Antimicrobial Compounds and Their Role in Plant Defense

13

Anupama Razdan Tiku

Abstract

Plants are important nutrient source for several organisms like microbes, heterotrophic plants, insects as well as vertebrates. Even though they lack a proper defense mechanism like animals, still they have developed a mixture of chemicals which are mainly protein based and are used as a means of defense by detecting attacking organisms and preventing them from causing major damage. In order to protect themselves from these microbes like fungi, bacteria, etc., plant cells have developed the capability to identify attacking pathogens and use inducible defense mechanism by producing toxic chemicals or antimicrobial compounds in the form of pathogen-degrading enzymes and secondary metabolites involved with plant defense. Secondary metabolites generally are grouped into three major classes of chemicals, i.e. terpenoids, phenolic and alkaloids. Some of these antimicrobial compounds are constitutive in nature, i.e. they occur in biologically active forms in healthy plants, whereas other metabolites are inductive in nature. Glucosinolates and cyanogenic glycosides exist in inactive form and are activated as a response to attack by pathogen or tissue damage. These compounds are activated by release of plant enzymes at the time of breakdown of cells. Preformed antimicrobial compounds are termed as "phytoanticipin", while "phytoalexins" are those antimicrobial compounds which are synthesized (as a result of synthesis of enzymes) from precursors as a response to attack by pathogen. Preformed inhibitors are usually tissue specific and are mainly present in the outer layers of the cells of plant organs. These inhibitors are mostly successful against comprehensive range of probable pathogens, and specific virulent pathogens might circumvent the effect of these secondary metabolites by eluding them or by enduring or by detoxification. Most of these constitutive plant compounds show antifungal activity, e.g. phenols, phenolic

---

A. R. Tiku (✉)
Department of Botany, Ramjas College, University of Delhi, Delhi, India
e-mail: anupama.tiku@yahoo.com

A. Singh, I. K. Singh (eds.), *Molecular Aspects of Plant-Pathogen Interaction*,
https://doi.org/10.1007/978-981-10-7371-7_13

glycoside, unsaturated lactones, sulphur compounds, saponins, etc. "Phytoalexins" are the most considered antimicrobial plant defense compounds. These compounds are pathogen specific and therefore more effective in plant defense mechanism. Transcriptional and translational activities in a plant are prerequisite for the production of phytoalexins. Examples of these antimicrobial phytoalexins are scopoletin, camalexin, glucosinolates, etc. This chapter will mainly discuss the role of both phytoanticipins and phytoalexins as plant defense antimicrobial compounds and also their use as "antibiotic potentiators" and virulence attenuators along with their role in crop protection/phytoprotection.

Keywords
Defense mechanism · Secondary metabolites · Phytoalexins · Phenolics · Toxins and alkaloids

13.1 Introduction

All plants growing in natural habitats are surrounded by a large number of antagonists including variety of microbes, nematodes, insects and herbivores and various kinds of abiotic environmental stress which are major sources of hindrance for crop yield. Plants have evolved with a wide variety of inherent and stimulative defense mechanisms to shield themselves from their potential enemies (Cowan 1999). Inherent defense includes many preformed obstructions including cell wall, bark and cuticular wax to protect the plant from attack of pathogens. Plants use stimulative defense mechanism including production of toxic chemicals, enzymes which degrade pathogens and cell suicide to protect themselves from attacking pathogens. Research on the role of these chemicals acting as plant defense compounds took off in the mid of the nineteenth century, e.g. Muller and Borger introduced phytoalexins based on their observation of *Phytophthora infestan* infection in potato tubers. Plant cells showed inhibition towards the pathogen by producing a chemical which reacted hypersensitively and was named as phytoalexin (Jeandet et al. 2014a, b; Jeandet 2015).

Plant compounds are identified as primary metabolites and secondary metabolites.

Primary metabolites (sugars, amino acids, nucleic acids) are involved in growth, development or reproduction, whereas secondary metabolites serve as toxic chemicals or defense-related proteins (Freeman and Beattie 2008). Plants produce diverse varieties of secondary metabolites using resultants of primary metabolisms including enzymes involved in various metabolic pathways and major biomolecules of the cell (Table 13.1) (Fig. 13.1). These are used to protect plants against invading microbial pathogens on account of their toxic nature. Some of them are as well involved in protection of plants from abiotic stress (such as UV-B exposure) and also used for communication between plants and other organisms (including attracting useful organisms like pollinators) or hostile interactions (such as restraint against pathogens and herbivores) (Schafer and Wink 2009).

Table 13.1 Plants containing antimicrobial compounds

Common name	Scientific name	Compound	Class	Activity
Allspice	*Pimenta dioica*	Eugenol	Essential oil	General
Aloe	*Aloe barbadensis*, *Aloe vera*	Latex	Complex mixture	*Corynebacterium*, *Salmonella*, *Streptococcus*, *S. aureus*
Apple	*Malus sylvestris*	Phloretin	Flavonoid derivative	General
Ashwagandha	*Withania somnifera*	Withaferin A	Lactone	Bacteria, fungi
Bael tree	*Aegle marmelos*	Essential oil	Terpenoid	Fungi
Barberry	*Berberis vulgaris*	Berberine	Alkaloid	Bacteria, protozoa
Basil	*Ocimum basilicum*	Essential oils	Terpenoids	*Salmonella*, bacteria
Bay	*Laurus nobilis*	Essential oils	Terpenoids	Bacteria, fungi
Betel pepper	*Piper betle*	Catechols, eugenol	Essential oils	General
Black pepper	*Piper nigrum*	Piperine	Alkaloid	Fungi, *Lactobacillus*, *Micrococcus*, *E. coli*, *E. faecalis*
Blueberry	*Vaccinium* spp.	Fructose	Monosaccharide	*E. coli*
Brazilian pepper tree	*Schinus terebinthifolius*	Terebinthine	Terpenoids	General
Buchu	*Barosma betulina*	Essential oil	Terpenoid	General
Burdock	*Arctium lappa*		Polyacetylene, tannins, terpenoids	Bacteria, fungi, viruses
Buttercup	*Ranunculus bulbosus*	Protoanemonin	Lactone	General
Caraway	*Carum carvi*		Coumarins	Bacteria, fungi, viruses
Cascara sagrada	*Rhamnus purshiana*	Tannins	Polyphenols	Viruses, bacteria, fungi
			Anthraquinone	
Cashew	*Anacardium*	Salicylic acids	Polyphenols	*P. acnes* bacteria, fungi
	Pulsatilla			
Ceylon cinnamon	*Cinnamomum verum*	Essential oils, others	Terpenoids, tannins	General
Chamomile	*Matricaria chamomilla*	Anthemic acid	Phenolic acid	*M. tuberculosis*, *S. typhimurium*, *S. aureus*, helminths

(continued)

Table 13.1 (continued)

Common name	Scientific name	Compound	Class	Activity
Chaparral	*Larrea tridentata*	Nordihydroguaiaretic acid	Lignan	Skin bacteria
Chilli peppers, paprika	*Capsicum annuum*	Capsaicin	Terpenoid	Bacteria
Clove	*Syzygium aromaticum*	Eugenol	Terpenoid	General
Coca	*Erythroxylum coca*	Cocaine	Alkaloid	Gramnegative and grampositive cocci
Cranberry	*Vaccinium* spp.	Fructose	Monosaccharide	Bacteria
		Other		
Dill	*Anethum graveolens*	Essential oil	Terpenoid	Bacteria
Eucalyptus	*Eucalyptus globulus*	Tannin	Polyphenol	Bacteria, viruses
Fava bean	*Vicia faba*	Fabatin	Thionin	Bacteria
Gamboge	*Garcinia hanburyi*		Resin	General
Garlic	*Allium sativum*	Allicin, ajoene	Sulfoxide	General
			Sulfated terpenoids	
Ginseng	*Panax notoginseng*		Saponins	*E. coli*, *Sporothrix schenckii*, *Staphylococcus*, *Trichophyton*
Glory lily	*Gloriosa superba*	Colchicine	Alkaloid	General
Goldenseal	*Hydrastis canadensis*	Berberine, hydrastine	Alkaloids	Bacteria, *Giardia duodenalis*, trypanosomes
				Plasmodia
Gotu kola	*Centella asiatica*	Asiaticoside	Terpenoid	*M. leprae*
Grapefruit peel	*Citrus paradisi*		Terpenoid	Fungi
Green tea	*Camellia sinensis*	Catechin	Flavonoid	General
				Shigella
				Vibrio
				S. mutans
				Viruses
Hemp	*Cannabis sativa*	βResercyclic acid	Organic acid	Bacteria and viruses
Henna	*Lawsonia inermis*	Gallic acid	Phenolic	*S. aureus*

