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***REVIEW PAPER***

# Role of Secondary Metabolites in Defense Mechanisms of Plants

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In natural systems, plants face a plethora of antagonists and thus posses a myriad of defense and have evolved multiple defense mechanisms by which they are able to cope with various kinds of biotic and abiotic stress. Generally, it is difficult to assign a change in the physiology of metabolism of the crop to a specific stress factor as normally a complex variety of various stress factors affects the plant simultaneously. However, there are inter-connections that exist between distinct and opposing signaling response pathways for defense against pathogens and insect herbivores and there also appear to be multiple response pathways invoked, depending on the specific stress context (Kusnierczyk *et al*., 2007). Besides antimicrobial nature, some of which are performed and some of which induced by infection. There are various other modes of defense include the construction of polymeric barriers to pathogen penetration and the synthesis of enzymes that degrade pathogen cell wall (Hammond *et al*., 1996). In addition, plants employ specific recognition and signalling systems enabling the rapid detection of pathogen invasion and initiation of vigorous defensive responses (Schaller *et al*., 1996).

**Plant defense against pathogens**

Even though they lack an immune system, plants are surprisingly resistant to diseases caused by the fungi, bacteria, viruses, and nematodes. Some defenses are induced by herbivore attack or microbial infection. Defenses that are produced only after initial herbivore damage theoretically require a smaller investment of plant resources than defenses that are always present, but they must be activated quickly to be effective.

After being infected by a pathogen, plants deploy a broad spectrum of defenses against invading microbes. A common defense is the hypersensitive response, in which cells immediately surrounding the infection site die rapidly, depriving the pathogen of nutrients and preventing its spread. After a successful hypersensitive response, a small region of dead tissue is left at the site of the attempted invasion, but the rest of the plant is unaffected. The hypersensitive response is often preceded by the production of reactive oxygen species. Cells in the vicinity of the infection synthesize a burst of toxic compounds formed by the reduction of molecular oxygen, including the superoxide anion (O2•), hydrogen peroxide (H2O2) and the hydroxyl radical (•OH). An NADPH-dependent oxidase located on the plasma membrane (Figure 1) is thought to produce O2•–, which in turn is converted to •OH and H2O2.

