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### Secondary Metabolites of Plants and their Role: Overview

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### Abstract

Secondary metabolites (SM) are compounds that are not necessary for a cell (organism) to live, but play a role in the interaction of the cell (organism) with its environment. These compounds are often involved in plants protection against biotic or abiotic stresses. Secondary metabolites are from different metabolites families that can be highly inducible in response to stresses. Primary metabolites perform essential metabolic roles by participating in nutrition and reproduction. A few SMs are used as especially chemical such as drugs, flavours, fragrances, insecticides, and dyes and thus have a great economic value. These new technologies will serve to extend and enhance the continued usefulness of the higher plants as renewal sources of chemicals, especially medicinal compounds. A continuation and intensification efforts in this field is expected to lead to successful biotechnological production of specific, valuable and as yet unknown plant chemicals.

**Keywords:** Secondary metabolites, drugs, flavours, fragrances, biotechnology

### Introduction

Plants possess capacity to synthesize different organic molecules called secondary metabolites. Unique carbon skeleton structures are basic properties of plant secondary metabolites. Secondary metabolites are not necessary for a cell (organism) to live, but play a

role in the interaction of the cell (organism) with its surroundings, ensuring the continued existence of the organism in its ecosystems. Formation of SMs is generally organ, tissue and cell specific and these are low molecular weight compounds. These compounds often differ between individuals from the same population of plants in respect of their amount and types. They protect plants against stresses, both biotic (bacteria, fungi, nematodes, insects or grazing by animals) and abiotic (higher temperature and moisture, shading, injury or presence of heavy metals). SMs are used as especially chemical such as drugs, flavours, fragrances, insecticides, and dyes by human because of a great economic value.

In plants, SMs can be separated into three groups (Terpenoids, Polyketides and Phenypropanoids) based on their biosynthesis origin (1). Alkaloids are additional class of SMs, which are nitrogenous organic molecules biosynthesized mainly from amino-acids, e.g., tryptophan, tyrosine, phenylalanine, lysine and arginine using many unique enzymes (2). Many of the most important therapeutic agents are alkaloids. The sites of biosynthesis are compartmentalised at cellular or sub-cellular level. However SMs can be transported long distances and accumulate from their location of synthesis.

**Primary Vs Secondary Metabolites :** Primary metabolites are found in all plants and execute vital metabolic responsibilities, by participating in

nutrition and reproduction (2). Sometimes it is hard to discriminate primary and secondary metabolites. For example, both primary and secondary metabolites are found among the terpenoids and the same compound may have both primary and secondary roles. Secondary metabolites are broad range of compounds from different metabolite families that can be highly inducible in stress conditions. Carotenoids and flavonoids are also involved in cell pigmentation in flower and seed, which attract pollinators and seed dispersers. Therefore, they are also involved in plant reproduction (3). Plant primary products refer to the compounds of nucleic acids, proteins, carbohydrates, fats and lipids and are related to structure, physiology and genetics, which imply their crucial role in plant development. In contrast, secondary metabolites usually take place as minor compounds in low concentrations. Primary metabolism refers to the processes producing the carboxylic acids of the Krebs cycle. Secondary metabolites, on the other hand, are non-essential to life but contribute to the species' fitness for survival. In fact, the specific constituents in a certain species have been used to help with systematic determination, groups of secondary metabolites being used as markers for botanical classification (chemotaxonomy). Plants secondary metabolites can be divided into three chemically distinct groups viz: Terpenes, Phenolics, N (Nitrogen) and S (sulphur) containing compounds.

