IN VITRO PROPAGATION AND SECONDARY METABOLITE PRODUCTION FROM MEDICINAL PLANTS:

CURRENT TRENDS (PART 1)

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(Part 1)

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FOREWORD

Biotechnology will continue to be the frontline area for research and application in the 21st century. The potential of biotechnology is enormous, and numerous breakthroughs have benefited the humankind. Plant science research holds tremendous potential to address pressing global issues, including climate change, food insecurity and sustainability. Necessary research to generate innovative discoveries that solve urgent problems is at risk.

India is rich in bioresources but has been slow in converting them into economic wealth through appropriate technologies. Medicinal plants are active biochemical factories of a vast group of secondary metabolites (SMS), and these are indeed basic sources of various pharmaceutical drugs. It is believed that 80% of the world's population utilises herbs, and in developing nations, its percentage could be as high as 95%. The Ayurveda market in India has been valued at INR 335 billion in 2019 and is expected to reach INR > 1000 billion by 2025. There is a growing interest in the world over photomedicines and photo-chemicals. Quite frequently, unique genotypes have to be multiplied in a pure form. Also, several medicinal plants worldwide are under threat of extinction due to climate change. Clonal propagation is important to multiply elite planting materials of selected medicinal tees and many prized herbs.

Plant tissue culture has been viewed as a key technology for enhancing the capability for the production of large quantities of planting material of selected elite high-yielding varieties to boost production and productivity. This technology holds enormous potential for meeting the demands of both domestic and export markets in terms of high-quality planting material.

In this context, the present book titled "*In vitro* Propagation and Secondary Metabolite Production from Medicinal Plants: Current Trends" edited by Professor (Dr) Mohammad Anis, Former Dean, Faculty of Life Sciences, Aligarh Muslim University, is a timely one. The book will certainly appeal to postgraduate students, researchers, biotechnologists, and industry and can be used as a reference book. We must convert our biological wealth into economic wealth and job opportunities. The book will be an important step in this era.

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PREFACE

Plant tissue culture is one of the most important and useful areas of plant biotechnology, having both fundamental and applied significance. Secondary metabolites hold the key to many medicinal properties present in plants, which thus makes their production a commercial prospect. Optimization of various factors responsible for enhanced cell growth and production of secondary metabolites has become a pre-requisite for the use of bioreactors commercially. Plant cell culture provides a viable alternative over whole plant cultivation for the production of secondary metabolites. The discovery that these metabolites could be extracted from callus came at a time when there was a concern about dwindling plant resources. A continuous callus is used from such medicinal plants, and there would be no need to use field-grown plants to obtain secondary metabolites.

There are fluctuations in the concentrations and quantities of secondary metabolites in field-grown plants as the biosynthesis of secondary metabolites, although controlled genetically, is affected strongly by environmental influence. Moreover, very little is known about the biotransformation that takes place once the crop is harvested. To overcome these limitations biotechnologist suggested the use of plant cell and tissue culture rather than to use whole plants for the extraction of certain secondary metabolites. Plant tissue culture may very well contain metabolic pathways that have been modified and/or abbreviated from that of the plant. The impact of rapid climate changes may also have an adverse effect on wild plant species leading to the loss of useful genetic material.

In vitro cell culture and controlled environment production systems offer excellent opportunities for the selection and seasonal independent propagation of elite lines with specific, consistent levels of medicinal metabolites with minimum contamination. Additionally, the plant materials produced by *in vitro* techniques allow efficient application of emerging analytical methods. The impact of these techniques perhaps may be greatest in the improvement of medicinal plants since the resulting genetic diversity may open avenues for the discovery of new medicinal metabolites and treatments.

The continued rise in consumer demand or plant-based medicines and the expanding world population have resulted in the indiscriminate harvest of wild species of medicinal plants. The impact of rapid climate changes may also have an adverse effect on wild plant species leading to the loss of useful genetic material.

The biomass is mainly obtained through conventional methods, which pose quality issues mainly due to poor seed germination, low viability of the seeds and mainly because of environmental factors. Therefore, there is a great need to develop alternative and stable approaches by using different biotechnological approaches like cell, tissue and organ culture, which are independent of all the environmental variations and obtain uniform biomass of high quality for pharmaceutical purposes. During the last 2 decades, a large number of *in vitro* propagation procedures through direct and indirect organogenesis, somatic embryogenesis and also using synthetic seeds have been developed for the conservation aspects.

Several attempts have been made to optimize the protocol for the production of biomolecules using tissue culture and also through hairy root culture (*Agrobacterium rhizogenes* mediated transformation). These cultural techniques can be achieved by monitoring environmental conditions like media optimization, physical conditions, use of biotic and abiotic elicitors, *etc.*, where improved production of secondary metabolites for commercial scale can be achieved. With the significant information on gene regulation and the key enzymatic steps

involved in the biosynthetic pathways, modulation in the production of bioactive content can be achieved to the levels required for the pharmaceutical industry. The present book will provide a comprehensive report on the research carried out in the area and will emphasize the future prospects that can be considered for the production of secondary metabolites or the development of elite germplasm with higher contents of secondary metabolites, which would benefit the mankind in an endurable way.

The purpose of this book is to provide important, state-of-the-art findings on secondary metabolite production in valuable medicinal plants. We are extremely grateful to all the contributors who warmly welcomed our invitation and agreed to contribute chapters to embellish information on the subject, thus helping in this endeavor. We also appreciate their patience and cooperation in meeting deadlines and revising their manuscripts when required. We would also like to place on record our sincere thanks to Mr. Zohaib Siddiqui for preparing the layout of the contents. As usual, my wife, Humera Anis, provided much needed morale support, and grand daughter, Haniya, provided moments of joy during a bit tiring and monotonous work.

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CHAPTER 1

Secondary Metabolite Production through Elicitation: Biotic, Abiotic, MeJA, PGRs and Stress Signaling in Improving Compounds in Select Medicinal Plants

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Abstract: Plants in addition to primary metabolites produce secondary metabolites which are of immense pharmaceutical importance and other industrial uses. Secondary metabolites are produced due to the stress experienced by plants in response to external triggers/agents like elicitors. Elicitation involves two types of elicitors namely biotic and abiotic. Elicitors have a vital role in plant tissue culture as these improve secondary metabolite content in cultures. Other culture conditions including volume and types of medium, duration, *etc.*, also affect the yield of alkaloids. Extensive research has been carried out for the enhanced level of alkaloids in *in vitro* cultured plants. Various common elicitors used in media are methyl jasmonate (MeJA), yeast extract (YE), fungal extract, ions from various salts like $CdCl_2$, heavy metal ions, and ionic, nonionic radiations, *etc.* The fungal cell wall components oligosaccharides and peptides have also been used as elicitors for the induction/enhancement of secondary metabolites in plant cell/organ cultures. The influence of sample representation of biotic and abiotic elicitors, *i.e.*, YE, *Aspergillus flavus*, MeJA, $CdCl_2$, $CaCl_2$, has been discussed taking a few medicinals and oil yielding plants from authors' laboratory. A direct link of stress with elicitors including plant growth regulators (PGRs) has been established showing over accumulation of proline, protein, SOD, APX and other antioxidant enzyme activity with increased levels of elicitation. Increasing demand forces researchers to conduct further investigation in this area for the production of phyto-compounds and even for viable commercial exploitation.

Keywords: Alkaloids, *Catharanthus roseus*, *Colchicum luteum*, Colchicine, Elicitor.

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Mohammad Anis & Mehrun Nisha Khanam (Eds.)

INTRODUCTION

The plant has been used to treat a wide range of diseases like asthma, fever, stomach ache, arthritis, menstrual disorders, toothache, migraine, insect bites and helminthiasis [1]. Plants are a rich source of metabolic products, attracting the attention of workers in amending the quantity and quality of traits. The secondary metabolites are the diverse group of organic compounds produced by plants to facilitate interaction with biotic and abiotic environments in establishing defense mechanisms [2]. These products are unique sources of pharmaceuticals, food additives, flavors and industrially important biochemicals [3]. The secondary metabolites have complex structures to be manufactured by chemical synthesis and semi-synthesis, and are frequently extracted from naturally and *in vitro* grown cultivated plants [4]. These metabolites are produced and even over-synthesized from different medicinal plants under biotic and abiotic influences [5]. Most commonly, the strategies adopted are optimization of medium component and environmental cultural conditions, the use of high producing superior cell lines, addition/feeding of precursors, overexpression of key enzymes in biosynthetic processes, and other biotechnological and cell culture techniques [6]. *In vitro* methodology may offer an alternative, promising technique for the production of phyto-compounds which can further be improved by the application of elicitors [7]. Elicitors of biotic and abiotic origin have been used extensively for the enriched synthesis of a wide variety of secondary metabolites. There are several reports of enhanced synthesis of secondary metabolites in cell cultures by using elicitors, PGRs, medium, precursor feeding and other biotechnological techniques [8]. Elicitors were used as an enhanced biomass production in different *in vitro* cultures such as *Ophiorrhiza mungos* [4], *Silybum marianum* [9], *Glycyrrhiza uralensis* [10], *Eruca sativa* [11], *Isatis tinctoria* [12] and *Centella asiatica* [13]. As an external stimuli, the elicitors are added to the medium, change cell metabolism, cause stress in culture and activate secondary metabolite synthesis [14]. Elicitors are known to activate a range of defense mechanisms including the synthesis and accumulation of diverse defensive secondary metabolites in plants [14]. The activating mechanisms of elicitors are considered to be different, complex and unpredictable at times, in relation to metabolite synthesis [15].

ELICITOR

Elicitation is an important current technique in which various elements or compounds are amended to the media for improving secondary metabolites in cultures. These molecules induce stress to cultivated cells and in response to adverse situations accumulate and synthesize improved amounts of phyto-compounds [16].

Classification of Elicitor

Elicitors can be categorized into two different types biotic and abiotic elicitors. The biotic elicitors originate from the living cells of lower/prokaryotic organisms fungi and bacteria; simple sugars, polysaccharides, chitin, glucans, pectin, cellulose, MeJA, glycoproteins or intracellular proteins and peptides in are added as external regulatory molecules which regulate a number of enzymes or ion channels through receptor binding mechanism by activating/deactivating gene expression in evoking stress response [17, 18] (Table **2**).