The hydroxyl radical is the strongest oxidant of these

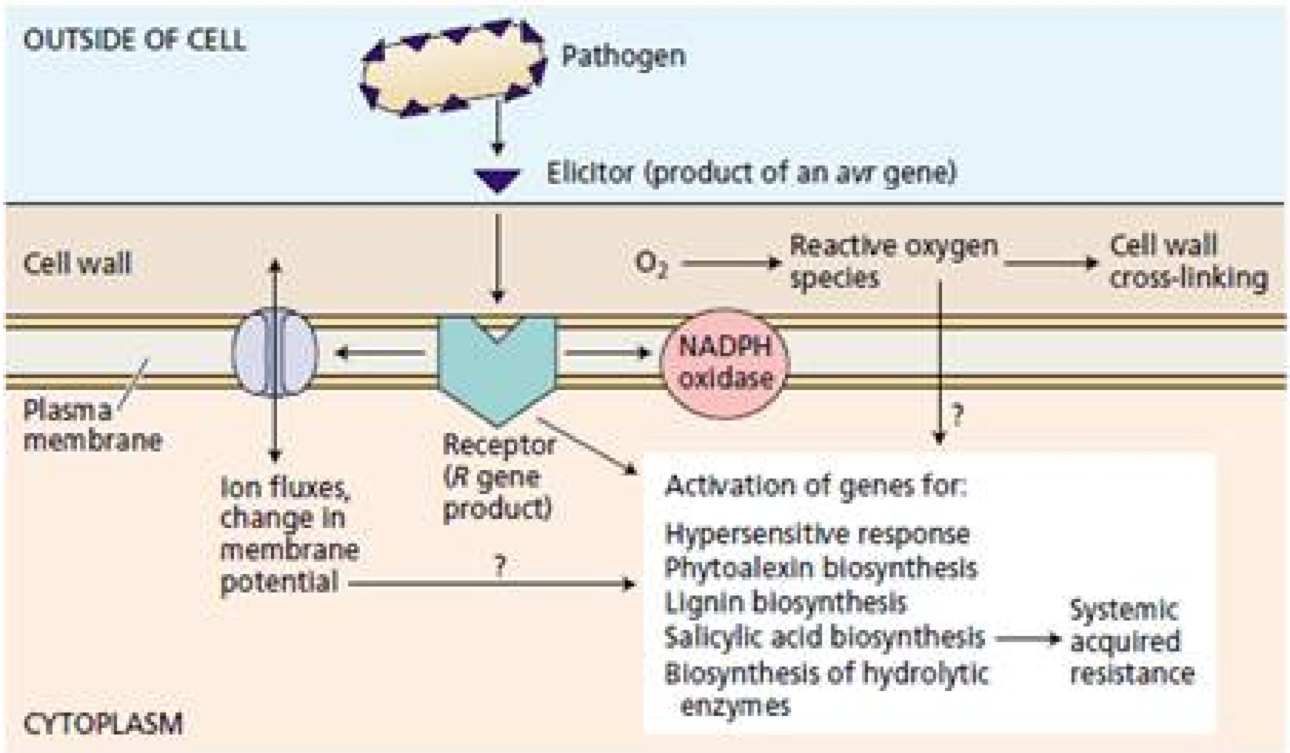



Fig 1. Many modes of anti-pathogenic defence are induced by infection. Fragments of pathogen molecules called elicitors initiate a complex signalling pathway leading to the activation of defence responses.

active oxygen species and can initiate radical chain reactions with a range of organic molecules, leading to lipid peroxidation, enzyme inactivation, and nucleic acid degradation (Lamb and Dixon 1997). Active oxygen species may contribute to cell death as part of the hypersensitive response or act to kill the pathogen directly.

Many species react to fungal or bacterial invasion by synthesizing lignin or callose. These polymers are thought to serve as barriers, walling off the pathogen from the rest of the plant and physically blocking its spread. A related response is the modification of cell wall proteins. Certain proline-rich proteins of the wall become oxidatively cross-linked after pathogen attack in an H2O2-mediated reaction (Bradley *et al*. 1992). This process strengthens the walls of the cells in the vicinity of the infection site, increasing their resistance to microbial digestion. Most of the R genes are thought to encode protein receptors that recognize and bind specific molecules originating from pathogens. This binding alerts the plant to the pathogen's presence This binding alerts the plant to the pathogen's presence. The specific pathogen molecules recognized are referred to as elicitors. Within a few minutes after pathogen elicitors have been recognized by an R gene, complex signaling pathways are Within a few minutes after pathogen elicitors have been recognized by an R gene, complex signaling pathways are set in motion that lead eventually to defense responses (see Figure 1). A common early element of these cascades is a transient change in the ion permeability of the plasma membrane. R gene activation stimulates an influx of Ca2+ and H+ ions into the cell and an efflux of K+ and Cl– ions. The influx of Ca2+ activates the oxidative burst that may act directly in defense (as already described), as well as signaling other defense reactions. Other components of pathogen-stimulated signal transduction pathways include nitric oxide, mitogen-activated protein (MAP) kinases, calcium-dependent protein kinases, jasmonic acid, and salicylic acid.

**Secondary metabolites**

Plants produce a large and diverse array of organic compounds that appear to have no direct functions in growth and development i.e. they have no generally recognised roles in the process of photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation. They have a very restricted distribution than primary metabolites in the whole plant kingdom i.e. they are often found only in one plant species or a taxonomically related group of species. High concentrations of secondary metabolites might result in a more resistant plant. Their production is thought to be costly and reduces plant growth and reproduction (Mazid *et al*., 2011).

Important ecological functions of Secondary metabolites in plants:

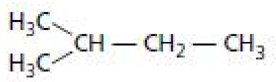
- Provides protection against herbivores and pathogens.
- They serve as attractants (smell, color, taste) for pollinators and seed dispersing animals.
- They function as agents of plant-plant competition and plant-microbe symbiosis.

**Classification of secondary metabolites**

Plant secondary metabolites can be divided into three chemically distinct groups: terpenes, phenolics, and nitrogen- containing compounds.

**TERPENES**

The terpenes, or terpenoids, constitute the largest class of secondary products, which are generally insoluble in water. They are biosynthesized from acetyl-CoA or glycolytic intermediates. All terpenes are derived from the union of five-carbon elements that have the branched carbon skeleton of isopentane.




Terpenes are classified by the number of five-carbon units they contain, although extensive metabolic modifications can sometimes make it difficult to pick out the original five-carbon residues. Ten-carbon terpenes, which contain two C5 units, are called monoterpenes; 15-carbon terpenes (three C5 units) are sesquiterpenes; and 20-carbon terpenes (four C5 units) are diterpenes. Larger terpenes include triterpenes (30 carbons), tetraterpenes (40 carbons), and polyterpenoids ([C5]n carbons, where n > 8).

**There Are Two Pathways for Terpene Biosynthesis**

Terpenes are biosynthesized from primary metabolites in at least two different ways. In the well-studied mevalonic acid pathway, three molecules of acetyl-CoA are joined together stepwise to form mevalonic acid (Figure 2). This key six-carbon intermediate is then pyrophosphorylated, decarboxylated, and dehydrated to yield isopentenyl diphosphate (IPP2). IPP is the activated five-carbon building block of terpenes. Recently, it was discovered that IPP also can be formed from intermediates of glycolysis or the photosynthetic photosynthetic carbon reduction cycle via a separate set of reactions called the methylerythritol phosphate (MEP) pathway that operates in chloroplasts and other plastids( Lichtenthaler, 1999). Although all the details have not yet been elucidated, glyceraldehyde-3-phosphate and two carbon atoms derived from pyruvate appear to combine to generate an intermediate that is eventually converted to IPP.