Hops	*Humulus lupulus*	Lupulone, humulone	Phenolic acids	General
Horseradish	*Armoracia rusticana*		Terpenoids	General
(Japanese) herb	*Rabdosia trichocarpa*	Trichorabdal A	Terpene	*Helicobacter pylori*
Legume (West Africa)	*Millettia thonningii*	Alpinumisoflavone	Flavone	*Schistosoma*
Lemon balm	*Melissa officinalis*	Tannins	Polyphenols	Viruses
Lemon verbena	*Aloysia triphylla*	Essential oil	Terpenoid	Ascaris
Licorice	*Glycyrrhiza glabra*	Glabrol	Phenolic alcohol	*S. aureus*, *M. tuberculosis*
Mountain tobacco	*Arnica montana*	Helenalins	Lactones	General
Oak	*Quercus rubra*	Tannins	Polyphenols	General
		Quercetin (available commercially)	Flavonoid	
Olive oil	*Olea europaea*	Hexanal	Aldehyde	General
Onion	*Allium cepa*	Allicin	Sulfoxide	Bacteria, *Candida*
Oregon grape	*Mahonia aquifolium*	Berberine	Alkaloid	*Plasmodium*
				Trypanosomes, general
Pau d'arco	*Tabebuia*	Sesquiterpenes	Terpenoids	Fungi
Papaya	*Carica papaya*	Latex	Mix of terpenoids, organic acids, alkaloids	General
Pasqueflower	*Anemone pulsatilla*	Anemonins	Lactone	Bacteria
Peppermint	*Mentha piperita*	Menthol	Terpenoid	General
Periwinkle	*Vinca minor*	Reserpine	Alkaloid	General
Peyote	*Lophophora williamsii*	Mescaline	Alkaloid	General
Poppy	*Papaver somniferum*	Opium	Alkaloids and others	General
Purple prairie clover	*Petalostemum*	Petalostemumol	Flavonol	Bacteria, fungi
Quinine	*Cinchona* sp.	Quinine	Alkaloid	*Plasmodium* spp.

(continued)

Table 13.1 (continued)

Common name	Scientific name	Compound	Class	Activity
Rauvolfia, chandra	*Rauvolfia serpentina*	Reserpine	Alkaloid	General
Rosemary	*Rosmarinus officinalis*	Essential oil	Terpenoid	General
Sainfoin	*Onobrychis viciifolia*	Tannins	Polyphenols	Ruminal bacteria
Savory	Satureja montana	Carvacrol	Terpenoid	General
Senna	*Cassia angustifolia*	Rhein	Anthraquinone	*S. aureus*
Cabbage	*Brassica oleracea*	Quercetin	Flavonoids	General
Rape seed	*B. rapa*	Cyanidin	Hydroxycinnamic acid	
Nabicol	*B. napus*	p-coumaric acid		
		Ferulic acid		
Broad bean	*Vicia faba*	Quinolizidine	Alkaloids	Bacteria
		Piperidine	Amines	Fungi
		Pyridine		
		Indolizidine		
Wild barley	*Hordeum chilense*	Hydroxamic acid	Aglucones	Pests
	H. brevisubulatum			Pathogens
	H. bulbosum			
Grapes	*Vitis vinifera*	Gallic acid	Polyphenols	Generals
		Catechin		
Makoi	*Solanum nigrum*	Luteolin	Flavonoids	Bacteria
		Oleuropein glycoside	Alkaloids	Fungi
		Veremivirine		
		Myristic acid		

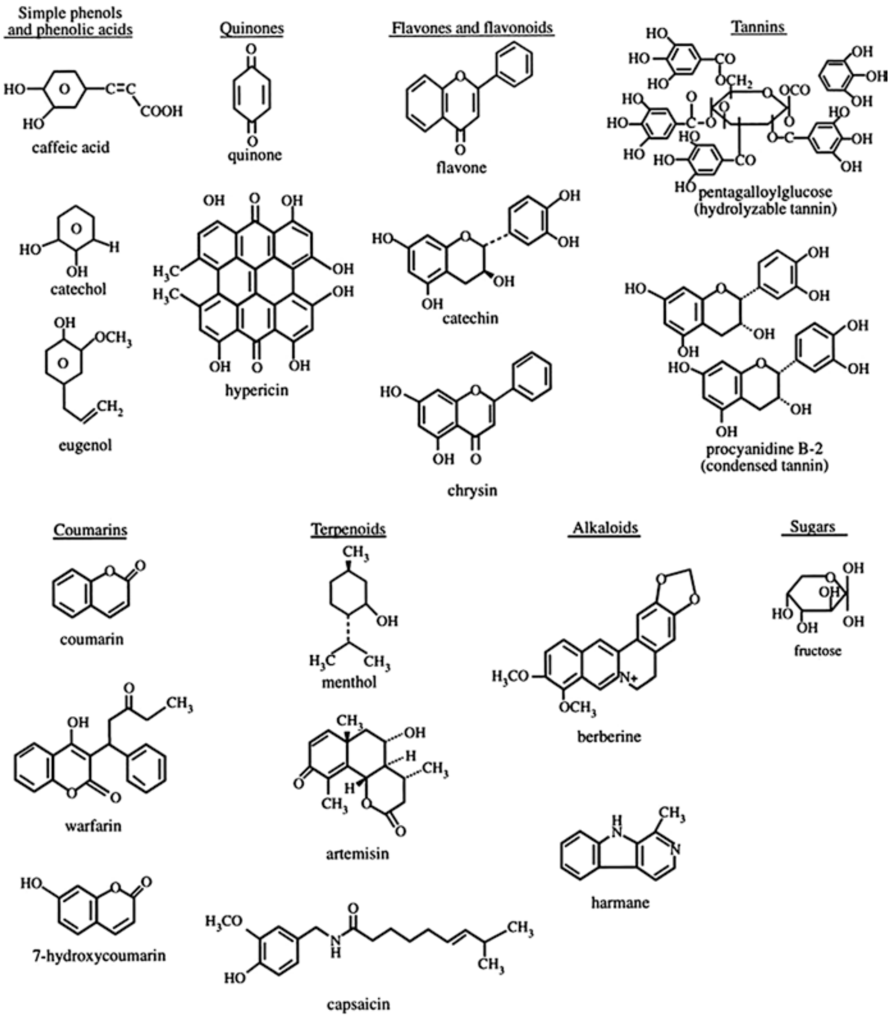


Fig. 13.1 Structures of common antimicrobial plant chemicals

The three major types of secondary metabolites are terpenes/terpenoids, phenolics and nitrogen- and sulphur-containing compounds produced mostly from common amino acids. Terpenes are formed from *5-C isopentanoid* units, which are toxic in nature and hence discourage many herbivores from eating the plants. Phenolics produced mainly from *shikimic* acid pathway are vital to protect the plants from invading microbial pathogens and fungi. Their defensive roles have been confirmed by in vitro experiments conducted by changing expression of secondary metabolites of plants using modern molecular methods (Mes et al. 2000; Van Etten et al. 2000). Studies have shown that around 200,000 secondary metabolites produced by plants are part of biochemical protective systems of plants, evolved over millions of years during which the respective plants and their foes have existed together (Wink 1999). Due to high energy and nutrient requirements required for production and maintenance of secondary metabolites, plants normally wait till the time pathogens are detected to produce toxic chemicals/defense-related proteins. Hence, plant defense chemicals can be grouped into two types which are constitutive metabolites and induced metabolites. Constitutive metabolites are also known as “prohibitins” or phytoanticipins. Induced metabolites, also known as phytoalexins, are formed to protect the plant from infection involving synthesis of enzymes (complex molecules) from simple molecules (Van Etten et al. 1994; Grayer and Harborne 1994).

