, an increase in total phenolic content of eggplant roots by the elicitor was significantly higher along with lignin deposition in the cell wall of eggplant roots. Phenylalanine ammonia-lyase (PAL) activity also showed an increase of 3.5 times by MeJA at 36 h of elicitation, respectively. The activities of polyphenol oxidase (PPO), cinnamyl alcohol dehydrogenase (CAD) and catalase (CAT) were also increased several folds by the elicitors. Accumulation of phenolics and lignin in high amounts, together with higher level activity of major defense enzymes in response to the elicitors were suggested to bolster eggplants in mounting practical and effective resistance against *Ralstonia solanacearum*, the devastating wilt pathogen[130].

Three blackberry cultivars (Chester Thornless, Hull Thornless and Triple Crown) were used in elicitation experiments. Blackberries treated with MeJA (0.01 and 0.1 mM) had an enhanced content of flavonoids and increased antioxidant capacity than untreated fruit. Extracts of treated fruits showed enhanced inhibition of A549 cell and HeLa 60 leukemia cell proliferation and induced the apoptosis of HeLa 60 cells[131].

Chitosan

Chitosan and its derivatives have been reported to elicit secondary metabolite formation in plants and plant cell cultures. Application of chitin and chitosan to soybean leaf tissues were reported to cause increased activity of PAL and tyrosine ammonia lyase (TAL) enzymes involved in the shikimate pathway. Total phenolic content of soybean leaves increased following chitosan and chitin oligomer treatments, showing a positive correlation between enzyme activity and total phenolic content[132].

The addition of chitosan, to the culture of *Panax ginseng* hairy roots caused growth to decrease for a less extent and an increase in total ginseng saponin with the increase in elicitor concentration[133].

Chitosan induced elicitation responses in dark-incubated *Cocos nucifera* endosperm cell suspension cultures led to the rapid formation of phenylpropanoid derivatives. An enhanced accumulation of p-hydroxybenzoic acid as the major wall-bound phenolics was evident. This was followed by p-coumaric acid and ferulic acid. Along with enhanced peroxidases activities in elicited lines, the increase in activities of the early phenylpropanoid pathway enzymes such as PAL, p-coumaroyl-CoA ligase (4CL) and p-hydroxybenzaldehyde dehydrogenase (HBD) in elicited cell cultures were also observed[134].

Modified MS medium supplemented with 100 mg/L chitosan induced significant increment in the cell biomass while higher amount of chitosan (150 mg/L) was found to induce the highest production of 9-hydroxycanthin-6-one (0.44%) when studied for the effect of different elicitor on cell biomass and alkaloid production in *Eurycoma longifolia* cells[135]. Chitosan was also reported to increase the content of root scopolamine and hyoscyamine and promoted the release of both alkaloids in hairy root cultures of *Brugmansia candida*[136].

Anthraquinones obtained from madder roots have been used for various pharmaceutical purposes. Chitosan was found to be the best biotic elicitor among nine plant derived and microbial derived polysaccharides tried. When elicited with 25 mg/L chitosan, the total production was increased approximately two times in a seven day culture as compared to that in the non elicited cells. Maximum anthraquinone colorants were obtained with 3 day treatment of chitosan[137].

Indirubin and indigo-related compounds are of significant interest as natural colorants and for the treatment of chronic granulocytic leukemia. The effect of chitosan on indirubin production in *Polygonum tinctorium* cells was investigated. The optimum concentration of chitosan for indirubin production was found to be 200 mg/L, at which the specific indirubin concentration (indirubin concentration per unit weight of cells) was 5.17 mg/gm dry cell weight (DCW). Indirubin production was enhanced by 72% when the cells were cultured in the presence of 200 mg/L of chitosan for 5 days using SH[138] medium supplemented with 2.72 mM calcium chloride and 5 mM indole[139].

Chitosan has recently been reported to act as a plant growth promotor in some species including orchids. The degree of deacetylation and concentrations of chitosan have varying effects on the growth and development of orchid cultured *in vitro*. It has also been shown that application of chitosan to *Dendrobium* orchid plants tended to increase the size of open florets and length of the inflorescences but did not affect the display life of cut orchids[140].

