International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(5): 970-975

Received: 21-07-2018
Accepted: 22-08-2018

Akhil Kumar
Department of Biotechnology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Vikrant
Department of Biotechnology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Anish Kumar Sharma
Department of Biotechnology, School of Science, P P Savani University, Kosamba, Surat, Gujarat, India

Correspondence
Akhil Kumar
Department of Biotechnology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Beneficial plant product, their uses and their evolutionary perspectives: A review

Akhil Kumar, Vikrant and Anish Kumar Sharma

Abstract
Plants are the natural source of development of new products which has medicinal value for drug discovery. Plants secondary metabolites are unique sources for pharmaceuticals food additives, flavors and others industrial values. A metabolic intermediates or product found as a differentiation product in restricted taxonomic group not essential to growth and life of the producing organisms and biosynthesis from one or more general metabolites by wider variety of pathways, which can be altered and can be use to produce beneficial products. They had created strategies of defense against pathogens and herbivores attacks. The commercial importance of these secondary metabolites has resulted in its production and exploring possibilities of enhancing its production by using tissue culture technology. The present review shows the brief introduction, classification, improvements of production and importance of secondary metabolites.

Keywords: Defense, secondary metabolites, food additives, tissue culture

Introduction
Plants are the source of various useful industrial compounds. Many plants are wide sources of natural products used in pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides (Balandrin and Klocke, 1988) [3]. Biotechnological approaches such as plant tissue cultures are found to supplement with traditional agriculture for the production of plant metabolites (Ramachandra Rao and RaviShankar, 2002) [18]. Plant tissue culture techniques can be established under sterile conditions for extraction of secondary metabolites. *In Vitro* production of secondary metabolites in the cell suspension cultures has been reported from commercially important medicinal plants**.** Advances in the plant tissue culture techniques combined with genetic engineering, specifically transformation technology have opened new ways for high volume production of pharmaceuticals, nutraceuticals and other beneficial substances (Hansen and Wright, 1999) [8]. Recent advancement in the molecular biology, enzymologist and fermentation technology of plant cell cultures suggests that these systems will become a viable source of industrially important secondary metabolites.

Definition
Secondary metabolites are substances which are produced by plants as defense chemicals; their absence does not affect the plants. They include alkaloids, phenolics, steroids, essential oils, lignins, resins and tannins etc. Secondary metabolites are compounds biosynthetically derived from primary metabolites. Secondary metabolites or secondary compounds are compounds that are not required for normal growth, development and are not made through metabolic pathways common to all plants. In plants, they are limited to the occurrence and may be restricted to a particular taxonomic group genus, species or family. Secondary metabolites are accumulated by plant cells in smaller qualities. These secondary metabolites are synthesized in the specialized cells at particular developmental stages.