Phytoanticipins consume large amount of carbon and energy exhibiting fitness cost under natural conditions. They are accepted as the primary chemical defense that a pathogen has to overcome, whereas production of phytoalexin takes up to 2–3 days, as enzyme synthesis is required for converting their precursors into the desired defense protein (Hammond-Kosack and Jones 1996). Moreover the same type of secondary metabolites/toxic chemicals exists in plants belonging to the same species or taxonomically related species. We will discuss possible types/groups of secondary metabolites acting as antimicrobial compounds used in plant defense mechanism in this chapter.

13.2 Terpenes/Terpenoids

They exist in almost all the plants representing the biggest group of secondary metabolites. There are more than 22,000 compounds described as terpenes, and they are grouped together based on their common biosynthetic origin from glycolytic intermediates or acetyl-CoA (Mazid et al 2011). Hydrocarbon isoprene (C5H8) which is a volatile gas produced during photosynthesis can protect cell membrane from damage due to extreme temperature or light conditions. It is the simplest form of terpenoid. Terpenoids are classified on the basis of number of the isoprene units which are used in their construction, for example, monoterpenoids (two isoprene units), sesquiterpenoids (three isoprene units), diterpenoids (four isoprene units) and triterpenoids (six isoprene units). Terpenes are assumed to be intricate part of plant defense mechanism in the form of toxins and feeding restraint to many insects and herbivores (Gershenzon and Croteau 1991). They are also active against bacteria, fungi and protozoa, for example, pyrethrins, menthol, camphor, farnesol and artemisinins. Capsaicin present in chilli peppers has bactericidal properties. Below several examples will be discussed from five major subclasses of terpenes.

13.2.1 Monoterpenes (C_{10})

Monoterpenes along with its derived compounds are vital representatives of insect toxicity, for example, pyrethroids/pyrethrins (monoterpene esters) exist in the leaves and flowers of chrysanthemum species presenting robust insecticidal reactions (neurotoxin) towards insects like bees, beetles, wasps, moths, etc. They are key elements of commercial insecticides. Monoterpenoids as well as sesquiterpenoids are the key elements of highly volatile compounds and essential oils, which are the reason for the fragrance of plants producing them. They are insect toxins and prevent bacterial and fungal infections in the plant. For example, mint plants (*Mentha* spp.) produce huge quantity of monoterpenes (menthone and menthol), formed and stored in glandular trichrome present in epidermal cells. In gymnosperms (conifers) like fir, lime and pine, monoterpenes collect in resin ducts of needles, twigs and trunk primarily in the form of alpha-pinene, beta-pinene, limonene and myrcene which are potent insect repellents effecting bark beetles and other serious pests of conifer species (Turlings et al. 1995).

13.2.2 Sesquiterpenes (C_{15})

Many sesquiterpenes have been reported till now, and the part they play in plant defense is mainly of anti-herbivore representatives belonging to Asteraceae family classified by a lactone ring with five members (a cyclic ester) having tough feed repelling properties affecting several insects and herbivores.

ABA is a well-known example of sesquiterpene playing important role as a plant hormone. In cotton four different sesquiterpenoid phytoalexins, desoxyhemigossypol, hemigossypol, desoxy-6-methoxygossypol and 6-methoxygossypol (Garas and Waiss 1986), help the plant to fight against fungus *Verticillium dahliae*. Resistant varieties of tobacco with *Phytophthora* had induction of sesquiterpenoid phytoalexins like capsidiol, rishitin, etc.

13.2.3 Diterpenes (C_{20})

Abietic acid, a significant diterpene, mostly exists in resin canals of tree trunks of legumes and pine trees. Toxic resin serves as chemical deterrent against the feeding insects which pierce these canals. Phorbol (an ester) is another example of diterpene which is present in members of Euphorbiaceae and proves to be a skin irritant as well as toxic for feeding herbivores. Gossypol (Fig. 13.2) which has tough antifungal and antibacterial characteristics is produced by cotton (*Gossypium hirsutum*) (Bennett and Wallsgrove 1994).

13.2.4 Triterpenes (C_{30})

Triterpene structures are quite similar to plant and animal steroidal hormones and sterols. The milkweeds generate numerous bitter-tasting glycosides, i.e. sterols which protect the plants against insects and herbivores. Limonoid is a bitter substance in citrus fruits and serves as anti-herbivore compounds present in family Rutaceae. Azadirachtin (Fig. 13.3), a complex limonoid, is present in *Azadirachta indica*, which restrains some insects from feeding on the plant and also protects the

Fig. 13.2 Structure of diterpene gossypol

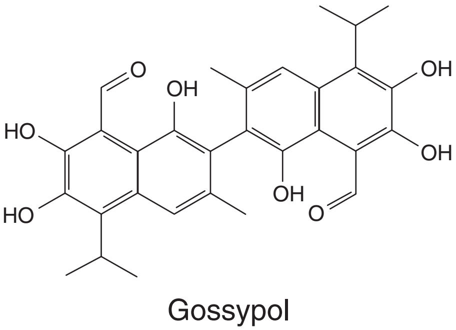


Fig. 13.3 Structure of triterpene azadirachtin

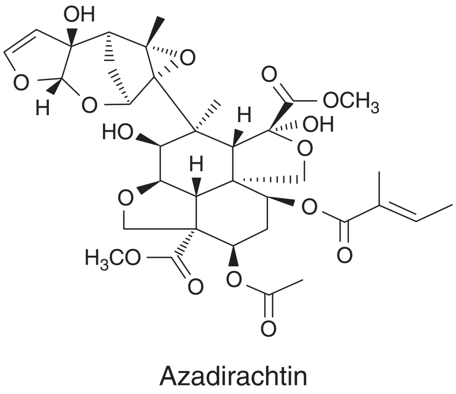
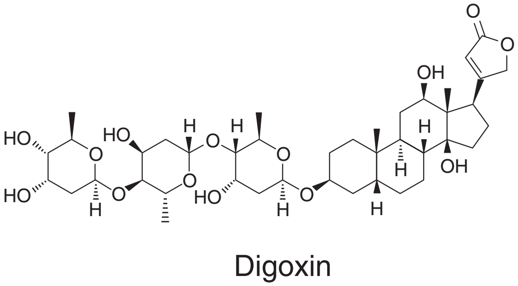


Fig. 13.4 Structure of triterpene digoxin



plant from microbial infections (Mordue and Blackwell 1993). Foxglove (*Digitalis purpurea*) also produces glycosides known as digitoxin and digoxin (Fig. 13.4).

13.2.5 Polyterpenes $(C_5)_n$

These are high molecular weight terpenes, for example, rubber has many polyterpenes showing antimicrobial properties. Rubber is found in long vessels known as laticifers made up of 1500–15,000 isopentenyl units having nearly all C-C double bonds with cis (Z) configuration. Another example is gutta rubber which has its double bonds in trans (E) configuration (Eisner and Meinwald 1995). Few polyterpenes (tetraterpenes) effect growth, e.g. hormone gibberellic acid, and also contribute to red, yellow and orange pigments (carotenoids).

13.3 Saponins

Saponins exist in large concentration in healthy plants exhibiting strong antifungal activity. These molecules have been found to be determinants of plant resistance to fungal attack (Osbourn 1996). These compounds also exhibit other properties like piscicidal, insecticidal and molluscicidal. Saponins are triterpenoids with attached sugar group, i.e. glycosylated triterpenoids which exist in membranes of cell of several species of plants. Having detergent properties they disrupt the membranes of cells of attacking fungal pathogens. Saponins are classified into three major groups based on the types of aglycone which are triterpenoid, a steroid or a steroidal glycoalkaloid. Saponins exist in the form of *triterpenoid saponins* mostly in dicotyledonous plants and also in few monocots, while *steroid saponins* exist mostly in monocots, for example, plants belonging to family Agavaceae, Liliaceae, Dioscoreaceae, etc. Saponins in the form of *saponin digitoxin* mostly exist in dicots, for example, foxglove. *Avena* has both the types mentioned above (Price et al. 1987). Saponins in the form of steroidal glycoalkaloids exist mostly in Solanaceae family (potato, tomato, etc.) along with Liliaceae family. Saponins found in oats and tomato and their function in defense of plants against phytopathogenic fungi have been studied in detail (Osbourn 1996).