**Terpenes Defend against Herbivores in Many Plants**

Terpenes are toxins and feeding deterrents to many plantfeeding insects and mammals; thus they appear to play important defensive roles in the plant kingdom. For example, the monoterpene esters called pyrethroids that occur in the leaves and flowers of *Chrysanthemum* species show very striking insecticidal activity. Both natural and synthetic pyrethroids are popular ingredients in commercial insecticides because of their low persistence in the environment and their negligible toxicity to mammals.

Many plants contain mixtures of volatile monoterpenes and sesquiterpenes, called essential oils that lend a char acteristic odor to their foliage. Peppermint, lemon, basil, and sage are examples of plants that contain essential oils. The chief monoterpene constituent of peppermint oil is menthol; that of lemon oil is limonene (Figure 3). Essential

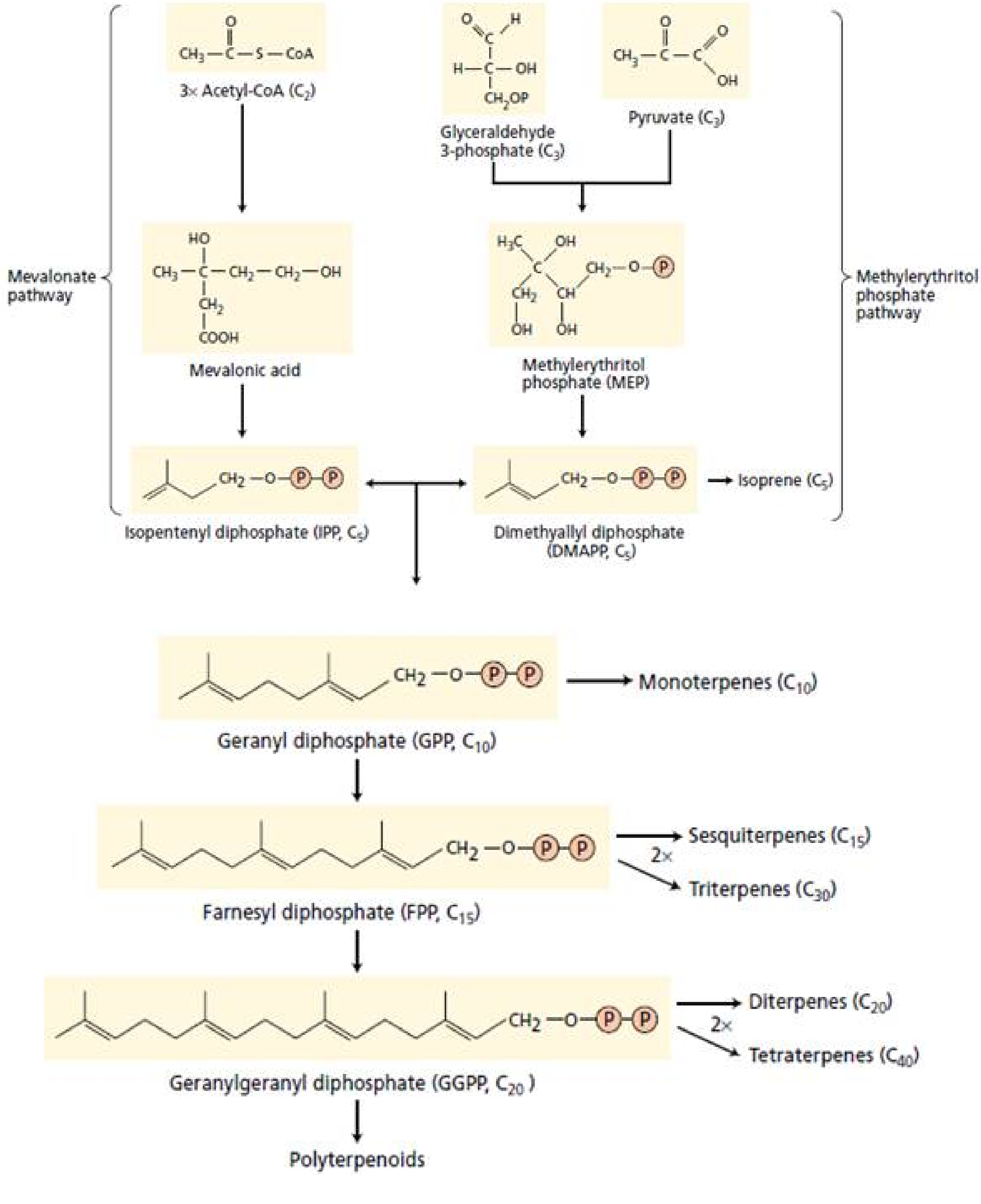



Fig. 2. Outline of terpene biosynthesis. The basic 5-carbon units of terpenes are synthesized by two different pathways. The phosphorylated intermediates, IPP and DMAPP, are combined to make 10-carbon, 15-carbon and larger terpenes.

oils have well-known insect repellent properties. They are frequently found in glandular hairs that project outward from the epidermis and serve to "advertise" the toxicity of the plant, repelling potential herbivores even before they take a trial bite.