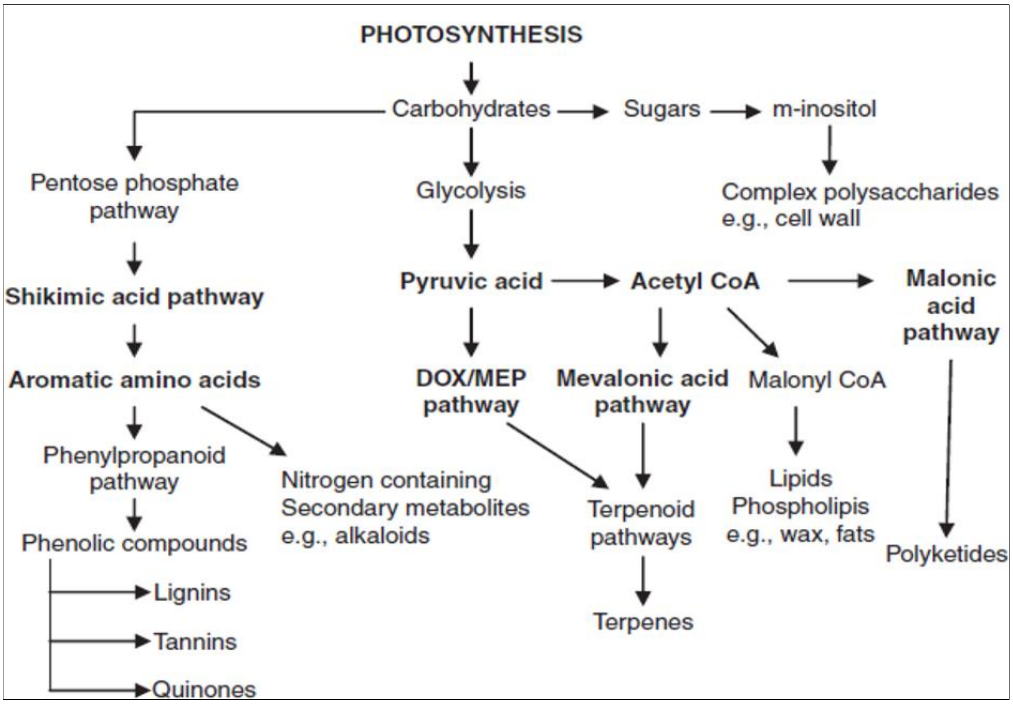

Fig 1: Biosynthetic relationships in the synthesis of major secondary metabolites (Ghasemzadeh and Jaafar, 2011)

Classification

Terpenes

Terpenes, also known as isoprenoids are the largest class of known secondary metabolites, containing about 50,000 identified substances (Vranova *et al*., 2012) [26]. These substances are formed by the fusion of five carbon units which have a branched backbone. These can be divided into monoterpenes (10 carbons), sesquiterpenes (15 carbons), and diterpenes (20 carbons). The largest terpenes include triterpenes, tetraterpenes and poly-terpenoids with 30, 40 and > 40 carbons respectively (Taiz and Zeiger, 2010) [10]. Terpenes are synthesized from primary metabolites by at least two different pathways: the mevalonate pathway (joining three molecules of Acetyl-CoA) and the non-mevalonate pathway, which both produce Isopentenyl diphosphate (IPP) and Dimethylallyl diphosphate (DMAPP). Terpenes show important functions on growth and development of plant metabolites such as gibberellins (diterpenes) and brassinosteroids (triterpenes). Although some terpenes shows metabolic importance in various plant metabolic pathways. They are generally associated with the defense of several plants against herbivores as insects (Trapp and Croteau, 2001; Veitch *et al*., 2008) [24, 25]

Flavonoids

Flavonoids are a large family of polyphenolic compounds. With more than 4500 different representatives are known. Thus the flavanoids constitute an enormous class of various natural phenolic products. It is present in mostly the plant tissues, often in vacuoles. Flavonoids can occur as monomers, dimmers and oligomers in plants. Flavonoids comprise a diverse set of compounds and perform a wide range of functions. Specific flavonoids can also provide protection to plants against UV-B irradiation. The flavonoids consist of various groups of plant metabolites which include chalcones, aurones, flavanones, isoflavonoids, flavones, flavonols, leucoanthocyanidins, Catechins and anthocyanins. Many flavonoids have been known to have antioxidant, anti-inflammatory and anti-tumour property. In one experimental study it was found that these secondary metabolites were also shows the antibacterial activity. The Phytochemical flavonoids were found in the aqueous plant extract of *Zapoteca portoricensis* leaves. These secondary metabolites could take part in the anti- *Pseudomonas aeruginosa* activity (Agbafor *et al*., 2011) [11].

Tannins

Tannins are polymeric phenolic substances found almost in all plant parts. In previous studies, tannins have been known to display different biological activities including antifungal and antibacterial (Carson and Hammer, 2010; Savoia, 2012; Ramawat, 2007) [4, 21, 7]. For occurrence, the antibacterial activity of ethanol and aqueous stem bark extracts from *Psidium guajava* (Myrtaceae) was evaluated against *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella sp.* (Abdulhamid *et al*., 2014) [1]. Tannins are present in high concentrations for both the ethanol and aqueous extracts. Ethanol and aqueous extract of the bark of *Psidium guajava* shows antimicrobial activities against all microorganisms tested. However, the ethanol extract displayed larger zones of inhibitions than the aqueous extract for the most susceptible microorganism being *S. Faecalis* and the least susceptible to *Salmonella sp*.

Anthocyanins

Anthocyanins are one of the greatest importance flavonoids studied. Atphysiological level, it takes part mainly in the plant-pollinator-dispersers interaction, due to its ability to provide colour to plants. These compounds are essential for plants to shows tolerance against cold and pathogens (Sivankalyani *et al*., 2016) [22]. Although when it present in the human diet may act as antioxidant (Homoki *et al*., 2016) [9].