Some vital saponins involved in antimicrobial activity are avenacins and avenacosides (mainly present in oats and related species like *Arrhenatherum elatius*). Avenacins exist only in roots, while avenacosides exist in roots as well as shoots. Avenacosides which are inactive biologically are transformed into antifungal monodesmosidic saponin 26-desglucoavenacosides. Oats are able to resist root-infecting fungus because of the presence of triterpenoid avenacin saponins. *Gaeumannomyces graminis* var. *tritici* is not able to infect oat plant even though it wreaks havoc to wheat and barley plant because of the presence of avenacins (Fig. 13.5a).

One of the major saponin, α-tomatine (Fig. 13.5b), a monodesmosidic steroidal glycoalkaloid, exists in its biologically active form in a healthy plant. It safeguards the tomato plants from both *Verticillium albo-atrum* (Pegg and Woodward 1986) and vascular wilt fungi *Fusarium oxysporum* f.sp. *lycopersici* (Smith and Machardy 1982). Saponins prove to be toxic for fungi as a result of the ability of saponins to associate with sterols present in membrane leading to formation of pores (Price et al. 1987; Fenwick et al. 1992). Actions of saponins like α-tomatine are dependent upon pH, but some fungi render the saponins ineffective by altering the pH at the infection site. Plants have developed a mechanism to safeguard themselves from their own saponins by placing them in the vacuole or in other organelles, whose membranes can avoid lysis because of low or altered sterol composition.

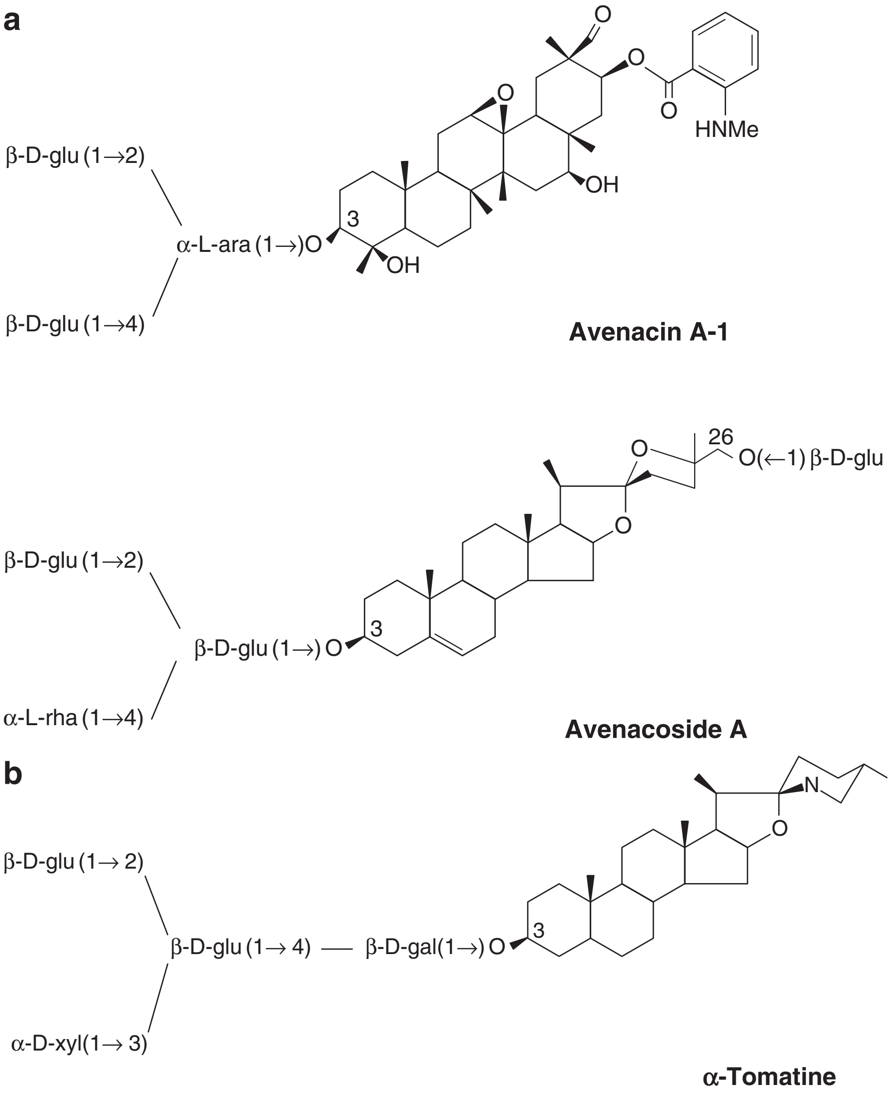
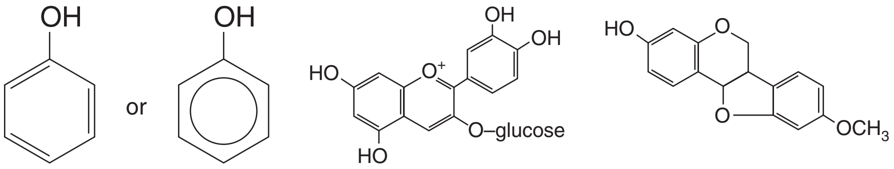


Fig. 13.5 (**a**) Structure of avenacin saponins present in oat (**b**) Structure of saponin present in tomato

13.4 Phenolics

Phenolics are a big class of secondary metabolites formed by plants to safeguard themselves against pathogens. They are created mostly via shikimic acid and malonic acid pathways in plants, comprising extensive variety of protective metabolites such as furanocoumarins, lignin, anthocyanins, flavonoids, tannins and



Phenol, the simplest phenolic compound

Cyanin glycoside, an anthocyanin

Medicarpin, a phytoalexin

Fig. 13.6 Structure of important phenolics

phytoalexins. These secondary products play a vital part in plant defense mechanism against threats like fungi, bacteria and nematodes as well as restraint herbivores from feeding the plant (Mazid et al. 2011). They contain phenol which is a hydroxyl functional group on an aromatic ring named phenol (Fig. 13.6).

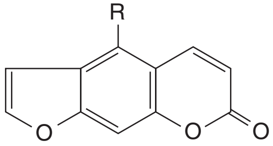
13.4.1 Coumarin

Simple phenolic compounds made up of fused benzene and α-pyrone rings occur extensively in vascular plants. They protect various plants in many ways against threats from insects and fungi. They are mostly derived through shikimic acid pathway and are involved actively against wide range of microbes (Brooker et al. 2008). These cyclic compounds behave as natural pesticides for plants and represent a point from where the exploration of new derivatives which possess a range of enhanced antifungal activity can start. Derivatives of halogenated coumarin operate efficiently against fungal growth, for example, 7-hydroxylated simple coumarin works against *Orobanche cernua* preventing its penetration and germination in host vascular system. Some coumarins also show high defense activity against soilborne plant pathogenic fungi (Brooker et al. 2008). Hydroxycinnamic acid, related to coumarins, shows inhibition towards gram-positive bacteria. Phytoalexins which are hydroxylated derivatives of coumarins formed in carrots show antifungal activity.

13.4.2 Furanocoumarin

A wide variety of plants produce furanocoumarins which are phenolic compounds. Plants belonging to family of Apiaceae usually produce these compounds. These phenolic compounds become toxic only when activated by light (UV-A), and they get integrated into the pyrimidine bases of DNA of vertebrate and invertebrate herbivore causing rapid cell death due to blocked transcription and repair mechanism. Psoralen is a basic furanocoumarin used against fungi (Fig. 13.7).

Fig. 13.7 Structure of furanocoumarin psoralen



Psoralen, furanocoumarin

13.4.3 Lignin

Branched polymer of phenylpropanoid groups exists primarily in the secondary cell walls of plants. Three different types of alcohols which are coniferyl, coumaryl and sinapyl oxidize to free radicals (ROS) by a ubiquitous plant enzyme – peroxidase – which reacts randomly as well as simultaneously to produce lignin. The reactive proportions of these three monomeric units in lignin fluctuate among species, plant organs and even different layers of single cell wall (Lewis and Yamamoto 1990). These phenolic monomers are present hundred or thousand times in the structure of lignin and constitute the main component of wood. It is insoluble, rigid and virtually indigestible therefore serving as exceptional physical wall against attack by pathogens. The physical toughness and chemical durability of lignin causes it to be impossible for herbivores and insect pathogens to digest it. Lignification prevents the development of pathogens and microbes and is formed frequently during infection or wounding.