Combination of MeJA and chitosan

There are reports on combined elicitor effects of cell cultures and some have turned successful than the individual elicitor treatments. Podophyllotoxin aryltetralin lignin serves as a commercial precursor of semi-synthetic etoposide and teniposide, important drugs in the treatment of testicular tumors, small cell lung cancer and acute leukemia[141]. Podophyllotoxin production was however increased in *Juniperus chinensis* cell suspension cultures by the combination of MeJA and chitopentaose (chito-oligosaccharide obtained by the partial hydrolysis of chitosan) rather than individual elicitor treatments. A 15 fold increase was observed than the control[142].

To stimulate the production of taxol (paclitaxel) by cell suspension cultures of *Taxus cuspiata* var. nana, two kinds of elicitors and a biogenetic precursor were used in Gamborg's B5 culture medium. The amount of taxol produced was greatest (16.6 mg/L) when the cell suspension cultures were treated with the combination of chitoheptaose (8 mg/L) and jasmonic acid (21 mg/L). The productivity was 4.1 fold greater than the control[143].

Taxus chinensis suspension cells were cultured in the modified Gamborg's B5 medium. Addition of 50 mg/L chitosan, 60 µM MeJA and 30µM Ag^+ resulted in the greatest paclitaxel production, at 25 mg/L in the cultures, being almost 40 times higher than that of the control culture, 10 times higher than that of the culture exposed to Ag^+, 6 times higher than that of the culture elicited by chitosan and almost double that of the culture elicited by MeJA[144].

THE NEW EPOCH - BASIL CELL CULTURES

Following reports on successful elicitation of plant cell cultures using MeJA and chitosan, efforts were made to do so the same in the *Ocimum* species. Cell suspension cultures of three *Ocimum* species *viz*., *O. basilicum* L., *O. sanctum* L. and *O. gratissimum* L. were newly established and their cell growth cycle was studied[145]. The growth characteristics of *Ocimum* cell suspension cultures when treated with elicitors, MeJA and chitosan individually and in combination were also studied. When used individually, MeJA and chitosan were found to enhance accumulated cell biomass but when administered in combination there was no significant enhancement. Reduction in cell growth was not observed in combined elicitor treatments. Of the two elicitors, MeJA was found to strongly influence cell growth with 25 µM accumulating highest biomass at

12 h for *O. basilicum* and 48 h for *O. sanctum*, while 50 µM at 8 h was found to be optimal for obtaining maximal enhancement of biomass for *O. gratissimum*. This study demonstrated that these elicitors could effectively enhance cell biomass content in lesser time and hence can be used for effective induction of phytochemicals considered economically important such as eugenol, methyl eugenol, methyl chavicol, linalool, thymol *etc*., from *O. basilicum* L., *O. sanctum* L. and *O. gratissimum* L. cell cultures[146].

CONCLUSION

In the light of total absence of research on plant cell cultures of *Ocimum*, this review could be one of the many resources that could be used to broaden the road for *Ocimum* plant cell culture technology. Having discussed the importance and need of cell culture technology for plants rich in secondary metabolites, the successful attempts achieved so far, the many number of species and great wealth of medicinal, culinary and aromatic properties found within the genus *Ocimum* along with the modest start of research conducted on three of its species namely, *O. basilicum* L., *O. sanctum* L. and *O. gratissimum* L., a significant volume of research could be opened in the field of production of secondary metabolites from *in vitro* cell cultures of *Ocimum* species alone. Successful establishment of cell cultures of various other *Ocimum* species could lead to attempts aimed at increased production of major secondary metabolites in less time. Screening and characterization of many economically important individual compounds from these improved cell cultures would again open up another whole world of significant research. A detailed and intricate study of the metabolic pathways will serve to hit on the targets in demand and increase their production on an industrial scale.