Lignins

Lignins are valuable in plants sustaining, due to its competence to provide greater rigidity to the plant cell wall. Although several synthetic polymers have wide range of applications, crude oil for its manufacture have resulted in different environmental impacts, despite the fact scientists find alternative raw materials for this purpose (Obaid *et al*., 2016) [14]. Lignins have specific structure which makes it different from other polymers. Now it is becoming an environmentally friendly substance with large number application in the industry such as manufacturing of moulds, thermoplastic reinforcement and integration with natural fibers to obtain the most varied materials (Saheb *et al*., 1999) [20].

Production of secondary metabolites by using tissue culture techniques

The field of plant tissue culture technology has resulting in the production of wide range of pharmaceutical substances for new therapeutics. Advancement in the field of plant cell cultures for the production of secondary compounds has made possible the production of a wide variety of pharmaceuticals consignment like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids, and amino acids. Successful attempts to produce some of these valuable pharmaceutical relatively in large quantities by cell cultures are demonstrated. Large-scale production of secondary compounds by plant tissue culture is found to be an attractive alternative approach as compared to traditional methods of plantation as it offers a controlled supply of biochemical independent of plant availability. Kieran *et al*. (1997) [12] shows the impact of genetic engineering related factor on cell suspension cultures. Currently advancement in plant tissue culture technology directs that transcription factors are new molecular tools for plant metabolic engineering to increase the production of valuable compounds fig. 1.