The abiotic elicitors, on the other hand, comprise of non-biological regulatory elements. These abiotic compounds contain a wide variety of chemicals or agents including metal ions. Various metals like Cd, Pb, Ni, Ag, Fe, Co, Al, Ca have been added to the media and are noted to be very efficient in improving the yield of alkaloids in several investigated plant materials [19, 20] (Table **1**). Abiotic elicitors like pH, extreme temperature, ultraviolet (UV) rays, X-rays, and Gamma rays have also been tested in several plants. Other signaling compounds often tried are salicylic acid, methyl jasmonates and NO_2 for enhanced synthesis and accumulation of phyto-compounds in tested samples [21].

Table 1. Various compounds (biotic, abiotic and other) and explant/ tissue used for elicitation.

Biotic Elicitor Used	Abiotic Factors Added	Explant/Tissue Used
Fungi: *Aspergillus, Pythium, Fusarium etc*; Cell wall components of fungi, sugars, pectin *etc.* Bacteria: *Pseudomonas* extract; Yeast extract; Casin hydrolysate; MeJA; Salicyclic acid; Peptides;	NaCl, KCl, $CdCl_2$, $CaCl_2$,, $AlCl_2$, $AgNO_3CuCl_2$, pH, temperature, UV ray, X-rays, Gamma rays.	Non-, embryogenic callus, suspension, different stages of somatic embryos, nodal stem, protoplast derived tissues, plantlets.

Table 2. Biotic and other factors' induced elicitation targeting different alkaloids: A few successful cases in medicinal plants.

Elicitor	Plant Species	Culture Type	Secondary Metabolite	References
Aspergillus flavus fungal extract	*Catharanthus roseus*	Embryogenic callus, somatic embryos	Vincristine and vinblastine	Dipti *et al.* 2016

CHAPTER 2

In Vitro Multiplication and Metabolite Variations through GC-MS of a Medicinal Plant *Scaevola Taccada* (Gaertn.) Roxb.

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Abstract: The present study investigated the difference in the phytoconstituents in the methanolic extract of mother and tissue cultured plants of *Scaevola taccada* (Gaertn). Roxb., an important medicinal plant of the Goodiniaceae family. An efficient protocol was established to rapidly multiply *S. taccada* using nodal explants. The explants were cultured on MS medium supplemented with different concentrations of BAP (0.5 mg/l, 2.5 mg/l, 5.0 mg/l, 10.0 mg/l), IAA (1.0 mg/l), Kinetin (1.0 mg/l), ascorbic acid (100 mg/l) and citric acid (25 mg/l). The maximum number of multiple shoots were obtained in MS medium supplemented with BAP (5.0 mg/l) in combination with Kinetin (1.0 mg/l) and additives ascorbic acid (100 mg/l) and citric acid (25 mg/l). Subculturing multiple shoots at periodic intervals of every 4 weeks produced the maximum number of shoots. The *in vitro* generated shoots were rooted in half-strength MS medium supplemented with IBA (0.5,1.0,1.5,2.0,2.5) mg/l NAA (0.5,1.0,2.0,2.5) mg/l. Among these, the highest root induction was obtained in IBA (1.5 mg/l) and NAA (0.1 mg/l). The rooted plantlets were transferred to pots containing a mixture of vermiculite and perlite for acclimatization for three weeks. The plants were hardened in a greenhouse and planted in open fields. Phytochemical analysis shows the methanolic extracts of the tissue cultured plants produced more bioactive compounds having various pharmaceutical importance than the mother plant.

Keywords: Acclimatization, BAP, Gas chromatography-mass spectrometry, Growth regulators, IBA, NAA.

INTRODUCTION

Scaevola taccada (Gaertn.) Roxb is a hemi-sclerophyllous littoral shrub of the family Goodeniaceae. The name "*Scaevola*" is derived from the Greek word "Scaevus" meaning "left-handed [1], also "*Scaevola*" means "little hand" [2]. Traditionally, different parts of *S. taccada* are used for the treatment of various ailments. It has been reported to possess antiviral properties against vesicular

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stomatitis virus and herpes simplex virus-1 and 2 and also anti-fungal, digestive, diuretic, carminative and anti-cancer properties [3 - 5]. The fruit juice promotes menstruation and treats ringworm tinea [6]. The root extracts are employed in treating beriberi, dysentery and syphilis [7]. Due to anthropogenic pressures of deforestation activities, *S. taccada* has been classified as a species under the regionally extinct (RE) category [8]. Conventional propagation methods using stem cuttings and seed germination are rather slow and also depend on the healthy mother plants [9, 10]. Therefore, to reinforce the conservation, development and utilization of this valuable plant species, it is essential to establish a high-frequency *in vitro* regeneration protocol. Gas chromatography mass spectrometry (GC-MS) is a key technological platform for secondary metabolite profiling [11 - 13]. The present study aimed to identify the chemical components present in the leaf, stem and root of tissue culture raised plants and its comparison with the mother plant using GC-MS.

MATERIALS AND METHODS

Plant Material and Sterilization

Healthy shoots of *S. taccada* were collected from plants grown in the Botanical garden, University of Calicut, Kerala. Young shoots with 2-3 nodes were collected and cut into small pieces containing single nodes. The explants were kept in running tap water for 1 hour followed by washing with teepol (2% v/v) solution for 10 minutes followed by two or three washing with sterilized distilled water. The explants were kept in Bavistin (2%, w/v) solution for 30 minutes and then rinsed with sterile double distilled water. Surface sterilization was carried out using 0.1% (w/v) $HgCl_2$ for 5 minutes followed by washing with sterile double distilled water 7 to 8 times inside a laminar airflow cabinet. The surface sterilized explants were inoculated to MS medium containing different concentrations of growth hormones.

Culture Medium and Conditions

Murashige and Skoog's [14] medium was used for the initiation of cultures. Shoot proliferation medium was supplemented with varying concentrations of BAP (0.5mg/l-10mg/l), IAA (1.0 mg/l), Kinetin (1.0 mg/l) either singly or in combinations with additives (50 mg/l ascorbic acid and 25 mg/l citric acid). The pH of all the media was adjusted to 5.8 before autoclaving at 121 ºC for 20 minutes. All cultures were incubated at 25±2 ºC for 16 hr photoperiods with a light intensity of 1000 $\mu Em^{-2}S^{1}$. The frequency of shoot and root regeneration number and length of shoot and roots were recorded after 4 weeks from the culture initiation.

Multiplication of Shoot

For shoot induction, MS medium was supplemented with BAP (0.5mg/l-10mg/l), Kinetin (1.0 mg/l) in combination with IAA (1.0 mg/l) and additives ascorbic acid (100 mg/l), citric acid (25 mg/l). After 4 weeks, the regenerated multiple shoots were separated and transferred to a fresh medium of the same composition to and length obtain a maximum number of shoots at a periodic interval of 3 weeks. The number of shoots and roots regenerated and the length of and roots were recorded after 4 weeks from the culture initiation.

Rooting and Acclimatization

Rooting of the isolated shoots was carried out *in vitro* and *in vivo*. *In vitro* root induction was achieved in full and half strength MS medium containing IBA (0.5, 1.0, 1.5, 2.0, 2.50 mg/l) IBA (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) and NAA (0.5, 1.0, 2.0, 2.5 mg/l). Plantlets with fully expanded leaflets and well developed roots were removed from the culture bottle and washed thoroughly to remove adhered agar and transferred to sterile vermiculite and perlite mixture (50:50) moistened with 1/10 MS medium. Initially, the plants were covered with perforated plastic bags to retain moisture and kept in a growth room for 4 weeks. Successfully established plantlets were subsequently transferred to field conditions.

Gas Chromatography - Mass Spectrometry

Sample Preparation

The samples for the GC-MS analysis were collected from the mother plants which were identified and authenticated. The tissue culture raised plants and *in vitro* hardened plants of 3rd, 6th and 9th month old were used for GC-MS analysis. The plant materials were washed carefully under running tap water and shade dried at room temperature and made into a powder using mortar and pestle. The dried powdered material was subjected to methanolic extraction by incubating for 24hrs on a rotary shaker. After filtering the extract, the filtrate was evaporated and concentrated by air drying. The air-dried pellet was dissolved in HPLC-grade methanol and subjected to GC-MS analysis to find out the bioactive compounds. The analysis of the methanolic extract was carried out using a gas chromatography- mass spectrometry (GC-MS) system (Shimadzu GC- 22 MS, Model Number: QP2010S) equipped with an ELITE-5MS capillary column (0.25mm Xx 30m, 0.25µm film thickness). The operating conditions were as follows: the GC temperature program consisting of 80°C for 2min, incremented at 4.25°C/min from 80°C to 260°C and held at 280°C for 2min. The injector temperature was 260°C; the sample injection volume was 1:11; and the split ratio was 100:1. Mass spectrometry conditions were: electron impact as the ion source,

CHAPTER 3

Metabolic Engineering & Synthetic Biology of Monoterpenoid Indole Alkaloids Pathway in *Catharanthus Roseus*

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Abstract: The anti-neoplastic herb, *Catharanthus roseus* (L.) G. Don (Apocynaceae), is a high-value, low-volume medicinal herb, which is the focus of global attention in view of being the source of terpenoid indole alkaloids (MIAs). MIAs are one of the largest classes of phyto-alkaloids, and many of them are sources of important pharmaceutical products. *C. roseus* is known to harbour more than 130 different bioactive MIAs that make it an interesting plant, finding use in several traditional and modern medical therapies. The remarkable presence of cellular and subcellular compartmentations for the synthesis and storage of MIAs allows the accumulation of these medicinally important MIAs in leaves (viz. vindoline, catharanthine, vinblastine, vincristine) and stem and roots (viz. tabersonine, ajmalicine, reserpine, serpentine, vindoline, catharanthine, horhammericine, leurosine, lochnerine). Out of them, any medicinally active MIAs found in *Catharanthus roseus*, vinblastine and vincristine are special since they possess anticancerous properties, along with ajmalicine and serpentine, which possess antihypertensive properties. However, the low plant yield and nonavailability of alternative chemical synthesis methods have increased their demand and market cost. In the research era of more than three decades, a plethora of studies have been carried out on *C. roseus* to explore, understand, explain, improve and enhance the *Homo/Heterologous* biosynthesis of MIAs. Metabolic engineering (ME) and synthetic biology are two powerful tools that have played and contributed majorly to MIAs studies. This chapter concentrates mainly on the efforts made through metabolic engineering and synthetic biology of MIAs in plant and microbial factories in the last three decades.