*I)Terpenes :* Terpenes comprise the biggest group of secondary metabolites and are free by their common biosynthetic origin from acetyl-coA or glycolytic intermediates. An immense bulk of the diverse terpenes structures produced by plants as secondary metabolites that are supposed to be concerned in defense as toxins and feeding deterrents to a large number of plant feeding insects and mammals. Terpenes are divided into monoterpenes, sesquiterpenes, diterpene, Triterpenes and polyterpenes. The pyrethroid (monoterpenes esters) occur in the leaves and flowers of Chrysanthemum species show strong insecticidal responses to insects like beetle, wasps, moths, bees, etc and a popular ingredient in commercial insecticides because of low persistence in the environment and low mammalian toxicity. In Gymnoperms (conifers) á-pinene, â-pinene, limonene and myrecene are found. A number of sesquiterpenes have been till now reported for their role in plant defense such as costunolides are antiherbivore agents of family composite characterized by a five member lactone rings (a cyclic ester) and have strong feeding repellence to many herbivorous, insects and mammals. ABA is also a sesquiterpene plays primarily regulatory roles in the initation and maintenance of seed and bud dormancy and plants response to water stress by modifying the membrane properties and act as a transcriptional activator (4). Abietic acid is a diterpene found in pines and leguminous tress. It is present in or along with resins in resin canals of the tree trunk . Another compound phorbol (Diterpene ester), found in plants of euphorbiaceae and work as skin irritants and internal toxins to mammals. The milkweeds produce several better tasting glucosides (sterols) that protect them against herbivors by most insects and even cattle. Several high molecular weight polyterpenes occur in plants. The principal tetraterpenes are carotenoids family of pigments.

(II) Phenolic compounds : Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group on an aromatic ring called Phenol, a chemically heterogeneous group also. They could be an important part of the plants defence system against pests and disease including root parasitic nematodes (5). Elevated ozone (mean 32.4ppb) increased the total phenolic content of leaves and had minor effects on the concentration of individual compounds (6).Coumarin are simple phenolic compounds widespread in vascular plants and appear to function in different capacities in various plant defense mechanisms against insect herbivores and fungi. They derived from the shikimic acid pathway, common in bacteria, fungi and plants but absent in animals

(7). Some coumarin derivatives have higher antifungal activity against a range of soil borne plant pathogenic fungi and exhibit more stability as compared to the original coumarin compounds alone (7). Furano is Also a type of coumarin with special interest of phytotoxicity, abundant in members of the family umbelliferae including celery parsnip and parsley. Psoraline, basic linear furacoumarin, known for its use in the treatment of fungal defence and found very rarely in SO, treated plants (8). Ligin is a highly branched polymer of phenyl- propanoid groups, formed from three different alcohols viz., coniferyl, coumaryl and synapyl which oxidized to free radical (ROS) by a ubiquitous plant enzymeperoxidises, reacts simultaneously and randomly to form lignin. Its physical toughness deters feeding by herbivorous animals and its chemical durability makes it relatively indigestible to herbivorous and insects pathogens. Lignifications block the growth of pathogen and are a frequent response to infection or wounding. Flavanoids perform very different functions in plant system including pigmentation and defence. Two other major groups of flavanoids found in flowers are flavanones and flavanols function to protect cell from UV-B radiation because they accumulate in epidermal layers of leaves and stems and absorb light strongly in the UV-B region while letting visible (PAR) wavelengths throughout uninterrupted (9). In addition exposure of plants to increased UV-B light has been demonstrated to increase the synthesis of flavanones and flavanols suggesting that flavanoids may offer measures of protection by screening out harmful UV-B radiation (6). Isoflavanoids are derived from a flavanones intermediate, naringenin, ubiquitously present in plants and a play a critical role in plant developmental and defence response. They secreted by the legumes and play an important role in promoting the formation of nitrogen fixing nodules by symbiotic rhizobia (10). Moreover, it seems that synthesis of these flavanoids is an effective strategy against reactive oxygen species (ROS). The analysis of activity of antioxidant enzymes like SOD, CAT, POX, APX, GPX and GR suggested that peroxidases

were the most active enzymes in red cabbage seedlings exposed to Cu<sup>++</sup> stress (11).Tannins included in the second category of plant phenolic polymers with defensive properties. Tannins are general toxins that significantly reduce the growth and survivorship of many herbivores, and also act as feeding repellents to a great diversity of animals.

(III) Sulphur containing secondary metabolites: They include GSH, GSL, Phytoalexins, Thionins, defensins and allinin which have been linked directly or indirectly with the defence of plants against microbial pathogens (12,13,14). GSH is the one of the major form of organic sulphur in the soluble fraction of plants and has an important role as a mobile tool of reduced sulphur in the regulation of plant growth and development and as a cellular antioxidants in stress responses (15), reported as a signal of plant sulphur sufficiency that down regulates sulphur assimilation and sulphur uptake by roots.