13.4.4 Flavonoids

Flavonoids, which are one of the biggest classes of plant phenolics, perform diverse roles from pigmentation to defense mechanism of plants. Flavones and flavonols are two main groups of flavonoids present in flowers. They protect the cells from harmful UV-B radiation by accumulating in epidermal layers of stems and plants (Lake et al. 2009). Anthocyanins as well as colourful water-soluble flavonoid pigments are formed by plants to protect their foliage from UV damage. They are hydroxylated phenolic substances existing as C_{6}–C_{3} unit connected to an aromatic ring, produced by plants in response to microbial infection. Because of their capability to complex with extracellular and soluble proteins and bacterial cell wall proteins, they can act against extensive range of microorganisms. Lipophilic flavonoids, often pathogen specific in their toxicity, disrupt cellular structure, microbial membranes and pathogen metabolism. An example is catechin, a flavonoid occurring in leaves of oblong green tea. Tea leaves showing antimicrobial activity have mixture of catechin compounds working against various bacteria and microorganisms, for example, *Vibrio cholerae*, *Streptococcus mutans*, *Shigella*, etc. Phloretin existing in some varieties of apples displays action against many varieties of microorganisms. Galangin which is produced from *Helichrysum aureonitens* (a perennial herb) is effective against extensive array of gram-positive bacteria as well as microbes and fungi.

13.4.5 Isoflavonoids

Isoflavonoids play a vital role in development of plant as well as defense mechanism. Their precursor is naringenin which is a flavanone intermediate, present in plants. They play a vital part in the formation of nitrogen-fixing nodules by symbiotic rhizobia and are secreted by legumes (Sreevidya et al. 2006). Phytoalexins are isoflavonoids, produced in response to pathogen attack having antibiotic and antifungal characteristics. These pathogen-specific, toxic compounds unsettle cellular structure and metabolism of pathogen, for example, medicarpin which is formed by alfalfa (*Medicago sativa*), rishitin also a toxin formed in tomatoes as well as potatoes (family Solanaceae) and camalexin formed by *Arabidopsis thaliana*.

13.4.6 Tannins

Tannins are group of polymeric phenolic substances which have the property of astringency which gives the ability to tan leather or precipitate gelatin from solution. They exist mostly in various plant parts like roots, bark, wood, fruits and leaves. Tannins are grouped into two types, hydrolyzable and condensed tannins. Hydrolyzable tannins are based on gallic acid (multiple aster of D-glucose), while flavonoid monomers are precursors of condensed one which are also known by the alias “proanthocyanidins”. Condensed flavan derivatives in plant woody tissues and polymerization of quinone units are also known to form tannins. These phenolics are water soluble and mainly stored in vacuoles of cell. Tannins combine with salivary proteins and digestive enzymes of insects and herbivorous animals making the insect protein inactive. A herbivore or an insect having large intake of tannins does not gain weight and eventually dies. Tannins are capable of binding proteins, for example, protocatechuic and chlorogenic acids playing a vital role in disease resistance in certain plants. In onions smudge disease caused by *Colletotrichum circinans* (fungus) and growth of other fungi is restricted due to formation of these two tannins. Some tannin like chlorogenic acid is oxidized into effective quinones showing antifungal properties in case of some disease-resistant plants (Cowan 1999).

13.5 Nitrogen-Containing Secondary Metabolites

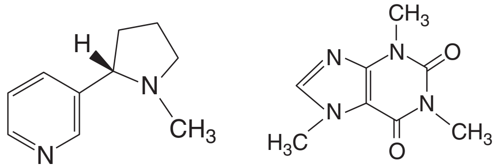
Nitrogen-containing secondary metabolites including alkaloids, cyanogenic glycosides and nonprotein amino acids are synthesized from common amino acids and are of significant interest due to their anti-herbivore defense role in plants.

13.5.1 Alkaloids

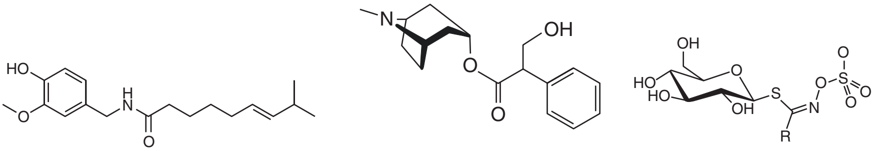
Alkaloids are the largest family of bitter-tasting nitrogenous compounds (Fig. 13.8) present in many vascular plants (20%) (Hegnauer 1988) mostly in herbaceous dicots and some gymnosperms and monocots. Pyrrolizidine alkaloids (Pas) are quite toxic and help in defense against infection caused by microbes and attack by herbivores. They are usually produced from few common amino acids like lysine, tyrosine, aspartic acid and tryptophan (Pearce et al. 1991). Cocoa, coffee and tea contain caffeine which is an alkaloid. These are toxic to both insects and fungi. Nicotine, an alkaloid, is formed by tobacco plant roots which is further transported to leaves of tobacco plant and is stored in vacuoles. Atropine, another neurotoxin and cardiac stimulant alkaloid, is formed by lethal nightshade plant (*Atropa belladonna*) and is highly toxic in large quantities. Capsaicin formed by members of genus *Capsicum* also has antimicrobial properties and works actively in plant defense mechanism. Their action involve effect on nervous system especially the chemical transmitters, membrane transport system, protein synthesis and miscellaneous enzyme activities (Creelman and Mullet 1997). Shikimic acid is a precursor for indole and its derivatives, amino acid tryptophan and its derivatives (psychedelic compounds, dimethyltryptamine), many alkaloids and other aromatic metabolites (Figs.13.9 and 13.10) which play an important part in resistance against microbes and fungi and nematodes.

13.5.2 Cyanogenic Glycosides

Hydrogen cyanide (HCN), a lethal chemical that halts cellular respiration in aerobic organisms, is produced by breaking down of cyanogenic glycosides (very deadly



Nicotine Caffeine Theobromine



Capsaicin Atropine Glucosinolate

Fig. 13.8 Structure of common alkaloids

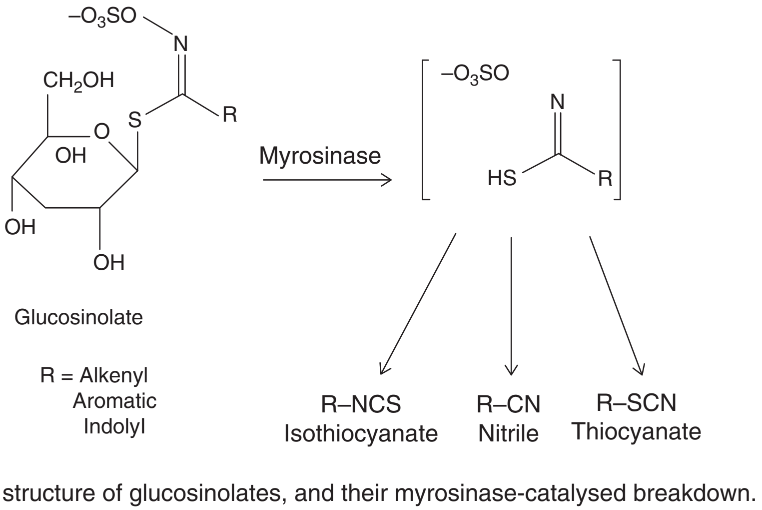
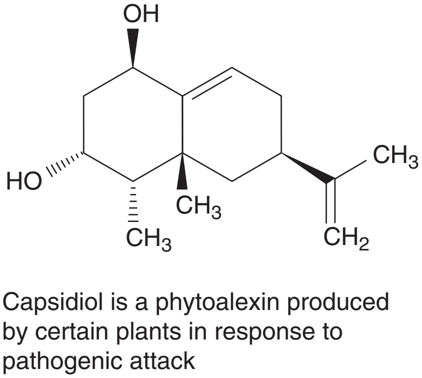


Fig. 13.9 Structure of glucosinolates and their myrosinase - catalysed breakdown

Fig. 13.10 Structure of phytoalexin capsidiol



nitrogenous compounds). When the herbivores feed on the plants that produce cyanogenic glycosides, the plant tissue gets damaged which leads to mixing of enzyme (including glycosidases and hydroxynitrile lyases) and substrate which are stored in separate sections of the healthy plant, producing HCN. HCN binds itself to the Fe-containing heme group of cytochrome oxidase as well as other respiratory enzymes causing cellular respiration poisoning. Cyanogenic glycosides are mainly present in families Gramineae, Rosaceae and Leguminosae (Seigler 1991). Seeds of almond, apricot, cherries and peaches contain a common glycoside called amygdalin, whereas *Sorghum bicolor* contains dhurrin (Poulton 1990). Cassava has a long shelf life because of the presence of cyanogenic glycosides. Lima bean (*Phaseolus lunatus* L.) is a classic example of a plant which may be researched to understand defense mechanism in plants showing inducible indirect anti-herbivore properties achieved by producing VOC, i.e. volatile organic compounds (Ballhorn et al. 2009).