Monoterpenes and sesquiterpenes, which are found in glandular hairs that project outward from the epidermis and serve as repellent against herbivores (Figure 4).

These substances repel ovipositing herbivores and attract natural enemies, including predatory and parasitic insects that kill plant-feeding insects and so help minimize further damage.

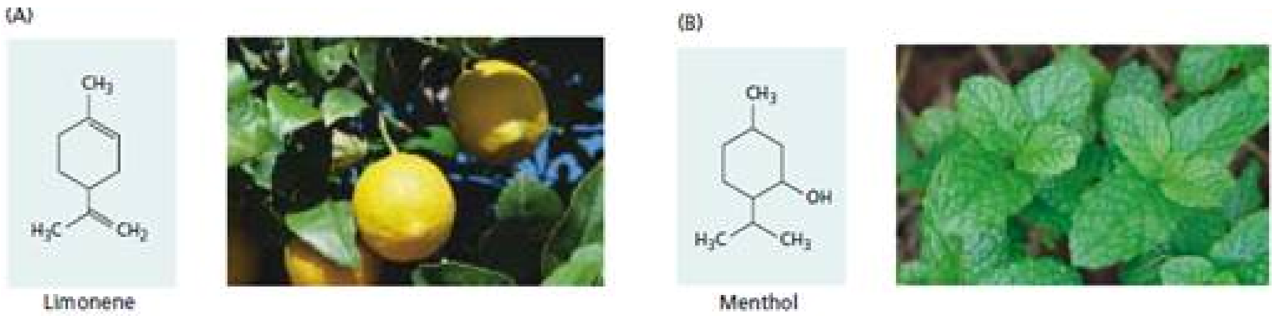



Fig. 3. Structures of limonene (A) and menthol (B).

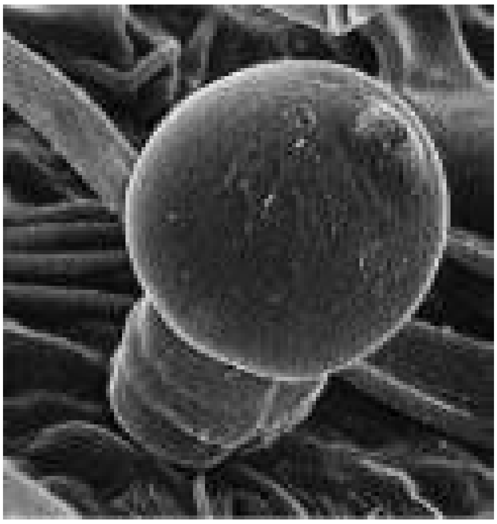


Fig. 4. Monoterpenes and sesquiterpenes are commonly found in glandular hairs on the plant surface.

Among the nonvolatile terpene antiherbivore compounds are the limonoids, a group of triterpenes (C30) well known as bitter substances in citrus fruit. Perhaps the most powerful deterrent to insect feeding known is azadirachtin (Figure 5A), a complex limonoid from the neem tree (*Azadirachta indica*) of Africa and Asia. Azadirachtin is a feeding deterrent to some insects at doses as low as 50 parts per billion, and it exerts a variety of toxic effects.

The phytoecdysones, first isolated from the common fern, *Polypodium vulgare*, are a group of plant steroids that have the same basic structure as insect molting hormones (Figure 5B). Ingestion of phytoecdysones by insects disrupts molting and other developmental processes, often with lethal consequences.

## PHENOLIC COMPOUNDS

Plants produce a large variety of secondary products that contain a phenol group—a hydroxyl functional group on an aromatic ring:

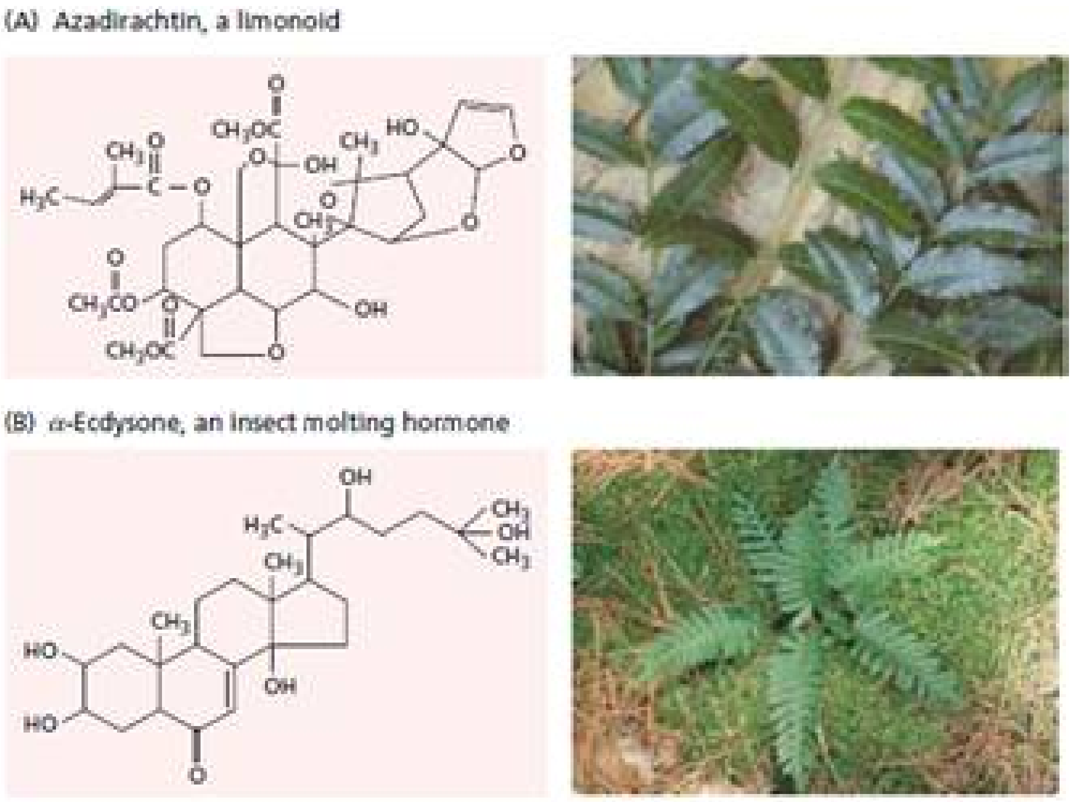
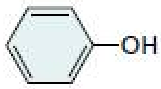



Fig. 5. Structure of two triterpenes, azadirachtin (A), and á-ecdysone (B), which serve as powerful feeding deterrents to insects.




These substances are classified as phenolic compounds. Plant phenolics are a chemically heterogeneous group of nearly 10,000 individual compounds: Some are soluble only in organic solvents, some are water-soluble carboxylic acids and glycosides, and others are large, insoluble polymers. Many serve as defense compounds against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or in reducing the growth of nearby competing plants.

**Biosynthesis of Plant Phenolics**

Plant phenolics are biosynthesized by several different routes and thus constitute a heterogeneous group from a metabolic point of view. Two basic pathways are involved the shikimic acid pathway and the malonic acid pathway (Figure 6). The shikimic acid pathway participates in the biosynthesis of most plant phenolics. The malonic acid pathway, although an important source of phenolic secondary products in fungi and bacteria, is of less significance in higher plants.

The shikimic acid pathway converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids.

The most abundant classes of secondary phenolic compounds in plants are derived from phenylalanine. This reaction is catalyzed by phenylalanine ammonia lyase (PAL), perhaps the most studied enzyme in plant secondary metabolism. Reactions subsequent to that catalyzed by PAL lead to the addition of more hydroxyl groups and other substituents. Trans-cinnamic acid, p-coumaric acid, and their derivatives are simple phenolic compounds called phenylpropanoids because they contain a benzene ring:


and a three-carbon side chain. Several transcription factors have been shown to regulate phenolic metabolism by binding to the promoter regions of certain biosynthetic genes and activating transcription. Some of these factors activate the transcription of large groups of genes (Jin and Martin 1999).

**Types of phenolic compounds**

**Coumarins:** They are simple phenolic compounds, widespread in vascular plants. They derived from the shikimic acid pathway, common in bacteria, fungi and plants but absent in animals. Also, they are a highly active group of molecules with a wide range of anti- microbial activity against both fungi and bacteria (Brooker *et al.*, 2008). It is believed that these cyclic compounds behave as natural pesticidal defense compounds for plants and they represent a starting point for the exploration of new derivatives