GSL is a group of low molecular mass N (nitrogen) and S (sulphur) containing plant glucosides that produced by higher plants in order to increase their resistance against the unfavourable effects of predators, competitors and parasites because their break down products are release as volatiles defensive substances exhibiting toxic or repelient effects for example, mustard oil glucosides in cruciferae and allyl cys sulfoxides in alllum (16). They are metabolised and absorbed as isothiocyanates that can affect the activity of enzymes involved both in the antioxidant defence system and in the detoxification from zenobiotics abd significantly affect GST activity and cell protection against DNA damage (17) whereas toxicity of glucosinolatic products is well documented but their mode of action has not yet been elucidated and results from experiments with Brassica plants modified in GSL content generated doubts about their contribution to plant defences.

Phytoalexins are synthesized in response to bacterial or fungal infection or other forms of stress that help in limiting the spread of the

invading pathogens by accumulating around the site of infection, appears to a common mechanism of resistance to pathogenic microbes in a wide range of plants. Many of these changes are linked to a rapid apoptotic response, resulting in death of one or a few invaded plant cells, known as the hypersensitive response (HR). Most plant families produce organic phytolexins of diverse chemistry; these groups are often associated with a family, for example sesquiterpenoids of Solanaceae, isoflavonoids of Leguminosae, while phytoalexins from Brassica have an indole or related ring system and one S atom as common structural features. Crucifereae appears to be the only plant family producing these S metabolites, which are clearly different from the other well- known GSL.Cruciferous crops are cultivated worldwide because they are extremely valuable and for the last decades, various research groups have investigated cruciferous phytoalexins as well as their biological activity. Typically, there are multiple responses involving several related derivatives such as up to nine wyerone (Furano-acetylenic derivatives) forms in Vicia fava and several forms of phaseollin in Phaseolus vulgaris and glyceollin in Glycine max, postin in Pisum sativum pods, Ipomearone in sweet pototo, orchinol in orchid tubers, trifolirhizin in red clover. Defensins, thionins and lectins are S-rich non-storage plant proteins synthesize and accumulate after microbial attack and such related situations. They inhibit growth of a broad range of fungi. Additionally defensins genes are partly pathogeninducible and others that are involved in resistance can be expressed constitutively. Some plant species produce lectins as defensive proteins that bind to carbohydrates or carbohydres containing proteins.

(IV) Nitrogen containing secondary metabolites: They include alkaloids, cyanogenic glucosides, and non-proteins amino-acids. Most of them are biosynthesized from common amino-acids. Alkaloids found in approximately 20% of the species of vascular plants, most frequently in the herbaceous dicot and relatively a few in

monocots and gymnosperms. Generally, most of them, including the pyrrolizidine alkaloids (PAs) are toxic to some degree and appear to serve primarily in defense against microbial infection and herivoral attack. Cyanogenic glucosides constitue a group of N-containing protective compounds other than alkaloids, release the poison HCN and usually occur in members of families viz., Graminae, Roosaceae and leguminosesae. They are not themselves toxic but are readily broken down to give off volatile poisonous substance like HCN and volatile H<sub>a</sub>S when the plant crushed; their presence deters feeding by insects and other herbivorous such as snails and slugs. Amygdalin, the common cynogenic glucoside found in the seeds of almonds, apricot, cherries and peaches while Dhurrin, found in Sorghum bicolar.

Many plants also contain unusual amino acids called non-protein amino-acids that incorporated into proteins but are present as free forms and act as protective defensive substance. For examples, canavanine and azetidine-2 carboxylic acid are close analogs of arginine and proline respectively. They exert their toxicity in various ways. Some block the synthesis of or uptake of protein amino acid while others can be mistakenly incorporated into proteins. Plants that synthesized non-protein amino acid are not susceptible to the toxicity of these compounds but gain defence to herbivorous animals, insects and pathogenic microbes.