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Keywords: *Catharanthus roseus*, Monoterpenoid indole alkaloids, Metabolic engineering, Synthetic biology.

INTRODUCTION

Alkaloids are a structurally assorted class of low molecular weight nitrogenous compounds that comprise 15.6% of all known natural products, but they account for more than 50% of the annual total sales of clinically used plant-based drugs (4-5 billion US $) [1]. Plants include self-defense mechanisms against insects and other herbivores. However, the biological activities of plant alkaloids may range from antihypertensive to anti-neoplastic, analgesic, CNS stimulant, vasodilatory, hypnotic, sedative, antihepatotoxic, mydriatic, cholinergic, antispasmodic, narcotic and antirheumatic. Around 53 plant alkaloids are currently used as drugs in different pharmaceutical preparations [2]. Monoterpenoid indole alkaloids (MIAs) are one of the major classes of alkaloids with over 3000 natural products containing a residue of tryptophan or tryptamine (*i.e.,* indole part) and terpenoid building blocks derived from secologanin [3]. MIAs majorly protect plants from pests and pathogens, and MIAs derivatives are also used as medicinally important phytomolecules acting as anticancer, antihypertensive, antimalarial, and antiarrhythmic agents (Table **1**). The well-known and well-studied plants producing MIAs are *Catharanthus roseus*, *Tabernaemontana divericata,* and *Rauwolfia serpentina.* However, *C. roseus* always attracts attention for remaining the only available source of anticancer phytomolecules, vinblastine, and vincristine. A large and diverse number of MIAs are biosynthesized in *C. roseus.* Being secondary metabolites, MIAs have very low plant yield (>3% of dry weight) [8]. For instance, extraction of 1 gm vinblastine and 1 kg of Paclitaxel requires 500 kg of dried Catharanthus leaves [9] and 10,000 kg of dry bark of taxol, respectively [10]. Therefore, the bark of 2-3 fully grown taxol trees is required to treat the cancer patient through paclitaxel. Being the only source of anticancer phytomolecules, vinblastine, and vincristine, *C. roseus* has been extensively studied. The extremely low (0.0001-0.0005% leaf dry weight) *in-plant* yields of vinblastine and vincristine makes their extraction difficult and exorbitantly expensive. Moreover, the extraction of vinblastine and vincristine from plant sources is extremely variable depending upon the environmental conditions and location of grown plants and harvest season. Also, the complex chirality of vinblastine and vincristine makes their chemical extraction very difficult and economically nonviable [11].

Table 1. Some important plant-derived alkaloids are under clinical use as drugs and phytomedicines.

Alkaloids	Alkaloid Class	Plant Source	Clinical Applications
Aconitine	MIA	*Aconitum variegatum*	Rheumatism, neuralgia, sciatica
Ajmalicine	MIA	*C. roseus, Rauwolfia* spp.	Vascular disorders
Ajmaline	MIA	*Rauwolfia serpentina*	Antiarrhythmic agent
Atropine	Tropane	*Atropa belladonna*	Antispasmodic, anti-Parkinson, cycloplegic drug
Berberine	BIA	*Berberis spp.*	Eye irritations, AIDS, hepatitis
Boldine	Aporphine	*Lindera aggregate*	Cholelithiasis, vomiting, constipation
Caffeine	Purine	*Coffea arabica,*	Neonatal apnea, atopic dermatitis
Camptothecin	MIA	*Camptotheca acuminate*	Antineoplastic
Canescine	MIA	*Rauwolfia spp.*	Antihypertensive agent
Cinchonidine	BIA	*Cinchona officinalis*	Increases reflexes, epileptiform convulsions
Cocaine	Tropane	*Erythroxylumnovogranatense*	Local anesthetic
Codeine	BIA	*Papaver somniferum*	Antitussive, analgesic
Colchicine	Tropane	*Colchicum autumnale*	Amyloidosis treatment, acute gout
Emetine	MIA	*Carapicheaipecacuanha*	Intestinal amoebiasis, expectorant drug
Ephedrine	Cathinon	*Ephedra sinica*	Nasal decongestant, bronchodilator
Ergometrine	Ergot	*Clavicepspurpurea*	Postpartum/postabortalhemorrhage
Ergotamine	Ergot	*C. purpurea*	Migraine treatment
Eserine	Tropane	*Physostigmavenenos*	Ophthalmology, antidote/poisoning
Galanthamine	Tropane	*Galanthus spp.*	Muscle relaxant, Alzheimer's
Hydrastine	BIA	*Hydrastis canadensis*	Gastrointestinal disorders
Hyoscine	Tropane	*Hyoscyamus niger*	Motion sickness
Hyoscyamine	Tropane	*Hyoscyamus niger*	Antispasmodic, antiparkinson, cycloplegic drug
Morphine	BIA	*Papaver somniferum*	Pain relief, diarrhoea
Narceine	BIA	*Papaver somniferum*	Cough suppressant
Nicotine	Tropane	*Nicotiana spp.*	Anti-smoking
Noscapine	BIA	*Papaver somniferum*	Cough suppressant
Papaverine	BIA	*Papaver somniferum*	Vasodilator, gastrointestinal disorders
Pilocarpine	Isopilocarpine	*Pilocarpus microphyllus*	Miotic in treatment of glaucoma, leprosy
Quinidine	MIA	*Cinchona spp.*	Ventricular arrhythmias, malaria, cramping

CHAPTER 4

Impact of Abiotic Stresses on *In Vitro* Production of Secondary Metabolites

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Abstract: Climate change conditions affect plant growth, net primary productivity, photosynthetic capability, and other biochemical functions that are essential for normal metabolism. The stimulation of biosynthesis of secondary metabolites is an important strategy developed by plants to cope with adverse environmental conditions. Many of these metabolites display a wide array of biological and pharmacological properties (*e.g.*, antioxidant, anti-inflammatory, antiproliferative, anti-allergic, antiviral, and antibacterial) and, thus, have valuable applications as pharmaceuticals, agrochemicals, cosmetics, fragrances, and food additives. The aim of this review is to present an overview of the impact of abiotic stress factors in the biosynthesis of secondary metabolites by *in vitro* cultures. Our literature survey showed that plant tissue culture has been an effective tool to understand plant response to abiotic stresses, such as drought, salinity, temperature, nutrient deficiency, or exposure to ultraviolet radiation, which is of particular interest in the actual scenario of climate change conditions. Furthermore, this technique appears as an environmentally friendly alternative for the production of high-value secondary metabolites for many applications.

Keywords: Drought, Environmental changes, *In vitro* cultures, Phenolics, Secondary metabolites, Temperature.

INTRODUCTION

Resisting adverse environmental conditions, which are now being exacerbated by climate change, is one of the major threats faced by plants. Climate change may occur due to natural processes or anthropogenic activities, and an increased incidence of coastal floods associated with sea-level rise and long-term droughts related to rainfall variations is expected. Also, by the end of the twenty-first cen-

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tury, global mean temperatures rising at least 5°C os expected [1, 2]. These extreme alterations expose plants to stressful abiotic factors that are outside of their physiological limits, negatively affecting plant growth and forcing them to adapt. Some plants have a great capacity to recognize environmental changes and adapt quickly, minimizing the adverse effects of these alterations. The accumulation of secondary metabolites (SM) is an example of an adaptive response by plants in coping with abiotic stress conditions [3, 4]. The changes in SM biosynthesis in plants under stress may be due to the "passive shift" mechanism, which occurs by the cell over-reduced status and the "active" mechanism, involving enzyme up-regulation [5]. SM like phenolics, alkaloids, and terpenes play an essential role in the interaction between the plant and its environment and are considered crucial defense compounds to scavenge reactive oxygen species (ROS) produced in response to environmental stresses, such as drought, salinity, ultraviolet (UV) radiation, extreme temperatures, nutrients deficiency, or heavy metals [5]. Many of these metabolites display a broad range of biological properties, namely antioxidant, anti-inflammatory, antitumoral, anti-allergic, antiviral, and antibacterial [6], and became useful compounds in several sectors, such as pharmaceutical, agrochemical, cosmetic, and food industries [7]. This is of particular relevance in the current context of increasing interest in natural products for industrial applications [8]. In some cases, natural supply is limited or chemical synthesis is unviable to produce SM, and, in this way, plant tissue culture (PTC) techniques emerge as a good alternative [9]. PTC techniques, besides providing a way to protect endangered species [7], allow the application of several strategies to improve or modify the production of SM of interest, such as the optimization of culture medium composition, changing environmental conditions, or inducting the SM pathways by elicitation and metabolic engineering, among other strategies. PTC techniques have been used in the last years to understand plant responses to several abiotic stresses. Hence, this chapter aims to make an overview of the last twenty years of literature about the impact of abiotic stress factors – drought, salinity, nutrient deficiency, UV radiation, and temperature – in the production of SM by *in vitro* plant cultures.

SECONDARY METABOLITES OF PLANTS

Higher plants synthesize a broad variety of low molecular weight SM derived from primary metabolites [10]. Contrary to the primary metabolism, which comprises all metabolic pathways that are essential to plants' growth and development, secondary metabolism is not essential for plants' life but is required for their survival [6, 11, 12]. SM serve as signals to attract pollinators and seed dispersers or protection against other abiotic and biotic stresses. Moreover, they can also contribute to the specific colors, odors, and tastes in plants [6, 10]. The accumulation of SM occurs in specific tissues, such as specialized glands,

vacuoles, and trichomes. Though most of these metabolites are genus and species-specific [11], there is a high chemical diversity with an estimated 200,000 compounds, being terpenoids, alkaloids, and phenolics the most diverse groups [12]. Terpenoids derive directly from the glycolysis of glucose *via* the methylerythritol phosphate (MEP) pathway or the mevalonic acid (MVA) pathway. Most alkaloids are originated from amino acid precursors and phenolic compounds from shikimic acid [13]. The production of these compounds depends on several factors (*e.g.*, plant species, physiology, developmental stages, or environmental conditions) and is generally stimulated by exposure to potential stress conditions [9, 14] (Fig. **1**).