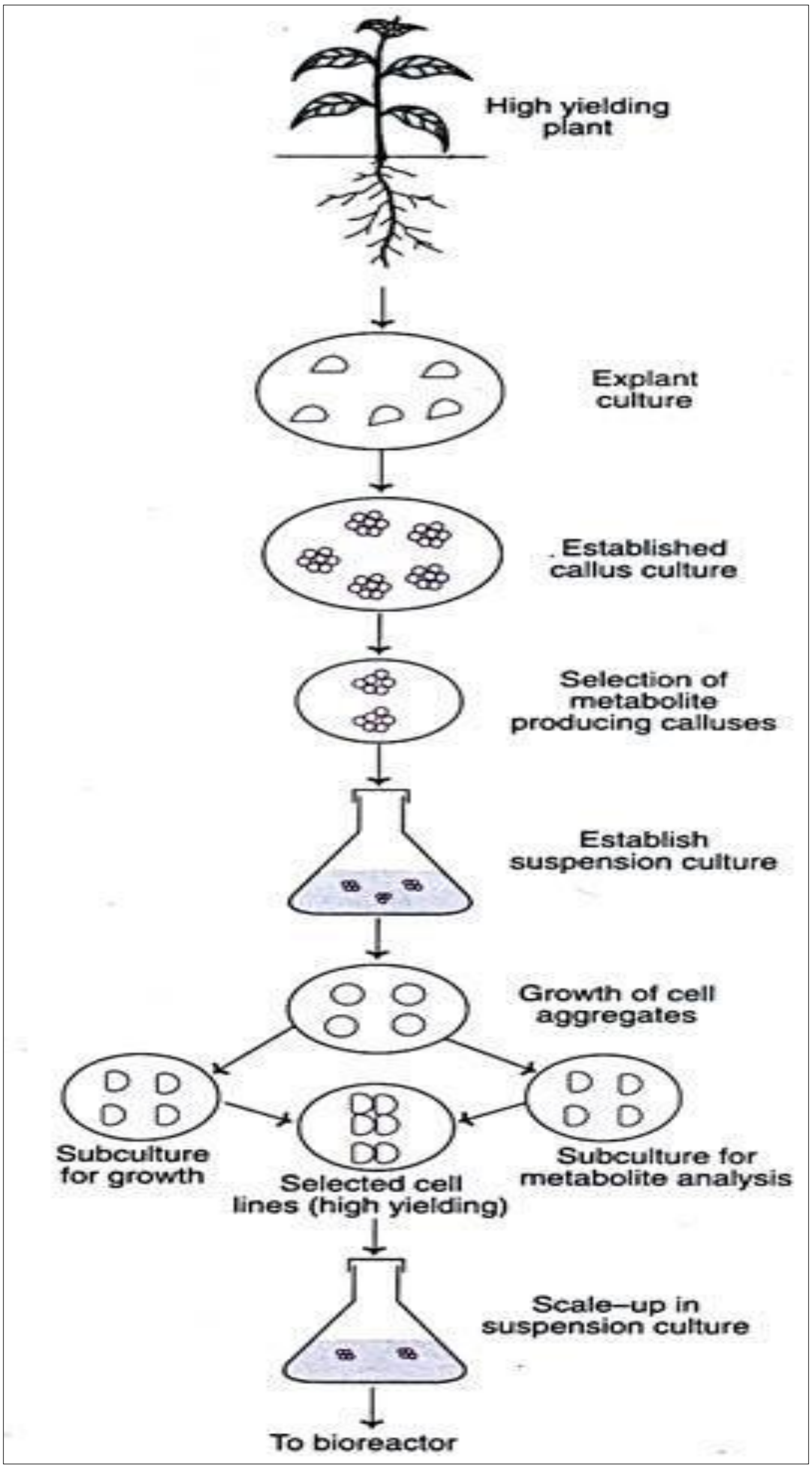

Fig 2: Shows production of secondary metabolites from plants cell**.**

Secondary metabolite production using genetically-engineered plant cell cultures

Genetic manipulation of cells cultures has great potential for altering the metabolic profile of plants, thereby making them profitable to the industry. Transgenic cell cultures hold promise for production of flavour components, food additives and colours. Identification of key regulatory enzymes of secondary metabolic pathways is necessary in order to manipulate them in culture cells generated cell suspension cultures from transgenic plants over – expressing bean PAL and alfalfa C_4 transgenic in tobacco which correspondingly increased the level of phenolic compounds with time in culture. Genetic engineering of metabolic pathways in plants is particularly applicable to such case where elicitation fails to provide the necessary specificity. Attempts at altering phenyl propanoids pathways flux by genetic engineering using plants cell culture (Dixon and Paiva, 1995) [6].

Table 1: List of some elicitor-induced secondary metabolite production in plant cell cultures.

Elicitor microorganism	Plant cell cultures	Secondary metabolites
Aspergilus niger	*Cinchona ledgeriana. Rubia tinctoria*	Anthraquinones
Alternaria sp	*Phaseolus vulgaris*	Phasolin
fusarium sp	*Apium graveolens*	Furocoumains
Dendryphion sp	*Papaver somniferum*	Sanguinarine
Penicillium expansum	*Sanguinaria canadensis*	Benophenanthridine

Evidence for evolutionary theory

The primary metabolites are the precursors of many secondary metabolite biosynthesis provides the starts of evidence for evolution. As primary metabolite are source of foundation for many known secondary metabolites. These are the acetate C2 unit (leading to polyketides and fatty acids), the phenylalanine/tyrosine derived C9 unit (leading to phenylpropanoids), the C5 isomeric units isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), and some amino acids. In plants, the terpenoid and phenylpropanoid pathways are predominant in the production of secondary metabolites, while in microorganisms the polyketide pathways is particularly well developed. The biosynthesis of phenylalanine and tryptophan, both involved in secondary metabolism, the shikimate pathway is also involved in some secondary metabolite biosynthetic pathways, with its end product chorismate. Secondary metabolites can be broadly classified into three main molecular families: the phenolics (including products from C2, C5, C91C2 pathways), terpenes (C10, C15, C20, C30, C40), and alkaloids, which are main key source of many products. There is a moderate evolutionary development of plant metabolic routes for the production of many classes of secondary metabolites, tissue types and organs. The metabolic pathways and traits appear to be conserved with the propensity of new pathways to be continuously built upon existing pathways and this provides the second point of evidence for evolution. For example, the production of chlorophyll appears to be conserved in aerial plants, evolved in photosynthesis.

The enzymes involved in the generation of secondary metabolites have also arisen as gradually adaptation of the primary enzymes themselves. The existing metabolic enzymes can be traced through ancestral proteins, and this gives rise to the conclusion that there was no emergence of new enzymes but there variation must occurs in the existing structures. This will leads to various examples of convergent, parallel, and divergent evolution can be seen in plant metabolic pathways and such examples include major enzymes such as cytochrome P450(CYP) enzymes, which a play a important role in the synthesis of various compounds including lignins, having relations to ancestral primary metabolic enzyme sterol 14-demethylase. Chalcone synthase, an enzyme involved in flavonoid synthesis in plants, is another example as it shares the same protein folds as the fatty acid synthesis enzyme β ketoacyl ACP synthase III. It is also shows that catalytic activity of plant enzymes, i.e., the ability to lodge many substrates of evolutionary diversity and hence explain the ability to evolve in new pathways which are responsible for environmental challenges.