REFERENCES

1. Fowler MW, Scragg AH Natural products from higher plants and plant cell culture. In: Pais MSS, Mavituna F, Novais JM, editors. Plant cell biotechnology. Vol.18. Berlin: Springer-Verlag, NATO ASI Series; 1988. p.165-177.
2. Rao SR, Ravishankar GA Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol Adv 2002; 20:101-153.
3. Heldt HW. Plant biochemistry. USA: Academic Press; 2005. p. 403.
4. Pezzuto JM Natural product cancer chemoprotective agents. In: Arnason JT, Mata R, Romeo JT, editors. Recent advances in phytochemistry. Phytochemistry of medicinal plants. Vol. 29. New York: Plenum Press; 1995. p. 19-45.
5. Rout SP, Choudary KA, Kar DM, Das L, Jain A Plants in traditional medicine system - Future source of new drugs. Int J Pharm Pharm Sci 2009; 1(1): 1-23.
6. Kieran PM, MacLoughlin PF, Malone DM Plant cell suspension cultures: some engineering considerations. J Biotechnol 1997; 59:39-52.
7. Mulabagal V, Tsay HS Plant cell cultures - An alternative and efficient source for the production of biologically important secondary metabolites. Int J Appl Sci Eng 2004; 2 (1): 29-48.
8. Dornenburg H, Knorr D Challenges and opportunities for metabolite production from plant cell and tissue cultures. Food Technol 1997; 51:47-54.
9. Ravishankar GA, Bhyalakshmi N, Ramachandra SR Production of food additives. In: Ramawat KG, Merillon JM, editors. Biotechnology: Secondary metabolites. New Delhi: Oxford IBH press; 1999. p. 89-110.
10. Pushpagandan P, Bradu L Basil. In: Chadha KL, Gupta R, editors. Medicinal and aromatic plants, India: Malhotra publishing House; 1995. p. 627-658.
11. Chandrappa SM, Harsha R, Dinesha R, Gowda TSS Antibacterial activity of *Coleus aromaticus* leaves. Int J Pharm Pharm Sci 2010; 2(3): 63-66.
12. Balyan SS, Pushpagandan P A study on the taxonomic status and geographical distribution of the genus *Ocimum*. PAFAI J 1988; 10(2):13-19.
13. Paton A A synopsis of *Ocimum* L. (Labiateae) in Africa. Kew Bul 1992; 47:403-435.
14. Heywood VH. Flowering plants of the world. Oxford: Oxford University Press; 1978. p. 239-240.
15. Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E Intraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry 1996; 43:1033-1039.
16. Simon JE, Quinn J, Murray RG Basil: A source of essential oils. In: Janick J, Simon JE, editors. Advances in new crops. Portland: Timber Press; 1990. p. 484-489.
17. Prakash P, Gupta N Therapeutic uses of *Ocimum sanctum* Linn (tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J Physiol Pharmacol 2005; 49(2):125-131.
18. Bunrathepl S, Palanuvej C, Ruangrungsi N Chemical compositions and antioxidative activities of essential oils from four *Ocimum* species endemic to Thailand. J Health Res 2007; 21(3):201-206.
19. Laskar S, Majumdar SG Variations of major constituents of essential oil of the leaves of *Ocimum sanctum* Linn. J Indian Chem Soc 1988; 65:301-302.
20. Harris B Methyl eugenol - The current bête noire of aromatherapy. Int J Aromather 2002; 12:193-201.
21. Siddique I Anis M Rapid micropropagation of *Ocimum basilicum* L. using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium. Biol Plantarum 2007; 51 (4): 787-790.
22. Chalchat JC, Ozcan MM Comparative essential oil composition of flowers, leaves and stems of sweet basil (*Ocimum basilicum* L.) used as herb. Food Chem 2008; 110:501-503.
23. Akgul A Volatile oil composition of sweet basil (*Ocimum basilicum* L.) cultivating in Turkey. Nahrung 1989; 33(1):87-88.
24. Marotti M, Piccaglia R, Giovanelli E Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. J Agric Food Chem 1996; 14:3926-3929.
25. Sanda K, Koba K, Nambo P, Gase A Chemical investigation of *Ocimum* species growing in Togo. Flavour Fragr J 1998; 13:226-232.
26. Guenther E. The essential oils. Florida: Krieger Publishing; 1949. p 399-433.
27. Topalov VD Basil. In: Topalov VD, editor. Essential oil and medicinal plants. Bulgaria: Hr. G. Danov Press; 1962. p 200-209.
28. Simon JE, Craker LE, Chadwick A (1984) Herbs: An indexed bibliography, 1971-1980. Hamden: Archon Books; 1984. p. 7-9.
29. Baritaux O, Richard H, Touche J, Derbesy M Effects of drying and storage of herbs and spices on the essential oil, Part I. Basil, *Ocimum basilicum* L. Flavour Frag J 1992; 7:267-271.
30. Khatri LM, Nasir MKA, Saleem R, Noor F Evaluation of Pakistani sweet basil oil for commercial explosion. Pak J Sci Ind Res 1995; 38:281-282.
31. Simpson M, Macintosh D, Cloughly J, Stuart A Past present and future untilisation of Myrica gale, Econ Bot 1996; 50: 122-129.
32. Simon J, Morales M, Phippen W, Vierra F, Hao Z Basil: A source of aroma compounds and a popular culinary and ornamental herb. In: Janick J, editor. Perspectives on new crops and new uses. Alexandria: ASHS Press; 1999. p. 499-505.
33. Abduelrahman AHN, El Hussein SA, Osman NA, Nour A Morphological variability and chemical composition of essential oils from nineteen varieties of basil (*Ocimum basilicum* L.) growing in Sudan. Int J Chem Technol 2009; 1(1):1-10.
34. Health HB. Source book of flavour. West Port: AVI publications ; 1981.
35. Machale KW, Niranjan K, Pangarkar VG Recovery of dissolved essential oils from condensate waters of basil and *Mentha arvensis* distillation. J Chem Tech Biot 1997; 69:362-366.
36. Ozcan M, Chalchat JC Essential oil composition of *O. basilicum* L. and *O. minimum* L. in Turkey. Czech J Food Sci 2002; 20(6):223-228.
37. Lawrence BM A review of the world production of essential oil. Perf & Flav 1985; 10:2-16.
38. Lee SJ, Umano K, Shibamoto T, Lee KG Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem 2005; 91:131-137.