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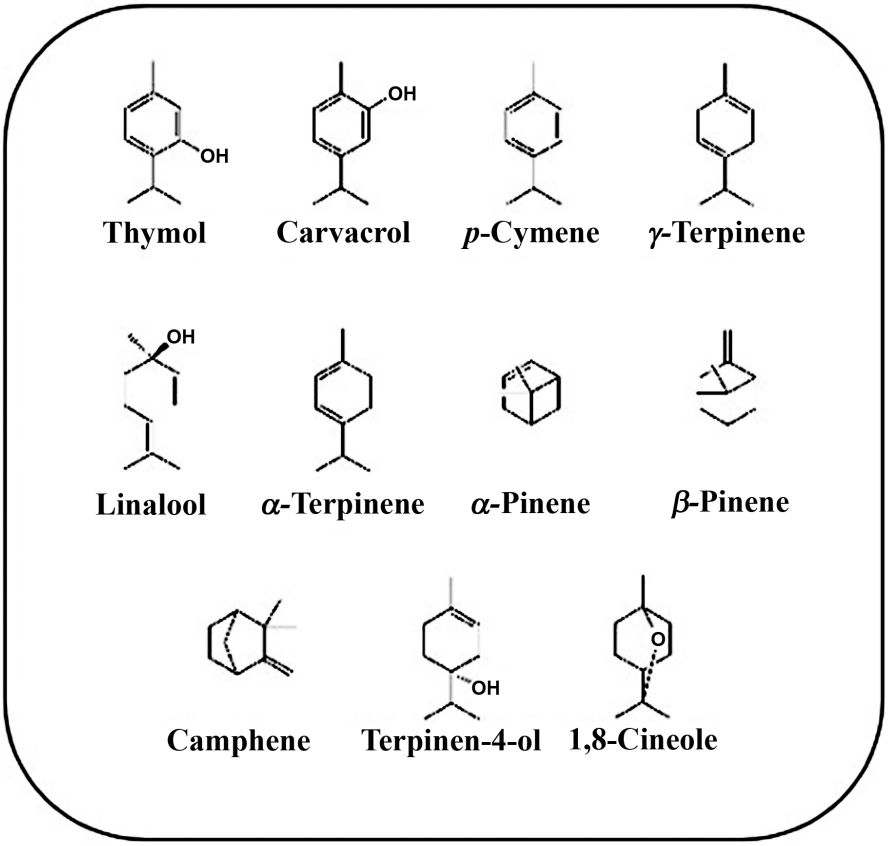

Fig. (1). Chemical structures of the most importante monoterpenes present in *Thymus* plants. Adapted from Li *et al*.(2019).

Presently there is a growing interest in products from natural sources to reduce the use of synthetic chemical substances with potentially toxic consequences [9].

CHAPTER 5

Glandular Trichomes: Bio-cell Factories of Plant Secondary Metabolites

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Abstract: Trichomes are specialised epidermal outgrowth that is present on the aerial parts of plants. On the basis of morphological and cellular variation, they are categorized into non-glandular trichomes (NGTs) and glandular trichomes (GTs). NGTs are known to be involved in the protective and defensive roles that attribute to provide structural and chemical corroboration to form specialized groups of secondary metabolites. GTs are specialized micro-organs that are considered factories for the biosynthesis of a considerable amount of different classes of bioactive metabolites. Conventionally these glandular and non-glandular trichomes are known for their protective roles against different biotic and abiotic stresses. Recently, they have attracted the interest of various researchers as a specialized organ for the production of various bioactive molecules of high pharmaceutical and commercial values. The major groups of secondary metabolites such as terpenoids, flavonoids, phenylpropanes, methyl ketones, acyl sugars and defensive proteins are reported in the trichomes of different plant species. However, the conception of the molecular regulation of their biosynthesis, storage and distribution during the development of trichomes is scattered. This review compiles structural and functional aspects of GTs and NGTs along with the molecular mechanism regulated for the production of secondary metabolite in these specialized organs. In addition, the role of several bio-physical parameters that affect the trichome biochemistry, which either directly or indirectly influence the biosynthesis of secondary metabolite, will also be focussed. The systemized knowledge of trichome biology, secondary metabolite pathway modulation and metabolic engineering at one platform will be helpful to explore recent advances in the field of trichome engineering in many medicinally important plants.

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Keywords: Glandular trichomes, Non-glandular trichomes, Secondary metabolites, Stress.

INTRODUCTION

Trichomes Greek "trichos" (hairs) are specialized epidermal outgrowth present on the aerial parts of plants. They originated by means of endo-reduplication [1 - 3]. Trichomes are the epidermal extensions, and in most of the plants, they are not connected with the vascular system of the plant, *i.e.*: they lack vasculature [4]. They exhibit great diversity in their morphological and mechanical characteristics, such as size, orientation, shape, density, and surface texture which influence the physiology and ecology of the plants [5]. Their spacial distribution also adds benefits for the identification of different plant species. More than 300 types of plant trichomes have been described by Wagner in the year 1991 [6]. These plant trichomes are specialised for performing the first line of defense mechanism against various biotic/abiotic stresses.

Trichomes can be a few micrometers to centimeters in size, unicellular or multicelluar, and exhibit enormous species-specific diversity among the plant Kingdom. Most acceptable criteria to classify these appendages is whether they are non-glandular (NGTs) or glandular (GTs). NGTs are predominantly present in angiosperms, but they are also reported in a few species of bryophytes and gymnosperms. *Arabidopsis thaliana* and cotton are two model plants that have unicellular, non-glandular structures and are used to study cell fate differentiation, cellular specification, morphogenesis and non-glandular trichome biology. However, very limited information about the synthesis of metabolites and their secretary behaviour can be obtained from NGT bearing plants.

GTs are multicellular structures containing apical, stalk and differentiated basal cells [7]. These GTs are also known for producing and reserving a significant amount of different classes of secondary metabolites. These metabolites are mainly synthesized in response to the defence mechanism and require specialized tissues/organs for their storage. Trichomes are one of specialized tissues which originate as small protrusions on the epidermis and serve as a sink for the storage of bioactive secondary metabolites in plants. The trichomes acquire these metabolites, which are not only beneficial for pharmaceutical industries but they also serve as valuable resources for the food and cosmetic industries. GTs show variability in the number of different cells as well as in their constituent of secondary metabolites and are classified into several types. The master groups of secondary metabolites that have been found to be fabricated in trichomes include terpenoids, flavonoids, phenylpropanes, methyl ketones, acyl sugars and defensive proteins. Among the two, *i.e.*; GT and NGT, GTs are special paradigms

to study the biosynthesis of metabolites, storage and their secretory mechanism. Many researchers studied metabolic regulation of secondary metabolites content in different trichome bearing plants viz. Mint, Basil, *Artemisia*, *Nicotiana, Cannabis*, *etc*., that serve as potential candidates for better understanding the biosynthetic pathway engineering in trichome biology [8 - 10].

Plants develop trichomes as a result of different defence responses against different abiotic and biotic stress responses. However, their morphology, location, metabolic constituent, ability to secrete and mode of secretion show considerable fluctuations among trichome bearing plant species. Trichome density is proportional to different environmental stimuli and their function is dependent on the trichome type and location. In the last few decades, several trichome bearing plant species have been paying attention to the accumulation of bioactive secondary metabolites and different stress related research where trichomes, either NGTs or GTs, act as active defence regulated responses to overcome different biotic/abiotic stresses. However, in several studies, NGTs are also known for their sink capacity; they act as a storage site for different toxic exudates and protect the plants from environmental and biological stresses. Although NGTs procured attention since they significantly contribute to different stress responses and the accumulation ability of metabolites [11 - 13], an update on the term 'glandular trichomes' was reported in 2020, which is based on a literature database Web of Science where a total of 2323 registered publications from 1919 to 2020 with a modest increment until 1990 were recorded. The study expeditiously plateaued at fifty publications per annum until around 2003 with the search for the very first genes involved in the biosynthesis of secondary metabolites produced in GTs of Mint. From 2005 until now, a sharp and constant speedy increase in the era of trichome research has been recorded, which reflects a large number of trichome bearing plant species such as *A. annua*, *C. sativa*, *Nicotiana*, *etc*., and their functional genomics and downstream processing were investigated [14]. A key and solitary property of GT is their ability to synthesize and secrete an enormous range of specialized metabolites relative to their size. Terpenoids, flavonoids, methyl ketones, phenylpropanoids, acyl sugars, defensive proteins and alkaloids, are the constituents mainly synthesized by the GTs [15]. These qualities make GTs an excellent experimental system for biosynthesis, mechanism of regulation of natural product pathways and other molecular genetic studies. In addition, various omics-techniques have greatly contributed to unveil the experimental protocols for the enhancement and biosynthetic of metabolites in the trichomes [16]. Hence, the present chapter is an attempt to compile the systematic knowledge available for some important GTs bearing plants toward their efficacy for secondary metabolite biosynthesis and storage. The molecular cascade regulating the production of metabolites and their cross-talk with different external biotic/abiotic factors will also be focussed in the present review.

CHAPTER 6

Role of Plant Growth Regulators for Augmenting Secondary Metabolites Production in Medicinal Plants

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Abstract: Plants are an important source of natural products for health care throughout the globe. Recent trends show an abrupt increase in the demand for medicinal plants due to their cost-efficiency, safety, and potency. The medicinal properties of the plants are attributable to the presence of secondary metabolites, which accumulate as the natural defense against herbivory and other interspecies defenses. Along with their medicinal uses, secondary metabolites are also used in flavorings, agrochemicals, fragrances, bio-pesticides, and food additives. The demand for secondary metabolites is mainly expedited through the collection of medicinal plants from the wild. This has provided an impetus for overharvesting medicinal plants from the wild, and many of them are threatened. The accumulation of secondary metabolites in medicinal plants is limited, and therefore diverse strategies for improving the production of secondary metabolites are a priority. Biotechnological applications, especially plant tissue culture techniques, offer a viable alternative for obtaining secondary metabolites. Along with the optimization of growth media and culture conditions, the role of plant growth regulators is vital in enhancing biomass and secondary metabolite accumulation in the culture medium. The present chapter demonstrates the types and uses of plant growth regulators with a focus on the application of plant growth regulators for the production of secondary metabolites from medicinal plants.

Keywords: *In-vitro* propagation, Medicinal plants, Natural products, Plant growth regulators, Plant tissue culture, Secondary metabolites.