Among members of mustard family (Brassicaceae), mustard oil glycosides are present which when broken down by thioglucosidase enzymes produce cyanide gas. Cyanogenic glycosides are the best example where defenses are produced only in reaction to damage to plant tissue or attack by pathogen like "phytoalexins".

13.5.3 Nonprotein Amino Acids

Several plant species have rare amino acids known as nonprotein amino acids that do integrate into proteins but are also present as free forms and act as shielding defensive substance (Johnson et al. 1989). For example, azetidine-2-carboxylic acid and canavanine block the synthesis or uptake of protein amino acids. They are close analogs of arginine and proline. After consumption, canavanine (NPA) is detected by herbivore enzyme that sticks arginine to arginine tRNA molecule and gets assimilated into protein in place of arginine. Resulting protein is a nonutility one due to its faulty tertiary structure or disrupted catalytic site (Rosenthal 1991). Plants which produce nonprotein amino acids do not get harmed themselves due to its toxicity, but they gain defense against a wide array of pathogenic microbes, insects and herbivorous animals.

13.6 Sulphur Containing Secondary Metabolites

These metabolites directly or indirectly defend plants against pathogen microbes. Defensins, thionins, GSH, phytoalexins and GSL are the examples of sulphur containing secondary metabolites (Saito 2004; Grubb and Abel 2006; Halkier and Gershenzon 2006).

13.6.1 GSH

GSH is the main form of organic S which is present as soluble fraction in plants. GSH gets amassed in the plant after it is attacked by fungi. Main cellular sections of the plant can carry between 3 and 10 mM of GSH. GSH displays properties of an antioxidant as well as reducing agent in an effort to diminish the oxidative stress (Kang and Kim 2007).

GSH targets and detoxifies xenobiotic and cytotoxins in vacuoles. For synthesis of GSH and other phytochelatins involved in heavy metal detoxification, enzymes are produced inside specialized epidermal cells like trichomes.

13.6.2 GSL (Glucosinolates)

GSL increases the resistance of higher plants to parasites, predators as well as competitors. They are a group of N and S with low molecular mass and holding plant glycosides. The breakdown products which are released in the form of protective volatile substances exhibit lethal or repelling effects (DeVos and Jander 2009), for example, allyl sulfoxides in *Allium* (Leustek and Saito 1999) and mustard oil glycosides in Cruciferae. Myrosinase catalyses the volatiles coming from GSL (Fig. 13.10) cleaving glucose from its bond with S atom. Resulting product (aglycone) reorganizes the loss of sulphate giving pungent as well as chemically reactive products, which includes isothiocyanates (R-N=C=S), thiocyanates and nitriles. These toxic and feed repelling products protect the plants from herbivores (Geu-Flores et al. 2009). It prevents various fungal diseases including light leaf spot (*Pyrenopeziza brassicae*), sclerotina stem rot (*Sclerotinia sclerotiorum*) and alternaria (*Alternaria brassicae*) from affecting the plants. The GSL become effective only when it comes in contact with myrosinase (a plant enzyme) which happens only if plant tissue gets damaged. (In healthy plants both enzymes and glucosinolate substrates are separated from each other due to compartmentalization.) The activity of enzymes involved in antioxidant defense mechanism may get affected by isothiocyanates, and defense of cell from DNA damage and GST activity may get affected because of detoxification from xenobiotic (Lipka et al. 2010). These three hydrolysis products are toxic for a wide array of fungi, and the breakdown products' nature depends upon the glucosinolate structure, myrosinase enzyme existing, the species of plant and variety of factors such as protein cofactors, temperature, metal ion concentrations and pH. Resistance of cabbage to *P. parasitica* and resistance of Indian mustard and oilseed rape to *L. maculans* have been associated with high glucosinolate levels. Breakdown products of glucosinolate also have effect on several nonpathogens of *Brassica*. These compounds are used as naturally available fungicides to regulate several pest harvest pathogens and cereal diseases related to fruits and vegetables (Mari et al. 1993).

13.6.3 Phytoalexin

Plants synthesize antimicrobial and often antioxidative elements de novo. These then accumulate quickly at infected areas affected by pathogen (bacteria or fungi) in order to limit the spread of invading microbes (Fig. 13.10). Organic phytoalexins produced by most of the plant families have a diverse chemistry, and mostly they are grouped under the classes including alkaloids, glycosteroids and terpenoids. The examples include isoflavonoids of Leguminosae and sesquiterpenoids of Solanaceae. S metabolites are only produced in Cruciferae plant family (Harborne 1999; Gross et al. 1994; Monde et al. 2000). The phytoalexins which are produced in the plants have a toxic effect on the attacking pathogens because they are responsible for rupturing the walls of the cell, delaying maturation, disrupting metabolism and preventing pathogen reproduction. The plant mutant which is incapable of producing phytoalexin experiences widespread presence of pathogens compared to wild plants which produce phytoalexin. Production of phytoalexins comes under systemic acquired resistance (SAR) or long-term resistance, which involves communication between the damaged tissue and the rest of the plant as well as usage and synthesis of plant hormones including salicylic acid, abscisic acid, ethylene and jasmonic acid. The entire mechanism involves genes that transcribe enzyme involved in synthesis of phytoalexins. Natural phenols such as isoflavonoids, polyphenols as well as associated substances have very important part to play in defense of plant, for example, in *Vitis vinifera* (grape) trans-resveratrol (a phytoalexin) plays a role in plant defense against fungal pathogens including *Botrytis cinerea*, and another phytoalexin *delta*-viniferin is present in grapevine formed during fungal infection by *Plasmopara viticola*. Sakuranetin is a flavonoid type of phytoalexin found in *Polymnia fruticosa* and rice against the pathogen *Pyricularia oryzae.* 6-Methoxymellein (a dihydroisocoumarin) is synthesized in carrot slices by UV-c gene against the pathogen *Botrytis cinerea* (Kurosaki and Nishi 1983). Phytoalexin danielone exists in papaya fruit displaying extraordinary antifungal activity against *Colletotrichum gloeosporioides* (Echeverri et al. 1997). Stilbenes are a phytoalexin produced in *Eucalyptus sideroxylon* during pathogen attacks.