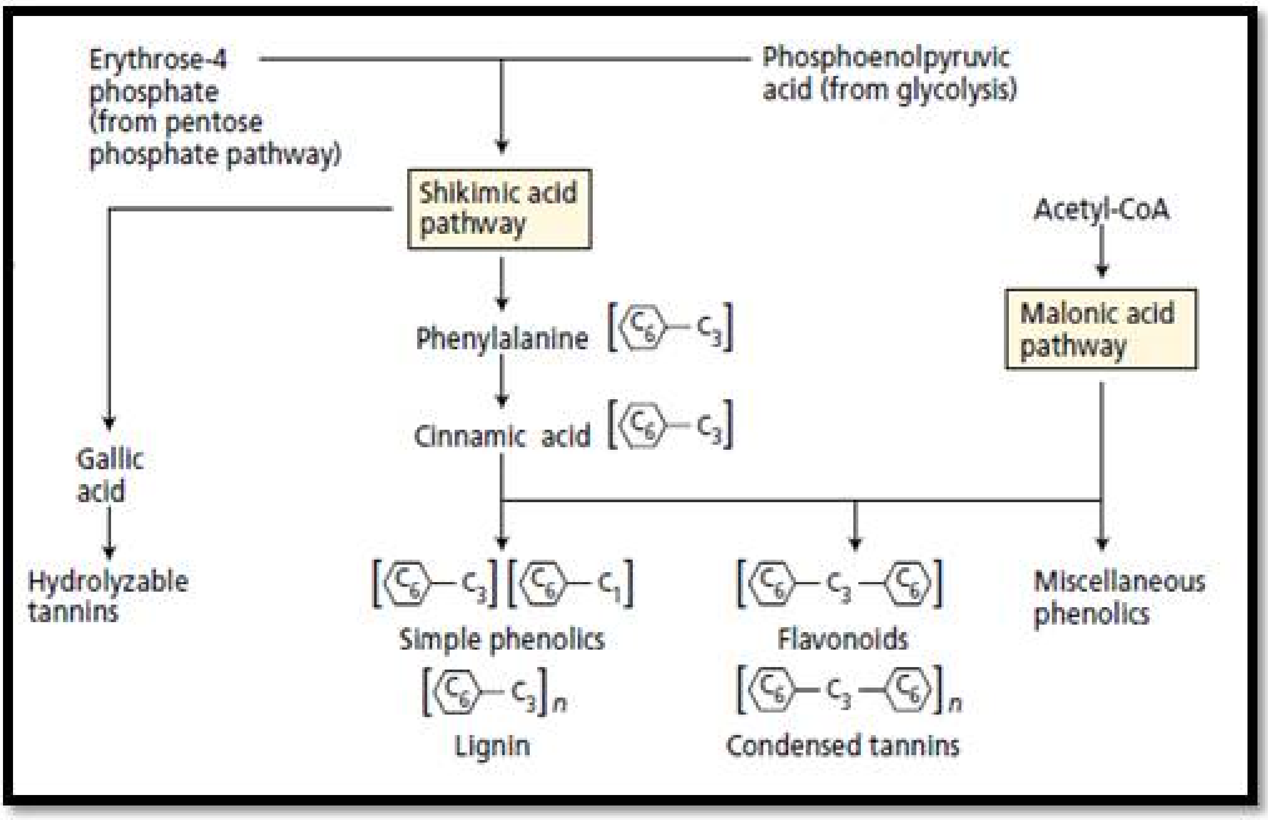



Fig. 6. Plant phenolics are biosynthesized in several different ways. In higher plants, most phenolics are derived at least in part from phenylalanine, a product of the shikimic acid pathway.

possessing a range of improved antifungal activity.

**Ligin:** It is a highly branched polymer of phenyl-propanoid groups, formed from three different alcohols viz., coniferyl, coumaryl and sinapyl which oxidized to free radicals (ROS) by a ubiquitous plant enzyme-peroxidase, reacts simultaneously and randomly to form monomeric units in lignin vary among species, plant organs and even layers of a single cell wall. Its physical toughness deters feeding by herbivorous animals and its chemical durability makes it relatively indigestible to herbivores and insects pathogens. Lignifications block the growth of pathogens and are a frequent response to infection or wounding (Hatfield and Vermerris 2001).

**Flavonoids:** One of the largest classes of plant phenolic, perform very different functions in plant system including pigmentation and defense. Two other major groups of flavonoids found in flowers are flavones and flavonols function to protect cells 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 (Lake *et al.*, 2009).

**Tannins:** They are included under the second category of plant phenolic polymers with defensive properties.. The defensive properties of tannins are generally attributed to their ability to bind proteins. Protocatechllic and chlorogenic acids probably have a special function in disease resistance of certain plants. They prevent smudge in onions, a disease caused by the fungus *Colletotrichum circinans* and prevent spore germination and growth of other fungi as well (Mayer, 1987).

**Nitrogen and Sulphur Containing Compounds**

A large variety of plant secondary metabolites have nitrogen in their structure. Included in this category are such well-known antiherbivore defenses as alkaloids and cyanogenic glycosides, which are of considerable interest because of their toxicity to humans and their medicinal properties. Most nitrogenous secondary metabolites are biosynthesized from common amino acids.

