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BIOCHEMISTRY OF SECONDARY METABOLITES: CURRENT APPROACHES

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ABSTRACT

Natural products have provided key guidelines for new drug discovery. The search for novel natural products with interesting bioactive properties is an ongoing discipline. Thousands of bioactive compounds have the potential or have already been established as pharmaceuticals, or nutraceuticals. The development of crops for bioactive compounds production presents both research and agronomic management challenges and market-related considerations. The population susceptible to develop chronic degenerative diseases represents more than 30% of the worldwide population, so the demand for new alternatives for control of these diseases has increased considerably in recent decades. Increasing consumption of functional foods with a high content of phytochemicals (bioactive compounds) is an option to prevent or correct chronic degenerative diseases. The increasing commercial importance of these chemical compounds has resulted in a great interest in secondary metabolism, particularly the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology and metabolomics. In today's world the use of bioactive compounds derived from plants plays an important role in pharmaceutical applications. This review presents information about these metabolites and their applications as well as their importance in agronomy and bioactive effects on human health as nutraceuticals.

KEYWORDS: Secondary metabolites, Bioactive compounds, Metabolomics engineering.

Plants produce a large, diverse array of organic compounds that appear to have no direct function in their growth and development. These compounds are known as secondary metabolites, secondary products, or natural products. Secondary metabolites also differ from primary metabolites in having a restricted distribution within the plant kingdom. That is, certain secondary metabolites are only found in one plant species or related group of species, whereas primary metabolites are found throughout the plant kingdom. For many years the adaptive significance of most secondary metabolites was unknown. These compounds were thought to be simply functionless end products of metabolism, or metabolic wastes. Study of these substances was pioneered by organic chemists of the nineteenth and early twentieth centuries who were interested in them because of their importance as medicinal drugs, poisons, flavors, and industrial materials (Grindberg *et al.,* 2007; Nunnery *et al.,* 2010).

Plant secondary metabolites are categorized in three main groups based on their biosynthetic pathways: phenolic compounds, terpenes and nitrogen-containing compounds (Rea *et al.,* 2010). Studies have demonstrated that terpenes are synthesized via the mevalonic pathway from precursor acetyl-CoA, while phenolic compounds are aromatic substances formed via the shikimic acid pathway or the mevalonic pathway. Nitrogen-containing secondary metabolites such as alkaloids are synthesized primarily from aliphatic amino acids derived from the tricarboxilic acid pathway or aromatic acids derived from the shikimic acid pathway, via acetyl-CoA, mevalonic acid (Parsaeimehr *et al.,* 2011).

Terpenoids

Terpenoids constitute a large family of phytoconstituents, composed by the most important group of active compounds in plants with over than 23,000 known structures. They are polymeric isoprene derivatives and the isoprene units are linked in head and tail fashion. The number of units incorporated into a particular terpene serves as a basis for their classification.

Terpenoids are classified according to the number of isoprene units they contain; isoprene, which itself is synthesized and released by plants, comprises 1 unit and is classified as a hemiterpene; monoterpenes, sesquiterpenes, diterpenes, sesterpenes, triterpenes, and tetraterpenes. Terpenes and terpenoids are the base constituents of many types of plant essential oils. It is proven that the terpenes have great biological activities

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such as: anti-cancers, anti-microorganisms and anti-inflammatory (Liu *et al.,* 2000).Volatility of terpenoids provides a tool for communication with other organisms such as neighboring plants, pollinators and foes of herbivores, via air-bone infochemicals. From a physiological standpoint, briefly, plant volatiles are involved in three critical processes, namely plant-plant interaction, the signaling between symbiotic organisms, and the attraction of pollinating insects. Their role in these "housekeeping" activities underlies agricultural applications that range from the search for sustainable methods for pest control to the production of flavours and fragrances.

Alkaloids

Alkaloids are the group of secondary metabolites that contain basic nitrogen atoms. In addition to carbon, hydrogen and nitrogen, this group may also contain oxygen, sulfur and rarely other element such as chlorine, bromine and phosphorus. Alkaloids are produced by a large variety of organisms, such as bacteria, fungi, animals but mostly by plants as secondary metabolites. Most alkaloids are classified chemically according to the nitrogen-containing ring system (pyrrolidine, piperidine etc.) and their biosynthetic origin, amino acids, amines, cyanogenic glycosides, and glucosinolates. Alkaloids have traditionally been of great interest because of their pronounced physiological and medicinal properties (for example, caffeine, nicotine, morphine, atropine, quinine) (Khadem and Marles, 2012).