39. Sahoo Y, Pattnaik SK, Chand PK *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation. In Vitro Cell Dev Biol Plant 1997; 33:293-29.
40. Phippen WB, Simon JE Shoot regeneration of young leaf explants from basil (*Ocimum basilicum* L.). In Vitro Cell Dev Biol Plant 2000; 36:250-254.
41. Martins AP, Salgueiro L, Vila R, Tomi F, Conigueral S, Casanova J Composition of the essential oils of *Ocimum canum* L., *O. gratissiumum* and *O. minimum*. Planta Med 1999; 65:187-189.
42. Pandey BP, Anita Economic Botany. New Delhi: Chand and Company Ltd.; 1990. p. 294.
43. Kothari SK, Bhattachary AK, Ramesh S Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L.f. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest. J Chromatogr A 2004; 1054:67-72.
44. Rajeshwari S (1992) *Ocimum sanctum.* The Indian home remedy. In: Rajeshwari S (ed.) Current Medical Scene, Cipla Ltd., Bombay
45. Sen P Therapeutic potential of Tulsi: From experience to facts. Drug News and Views 1993; 1:15- 21.
46. Nagarajun S, Jain HC, Aulakh GS (1989) Indigenous plants used in the control of diabetes. In: Atal CK, Kapoor BM, editors. Cultivation and utilization of medicinal plants. India: PID CSJR; 1989. p. 584.
47. Bhargava KP, Singh N Antistress activity of *Ocimum sanctum* Linn. Indian J Med Res 1981; 73:443-451.
48. Sarkar A, Pandey DN, Pant MC Changes in the blood lipid profile level after administration of *Ocimum sanctum* (Tulsi) leaves in the normal albino rabbits. Indian J Physiol Pharmacol 1994; 38(4):311–312.
49. Khanna N, Bhatia J Action of *Ocimum sanctum* (Tulsi) in mice: Possible mechanism involved. J Ethnopharmacology 2003; 88(2-3):293-296.
50. Sethi J, Sood S, Seth S, Thakur A Protective effect of Tulsi *(Ocimum sanctum)* on lipid peroxidation in stress induced by anemic hypoxia in rabbits. Indian J Physiol Pharmacol 2003; 47(1):115-119.
51. Sarkar A, Pandey DN, Pant MC A report on the effect of *Ocimum sanctum* (Tulsi) leaves and seeds on blood and urinary uric acid, urea and urine volume in normal albino rabbits. Indian J Physiol Pharmacol 1990; 34:61-62.
52. Mandal S, Das DN, De K, Ray K, Roy G, Chaudhiri SB, Sahana CC, Chowdhuri MK *Ocimum sanctum Linn* - A study on gastric ulceration and gastricsecretion in rats. Indian J Physiol Pharmacol 1993; 37:91-92.
53. Batta SK, Santhakumari G The antifertility effect of *Ocimum sanctum* and *Hibiscus rosa-sinensis*. Indian J Med Res 1971; 59:777-781.
54. Uhl S Ingredients: The building blocks for developing ethnic foods. Food Technol 1996; 6:79-84.
55. Archana R, Namasivayam A A comparative study on different crude extracts of *Ocimum sanctum* on noise stress. Phytother Res 2002; 16:579-580.
56. Gupta SK, Prakash J, Srivastav S Validation of claim of Tulsi, *Ocimum sanctum* Linn as a medicinal plant. J Expt Biol 2002; 40:65-773.
57. Onajobi FD Smooth muscle contracting lipidicsoluble principles in chromatographic fractions of *Ocimum gratissimum.* J Ethnopharmacol 1986; 18:3-11.
58. Ilori M, Sheteolu AO, Omonibgehin EA, Adeneye AA Antidiarrhoeal activities of *Ocimum gratissimum* (Lamiaceae). J Diarrhoeal Dis Res 1996; 14:283-285.
59. 59.Adebolu TT, Oladimeji SA Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. Afr J Biotechnol 2005; 4(7):682-684.
60. Nakamura CV, Ueda-Nakamura T, Bando E, Melo AF, Cortez DA, Dias Filho BP Antibacterial activity of *Ocimum gratissimum* L. essential oil. Mem Inst Oswaldo Cruz 1999; 94:675-678.
61. Lemos JA, Paula JR Antifungal activity of *Ocimum gratissimum* towards dermatophytes. Mycoses 2005; 48:172-175.
62. Ezekwesili CN, Obiora KA, Ugwu OP Evaluation of antidiarrhoeal property of crude aqueous extract of *Ocimum gratissimum* L. (Labiatae) in rats. Biokemistri 2004; 16(2):122-131.