INTRODUCTION

Natural products obtained from plants are valued for health care throughout the globe. About 60,000 plant species are used as medicine, nutritional supplement, and aromatic in the world [1]. Recent trends show an abrupt increase in the

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demand for medicinal plants [2] which is more likely attributable to cost-efficiency, safety, and potency. At present, there are about 90 plant species used to develop 121 clinically prescribed drugs in the market [3]. Several new plants are being screened for their pharmacological activities for developing new drugs. The global herbal market has increased from $1.2 billion in 1990 to $ 25.6 billion in 2015 [4, 5] with an expectation to reach $ 5 trillion by the year 2050 [6]. High demand has provided an impetus for overharvesting from the wild and about 21% of medicinal plant species used in herbal preparation are endangered [7].

Plants produce compounds with toxic and hallucinogenic properties as the natural defense against herbivory and other interspecies defenses. These plants often contain secondary metabolites which modulate a corresponding molecular target (neuroreceptor, enzymes that degrade neurotransmitters, ion channels, ion pumps, or the elements of the cytoskeleton) in animals and humans [8]. Large numbers of secondary metabolites are extracted and used in the synthesis of modern medicines. Along with their utility as medicine, secondary metabolites are also used as flavorings, pharmaceuticals, agrochemicals, fragrances, bio-pesticides, and food additives. As such, plants produce more than 100,000 secondary metabolites, this number may exceed over 500,000 once we know the structural characterization [9]. Plants continue to be the principal source of many important bioactive molecules/pharmacophores [10]. Interestingly, one-fourth of the prescribed drugs across the globe contain chemical compounds (secondary metabolites) directly, indirectly, or semi-synthetically obtained from plants [11].

Medicinal plants from the wild are the major source of raw material for obtaining secondary metabolites. The accumulation of secondary metabolites is limited, and alternative methods for the production of secondary metabolites are being focused by the pharmaceutical firms under controlled conditions. Plant tissue culture provides a promising outlet for the mass propagation of medicinal plants as well as the production of bioactive compounds (secondary metabolites) under *in vitro* conditions. Cell suspension cultures often accumulate desired natural target compounds. Advancements in molecular biology and fermentation technology of plant cells suggest that these techniques will be a viable source of obtaining secondary metabolites [12]. Along with the optimization of growth media and culture conditions, the role of plant growth regulators (PGRs) is vital for stimulating diverse secondary metabolites from medicinal plants [13, 14]. The mechanism of PGRs varies considerably and hence the production of secondary metabolites is unpredictable in terms of quality, quantity, and diversity [15]. Enhancement of secondary metabolites by optimizing PGRs has provoked their commercial application. Hence, it becomes imperative to review the application of PGRs for the production of secondary metabolites from medicinal plants.

PLANT GROWTH REGULATORS (PGRs)

PGRs can be defined as natural or synthetic compounds that regulate the development or metabolic activities of plants (mostly at low concentrations). These compounds can be associated primarily with an increase or reduction of plant growth along with effects on other physiological processes like flowering, fruit formation, fruit ripening, fruit drop, and defoliation or quality traits. PGRs may act at the site of synthesis or are translocated to the target sites. Meristems tissues and growing fruits are the major sites for their production in plants. Various groups of hormones often interact synergistically or antagonistically to control physiological activities in plants. This provokes the need for understanding their structure, function, and production complexities to get the desired performance of the plant. Unlike animals, plants produce hormones in many cells, playing multiple regulatory roles thereby affecting several aspects of plant development.

The credit for the discovery of plant hormones goes to Neljubow [16], who demonstrated the triple response (horizontal growth, inhibition of elongation, and radial swelling) of pea seedlings to ethylene. The systematic use of PGRs began in the 1930s with the use of ethylene for flower induction and fruit formation in pineapple [17]. Since then, PGRs have been used in agriculture, horticulture, and viticulture to improve the qualitative and quantitative traits of the plants.

Types of PGRs

Auxins, cytokinins, gibberellins, abscisic acid, and ethylene are the main PGRs (Fig. **1**) involved in the vital physiological processes of the plants, and hence this group is termed as the "classic group" of plant hormones. Brassinosteroids and jasmonates are also known for phytohormonal activities, but the classification of polyamines and other compounds is still controversial [18].

Auxins

Auxins are plant-growth regulators with some morphogen-like characteristics. Auxins are organic acids that play a cardinal role in the coordination of cell expansion, division, elongation, and differentiation, thereby affecting the shape and function of cells and tissues in plants [19]. The work of Charles Darwin and his son Francis in the 1880s, followed by Frits W. Went in the 1920s on photoperiodism, led to the discovery of auxin. The first chemical structure of plant auxin, indole-3-acetic acid (IAA) was deduced in the 1930s. Five auxins (indole-3-acetic acid, 4-chloroindole-3-acetic acid, phenylacetic acid, indole-3-butyric acid, and indole-3-propionic acid) are known to occur naturally in plants [20]; several others are synthetic auxin analogs (Fig. **1**). Auxins are known for

CHAPTER 7

Hassawi Rice (*Oryza Sativa* L.) Nutraceutical Properties, *In Vitro* Culture and Genomics

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Abstract: An indigenous reddish-brown landrace rice of the *indica* variety known as Hassawi rice (*Oryza sativa* L.) is cultivated in Saudi Arabia. This rice variety has both nutritive and non-nutritive bioactive components that have therapeutic potential and promote favorable metabolic profiles. Hassawi rice has health advantages that should be further investigated, especially for the treatment of diabetes and obesity. There is a direct need for the conservation and improvement of this important germplasm source. Breeding efforts are limited, although a couple of hybrids were developed. Biotechnology approaches offer effective tools for crop genetic improvement. In this direction, *in vitro* regeneration of this crop has been developed that enabled the evaluation of abiotic stress factors. Furthermore, recent genomic studies revealed that Hassawi rice harbors novel alleles for salinity tolerance. This chapter reviews the research carried out on Hassawi rice in relation to nutritional and health benefits as well as secondary metabolites bioactivity and progress made on *in vitro* culture and genomics.

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Keywords: Abiotic stress, Anti-diabetes, Anti-obesity, Breeding, Genomics, Nutrients, Secondary metabolites.

INTRODUCTION

Rice is one among the foremost important cereal crops and widely used for genetic studies. *Indica* and *japonica* are the two main subspecies of *Oryza sativa* L [1]. The cultivation of Hassawi rice began in Al-Ahsa oasis in Saudi Arabia, where it orginates from the wild-type of *indica* variety [2]. Its major characteristics are wide adaptability to drought and soil salinity [3]. However, it is susceptible to lodging, photoperiod sensitivity, and delayed maturity. Therefore, a breeding program was initiated to produce improved rice varieties by overcoming undesirable traits Two hybrids, Hassawi-1 and Hassawi-2, were developed by crossing local Hassawi rice and a Chinese inbred ancestor (IR1112) [2, 4]. The organelle genome sequences of Hassawi rice are very useful for understanding the inheritance of cultivars and their forthcoming breeding studies. There have been several publications on the whole genomes of the chloroplasts and mitochondria of indica and japonica plants [1, 5 - 7]. Comparative study revealed that many cp genomes had substantially conserved gene order and critical gene content. Two Hassawi rice cultivars' genomes were sequenced using next-generation sequencing technologies, according to Zhang *et al*. [2] (both cp and mt genomes). Hassawi-1, Hassawi-2, indica 93-11, and japonica Nipponbare all had single-nucleotide polymorphisms (SNPs), insertions or deletions (InDels), reverse complementary variation (RCV), simple sequence repeats (SSRs), and repeats in their genomes, according to a comparative analysis of the organellar genomes [2]. Hassawi rice, an underutilized food resource, is classified as pigmented rice and is characterized by its brown reddish color (Fig. **1**). It has lower carbohydrate and higher protein contents and also possesses considerable amounts of non-starch polysaccharides and phenolic compounds [8]. This characteristic of Hassawi rice points to its potential as a functional food that effectively supports and safeguards the human body against several chronic disorders. Therefore, Hassawi rice has significant potential economic benefits as well as nutritional value.

This chapter discusses nutritional and functional properties, beneficial health impact on the human body, *in vitro* culture, and genomics of Hassawi rice.

NUTRITIONAL COMPOSITION OF HASSAWI RICE

Both macro- and micronutrients of Hassawi rice have been studied and compared with the most commonly consumed rice types, such as Basmati rice and long

grain white rice. Details on the macro- and micronutrients of Hassawi rice are presented in Table **1**.

Fig. (1). Uncooked Hassawi rice grains (**a**) and cooked Hassawi rice in the popular local dish known as Kabsa (**b**). (Photos by Shareefa Q. Al-Mssallem).

Table 1. Nutritional composition of raw Hassawi rice, brown rice, long grain rice, and white Basmati rice size.

Components	Brown Rice	Long Grain White Rice	Hassawi Rice	White Basmati Rice
Nutrients (g/100g)				
Total carbohydrates	76.74	80.66	66.82	78.68
Non-starch polysaccharides	4.06	1.64	3.84	2.30
Total protein	8.22	6.90	10.49	7.97
Total fat	2.80	0.56	1.99	1.66
Macroelements (µg/g)				
Calcium, Ca	300.60	285.00	126.52	57.71
Phosphorus, P	3193.00	1130.00	1856.77	1250.53
Potassium, K	2515.33	1150.00	1522.65	1342.75
Sodium, Na	124.00	50.00	79.14	11.91
Microelements (µg/g))				
Zinc, Zn	23.00	10.90	39.04	15.58
Iron, Fe	14.57	7.78	13.25	8.93

CHAPTER 8

In Vitro Rapid Regeneration of Plantlets from Shoot Tip Explants of *Allamanda Cathartica* L. and Characterization of Phytochemicals in Regenerants

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Abstract: This study demonstrates a rapid, economic and efficient plantlet regeneration protocol for an exotic ornamental and medicinal plant *Allamanda cathartica* L. by using shoot tip explants. Interaction of various PGRs (mT, IAA, IBA or NAA) and sucrose was tested in MS medium to obtain maximum shoot regeneration from shoot tip explants. *m*T (3.0 µM) + NAA (0.5 µM) + 4% sucrose was found to be an optimum combination for maximum shoot proliferation with 20.80 mean shoot number and 7.60 cm mean shoot length after 12 weeks of culture based on 93.20% responsive explants. Microshoots (4-5 cm) showed maximum rhizogenic response as they produced 4.20 mean root number with 4.90 cm root length after 4 weeks of culture on ½ MS medium when supplemented with 0.5 µM NAA. Well-developed rooted plantlets were acclimatized successfully with a 96% survival rate. The primary phytochemical screening of aqueous leaf extract in the regenerants revealed the presence of proteins, carbohydrates, lipids, phenols, proteins, and saponins. Quantification of phytochemical constituents showed that the amount of phenols was highest, followed by lipids, proteins, carbohydrates, saponins and alkaloids in the micropropagated plants. These phyto-constituents are known to cure numerous ailments.