**Transport, Storage and Turnover:** SMs can be water soluble (hydrophilic) compounds or lipophilic (needs organic solvents), therefore needs different cellular mechanism for their transport, storage and turnover. Most substances are synthesized in the cytoplasm, the ER or in the organelles. Hydrophilic SMs are usually stored in the vacuole after their formation in cytoplasm, whereas lipophilic substances are sequestered in resin ducts, laticifers, glandular hairs, trichomes, thylakoid membranes or on the cuticle. Hydrophilic SMs have to pass the tonoplast, which is impermeable to many of the polar secondary metabolites. For some alkaloids

and flavanoids, a specific transporter has been described, which pumps the compounds into the vacuole. In order to avoid autotoxicity, plants cannot store these compounds in the vacuole but usually sequester them on the cuticle, in dead resin ducts or cells which are lined by a biomembrane but an impermeable solid barrier. In many instances, the site of biosynthesis is restricted to a single organ such as roots, leaves or fruits, but an accumulation of the corresponding products can be detected in several other plant tissues. Long distance transport must take place in these instances. The xylem or phloems are likely transport routes but an apoplastic transport can also be involved. Storage can also be tissue and cell- specific, depending upon the protection providing to the plants.In a number of plants, specific idioblasts have been detected that contain tannins, alkaloids or glucosinolates. More often, SMs are concentrated in trichomes or glandular hairs (many terpenoids in Labiatae, Asteraceae), stinging hairs (many amines in urticaceae) or the epidermis itself (many alkaloids, flavanoids, anthocyanins, cynogenic glycosides, coumarins, etc.) flowers, fruits and seeds are usually rich in SMs, especially in annual plants. In perennial species, high amounts of SMs found in bulbs, roots, rhizomes and the bark of roots and stems. It is well-established that profiles of SMs vary with time, space and developmental stage. Since related plant species often show similarities in the profiles of their SMs, they have been used as taxonomic tool in plant systematic. However, profiles of closely- related plants quite often differ substantially or those of unrelated plant group show strong similarities; this clearly shows that SM patterns are not unambiguous systematic markers but that convergent evolution and selective gene expression are common themes.

**Extraction of Secondary Metabolites from Plant :** Plant secondary metabolites are currently the subject of much research interest, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process. Successful extraction begins with careful selection and preparation of plant samples. During the extraction of plant material, it is important to minimize interference from compounds that may coextract with the target compounds, and to avoid contamination of the extract, as well as to prevent decomposition of important metabolites or artifact formation as a result of extraction conditions or solvent impurities. Researchers from a variety of scientific disciplines are confronted with the challenge of extracting plant material with solvents, often as a first step toward isolating and identifying the specific compounds responsible for biological activities associated with a plant or a plant extract. The impetus for this research arises largely because plants form the foundation of traditional pharmacopeias, and because many of our currently important pharmaceutical drugs are obtained from plants. Further interest arises from the growing awareness that many of the secondary metabolites of organisms, including plants, serve important biological and ecological roles, mainly as chemical messengers and defensive compounds. Investigators engaged in the isolation of secondary metabolites from plants soon discover the need for considerable laboratory finesse in the apparently routine "sample preparation" steps that convert crude plant material into an extract suitable for chemical analysis, biological testing, or chromatographic separation.

**Major Secondary Metabolite Pathways :** In plants particularly three pathways are the source of most secondary metabolites: The shikimate pathway, the isoprenoid pathway and the polyketide pathway. After the formation of the major basic skeletons, further modifications result in plant species specific compounds. The shikimate pathway is the major source of aromatic compounds. It is found in microorganisms and plants, but not in mammals, making it an interesting target for herbicides and antibiotics, as these compounds are expected not to have any effect on the mammalian system. Glyphosate is a well known example. The

enzymes channeling chorismate into the aromatic amino acids pathways are chorismate mutase and anthranilate synthase. Although, in several plant species for both chorismate mutase and anthranilate synthase more than one gene has been cloned, only in case of chorismate mutase a plastidial and a cytosolic enzyme have been found. The phenylpropanoid pathway is one of the most important metabolic pathways in plants in terms of carbon flux. In a cell more than 20% of the total metabolism can go through this pathway, the enzyme chorismate mutase is an important regulatory point. The importance of this pathway is due to the fact that it leads to among others lignin, lignans, flavonoids, and anthocyanins. Key to these products is the enzyme phenylalanine ammonia lyase (PAL), which converts phenylalanine into trans-cinnamic acid by a non-oxidative deamination. This enzyme can be found in all plants, in some plants a single enzyme is found, whereas others may have several iso-enzymes. The other important pathway in plants is that of the terpenoids, also known as isoprenoid pathway. Terpenoids include more than one third of all known secondary metabolites. Moreover, the C5-building block is also incorporated in many other skeletons, e.g. in anthraquinones, naphtoquinones, cannabinoids, furanocoumarines, and terpenoid indole alkaloids. In the "decoration" type of reactions in various types of secondary metabolites C5-units are attached to the basic skeleton, e.g. hop bitter acids, flavonoids and isoflavonoids.