63. Holets FB, Ueda-Nakamura T, Filho BPD, Cortez DAG, Morgado-Diaz JA, Nakamura CV Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid *Herpetomonas* samuelpessoai. Act Protonzool 2003; 42:269-276.
64. Obaseiki-Ebor EE, Odukoya K, Telikepally H, Mitscher LA, Shankel DM Antimutagenic activity of extracts of leaves of four common edible vegetable plants in Nigeria (West Africa). Mutat Res 1993; 302:109-117.
65. Nakamura CV, Ishida K, Faccin LC, Filho BPD, Cortez DAG, Rozental S, Souza WD, Ueda-Nakamura T *In vitro* activity of essential oil from *Ocimum gratissimum* L. against four *Candida* species. Res Microbiol 2004; 155:579-586.
66. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol 2004; 35:275-280.
67. Silva MR, Oliveira Jr JG, Fernandes OF, Passos XF, Costa CR, Souza LK, Lemos JA, Paula JR Antifungal activity of *Ocimum gratissimum* towards dermatophytes. Mycoses 2005; 48:172-175.
68. Prakash J, Gupta SK, Singh N, Kochupillai V, Gupta YK Antiproliferative and chemo preventive activity of *Ocimum sanctum* Linn. Int J Med Biol Environ 1999; 27:165.
69. Nangia-Makker P, Tait L, Shekhar MPV, Palomino E, Hogan V, Piechocki MP, Funasaka T, Raz A Inhibition of breast tumor growth and angiogenesis by a medicinal herb: *Ocimum gratissimum*. Int J Cancer 2007; 121:884-894.
70. Sulistiarini D, Oyen LPA, Nguyen XD *Ocimum gratissimum* L. Plant Resources of South-East Asia. No. 19: Essential oils Plants, Prosea Foundation, Bogor, Indonesia, 1999. p. 140-142.
71. Dubey NK, Tiwari TN, Mandin D, Andriamboavonjy H, Chaumont JP Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoterapia 2000; 7(15):567-569.
72. Chitwood DJ Phytochemical based strategies for nematode control. Annu Rev Phytopathol 2003; 40:221-249.
73. Gaj MD Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.). Bull Acad Sin 2004; 42 :9-16.
74. Zhong JJ Biochemical engineering of the production of plant-specific secondary metabolites by cell suspension cultures. Adv Biochem Eng Biotechnol 2001; 72:1-26.
75. Bhojwani SS, Razdan MK. Plant tissue culture: Theories and practice, a revised edition. The Netherlands: Elsevier Science; 1996.
76. Hall RD (1999) Plant Cell Culture Protocols. New Jersey: Humana Press Inc.; 1999.
77. Haberlandt G Culturversuche mit isolierten Pflanzenzellen. Sitz Akad Wiss Wien 1902; 111:69-92.
78. White PR Potentially unlimited growth of excised tomato root tips in a liquid medium. Pionr Physiol 1934; 9:585-600.
79. Gautheret RJ Sur la possibilite de realiser la culture indefinee de tissus de tubercules de carotte. C R Acad Sci 1939; 208:118-130.
80. Street HE Plant Tissue and Cell Culture. Oxford: Blackwell Scientific Publications; 1977.
81. Staba EJ Production of useful compounds from plant tissue culture. In: Fujiwara A, editor. Plant Tissue Culture. Tokyo: Japanese Association for Plant Tissue Culture; 1982. p. 25-30.
82. Dornenburg H, Knorr D Strategies for the improvement of secondary metabolite production in plant cell cultures. Enzyme Microb Tech 1995; 17:674-684.
83. Dornenburg H, Knorr D Production of the phenolic flavour compounds with cultured cells and tissues of *Vanilla planifolia* species. Food Biotechnol 1996; 10:75-92.
84. Chung IS, Kang YM, Oh JM, Kim T, Lee HJ, Chae YA Continuous suspended cultures of *Mentha piperita* in cell recycled airlift bioreactor. Biotechnol Tech 1994; 8:789-792.
85. Hippolyte IB, Marin J, Baccou C, Jonard R Growth and rosmarinic acid production in cell suspension cultures of *Salvia officinalis* L. Plant Cell Rep 1992; 11:109-112.
86. Fujita Y (1988) Industrial production of shikonin and berberine. In: Bock G, Hansh J, editors. Applications of plant cell and tissue culture. Vol. 137. Chichester: John Wiley and Sons; 1988. p. 228-238.