Keywords: Acclimatization, *In vitro* rooting, Micropropagation, PGRs, Sucrose.

INTRODUCTION

Ornate plants are grown worldwide for green industries, aesthetic values and decorative purposes in gardens and roadsides. The economic values of decorative plants have considerably increased over the last few decades and are expected to

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continue further escalation in both domestic and international markets. The growth in floriculture cultivation has been phenomenal in the last decade, the area under flower cultivation has doubled in India [1]. One such potential plant is *Allamanda cathartica* (Apocyanaceae) which possesses both ornamental and medicinal values. It is a woody climber, grown for its attractive funnel shaped yellow colored flowers and is commonly known as the golden trumpet vine. It is native to South America and West Indies, distributed throughout the tropics [2]. The plant was introduced to many countries owing to its ornamental value. The leaves of the plant are of high medicinal importance due to the presence of various phytochemicals like alkaloids, carbohydrates, lipids, phenols, proteins and saponins. These phyto-constituents have displayed anti-inflammatory and healing activities [3]. Leaves are also used as an antidote and for relieving coughs and headaches. *A. cathartica* is widely cultivated in the gardens of Australia and some local businesses in the region even include it in their corporate identity. *Allamanda spp* regroups 15 valid species that grow up to 6m [4], some being utilized for ornamental or medicinal purposes. This plant species is reproduced by seeds and can also be propagated*via* stem segments. Under natural conditions, the plant has to cope up with numerous environmental challenges, particularly season dependency, heat, light, humidity and various other biotic and abiotic stresses. Besides these various factors, a major problem is the seed setting which does not take place in the regions of Uttar Pradesh, India. So the species is propagated through stem cutting, which takes a very long time to develop into an entire plant. Therefore, there is an urgent need to develop an alternative, efficient and cost-effective method to propagate this exotic ornamental plant on a mass scale.

Phytochemical screening is a significant source in identifying new medicinally and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids *etc.* Due to recent advancements in the field of pharmacognosy, several methods have been developed for the standardization and identification of crude drugs. It has been emphasized that complete phytochemical investigations of medicinal plants are necessary because the secondary metabolites present in the plants are responsible for medicinal activity [5].

In the present study, an attempt has been made to test the response of cytokinins and auxins for developing a simple but rapid and efficient protocol for large scale propagation of *A. cathartica* from shoot tip explant of a 3-year-old plant and also the quantification in the leaf extracts.

MATERIALS AND METHODS

Explants Collection

Shoot tips of *A. cathartica* were collected in the month of July from a 3-year-old plant maintained at the botanical garden of the university in Aligarh, India.

Explant Preparation

Shoot tip explants were washed under running tap water for 20 min to remove the adherent dust particles prior to immersion in an aqueous solution of 5% (v/v) labolene detergent (Qualigens, India) for 10 min, followed by thorough washing with double distilled water (DDW). They were then surface sterilized by soaking in 0.1% (w/v) $HgCl_2$/water solution and rinsed 4-5 times with sterile DDW to remove the disinfectant. Under the laminar flow hood, shoot tip explants of 0.5 cm were excised and placed on shoot bud induction media in a vertical position.

Media and Culture Condition

All explants were cultured on MS medium [6] supplemented with organic components (Myo-inositol, Thiamine HCl, Pyridoxine HCl, Nicotinic acid and Glycine) containing 10-50 g/l sucrose with different concentrations of Benzyladenine (BA), Kinetin (Kn) meta-Topolin (mT) alone (Table **1**) and in combination with auxins (Indole-3- acetic acid (IAA), Indole-3-butyric acid (IBA) or α-Naphthalene acetic acid (NAA). The pH was adjusted to 5.8 using 1N NaOH and the medium was gelled with 0.8% (w/v) bacteriological grade (Fischer Scientific, India) before autoclaving at 121ºC for 15 min. Cultures were maintained at 25 ± 2ºC under 16/8 light/dark photoperiodic cycles with light intensity of 50 $\mu molm^{-2}s^{-1}$ provided by cool white fluorescent lamps (2 x 40W, Philips, India).

Table 1. Effect of various cytokinins on multiple shoots regeneration from shoot tip explants in MS medium after 8 weeks of culture.

Cytokinins (µM)			Response (%)	No. of Shoots/Explant	Shoot Length (cm)
BA	**Kn**	***m*T**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
00	00	00	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}
1.0	-	-	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}
2.0	-	-	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}
3.0	-	-	11.20 ± 0.32^{j}	1.60 ± 0.16^{g}	1.35 ± 0.15^{g}
4.0	-	-	36.10 ± 0.23^{g}	2.50 ± 0.22^{ef}	2.30 ± 0.15^{f}
5.0	-	-	60.90 ± 0.23^{c}	4.60 ± 0.16^{c}	3.45 ± 0.13^{c}

CHAPTER 9

Production of Secondary Metabolites from Endangered and Commercially Important Medicinal Plants Through Cell and Tissue Culture Technology

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Abstract: Pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, monoterpenes, flavonoids and amino acids are now being produced using plant cell culture technologies. The standardization of plant metabolite processing technologies using *in vitro* cultures assists in the understanding of their biosynthesis and accumulation biology. The development of metabolites in plant cell cultures is affected by a number of factors, including physical, chemical, nutritional and genetic factors. The controlled production of plant metabolites in cell cultures is a viable alternative not only for reducing pressure on the natural habitats of plant species but also for providing year-round conditions for metabolite production. Exposure of cultured cells to biotic and abiotic elicitors increased the production of plant metabolites. Hairy root induction has recently been discovered to be effective in the production of metabolites synthesized in various parts of plants.

Keywords: Callus cultures, Elicitation, Hairy root cultures, Suspension cultures, Secondary metabolites.

INTRODUCTION

Anthropogenic activities, which increase with the rapid increase in the world population, are rapidly increasing the destruction of the natural habitat of many plant species. It also affects the ecosystem negatively. Many commercially used medicinal and endemic plants endanger the extinction of plants due to over-collection from their habitats. The production of plants by traditional methods brings with it many restrictions. Most plants grown from seed are highly heterozy-

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gous and can vary greatly in growth and yield. In addition, the production of desired varieties is limited as many of the plants are not suitable for vegetative propagation by cutting and grafting. One of the most important methods to deal with this alarming situation is plant tissue culture, which is a biotechnological application [1]. People have been using plants for various purposes for ages. Primary metabolite needs such as carbohydrates, proteins and fats are met by plants. In addition to these primary metabolites, which are of great importance to humans, there are some substances that do not have a direct role in transport, energy, growth and differentiation events in the plant, but provide the social communication of the plant. These substances are called secondary metabolites. Secondary metabolites are chemicals that enable the plant to adapt to its environment and to be aware of its environment. Secondary metabolites in plants have important ecological functions such as resisting environmental stress factors such as drought, salinity, UV, protecting the plant against herbivores and microorganisms, pollination and seed dispersal. Plant secondary metabolites are generally classified according to their biosynthetic production pathways [2]. According to these pathways, secondary metabolites are; They are classified into three main families: phenolic compounds, terpenes and alkaloids. The largest group is phenolic compounds. Alkaloids are less common in the plant kingdom than phenolics. These chemicals, which differ in terms of amount and composition in plants, have led to the emergence of the disciplines of chemical taxonomy and chemical ecology [3]. These metabolites have very important benefits in the fields of food, cosmetics, agriculture and medicine. Perhaps the most important of these areas is the use of secondary metabolites in medicine, as it is very important for human health [2]. Plants from which drug-active ingredients are obtained are called medicinal plants. Many of the medicinal plants contain pharmaceutically highly active secondary compounds. *Betula lenta* L. plant is the source of aspirin, which is recommended because it provides blood thinner and protection against heart attack. *Hypericum perforatum* L. is a medicinal plant that is the source of hypericin, pseudohypericin, perforin, adiperforin active compounds and has an antidepressant effect. Before the chemistry of these compounds was known and their existence was revealed, people started to use plants to treat various diseases. This preference formed the basis for phytotherapy applications [2]. Plant tissue culture studies are of great importance in preventing the extinction of medicinal plants and reducing habitat destruction. In addition, many plants with secondary metabolites that the pharmaceutical industry is interested in are endangered plants due to the rapid consumption of natural populations. For this reason, it is imperative to find new approaches for the sustainable production of high-value molecules [4].

Micropropagation is a popular biotechnological method based on direct or indirect methods of reproduction, rooting and then growing of immature or mature plant

parts [5]. This technique exhibits high potential for rapid propagation from plants and the production of high-quality products. Therefore, in *in vitro* culture, it is possible to increase and produce the desired compounds in line with consumer needs in controlled and sterile conditions independent of the environment. In recent years, micropropagation has been used for rapid and mass reproduction of medicinal plants through tissue culture, year-round production, pathogen-free production, increased productivity and yield, conservation of endangered species, and *in vitro* production of secondary metabolites [6]. In conventional production, many plants do not sprout, bloom, produce seeds in certain climatic conditions, or have long growth and reproduction times. Micropropagation can provide a steady supply of plants using minimal space and time. The benefits of *in vitro* micropropagation of the plant to the plant are listed below [7, 8]:

• To increase the plant's reproduction rate.

• To ensure the production of secondary metabolites.

• Meeting the needs of the plant for its controlled production.

• Ensuring that the plant reproduces independently of seasonal changes throughout the year.

• Production of clones with desired characteristics.

• Production of plants by ensuring their genetic development.

• To ensure the reproduction and protection of endangered plant species.

• To ensure the preservation of genetic material by cryopreservation.

As the need for medicinal plants increases, some of their natural habitats are more threatened [9]. Due to their medicinal use in the Ayurvedic, Unani and Siddha systems of medicine, the collection of wild plants from their native habitat has led to the species' extinction. Therefore, this study describes an alternative *in vitro* culture approach for the isolation and characterization of plant secondary metabolites [10]. A multi-dimensional approach is required for the selection of higher quality genotypes and *ex-situ*, as well as for the maintenance of *in-situ* conservation and subsequent reproduction by both conventional and biotechnological methods that can provide a solution to the current problem. *In vitro* plantlet regeneration is an effective method for plant protection [11]. Many techniques of somatic haploid embryos are needed in academic and applied plant science.