Recent trends and future prospective of secondary metabolites

Precursor addition for improvement of secondary metabolite production

The accumulation of secondary metabolites in plants is part of the defense response against pathogenic attack, which is triggered and activated by elicitors, the signal compounds of plant defense responses. Therefore, the treatment of plant cells with biotic and/or abiotic elicitors has been a useful strategy to enhance secondary metabolite production in cell cultures. The most frequently used elicitors in previous studies were fungal carbohydrates, yeast extract, MJ and chitosan. MJ, a proven signal compound, is the most effective elicitor of taxol production in *Taxus chinensis* Roxb. and ginsenoside production in *Panax ginseng* C.A. Meyer cell/organ culture. In the present study, the effect of different concentrations of MJ on embryogenic cell growth and eleutheroside accumulation was tested and results revealed that addition of 200 _M MJ was suitable for optimum accumulation of eleutheroside B, E, E1 and chlorogenic acid. However, addition of MJ at higher concentration (above 100 _M) was detrimental for biomass accumulation. The involvement of amino acids in the biosynthesis of hyperforin and adhyperforin was reported in *H perforatum* shoot cultures. Valine and isoleucine, upon administration to the shoot cultures, were incorporated into acyl side chain of hyperforin and adhyperforin, respectively. Feeding the shoot cultures with unlabelled lisoleucine at a concentration of 2 mM induced a 3-7-fold increase in the production of adhyperforin. The addition of 3 mM threonine, a precursor of isoleucine, stimulated a 2-fold increase in the accumulation of adhyperforin. Production of triterpenes in leaf derived callus and cell suspension cultures of Centella asiatica was enhanced by the feeding of amino acids. In the callus culture manifold increase of asiaticoside accumulation was reported with the addition of leucien.The effect of coniferyl alcohol as a precursor of flavonolignan biosynthesis on silymarin components production in *Silybum marianum* suspension culture was reported. Coniferyl alcohol showed the changes in silymarin complex production. A significant increase of silydianin was observed only after 72 hrs of the application of 46 _M coniferyl alcohol. The same precursor coniferyl alcohol in the form of complex with cyclodextrin was used as precursor for podophyllotoxin accumulation in Podophyllum hexandrum cell suspension cultures.

Hairy root cultures as a source of secondary metabolites

The hairy root system based on inoculation with *Agrobacterium rhizogenes* has become popular in the two last decades as a method of producing secondary metabolites synthesized in plant roots. Unorganized plant tissue cultures are frequently unable to produce secondary metabolites at the same levels as the intact plant. This is also the case of scopolamine production in undifferentiated *in vitro* cultures of Solanaceae, probably due to the specific location of some of the key enzymes involved in this biosynthetic pathway have demonstrated that the expression of the *pmt* gene was pericycle-specific, and it has also been shown that H6H is localized in the root pericycle. In addition, have observed that TR proteins accumulate in the lateral roots of *Hyoscyamus niger*. Another possible reason for the low production of scopolamine in undifferentiated *in vitro* cultures could be that the auxin added to the callus and cell culture media for normal growth inhibit the activity of some of the key enzymes involved in scopolamine biosynthesis, such as PMT.

Bioreactors scaling up of production of secondary metabolites

This is the application of bioreactor system for large-scale cultivation of plant cells for the production of valuable bioactive compounds in an active field. Plant cells in liquid suspension offer a unique combination of physical and chemical environments that must be accommodated in large-scale bioreactor process. Some of the well known drawbacks of the cell suspension cultures include the instability of the productive cell lines, the slowness of the cell growth and limited knowledge about the metabolic pathway. Sufficient oxygen supply and proper mixing in airlift bioreactors may not be suitable for high density plant cell suspension cultures. Well known problem shear sensitivity and rapid setting characteristics of plant cell aggregates and cell floating tendencies of the cell cultures have to be solved when bioreactors are designed. The main constraint for commercial exploitation of *in vitro* cultures is the scaling up at industrial level. Hairy roots, callus and suspension cultures are complicated when it comes to scaling up and pose unique challenges. Mechanical agitation causes wounding of hairy roots and leads to callus formation. With a product of sufficiently high value it is feasible to use batch fermentation, harvest the roots, and extract the product. For less valuable products it may be desirable to establish a packed bed of roots to operate the reactor in a continuous process for extended periods collecting the product from the effluent stream. Scale up becomes difficult in providing nutrients from both liquid and gas phases simultaneously. Meristem dependent growth of root cultures in liquid medium results in a root ball with young growing roots on the periphery and a core of older tissue inside. Restriction of nutrient oxygen delivery to the central mass of tissue gives rise to a pocket of senescent tissue. Due to branching, the roots from an interlocked matrix that exhibits a exploit hairy root culture as a source of bioactive chemicals depends on development of suitable bioreactor system where several physical and chemical parameters must be taken into consideration.