87. Ravishankar GA, Venkataraman LV (1993) Role of plant cell culture in food biotechnology: Current trends, limitations and future prospects. In: Prakash J, Pierik RLM, editors. Plant biotechnology: Commercial prospects and problems. New Delhi: Oxford IBH Press; 1993. p. 255-274.
88. Kim DJ, Chang HN Enhanced shikonin production from *Lithospermum erythrorhizon* by *in situ* extraction and calcium alginate immobilization. Biotechnol Bioengin 1990; 36(5):460-466.
89. Curtin C, Zhang W, Franco C Manipulating anthocyanin composition in *Vitis vinifera* suspension cultures by elicitation with jasmonic acid and light irradiation. Biotechnol Lett 2003; 25(14):1131-1135.
90. Rhodes MJ, Spencer A, Hamill JD Plant cell culture in the production of flavour compounds. Biochem Soc Trans 1991; 19(3):702-706.
91. Rao RS, Ravishankar GA Biotransformation of isoeugenol to vanilla flavour metabolites and capsaicin in freely suspended and immobilized cell cultures of *Capsicum frutescens*: study of the influence of *ß*-cyclodextrin and fungal elicitor. Process Biochem 1999; 35:341-348.
92. Kurata H, Achioku T, Okuda N, Furusaki S Intermittent light irradiation with a second-scale interval enhances caffeine production by *Coffea arabica* cells. Biotechnol Prog 1998; 14(5):797-799
93. Tabata H Production of paclitaxel and the related taxanes by cell suspension cultures of *Taxus* species. Curr Drug Target 2006; 7(4):453-461.
94. Ogino T, Hiraoka H, Tabata M Selection of high nicotine producing cell lines of tobacco callus by single cell cloning. Phytochemicals 1978; 22:2447-2450.
95. Fujita Y, Takahashi S, Yamada Y Selection of cell lines with high productivity of shikonin derivatives through protoplasts of *Lithospermum erythrorhizon*. Proc Euro Congr Biotechnol 1984; 1:161-166.
96. Stafford A, Morris P, Fowler MW Plant cell biotechnology: A perspective. Enzyme Microb Technol 1986; 8:19-23.
97. Misawa M Production of useful plant metabolites. In: Fiechter A, editor. Advances in biochemical engineering and biotechnology. Berlin; Springer-Verlag; 1985. p. 59-88
98. Bohm H, Rink E Betalaines. In: Constabel F, Vasil I, editors. Cell culture and somatic cell genetics of plants. Vol. 5. New York: Academic Press; 1988. p. 449-63.
99. Rajendran L, Ravishankar GA, Venkataraman LV, Prathiba KR Anthocyanin production in callus cultures of *Daucus carota* L. as influenced by nutrient stress and osmoticum. Biotechnol Lett 1992; 14:707-714.
100. Ibrahim RK Regulation of synthesis of phenolics, In: Constabel F, Vasil I, editors. Cell culture and somatic cell genetics of plants. Vol. 4. SanDiego: Academic Press; 1987. p. 77-95.
101. Kreis W, Reinhard E 12ß-hydroxylation of digitoxin by suspension cultured *Digitalis lanata* cells: Production of digoxin in 20-litre and 300-litre airlift reactors. J Biotechnol 1992; 26:257-273.
102. Knorr D, Miazga SM, Teutonica RA Immobilization and permeabilization of cultured plant cells. Food Technol 1985; 39:139-142.
103. Beaumont MD, Knorr D Effects of immobilizing agents and procedures on viability of cultured celery (*Apium graveolens*) cells. Biotechnol Lett 1987; 9:377-382.
104. Robbins RJ, Rhodes MJC The stimulation of anthraquinone production by *Cinchona ledgeriana* cultures with polymeric adsorbents. Appl Microbiol Biotechnol 1986; 24:35-41.
105. Beiderbeck R, Knoop B Two-phase culture. In: Constable F, Vasil I, editorsCell culture and somatic cell genetics of plants. Vol. 4. San Diego: Acedemic Press; 1987. p. 255-266.
106. Beiderbeck R, Knoop B (1988) Enhanced production of secondary substances: Addition of artificial accumulation sites to cultures. In: Bajaj YPS (ed.) Biotechnology in agriculture and forestry. Medicinal and aromatic plants. Vol. 4. Berlin: Springer-Verlag; 1988. p.123-135.
107. Roberts SC, Shuler ML Large scale plant cell culture. Curr Opin Biotechnol 1997; 8:154-159.