**Functions of Secondary Metabolites :** Many secondary compounds have signalling functions influence the activities of other cells, control their metabolic activities and co-ordinates the development of the whole plant. Other substances such as flower colours serve to communicate with pollinators or protect the plants from feeding by animals or infections by producing specific phytoalexines after fungi infections that inhibit the spreading of the fungi mycelia within the plant (18). Plants use secondary metabolites (such as volatile essential

oils and colored flavonoids or tetraterpenes) also to attract insects for pollination or other animals for seed dispersion, in this case secondary metabolites serve as signal compounds. Compounds belonging to the terpenoids, alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases (19) and in particular some of these compounds seem to be efficient in preventing and inhibiting various types of cancer (20, 21). It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use of the plants (22). Secondary metabolites are a metabolic intermediates or product, found as a differentiation product in restricted taxonomic groups, not essential to growth and life of the producing organism and biosynthetized from one or more general metabolites by wider variety of pathways than is available in general metabolism.

Presence of volatile monoterpenes or essential oils in the plants provides an important defense strategy to the plants, particularly against herbivorous insect pests and pathogenic fungi. These volatile terpenoids also play a vital role in plant-plant interactions and serve as attractants for pollinators (23). They act as signalling molecules and depict evolutionary relationship with their functional roles. Soluble secondary compounds such as cyanogenic glycosides isoflavoids and alkaloids can also be toxic to animals.

**Biotechnology and Secondary Metabolites :** Since SM have evolved as compounds that are important for the ûtness of the organisms producing them, many of them interfere with the pharmacological targets, which make them interesting for several biotechnological applications. Controlled clinical studies have shown the efficacy of several, for example extracts from *Ginkgo biloba*, *Hypericum perforatum*, *Piper methysticum*, *Chamomilla recutita*, *Crataegus monogyna*, *Silibum* 

# marianum, Melissa ofûcinalis, Mentha piperita, Valeriana ofûcinalis.

The use of stimulants (such as caffeine, nicotine, ephedrine), fragrances (several essential oils), ûavours (essential oils, capsaicin, piperine, etc.), natural dyes, poisons (strychnine) and hallucinogens (morphine, heroin, cocaine, tetrahydro cannabinol) is based on SM. Since many SM are insecticidal, fungicidal and phytotoxic, they may be used in agriculture as natural plant protectants. Before the advent of synthetic pesticides about 60 years ago, plantderived insecticides (including nicotine, rotenone, quassin, ryanodine, pyrethrins and azadirachtins) were a common theme. Applications unequivocally showed that these natural insecticides worked. One ecological advantage is that SM are readily degraded in plants and in soil, is also their disadvantage and synthetic pesticides are more resistant and persistent. Moreover, modern pesticides are usually more potent than biopesticides. On the other hand, plants are easy to grow and biopesticides could be a sustainable source of plant protectants for farmers in countries that do not have access to Western synthetic pesticides. Unfortunately, legislation does not favour mixtures of compounds to be used as pesticides; therefore, the development of biorational pesticides has to face many obstacles. Nevertheless, natural compounds do provide an underexplored alternative. As a consequence of these various applications, a world market for plant extracts and isolated SM exists, which exceeds 10 billion US dollars annually. Therefore, it is a challenge for biotechnologists to find ways to produce these compounds in sufucient quantity and quality.

The main and traditional way is to grow the respective plants in the ûeld or in greenhouses and to extract the products from them. For several species, new varieties have been selected with improved yields and quality. In this context, cell and organ culture are important techniques for in vitro propagation. In a few instances, genetic engineering of secondary metabolism has already had a direct inûuence for example, when *Atropa belladonna* plants were transformed with the gene that encodes the enzymes converting L-hyoscyamine into Lscopolamine, new plants were generated which produced scopolamine as the major product. More often, ûavonoid metabolism has been altered genetically, producing plants with different ûower colours. It is a challenge for future research to isolate the genes of biosynthetic pathways and to express them either in transgenic plants or in microbes.