Allicin with the structure 3-hydroxy-5-methoxy-6-methyl-2-pentyl-4H-pyran-4-one is the first phytoalexin isolated from garlic (Illic et al. 2011). Allicin is another phytoalexin obtained from garlic. It is an organosulfur compound formed when alliinase enzyme converts alliin into allicin which also gives aroma to the fresh garlic. Allicin being an unstable compound quickly changes into a series of other sulphur-containing compounds such as diallyl disulphide having antibacterial, antifungal, antiviral or antiprotozoal activity. Other examples of associations of phytoalexin induction with the resistance in plants are displayed in Table 13.2. Production and function of phytoalexins for disease resistance can be restored or enhanced through genetic engineering techniques by introducing simple genetic constructs carrying phytoalexin synthesizing genes, for example, in grapevine, phytoalexin "resveratrol" is synthesized by stilbene synthase (STS) gene. STS genes (Vst1 and Vst2) from grapevine were transferred to tobacco through genetic transformation; and when the assays were performed in transformed plants, they showed higher resistance to *B. cinerea* (Hain et al. 1993). STS gene responsible for phytoalexin production was later transformed from different sources into many plant systems like *Arabidopsis*, papaya, tomato, wheat, barley, rice and alfalfa conferring resistance to various pathogens (Jeandet et al. 2013). In alfalfa, overexpression of enzyme isoflavone 7-O-methyltransferase played a critical part in biosynthesis of phytoalexin "maiackiain" which was associated with resistance of the plant to *Phoma medicaginis He and Dixon (2000).* Transformation of soya bean hairy root with both AhRS3 (peanut resveratrol synthase 3) and ROMT (resveratrol-0-methyltransferase) gene resulted in the resistance of transformed plant against *Rhizoctonia salami* (Zernova et al. 2014). Cytokinin overexpression in tobacco plant resulted in surge of resistance of plant against the pathogen *Phytophthora syringae* due to up-regulated synthesis of capsidiol and scopoletin, the two phytoalexins which we involved in the process (Grosskinsky et al. 2011). Similarly in the case of *Arabidopsis*, production of camalexin as well as resistance to *B. cinerea* was severally affected because of the mutation occurring in two MAP kinases (MPK3 and MPK6) (Ren et al. 2008). Some genes which function as phytoalexin biosynthesis regulators have also been identified, for example, overexpression of Rac protein in rice leads to accumulation of phytoalexin momilactone (Ono et al. 2001) and resistance of plant against bacterial blight. Overexpression of non-expressor of pathogenesis-related gene 1 induced the biosynthesis of phytoalexin gossypol (Parkhi et al. 2010).

Table 13.2 Phytoalexin induction and resistance to plant pathogens

Plant	Pathogen	Phytoalexin
Grapevine {*Vitis* spp.)	*Plasmopara viticola*	Viniferins
Grapevine (*Vitis* spp.)	*Botrytis cinerea*	Viniferins
Carnation (*Dianthus* spp.)	*Fusarium oxysporum*	Dianthalexin
		Methoxydianthramide S
Pea *(Pisum sativum)*	*Pseudomonas syringae*	Pisatin
Chickpea *(Cicer arietinum)*	*Ascochyta rabiei*	Medicarpin
		Maakiain
Citrus spp.	*Phytophthora citrophthora*	Scoparone
Oat *(Avena sativa)*	*Puccinia coronata*	Avenalumins

13.7 Proteins and Enzymes

Several plants and their seeds have protein that impedes pathogen and best enzymes after developing complexes that would block active sites or modify enzyme conformations. These proteins have small structure and mostly consist of amino acid cysteine. They include proteinase inhibitors, lectins, anhydrase inhibitors and defensins. Unlike simple chemicals like phenolics, alkaloids, terpenoids, etc. they need large amount of energy and resources of plant for their production. They are usually produced in significant concentration only after the attack by pest or pathogen. On activation these defensive protein and enzymes would effectively impede the growth of pathogens including fungi, bacteria as well as insect herbivores and nematodes.

13.7.1 Defensins

Small cysteine-rich protein displaying comprehensive antimicrobial activity was first isolated from endosperm of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). They are best characterized in seeds but are also present in other plant tissues like leaves, pods, tubers, fruits, barks, etc. They display extensive array of biological activities and impede the spread of wide-ranging fungi and bacteria (Thomma et al. 2002). They also impede digestive protein in herbivores. The exact mechanism of their action is still under consideration; it looks like that they attack molecular targets in the pathogens' plasma membrane. They disrupt cellular ion balance by forming new membrane pores as well as impede pre-existing ion channels.

13.7.2 Digestive Enzyme Inhibitors

Digestive enzyme inhibitors inhibit the digestion and disrupt the nutrient absorption by herbivores. Alpha-amylase inhibitor proteins bind to amylase enzyme impeding digestion of starch in legumes. Glycoproteins and non-enzymatic proteins like lectins bind to carbohydrates displaying various properties, for example, in invertebrates they cause lumping of blood cells and in insects they disrupt digestion (Peumans and Van-Damme 1995). Ricin combines lectin molecule with an N-glycoside hydrolase to produce a very powerful toxin in *Ricinus*. It impedes protein synthesis in animal cells.

13.7.3 Protease Inhibitors

Protease inhibitors impede digestive enzymes like chymotrypsin and trypsin. They are produced by plants as a defense mechanism to protect them from attack by herbivores. They have wide distribution but mainly seen in legumes, members of Solanaceae and grasses.

13.7.4 Hydrolytic Enzymes

When pathogens attack plants, some of the plants in order to defend themselves produce hydrolytic enzymes. These enzymes act upon pathogenic fungi by degrading their cell walls in the extracellular spaces where the enzymes get accumulated. For example, the degradation of chitin in cell wall of fungi is catalysed by chitinases. The degradation of glycosidic linkages in glucans existing in several oomycete (water moulds) cell walls is catalysed by glucanases. Another example of a hydrolytic enzyme with the ability to degrade cell walls of bacteria is lysozymes.

In this review we have discussed the utility of secondary metabolites occurring in plant in their defense system. We have tried our best to portray the role of different chemical compounds produced by plants itself against various plant pathogens. Several factors are involved in the interaction of a species of plants with its respective pest and non-pest and hence quite complex. Synthesis and accumulation of these compounds occur in tissues which are young and developing like leaves as well as in tissues of reproduction like seeds and flowers, thereby protecting young plant tissues. Some of the antimicrobial secondary metabolites are preformed, while others are induced by infection itself (phytoalexins, cyanogenic glycosides, glucosinolates, etc.). Organic farming can be made sustainable by identifying and properly using the natural chemical compounds to combat the pathogens, thereby eliminating or at least minimizing the use of fungicides in agriculture. Further research is required for development of natural pesticides. The genes required for producing these valuable defensive compounds can be isolated and then synthesized in bulk for the crop plants to be reengineered metabolically. This will make the plants more resistant towards threats posed by microbial pathogens, various herbivores and several environmental stresses.

References

Ballhorn DJ, Kautz S, Heil M, Hegeman AD (2009) Cyanogenesis of wild lima bean (Phaseolus lunatus L.) is an efficient direct defence in nature. Plant Signal Behav 4(8):735–745

Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127:612–633

Brooker N, Windorski J, Blumi E (2008) Halogenated coumarins derivatives as novel seed protectants. Commun Agric Appl Biol Sci 73(2):81–89

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12(4):564

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Ann Rev Plant Physiol Plant Mol Biol 48:355–381

DeVos M, Jander G (2009) Myzus persicae (Green peach aphid) salivary components induce defence responses in Arabidopsis thaliana. Plant Cell Environ 32(11):1548–1560

Echeverri F, Torres F, Quinones W, Cardona G, Archbold R, Roldan J, Brito I, Luis JG, LahlouU EH (1997) Danielone a phytoalexin from papaya fruit. Phytochemistry 44(2):255256. INIST: 2558881 (http://cat.inist.fr/?aModele=afficheN&cpsidt=2558881)

Eisner T, Meinwald J (1995) Chemical ecology: the chemistry of biotic interaction. In: (eds). National Academy Press, Washington, DC

Fenwick GR, Price KR, Tsukamota C, Okubo K (1992) Saponins. In: DMello JP, Duffus CM, Duffus JH (eds) Toxic substances in crop plants. Royal Society of Chemistry, Cambridge, pp 285–327

Freeman BC, Beattie GA (2008) An overview of plant defences against pathogens and herbivores. Plant Health Instr 0226-01.