108. Angelova Z, Georgiev S, Roos W Elicitation of plants. Biotechnol & Biotechnol Eq 2006; 20:72-83.
109. Dixon RA, Paiva NL Stress-induced phenylpropanoid metabolism. Plant Cell 1995; 7:1085-1097.
110. Smetanska I Production of secondary metabolites using plant cell cultures. Adv Biochem Engin/Biotechnol 2008; 111:187-228.
111. Thaler JS, Fidantsef AL, Bostock RM Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defence-related proteins, herbivore, and pathogen performance in tomato. J Chem Ecol 2002; 28:1131-1159.
112. Herrmann KM The shikimate pathway as an entry to aromatic secondary metabolism. Plant Physiol 1995; 107:7-12.
113. Bartel B Auxin biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 1997; 48:51-66.
114. Vogt T Phenylpropanoid biosynthesis. Mol Plant 2010; 3:2-20.
115. Devoto A, Ellis C, Magusin A, Chang H, Chilcott C, Zhu T, Turner J Expression profiling reveals COI1 to be a key regulator of genes involved in wound and methyl jasmonate induced secondary metabolism, defense, and hormone interactions. Plant Mol Biol 2005; 58: 497-513.
116. Yan Y, Stolz S, Chetelat A, Reymond P, Pagni M, Dubugnon L, Farmer EE A downstream mediator in the growth repression limb of the jasmonate pathway Plant cell 2007; 19:2470-2483.
117. Creelman R, Mullet JE Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 1997; 48: 355-381.
118. Turner JG, Ellis C, Devoto A The jasmonate signal pathway. Plant Cell 2002; 14 Suppl: 153-164.
119. Kim HJ, Chen F, Wang X, Rajapakse NC Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). J Agri Food Chem 2006; 54:2327-2332.
120. Gundlach H, Miller MJ , Kutchan TM, Zenk MH Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci USA 1992; 89:2389-2393.
121. Blechert S, Brodschelm W, Holder S, Kammerer L, Kutchan TM, Mueller MJ, Xia MQ, Zenk MH The octadecanoic pathway: signal molecules for the regulation of secondary pathways. Proc Natl Acad Sci USA 1995; 92: 4099-4105.
122. Li Z, Wang X, Chen F, Kim HJ Chemical changes and overexpressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. J Agric Food Chem 2007; 55(3):706-713.
123. Horbowicz M, Chrzanowski G, Koczkodaj D, Mitrus J The effect of methyl jasmonate vapors on content of phenolic compounds in seedlings of common buckwheat (*Fagopyrum esculentum* Moench). Acta Soc Bot Pol 2011; 80(1):5-9.
124. Sudha G, Ravishankar GA Influence of methyl jasmonate and salicylic acid in the enhancement of capsaicin production in cell suspension cultures of *Capsicum frutescens* Mill. Curr Sci 2003; 85(8, 25):1212-1217.
125. Hasanloo T, Khavari-Nejad RA, Majidi E, Shams Ardakani MR Flavonolignan production in cell suspension culture of *Silybum* marianum. Pharm Biol 2008; 46(12):876-882.
126. Shohael AM, Murthy HM, Hahn EJ, Paek KY Methyl jasmonate induced overproduction of eleutherosides in somatic embryos of *Eleutherococcus senticosus* cultured in bioreactors. Electron J Biotechn 2007; 10(4):633-637.
127. Gaines JL Increasing alkaloid production from *Catharanthus roseus* suspensions through methyl jasmonate elicitation. Pharm Eng J 2004; 24:24-35.
128. Ketchum REB, Gibson DM, Croteau RB, Shuler ML The kinetics of taxoid accumulation in cell suspension cultures of *Taxus* following elicitation with methyl jasmonate. Biotechnol Bioeng 1999; 62(1):97-105.
129. Thanh NT, Murthy HN, Yu KW, Hahn EJ, Paek KY Methyl jasmonate elicitation enhanced synthesis of ginsenoside by cell suspension cultures of *Panax ginseng* in 5-l balloon type bubble bioreactors. Appl Microbiol Biotechnol 2005; 67:197-201.
130. Mandal S Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. Afri J Biotechnol 2010; 9(47):8038-8047.
131. Wang SY, Bowman L, Ding M Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus* sp.) and promotes antiproliferation of human cancer cells. Food Chem 2008; 107: 1261-1269.