Phenolics

Phenolic compounds are secondary metabolites that encompass several classes of structurally diverse products biogenetically arising from the shikimate-phenylpropanoids flavonoids pathways (Krzyzanowska *et al.,* 2010). Plants need phenolic compounds for growth, reproduction, pigmen-tation, resistance to pathogens, resistance to influences of heavy metal-salts or in a general form to biotic and abiotic stresses (Ferrari, 2010). Phenolics can be broadly divided into non-soluble compounds such as condensed tannins, lignins, and cell-wall bound hydroxycinammic acids, and soluble phenolics such as phenolic acids, flavonoids and quinones (Krzyzanowska *et al.,* 2010).

Agriculture approaches

It is obvious from the knowledge of biochemistry that secondary metabolites have a wide range of biological activities and enormous potential for uses in agriculture that requires indepth investigation and evaluation in the context of domesticated livestock production, particularly the use of conventional antibiotics is being reduced or eliminated from the diets of food and fibre producing livestock. The complexity and breadth of the bioactivity of secondary metabolites have the potential to reduce the likelihood that microorganisms or parasites will develop resistance, and their effectiveness is such that concentrations as low as 0.1g/kg feed may be sufficient. Nevertheless, issues such as toxicity, photosensitivity, residues, taint, allergenicity and cost effectiveness still need to be addressed before these compounds will gain widespread acceptance in the agricultural industries (Acamovic and Brooker, 2005).

Biotic and abiotic stresses

Biotic and abiotic stresses are well known as inductors of different responses in plants. Stress response is initiated when plants recognize a disorder at a cellular level, activating signal transduction pathways that transmit information within individual cell and throughout plant, leading to changes in expressing many gene networks (Gorovits and Czosnek, 2007). One way to generate resistance in plants to stress conditions is the use of elicitors, because their application on plant surface activates multiple signaling pathways of intracellular defense (Bent and Mackey, 2007). Elicitors are very stable molecules that induce an immune defense response in plants. Elicitor needs to be recognized on plant by a receptor (usually a protein), which activates the expression of defense genes. There are two groups of elicitors, the biogenic and abiogenic (Spoel and Dong, 2012). Biogenic elicitors are divided into two groups, exogenous and endogenous. The exogenous elicitors are isolated from pathogens or culture medium, while endogenous elicitors are isolated from some plants (Mejía-Teniente *et al.,* 2010). On the other hand, the abiogenic elicitors are heavy metal ions, inhibitors of certain metabolic stages, UV radiation, some kinds of antibiotics and fungicides. An important elicitor is salicylic acid, a phenolic endogenous growth regulator which participates in the regulation of physiological processes in plants, and it is also important in immunity to diseases (Ferrari, 2010). It is involved in endogenous signaling and in the plant defense response against pathogens. Jasmonic acid is an important signaling molecule in plant defense responses (Dombrecht *et al.,* 2007).

Alleopathic approaches

Various secondary metabolites produced by plants and microorganisms have been considered as potential allelochemicals and to play an important role due to their bioactivity (Latkowska *et al.,* 2008). For example, the phenolic compounds are distributed in plants and very common in vegetative decomposition products, and they are important precursors of humic substances in soils (John and Sarada, 2012). Phenolic allelochemicals can also inhibit plants from absorbing nutrients from surroundings and affect the normal growth of plants. In a study of allelopathic effects of phenolic acids such as benzoic, hydrobenzoic, vanillic, and caffeic were reported to have antimicrobial and antifungal activity, probably due to enzyme inhibition. Hydroxycinnamic acid derivatives such as caffeic, chlorogenic, sinapic, ferulic, and p-coumaric acids possess strong antioxidant activity due to inhibition of lipid oxidation and scavenging reactive oxygen species (Hounsome *et al.,* 2008). Plants use a variety of mechanisms to release secondary compounds into their surrounding environment. Each of these processes may release chemicals that mediate allelopathic interactions between plants. Therefore these compounds are being important for the pharmaceutical industry (Chen *et al.,* 2009).