**Types**

**Alkaloids**: The alkaloids are a large family of more than 15,000 nitrogen- containing secondary metabolites found in approximately 20% of the species of vascular plants. The nitrogen atom in these substances is usually part of a heterocyclic ring, a ring that contains both nitrogen and carbon atoms. As a group, alkaloids are best known for their striking pharmacological effects on vertebrate animals. Alkaloids are usually synthesized from one of a few common amino acids—in particular, lysine, tyrosine, and tryptophan. Several different types, including nicotine and its relatives (Figure 7), are derived from ornithine, an intermediate in arginine biosynthesis.

The role of alkaloids in plants has been a subject of speculation for at least 100 years. Alkaloids were once thought to be nitrogenous wastes (analogous to urea and uric acid in animals), nitrogen storage compounds, or growth regulators, but there is little evidence to support any of these functions. Most alkaloids are now believed to function as defenses against predators, especially mammals, because of their general toxicity and deterrence capability.

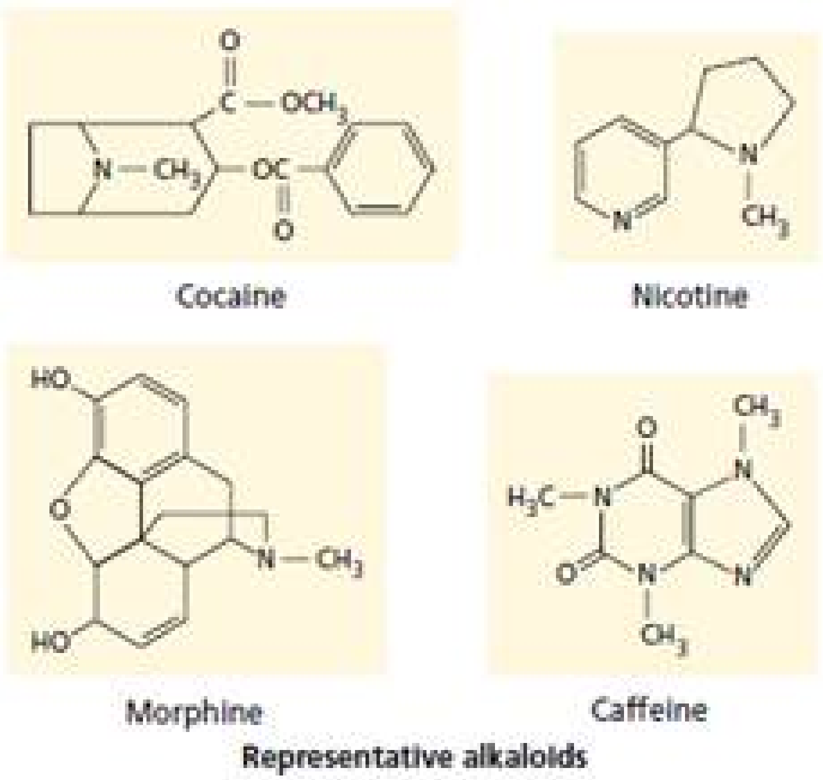



Fig. 7. Examples of alkaloids, a diverse group of secondary metabolites that contain nitrogen, usually as part of a heterocyclic ring

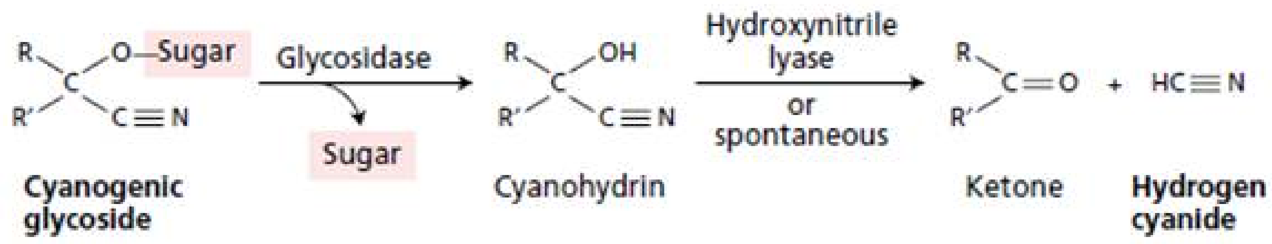



Fig. 8. Enzyme-catalyzed hydrolysis of cyanogenic glycosides to release hydrogen cyanide. R and R2 represent various alkyl or aryl substituents. For example, if R is phenyl, R2 is hydrogen, and the sugar is the disaccharide â-gentiobiose, the compound is amygdalin

**Cyanogenic Glycosides:** They constitute a group of N-containing protective compounds other than alkaloids, release the poison HCN and usually occur in members of families viz., Graminae, Rosaceae and Leguminosae (Seigler, 1991). They are not in themselves toxic but are readily broken down to give off volatile poisonous substances like HCN and H2S when the plant is crushed; their presence deters feeding by insects and other herbivores such as snails and slugs.