CHAPTER 10

Antimicrobial Efficacy of *In Vitro* Cultures and their Applications

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Abstract: Treatment of microbial infections has become more challenging with the evolution of antibiotic resistant microbes and indiscriminate use of antibiotics. Several phytochemicals have shown potential inhibitory action against such microbes. These antimicrobials have shown their efficacy in treating such infections. These natural products also played significant role in restoration of activity of less effective antibiotics, when used in combination with antibiotics. But still, scientists are facing some major challenges in using such metabolites for medicines- there is urgent need to explore more plants showing microbial inhibition activity, plant products from field grown plants are not sufficient to meet the growing demand and purification of antimicrobial compounds, so that dosage for patients can be finalized. Tissue culture has emerged as great technology not only in the conservation of such medicinal plants but it provides major application for the production of secondary metabolites. Various micropropagules such as calli, *in vitro* cultures, and cell suspensions have shown their potential for the production of pharmaceutically active compounds similar to mature plants. Production of such phytochemicals can be enhanced by manipulating media supplements, culture conditions and elicitations. As, in nature production of antimicrobials is the result of interaction between the plants and microbes, therefore, such interaction can be provided to *in vitro* cultures by biotic elicitation. *In vitro* production of antimicrobial compounds has been reported in many plants such as *Ricinus communis*, *Calendula officinalis*, *Abrus precatorius*, *etc.* Thus, plant tissue culture paves an efficient and feasible method of production of such natural compounds as an alternative of antibiotics.

Keywords: Antibiotic, Antimicrobial, Antioxidant, Elicitation, Inhibition, Metabolites, Micropropagules, Pathogenic, Precursor.

INTRODUCTION

Plant secondary metabolites display great applications in the field of human health, nutrition and many industries. Plant cell and organ culture systems have

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become an effective means for the production of pharmaceutically important compounds, phytochemicals used as industrial products and food additives. Secondary metabolites are natural products synthesized by plants and microbes. Primary metabolites of plants are directly involved in their growth and development, whereas secondary metabolites or better termed specialized metabolites are required for their survival. These compounds develop defense mechanism in the plants to combat stress induced by pathogenic attack or environmental variations. Elicitation of *in vitro* cultures is nowadays being used as an efficient method for the production of secondary metabolite compounds. Elicitors promote secondary metabolite synthesis and their accumulation by *in vitro* cultures. Elicitors are the chemical compounds from abiotic and biotic sources that can stimulate stress responses in plants, leading to enhanced synthesis and accumulation of secondary metabolites or the induction of novel secondary metabolites. Elicitor type, dose, and treatment methods are major factors determining the production of secondary metabolites. Other important parameters, which affect the production of such compounds are duration of exposure to cell lines, nutrient composition, age or stage of the culture, *etc.*The collections of these compounds from field grown plants is unable to meet the growing demand of plant based pharmaceutical products, as the metabolites produced by an individual plant is very less. As, both quality and quantity of these compounds are not uniform in nature, it is difficult to fix the dosage for the patients. The production of novel antimicrobial agents is still highly dependent on the discovery of new natural products. At present, most antimicrobial drugs used in medicine are of natural origin.

Antibiotic-resistant microorganisms have emerged as a great challenge for the treatment of patients. Hence, several researchers are focusing their attention on the discovery of new antimicrobial compounds as efficient alternative of antibiotics. Although several plant secondary metabolites and their derivatives have been identified as possible antimicrobial agents, they are not enough and there is a need to explore more such compounds [1, 2]. Secondary metabolites such as alkaloids and polyphenols have shown strong antimicrobial activity [3]. Polyphenols are considered one of the most diverse groups of secondary metabolites. Their strong antioxidant efficacies also contribute antimicrobial effects. Similarly, many alkaloids have shown their antimicrobial potential. These phytochemicals have become greatest tool in overcoming the problem of antibiotic resistance. Several antimicrobials show wide spectrum antimicrobial function, thus they can serve as efficient alternative of antibiotics. To make it cost-effective and commercially feasible, there is a need to optimize various factors, and a standardized protocol can be used for enhanced production of these phytochemicals.

There are different steps:

Culture Initiation

There is a need to grow cells or tissues of medicinal plants. Development of their regeneration protocols is also required to provide a continuous source of materials. *In vitro* cultures are being grown in aseptic and fully controlled condition thus, it provides contamination-free and more uniform material. Secondary metabolite production from *in vitro* cultures at commercial level is feasible by using bioreactors. However, it requires optimization of various factors: growth and maintenance of cell lines or other *In vitro* cultures, identification of suitable elicitor, and extraction of metabolites [4]. The establishment of cultures for phytochemical production undergo mainly two phases- first proliferation and another production phase. After culture initiation, cultures are transferred on a favorable medium which supports the growth and multiplication, but less metabolites are produced by growing cultures. However, production medium favors secondary metabolite production, but not the growth of the cultures.

Type of Cultures

Generally, cell supensions or calli are used for the production of these compounds. But some phytochemicals are organ specific, and their synthesis take place from differentiated organs such as *in vitro* leaves, shoots, somatic embryos, roots, plantlets, *etc.* Among differentiated tissues, hairy root cultures (Fig. **1**) are highly efficient in producing such metabolites at the commercial level [5]. Many researchers have observed the significant antimicrobial potential of different micropropagules of plants (Table **1**). *In vitro* cultures of many plants have also shown their broad spectrum of action against several pathogenic microbes.

Fig. (1). Hairy root culture of *Wedelia chinensis*.

CHAPTER 11

The Contemporary Facts Towards *In Vitro* Production of the Plant-derived Medicinal Metabolites

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Abstract: Plants are active biochemical factories of a vast group of secondary metabolites (SMs) and these SMs are indeed a basic source of various commercial pharmaceutical drugs. From the prehistoric time, plants have been used for therapeutic resolutions. Medicinal and aromatic plants are the biogenic pond of diverse forms of SMs, which results in their overexploitation. There is an increasing need for the natural phytochemicals from plants for sustainable and economical value forces their mass production through *in vitro* plant tissue culture (PTC) methods. A vast quantity of medicinal plants and their metabolites have been developed by *in vitro* culture techniques in a small time period related to conventional methods. *In vitro* plant cell cultures assist in a potential role in the commercial production of SMs. The novel prime practices of *in vitro* techniques facilitate transgenic cultures and enlighten the understanding lane of regulation and expression of biosynthetic pathways. SMs have composite chemical alignment and are created in response to different forms of stress to accomplish various physiological tasks in the plant host system. They are immensely utilized in pharmaceutical industries, dietary supplements, cosmetics, fragrances, dyes, flavors, *etc.* SMs are also termed specialised metabolites, secondary products, toxins or natural products; these are basically organic compounds produced by plants and are not directly involved in the growth and development of the plant. Instead, they usually intervene with ecological interactions and conceivably produce selective support for the plant host by increasing its survivability or productivity. Few SMs are specific for a narrow set of plant species within a phylogenetic group. SMs habitually play a vital role in the defense systems of plants against herbivory and other interspecies defences. Human beings uses SMs mainly for medicines, pigments, flavourings and recreational drugs. Prolonged use of these SMs in several industrial areas still needs to be focused to enhance the fabrication by using *in vitro* PTC practices and optimizing their large-scale fabrication using bioreactors. The present book chapter intends to highlight the rationale of the *in vitro* production of SMs from medicinal plants and their progress in the modern epoch for the mass production facts toward the step of commercial and economical forte.

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Keywords: Bioreactor, Micropropagation, Phytochemicals, Secondary metabolites.

INTRODUCTION

Secondary metabolites (SMs) of plant species are the chemical building blocks for their therapeutically beneficial effects. They are also important indicators for determining the quality of pharmaceutical materials [1]. Plant SMs are naturally occurring sources of biologically active compounds that are used in a healthy diet, traditional medicine, and a variety of industrial applications [2]. In response to various forms of stress, plants produce SMs with a complex chemical composition. These metabolites are used to perform various physiological tasks. There is a wide range of applications for these chemicals in the pharmaceutical and cosmetic industries, cosmetics, dietary supplements, fragrances, flavors, dyes, *etc.* It has become necessary to focus research efforts on increasing the production of these metabolites by employing plant tissue culture (PTC) techniques and optimizing their large-scale production using bioreactors [3]. When it comes to health benefits, medicinal plants are superior to synthetic drugs in terms of safety and cost [4 - 6]. The herbal medicine industry has recently emerged as one of the fastest-growing in the world. It is estimated that the global trade in medicinal plants and their metabolites will reach US$ 5 trillion by 2050, with an average annual growth rate of 7% [7]. It takes a long time for the plant to grow to the point where it can produce the amount of metabolites desired. To overcome this situation, one alternative method is to use PTC techniques for the rapid production of SMs for commercial use [8].

Because of the wide variety of SMs, most of the plants have medicinal properties that can be attributed to a diverse group of naturally occurring metabolic products. Although these metabolites are not essential for the growth and development of plants [9, 10], they play an important role as signaling molecules and defense agents. They're viewed as economically significant products as insecticides, dyes, flavors, and fragrances are just a few of the many things they're used [11]. When developing SMs for the market, consistency and high yield are critical factors [12, 13]. Secondary metabolite production has been improved using traditional and metabolic engineering methods [14]. Elicitor-mediated augmentation of SMs in plants *ex vitro* and *in vitro* is reported to be a very effective and highly reproducible strategy [15 - 18]. Microbes, considered biotic elicitors, activate a wide range of secondary metabolic pathways (fungi, bacteria, viruses, yeast, *etc.*). Bacterial, fungal, and yeast extracts are increasingly being used to induce SMs because of their better yield and quality [18 - 20]. Fungal elicitors are the best way to increase the production of SMs in PTC [21]. It activates secondary metabolic pathways and phytoalexins in plant disease resistance physiological

progressions [3]. Alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids and amino acids are just a few examples of the many SMs that are used in pharmaceutical preparations. Bioreactor design, nutrient optimization, selection of highly productive lines, elicitation, two-phase cultivation, and metabolic engineering have all been employed in PTC to augment the SMs production in a large scale [22 - 24].