Perspectives in human health applications

The use of natural compounds as inhibitory agents for virulence factor production is a new approach to overcome increased antimicrobial resistance in pathogenic bacteria [Niraula *et al*., 2010] [13]. Medicinal plants are the most exclusive source of lifesaving drugs for majority of the world's population. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past decades. The secondary metabolites are known to play a major role in the adaptation of plants to their environment and also represent an important source of pharmaceuticals. Increasing epidemiological evidence associates diets rich in fruits and vegetables with reduced risk of heart disease, cancer, and other chronic diseases. A major benefit from such a diet may be increased consumption of antioxidants, including carotenoids, ascorbate, tocopherols, and phenolics. fraction, fl avonoids, are potent antioxidants and include compounds such as flavones, isofl avones, flavonones, catechins, and the red, blue and purple pigments known as anthocyanins [Cote *et al*., 2010] [5]. Dietary flavonoids (quercetin, kaempferol, and isorhamnetin) possess antiviral, anti-infl ammatory, antihistamine, and antioxidant properties. They have been reported to inhibit lipid peroxidation, to scavenge free radicals, to chelate iron and copper ions (which can catalyze production of free radicals), and to modulate cell signaling pathways [Cote *et al*., 2010] [5]. Production of peroxides and free radicals, which damage lipids, proteins, and DNA, has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases (Parkinson and Alzheimer). Flavonoids protect low-density lipoprotein cholesterol from being oxidized, preventing the formation of atherosclerotic plaques in the arterial wall. They stimulate enzymes involved in detoxifi cation of cancerogenic substances and inhibit inflmammation associated with local production of free radicals [Hounsome *et al*., 2008; Aires *et al*., 2009] [10, 2]. There are also reports about the usage of alkaloids in pharmaceuticals and about *Rauwolfia canescens* being a central nervous stimulator. Dietary saponins cause a reduction of blood cholesterol, inhibit growth of cancer cells, and stimulate the immune system. Some saponins, such as sapotoxin, can be toxic for humans, causing irritation of membranes of the respiratory and digestive tract, and increase the membrane permeability of red blood cells and urticaria (skin rash). A key to the evaluation of strategies to improve productivity is to carry out that all the problems must be seen in a holistic context. At any rate, substantial progress in improving secondary metabolite production from plant cell cultures has been made within last few years. These new technologies will serve to extend and enhance the continued usefulness of higher plants as renewable sources of chemicals, especially medicinal compounds. We hope that a continuation and intensify cation efforts in this field will lead to controllable and successful biotechnological production of specific, valuable, and as yet unknown plant chemicals.

Application of secondary metabolites

Secondary metabolites are important characteristic feature of plants. It can protect the plants from wide variety of microorganisms (viruses, bacteria, fungi) and herbivores (arthropods, vertebrates). All the defense systems of plants and animals provide protections at different developmental stages. A few specialized pathogens have evolved in plants; have overcome the chemical defense barrier including antibiotics which are produced in nature. Secondary metabolites serve (I) as competitive weapons used against other bacteria, fungi, amoebae, plants, insects, and large animals (ii) Use as metal transporting agents (iii) Use as agents of symbiosis between microbes and plants, nematodes, insects, and higher animals (IV) Act as sexual hormones (v) Behave as differentiation effectors. Although antibiotics are not obligatory for sporulation, some secondary metabolites (including antibiotics) stimulate spore formation and inhibit or stimulate germination. Formation of secondary metabolites and spores are regulated by similar factors. Thus the secondary metabolite can (I) Slow down germination of spores until a less competitive environment and more favourable conditions for growth exist(ii) Protection against the dormant or initiated spore from consumption by amoebae(iii) Wipe out the immediate environment of competing microorganisms during germination.

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