If successful, recombinant bacteria or yeasts might be grown someday, which will produce valuable plant SM. Combinatorial biosynthesis might then be an open field. Using genes encoding enzymes for the biosynthesis of antibiotics, this strategy has already been successful. It has also brought about renewed interest in the regulation of SM synthesis and in the location and means of sequestration of these substances within the plant. In recent years, attempts have been made to express the genes of alkaloid biosynthesis in microorganisms. Ultimately, it might be possible to produce valuable alkaloids from recombinant bacteria or yeast. If the corresponding SM (both from plant or microbial origin) confers resistance to insects or pathogens, genetic transformation of susceptible crop plants could be another valuable avenue for Exploitation. For more than two decades, scientists around the world have tried to produce valuable SM in cell or organ cultures. Whereas undifferentiated cell cultures have often failed to produce such a compound in reasonable yields, differentiated organ cultures (e.g. transformed root cultures) are often as active as the intact plant .Cell- and tissue-speciûc gene expression appears to control these processes. In addresses the production of SM in vitro (Table 1).

It is possible that genetic engineering may help to improve plant cell cultures as biotechnological production systems in the future. Table1. Secondary Metabolites from Plant Cell, Tissue and Organs Cultures

### **Plant Name**

Adhatoda vasica Agastache rugosa Ammi majus

Angelica gigas Arachis hypogaea Artemisia annua Aspidosperma ramiflorum Azadirachta indica Brucea javanica Bupleurum falcatum Camellia chinensis Capsicum annum Cassia acutifolia C. senna Catharanthus roseus

Cayratia trifoliata Centella asiatica

Drosera rotundifolia Eleutherococcus senticosus Eriobotrya japonica Fabiana imbricata Fagopyrum esculentum Fritillaria unibracteata Gentiana macrophylla Gentianella austriaca Glycyrrhiza glabra Gymnema sylvestre Hemidesmus indicus Hypericum perforatum Mentha arvensis Momordica charantia

### **Active Ingredient**

Vasine Rosmarinic acid Umbelliferone Triterpenoid Deoursin Resveratol Artemisinin Ramiflorin Azadirachtin Cathin Saikosaponins Flavones Capsiacin Anthraquinones Anthraquinone Indole alkaloids Vincristine Catharathine Stilbenes Asiaticoside 7-Methyljuglone Eleuthrosides Triterpenes Rutin Rutin Alkaloids Glucoside Xanthone Glycyrrhizin

Shoot culture(24) Hairy root(25) Shootlet(26) Suspension(27) Hairy root(28) Hairy root(29) Callus(30) Callus(31) Suspension(32) Suspension(33) Root(34) Callus(35) Callus(36) Suspension(37) Hairy root(38) Suspension(39) Suspension(40) Suspension(41) Suspension(42) Hairy root(43) Callus(44) Shoot culture(45) Suspension(46) Callus(46) Callus and Suspenson(47) Hairy root(48) Multiple shoot(49) Hairy root(50) Multiple shoot(51) Hairy root(52) Callus(53) Shoot culture(54) Multiple shoot(55) Shoot(56) Callus(57)

**Culture Type** 

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Gymnemic acid

Lupeol, Rutin

Hypericin

Terpenoid

Flavonoid

### Conclusion

This review has dealt with a small selection of plant secondary- metabolites and their potential roles in defence mechanisms and ecological adaptation, in addition to the topics we have covered. there is an enormous range of other compounds present in the plant kingdom, with a very varied distribution. Plant secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive. Secondary metabolites have important ecological functions in plants: They protect plants against being eaten by herbivores and against being infected by microbial pathogens. They serve as attractants (odor, color, taste) for pollinators and seeddispersing animals. They function as agents of plant-plant competition and plant-microbe symbioses. The ability of plants to compete and survive is therefore profoundly affected by the ecological functions of their secondary metabolites. Biotechnological approaches are also involved in production of secondary metabolites through genetic engineering process. Plant tissue culture may also play a major role for the same.

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