There are approximately 100,000 SMs in the plant kingdom, all of which are restricted to specific taxonomic groups. The three major groups of SMs in plants are nitrogen-containing compounds (cyanogenic glycosides, alkaloids, and glucosinolates), phenolic compounds (flavonoids and phenylpropanoids), and terpenes (isoprenoids) based on their biosynthetic pathways [25]. In the current book chapter, a few selected botanicals are overviewed for their SMs under *in vitro* conditions, like a callus, suspension cells, and root cultures. The Table **1** provides an overview of the growth and development of a few selected plants' secondary metabolites under *in vitro* conditions. A new solution for the production and conservation of natural products is to use PTC of higher plants as a renewable and environmentally friendly raw material source.

Table 1. List of selected plants and elicitors used to induce secondary metabolites under *in vitro* culture conditions.

S. No	Plant Species	Elicitors	Secondary Metabolites	Type of Culture	References
1.	*Artemisia absinthium*	MeJA, JA and GA	Total phenolic and Total flavonoid content	Suspension	[378]
2.	*Artemisia absinthium*	silver and copper nanoparticles	Secondary metabolites and antioxidants	Callus cultures	[379]
3.	*Artemisia vulgaris*	Farnesyl Diphosphate	ß-Caryophyllene	Callus and Hairy Root Cultures	[380]
4.	*Codonopsis pilosula*	Methyl Jasmonate	Atractylenolide, lobetyolinin and polysaccharide	Adventitious roots and multiple shoots	[381]
5.	*Achillea gypsicola*	MeJA and SA	Camphor and phenolic content	Cell suspension	[382]
6.	*Thevetia peruviana*	MeJA and SA	Phenolic content	cell suspension	[383]
7.	*Catharanthus roseus*	cyclodextrins and MeJA	Terpenoid indole alkaloid	Cell cultures	[128]
8.	*Catharanthus roseus*	artemisinic acid	Vindoline and vinblastine	Cell suspension	[384]
9.	*Catharanthus roseus*	Sodium Nitroprusside	Terpenoid Indole Alkaloid	Hairy Root Cultures	[385]

CHAPTER 12

Harnessing the Potential of Plant Tissue Culture Techniques for the Herbal Industry

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Abstract: Over the past few years, there has been a tremendous global shift of preference toward herbal medicine because of its affordability, accessibility, efficacy, and lesser side effects. The pharmacological and healing properties of the herbs are due to the presence of a wide array of secondary metabolites. These metabolites are biosynthesized through defined pathways and stored in various parts of the plant, like leaf, root, rhizome, bark, and floral parts. In recent years due to the growing realization of the pharmaceutical properties of medicinal plants, they have been subjected to indiscriminate exploitation. Further, the lack of agrotechnology in many cases and the nonavailability of broad genetic diversity provide impediments to their largescale cultivation and improvement. This situation has created a huge gap between the demand and supply of medicinal plants all over the world. Hence, rapidly propagating high valued medicinal plants through unconventional technologies is warranted and will provide high dividends to farmers and the herbal industry. Further, generating large-scale healthy, genetically uniform plants with defined chemical content will facilitate pre-clinical and translational studies. Therefore, efforts in the development of robust *in vitro* propagation systems for herbal plants can address the core concern of their conservation and large-scale utilization. Studies on cell suspension, hairy root culture, and genetic transformation have provided the desired impetus in metabolic engineering and enhanced their commercial value. The present article highlights some of these developments and provides a futuristic perspective on the subject.

Keywords: Cell suspension culture, Hairy root culture, Herbal medicine, Medicinal plants, Metabolic engineering, Micropropagation, Secondary metabolites.

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Mohammad Anis & Mehrun Nisha Khanam (Eds.)

INTRODUCTION

The herbal industry greatly relies on herbs that contain active ingredients derived from plant parts such as leaves, roots, or flowers to treat diseases and enhance general wellbeing [1 - 3]. Since ancient times, herbs have been considered an important part of society, well-known for their culinary as well as medicinal uses. Traditional herbal medicines form a major part of the healthcare system all over the world and have become a rising global commercial enterprise [4 - 6]. At present, almost 80% population in developing countries rely largely on herbal medicines for their basic healthcare requirements [7 - 9]. The past decade has witnessed a tremendous surge in the acceptance and renewed interest in the use of herbal medicines, owing to their safety and health-promoting effects, which has led to the rapid growth of the herbal drug industry [10, 11]. Medicinal plants are also gaining greater attention from the scientific community for novel drug discovery and development. The extensive exploitation of herbs and other plant-based natural products has resulted in a huge gap between their demand and availability [12]. In addition to that, information regarding cultivation practice is only available for a few medicinal plants (less than 10%) and agro-technology is available for about 1% of the total plants known globally, which further limits their cultivation [13]. An emerging concern is the rapid extinction of plant species worldwide, with experts predicting that we are losing at least one potential major drug every two years. Out of an estimated 422,000 medicinal and aromatic plant (MAP) species identified worldwide, 12.5% (52000) of them are used medicinally, and 8% (4160) of them are considered threatened species [14, 15]. To meet the escalating demand for herbal medicines and products, biotechnological interventions through plant cell, tissue, and organ culture offer a sustainable method for addressing some of the challenges in the propagation of valuable medicinal plants. *In vitro* propagation is a promising alternative that will facilitate the efficient and rapid mass propagation of plants to obtain uniform plant materials throughout the year, independent of the environmental conditions [16, 17]. Further, the therapeutically important secondary metabolites, which are otherwise synthesized in low amounts in plants, can be engineered to increase yields as well as to synthesize target compounds using *in vitro* systems and lay the foundation for their large-scale production in bioreactors [18, 19].

THE ECONOMIC IMPORTANCE OF HERBAL MEDICINE

The global market for herbal medicine and herbal products is estimated to have an annual growth rate of up to 15%, representing a significant share of the total world pharmaceutical market [20]. The international herbal medicine market was estimated at US$ 60 billion in 2010 and is expected to flourish with good growth potential to reach US$ 5 trillion by 2050 [21]. The annual turnover of herbal

medicines increased around 5-15% from the past decade in the year 2000 to an estimated US$ 30 billion in various developed countries, including the United States (USA), Australia, Canada, and parts of Europe. In Asia, with the increasing human population and commercial trade, the demand for herbal products almost doubled during the late 1990s [22]. In India, the export of herbal extracts and herbal products accounted for US$ 456.12 million during the year 2017-2018, with a growth rate of 12.23% over the previous year [23], while the annual herbal drug production in China is worth US$ 48 billion with the export of US$ 3.6 billion [24]. The demand for herbal products also increased as a result of the emergence of natural cosmetics, functional foods, herbal supplements, fragrances, and various other botanical products that represent multibillion-dollar industries. As per previous estimates, only 6% of existing plant species have been investigated for their potential medicinal properties [25 - 27]. However, with the advanced scientific developments, the percentage of characterized species is likely to be higher, but still, a large number of plants need to be studied for their potent bioactivities. Hence, there is a promising potential for future discoveries from plant-derived products for various industrial applications, including novel drug development.

The present article discusses the challenges of the herbal industry and possible technological interventions to address these pending issues. It is strongly realized that the large-scale production of medicinal plants and their bioactive compounds is essential to cater to the need of herbal industries. The use of *in vitro* propagation techniques, the establishment of cell suspension and hairy root culture system, and metabolic engineering are the key focus areas that hold promise in this direction.

LARGE SCALE PLANT PROPAGATION THROUGH PLANT TISSUE CULTURE TECHNIQUES

Plant tissue culture is considered a promising tool for the propagation of a large number of plants for commercial purposes [28]. It refers to the cultivation of plant cells, tissues, and organs on an artificial nutrient media under aseptic and optimum conditions [29]. Compared to conventional methods of propagation, it offers several unique advantages such as enormous multiplicative capacity, efficient ex-situ conservation of endangered plants, production of healthy and disease-free plants throughout the year, and use of cell, tissue and organ culture for the production of various bioactive compounds which is otherwise a difficult task. In the context of medicinal plants, the use of plant cell, tissue and organ culture techniques becomes more relevant due to the huge commercial angle attached to it. For a long time, many researchers have been working on the propagation of medicinal plants employing plant tissue culture protocols (Table **1**)

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Mohammad Anis

Prof. Mohammad Anis, is a Ph.D., has 38 years of teaching and research experience. He has served as dean, Faculty of Life Sciences and Chairman, Department of Botany at the Aligarh Muslim University, India. He has made significant scientific contributions in cytogenetics, morphogenesis and tissue culture. Prof. Anis has been the recipient of prestigious BSR Fellowship of the University Grants Commission, Government of India in 2018.

He visited the University of Georgia, Athens, USA (1994), the Frankfurt University, Germany (1998) and Institute of Plant Biotechnology, Nitra, Slovak Republic (2006). He is a recipient of Lifetime Achievement Award (2016) by Venus International Foundation, Chennai; Vigyan Ratan Samman (2010) of Council of Science and Technology, U.P.; Prof. P. Maheshwari Medal of Indian Botanical Society (2013); UGC Mid-Career Award (2015) and Prof, HE Street Memorial Award (2019). He has been a Visiting Professor to King Saud University, Riyadh from 2016-2019. He was elected President of Indian Botanical Society in 2020.

Prof. Anis has published over 220 research papers in reputed journals with "h" index 47; citations 6550, "i10" 148 and supervised research for over 25 PhDs, 10 M Phils and scores of MSc Projects. He has published three books on Plant Tissue Culture from Springer. He is a life member of National Academy of Sciences, India and holds fellowships of Indian Botanical Society (FBS), Indian Society of Genetics and Plant Breeding (FISG), International Society of Plant Morphologist (FISPM) and Linnean Society of London (FLS).

Mehrun Nisha Khanam

Mehrun Nisha Khanam is a Ph.D., earned her doctorate degree in botany from the Aligarh Muslim University, Aligarh in 2019 on the topic "Studies on Interactive effects of different PGRs on In-vitro regeneration and Biochemical Yield in Allamanda cathartica L." She then worked as assistant professor at the Department of Biotechnology and University Centre for Research and Development, Chandigarh University, Mohali (Punjab), India, before joining the Research Centre for Plant Plasticity, Seoul National University, Republic of Korea as Post-Doctoral Researcher in 2022.

She has published several research papers in journals of repute like Plant Cell, Tissue and Organ Culture (2018), Industrial Crops and Products (2020) and in-vitro cellular and develop Biol-Plant (2020) and received Prof. HC Arya Gold Medal during IBS Conference at Ranchi University, 2017.