132. Khan W, Prithiviraj B, Smith DL Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. J Plant Physiol 2003; 160:859-863.
133. Jeong GT, Park DH Enhancement of growth and secondary metabolite biosynthesis: Effect of elicitors derived from plants and insects. Biotechnol Bioprocess Eng 2007; 10:73-77.
134. Chakraborty M, Karun A, Mitra A Accumulation of phenylpropanoid derivatives in chitosan-induced cell suspension culture of *Cocos nucifera*. J Plant Physiol 2009; 166:63-71.
135. Keng CL, Wei AS, Bhatt A Elicitation effect on cell biomass and production of alkaloids in cell suspension culture of the tropical tree *Eurycoma longifolia*. Research Journal of the Costa Rican Distance Education University 2010; 2(2):239-244.
136. Pitta-Alvarez SI, Giulietti AM Influence of chitosan, acetic acid and citric acid on growth and tropane alkaloid production in transformed roots of *Brugmansia candida*, Effect of medium pH and growth phase. Plant Cell Tiss Org Cult 1999; 59:31-38.
137. Jin JH, Shin JH, Kim JH, Chung IS, Lee HJ Effect of chitosan elicitation and media components on the production of anthraquinone colorants in madder (*Rubia akane* Nakai) cell culture. Biotechnol Bioprocess Eng 1999; 4:300-304.
138. Shenk RU, Hildebrandt AC Medium and techniques for induction and growth of monocotyledonous plant cell cultures. Can J Bot 1972; 50:199-204.
139. Kim JH, Shin JH, Lee HJ, Chung IS, Lee HJ Effect of chitosan on indirubin production from suspension culture of *Polygonurn* tinctorium. J Ferment Bioeng 1997; 83(2):206-208.
140. Uthairatanakij A, Teixeira da Silva JA, Obsuwan K Chitosan for improving orchid production and quality. Orchid Sci Biotech 2007; 1(1):1-5.
141. Williams SD, Birch R, Eihorn LH Treatment of disseminated germ cell tumors with cisplatin, bleomycin and either vinblastine or etoposide. The New England J Med 1987; 316: 1433-1441.
142. Premjet D, Itoh K, Tachibana S Enhancement of podophyllotoxin production by biogenetic precursors and elicitors in cell suspension cultures of *Juniperus chinensi*. Pak J Biol Sci 2002; 5(11):1267-1271.
143. Tachinbana S, Muranaka T, Itoh K Effect of elicitors and a biogenetic precursor on paclitaxel production in cell suspension cultures of *Taxus cuspidate* var. nana. Pak J Biol Sci 2007; 10(17):2856-2861.
144. Zhang CH, Mei XG, Liu L, Yu LJ Enhanced paclitaxel production induced by the combination of elicitors in cell suspension cultures of *Taxus chinensis*. Biotechnol Lett 2000; 22: 1561-1564.
145. Mathew R, Sankar PD Growth characteristics of cell suspension cultures for secondary metabolite production in O*cimum basilicum* L., O*cimum sanctum* L. and O*cimum gratissimum* L. J Pharm Res 2011; 4(10):3323-3326.
146. Mathew R, Sankar PD Effect of methyl jasmonate and chitosan on growth characteristics of O*cimum basilicum* L., O*cimum sanctum* L. and O*cimum gratissimum* L. cell suspension cultures. Afr J Biotechnol 2012; 11(21):4759-4766.