Garas NA, Waiss AC (1986) Differential accumulation of antifungal sesquiterpenoids in cotton stems inoculated with Verticillium dahliae. Phytopathology 76:1011–1017

Gershenzon J, Croteau R (1991) Terpenoids. In: Rosenthal GA, Berenbaum MR (eds) Herbivores their interaction with secondary plant metabolites, Vol I: the chemical participants, 2nd edn. Academic, San Diego, pp 165–219

Geu-Flores F, Olsen CE, Halkier BA (2009) Towards engineering glucosinolates into noncruciferous plants. Planta 229(2):261–270

Grayer RJ, Harborne JB (1994) A survey of antifungal compounds from higher plants 1982–1993. Phytochemistry 37:19–42

Gross D, Porzel A, Schmidt J (1994) Phytoalexine mit Indolstruktur aus Kohlrabi [Phytoalexins with indole structure from Kohlrabi]. Z Naturforsch 49(5–6):281–285

Grosskinsky DK, Naseem M, Abdelmoshem UA, Plickert N, Engelke T, Griebel T, Zeier J, Novak O, Strand M, Pfeifhofer H et al (2011) Cytokinins mediate resistance against Pseudomonas syringae in tobacco through increased antimicrobial phytoalexin synthesis independent of salicylic acid signaling. Plant Physiol 157:815–830

Grubb C, Abel S (2006) Glucosinolate metabolism and its control. Trends Plant Sci 11:89–100

Hain R, Reif HJ, Krause E, Langebartels R, Kindl H, Vornam B, Wiese W, Schmelzer E, Schreier P, Stöcker R et al (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361:153–156

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Ann Rev Plant Biol 57:303–333

Hammond-Kosack KE, Jones JDG (1996) Resistance gene-dependent plant defence responses. Plant Cell 8:1773–1791

Harborne JB (1999) The comparative biochemistry of phytoalexin induction in plants. Biochem Syst Ecol 27:335–367

He XZ, Dixon RA (2000) Genetic manipulation of isoflavone 7-O-methyltransferase enhances biosynthesis of 4′-O-methylated isoflavonoid phytoalexins and disease resistance in alfalfa. Plant Cell 12(9):1689–1702

Hegnauer R (1988) Biochemistry, distribution and taxonomic relevance of higher plant alkaloids. Phytochemistry 27:2423–2427

Ilic Dusica P, Nikolic Ljubisa B, Stanojevic Ljiljana P, Nikolic Vesna D, Stankovic Mihajlo Z, Milorad C (2011) Allicin and related compounds: biosynthesis, synthesis and pharmacological activity(PDF). Facta Universitatis 9(1):9–20. https://doi.org/10.2298/FUPCT1101009I

Jeandet P (2015) Phytoalexins: current progress and future prospects. Molecules 20:2770–2774

Jeandet P, Clément C, Courot E, Cordelier S (2013) Modulation of phytoalexin biosynthesis in engineered plants for disease resistance. Int J Mol Sci 14:14136–14170

Jeandet P, Clément C, Courot E (2014a) Resveratrol production at large scale using plant cell suspensions. Eng Life Sci. https://doi.org/10.1002/elsc.201400022

Jeandet P, Hébrard C, Deville MA, Cordelier S, Dorey S, Aziz A, Crouzet J (2014b) Deciphering the role of phytoalexins in plant-microorganism interactions and human health. Molecules 19:18033–18056

Johnson R, Narvaez J, An G, Ryan C (1989) Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defence against Manduca sexta larvae. Proc Natl Acad Sci U S A 86:9871–9875

Kang SY, Kim YC (2007) Decursinol and decursin protect primary cultured rat cortical cells from glutamate-induced neurotoxicity. J Pharm Pharmacol 59(6):863–870

Kurosaki F, Nishi A (1983) Isolation and antimicrobial activity of the phytoalexin 6methoxymellein from cultured carrot cells. Phytochemistry 22(3):669. https://doi.org/10.1016/S00319422(00)869599

Lake JA, Field KJ, Davey MP, Beerling DJ, Lomax BH (2009) Metabolomic and physiological responses reveal multi-phasic acclimation of Arabidopsis thaliana to chronic UV radiation. Plant Cell Environ 32(10):1377–1389

Leustek T, Saito K (1999) Sulfate transport and assimilation in plants. Plant Physiol 120:637–643

Lewis NG, Yamamoto E (1990) Lignin: occurrence, biogenesis and biodegradation. Ann Rev Plant Physiol Plant Mol Biol 41:455–496

Lipka U, Fuchs R, Kuhns C, Petutschnig E, Lipka V (2010) Live and let die-Arabidopsis non-host resistance to powdery mildews. Eur J Cell Biol 89(2):194–199

Mari M, Lori R, Leoni O, Marchi A (1993) ln vim activity of glucosinolate-derived isothiocyanates against postharvest fruit pathogens. Ann Appl Biol 123:155–164

Mazid M, Khan TA, Mohammad F (2011) Role of secondary metabolites in defence mechanisms of plants. Biol Med 3(2):232–249

Mes JJ, Van Doorn AA, Wijbrandi J, Simons G, Cornelissen BJC, Haring MA (2000) Expression of the Fusarium resistance gene I-2 colocalizes with the site of fungal containment. Plant J 23:183–193

Monde K, Osawa SM, Harada N, Takasugi M, Suchy M, Kutschy P, Dzurilla M, Balentova E (2000) Synthesis and absolute stereochemistry of a cruciferous phytoalexin, (−)-Spirobrassinin. Chem Lett 598:886–887

Mordue AJ, Blackwell A (1993) Azadirachtin: an update. J Insect Physiol 39:903–924

Ono E, Wong HL, Kawasaki T, Hasegawa M, Kodama O, Shimamoto K (2001) Essential role of the small GTPase Rac in disease resistance of rice. Proc Natl Acad Sci U S A 98:759–764

Osbourn AE (1996) Preformed antimicrobial compounds and plant defences against fungal attack. Plant Cell 8:1821–1831

Parkhi V, Kumar V, Campbell LM, Bell AA, Shah J, Rathore KS (2010) Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing Arabidopsis NRP1. Transgenic Res 19:959–975

Pearce G, Strydom D, Johnson S, Ryan CA (1991) A polypeptide from tomato leaves induces wound inducible proteinase inhibitor proteins. Science 253:895–898

Pegg GF, Woodward S (1986) Synthesis and metabolism of a-tomatine in tomato isolines in relation to resistance to Verticillium albo-atrum. Physiol MOI Plant Pathol 28:187–201

Peumans WJ, Van Damme EJM (1995) Lectins as plant defence proteins. Plant Physiol 109:347–342

Poulton JE (1990) Cyanogenesis in plants. Plant Physiol 94:401–405

Price KR, Johnson IT, Fenwick GR (1987) The chemistry and biological significance of saponins in food and feeding stuffs. Crit Rev Food Sci Nutr 26:27–133

Ren D, Liu Y, Yang KY, Han L, Mao G, Glazebrook J, Zhang S (2008) A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in Arabidopsis. Proc Natl Acad Sci U S A 105:5638–5643

Rosenthal GA (1991) The biochemical basis for the deleterious effects of L-canavanine. Phytochemistry 30:1055–1058

Saito K (2004) Sulfur assimilatory metabolism. The long and smelling road. Plant Physiol 136:2443–2450

Schafer H, Wink M (2009) Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. Biotechnol J 4(12):1684–1703

Seigler DS (1991) Secondary metabolites and plant systematic. In: Conn EE (ed) The biochemistry of plants, Secondary plant products, vol 7. Plenum, New York/London, pp 139–176

Smith CA, MacHardy WE (1982) The significance of tomatine in the host response of susceptible and resistant tomato isolines infected with two races of Fusarium oxysporum f. sp. lycopersici. Phytopathology 72:415–419

Sreevidya VS, Srinivasa RC, Rao C, Sullia SB, Ladha JK, Reddy PM (2006) Metabolic engineering of rice with soyabean isoflavone synthase for promoting nodulation gene expression in rhizobia. J Exp Bot 57(9):1957–1969

Thomma BPHJ, Cammue BPA, Thevissen K (2002) Plant defenses. Planta 216(2):193–202

Turlings TCJ, Loughrin JH, Mccall PJ, Roese USR, Lewis WJ, Tumlinson JH (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc Natl Acad Sci U S A 92:4169–4174

Van Etten HD, Mansfield JW, Bailey JA, Farmer EE (1994) Two classes of plant antibiotics: phytoalexins versus “phytoanticipins”. Plant Cell 6:1191–1192

Wink M (1999) Functions of plant secondary metabolites and their exploitation in biotechnology, Annual plant reviews, vol 3. CRC Press, Boca Raton

Zernova OV, Lygin AV, Pawlowski ML, Hill CB, Hartman GL, Widholm JM, Lozovaya VV (2014) Regulation of plant immunity through modulation of phytoalexin synthesis. Molecules 19:7480–7496