Biotechnological approaches

Many biotechnological strategies have been hypothesized and used to enhance the production of secondary metabolites in plants such as: high yielding cell line screening, optimization of cultivation media, biosynthesis pathways engineering, usage of elicitors, large scale cultivation in bioreactor system, root culture, plant cell immobilization, and biotransformation (Peterhansel *et al.,* 2008). Several strategies have been followed to improve yields of secondary metabolites in plant cell cultures such as: the culturing of differentiated cells *i.e.,* shoots, roots and hairy roots, and induction by elicitors and metabolic engineering (Parsaeimhr *et al.,* 2011). In most cases the cultures of differentiated cells have been able to get productions of the desired compounds in levels comparable to the mother plant. However the culture of such differentiated tissues on a large-scale in bioreactors is a major constraint, but for studies of the secondary metabolites biosynthesis such systems are very useful (Niraula *et al.,* 2010). Plant cell cultures provide an excellent system to study biosynthesis of secondary metabolites for the large scale production of these compounds, but unfortunately in most cases production is too low for commercialization, therefore advances in biotechnology particularly in plant cell cultures methods, should provide new means for the commercial processing of even rare plants and the chemicals they provide (Du *et al.,* 2010).

Metabolomics engineering

Metabolic engineering involves the targeted and purposeful alteration of metabolic pathways found in an organism to achieve better understanding and use of cellular pathways for chemical transformation, energy transduction, and supramolecular assembly. This technique applied to plants will permit endogenous biochemical pathways to be manipulated and results in the generation of transgenic crops in which the range, scope, or nature of a plant's existing natural products are modified to provide beneficial commercial, agronomic, and/or postharvest processing characteristics (Du *et al.,* 2010). Metabolic profiing determines the consequences of a targeted change in gene activity and has the potential to provide information on gene function and its effects on the complex biochemical network (Vogt, 2010). Metabolomics is increasingly being used in a variety of health applications including pharmacology, pre-clinical drug trials, toxicology, transplant monitoring, newborn screening and clinical chemistry (Du *et al.,* 2010).

Pharmaceutical Approaches

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 (Krzyzanowska *et al.,* 2010). The demand for natural products has increased in recent years because of limitations in the process of obtaining drugs based on chemical synthesis (Vogt, 2010). Many carotenoids present in chili peppers, particularly capsanthin and β-carotene, have shown a strong antioxidant activity, and some others, like the yellow/orange β-cryptoxanthin, and β-carotene are vitamin A precursors having the highest activity. Furthermore, the antioxidant properties of carotenoids appear to provide protection against several heart diseases and different types of cancer (Rodriguez-

Burruezo *et al.,* 2009). On the other hand, bioactive compounds not only increased interest in soybean products but also led to the incorporation of soybean isoflavone extracts into a range of commercial functional foods and to the development of numerous non-prescription food supplements known as nutraceuticals. Isoflavones have been demonstrated to have antiatherosclerotic, antioxidative, antitumorial, and antiestrogenic properties that can be used in the treatment of important diseases such as cancer, heart and kidney diseases (Cheng *et al.,* 2010).

Human health applications

The consumption of antioxidants in foods is key to maintenance of human health because the body lacks adequate levels of biochemical compounds to provide sufficient protection against the constant and inevitable formation of reactive oxygen species, which are powerful oxidants (Vanamala *et al.,* 2006). Antioxidants are secondary metabolites present in plants, particularly fruits. Antioxidants are associated with a reduced rate of heart disease mortality and incidents of mouth, pharynx, esophagus, lung, stomach and colon cancers, and other degenerative diseases and aging (Bhattacharya *et al.,* 2010). The most important natural antioxidants present in green chilli are vitamin C, carotenoids, and phenols. Chilli pepper is reported to contain moderate to high levels of phenolics or flavonoids, phytochemicals that are important antioxidant components of a plant-based diet. Moreover, the metalloenzyme, superoxide dismutase, which is universally present in all plants and imparts defense against oxidative stress, converts superoxide radical anion into hydrogen peroxide (Matsufuji *et al.,* 2007).

CONCLUSIONS

Plant secondary metabolites have multiple functions throughout the plants life cycle. Interestingly, plant secondary metabolites have a great potential for industry, agriculture, medicine, and food sciences. Therefore, the understanding of their biosynthetic pathways is of a great significance for fundamental and applied sciences. With the development of science and technology the research methods to study biosynthetic pathways of plant secondary metabolites clearly evolved, but there are still some pitfalls. Plant secondary metabolism genetic engineering has developed rapidly in recent years, demonstrating the broad application possibilities. However, the diversity of plant secondary metabolites coupled with the complexity of related enzymes and the regulation of gene expression increase the difficulty of the secondary metabolic engineering. In future, all of the secondary metabolism-related enzymes will be characterized through the transcriptome sequencing, which will lay a solid foundation for the metabolic engineering.

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