The breakdown of cyanogenic glycosides in plants is a two-step enzymatic process. Species that make cyanogenic glycosides also make the enzymes necessary to hydrolyze the sugar and liberate HCN:

1. In the first step the sugar is cleaved by a glycosidase, an enzyme that separates sugars from other molecules to which they are linked (Figure 8).
2. In the second step the resulting hydrolysis product, called an á-hydroxynitrile or cyanohydrin, can decompose spontaneously at a low rate to liberate] HCN. This second step can be accelerated by the enzyme hydroxynitrile lyase.

**Glucosinolates:** A second class of plant glycosides, called the glucosinolates, or mustard oil glycosides, break down to release volatile defensive substances. Found principally in the Brassicaceae and related plant families, glucosinolates give off the compounds responsible for the smell and taste of vegetables such as cabbage, broccoli, and radishes.

The release of these mustard-smelling volatiles from glucosinolates is catalyzed by a hydrolytic enzyme, called a thioglucosidase or myrosinase, that cleaves glucose from its bond with the sulfur atom (Figure 9). The resulting aglycone, the nonsugar portion of the molecule, rearranges with loss of the sulfate to give pungent and chemically reactive products, including isothiocyanates and nitriles, depending on the conditions of hydrolysis. These products function in defense as herbivore toxins and feeding repellents. Like cyanogenic glycosides, glucosinolates are stored in the intact plant separately from the enzymes that hydrolyze them, and they are brought into contact with these enzymes only when the plant is crushed.

**Phytoalexins**

These are chemically diverse group of secondary metabolites with strong antimicrobial activity that accumulate around the site of infection. Phytoalexin production appears to be a common mechanism of resistance to pathogenic microbes in a wide range of plants. However, different plant families employ different types of secondary products as phytoalexins. For example, isoflavonoids are common phytoalexins in the legume family, whereas in plants of the potato family (Solanaceae), such as potato, tobacco, and tomato, various sesquiterpenes are produced as phytoalexins.

Phytoalexins are generally undetectable in the plant before infection, but they are synthesized very rapidly after microbial attack because of the activation of new biosynthetic pathways. The point of control is usually the initiation of gene transcription. Thus, plants do not appear to store any of the enzymatic machinery required for phytoalexin synthesis. Instead, soon after microbial invasion they begin transcribing and translating the appropriate mRNAs and synthesizing the enzymes de novo (Kombrink and Somssich, 1995).

**CONCLUSION AND FUTURE PROSPECTS**

Plants have evolved multiple defense mechanisms against microbial pathogens and various types of environmental stress. Besides anti-microbial secondary metabolite, some of which are performed and some of which

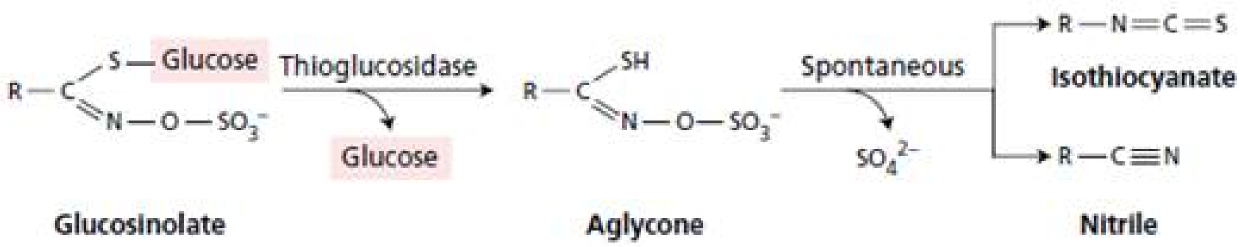



Fig. 9. Hydrolysis of glucosinolates to mustard-smelling volatiles. R represents various alkyl or aryl substituents

are induced by infection. Today, advanced tools are demanded to investigate the correct correlation between N and S fertilization and crop resistance management. In a number of previous research articles and review papers, it have shown that the N and S containing secondary metabolites are influenced by optimum supply of N and S and their good nutrition can enhance the capability of a plant to cope with biotic and abiotic stress.

The identification of the mechanisms causing Systemic Induced Resistance will be an important milestone for sustainable agricultural production, as the use of fungicides could then be minimized or eliminated. Thus, SIR may become an important strategy for efficiently combating with pathogens in organic forming system. Therefore, additional research in area of natural pesticides development is needed in current scenario. In the long term, it will probably be possible to generate gene cassettes for complete pathways, which could then be used for production of valuable defensive secondary metabolites in bioreactors or for metabolic engineering of crop plants. This will improve their resistance against herbivores and microbial pathogens as well as various environmental stresses.

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