



Role of Secondary Metabolites in Plant Defence Mechanism

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ABSTRACT

Secondary metabolites found in plants have a role in defence against herbivores, pests and pathogens. In this review we have discussed role of metabolites in plant defence. The role of secondary metabolites in defence may involve deterrent and anti-feedant activity, toxicity or acting as precursors to physical defence systems. Role of both cyanogenic glucosides and glucosinolates, phenolics are discussed as defensive compounds. Biochemistry of metabolites and their formation are discussed. An enormous variety of secondary metabolites are derived from shikimic acid or aromatic amino acids, many of which have important roles in defence mechanisms. Several classes of secondary products are induced through environmental stresses, infection, wounding or herbivory. Role of salicylates and jasmonates have been discussed as signals and in many other physiological processes.

KEYWORDS : Cyanogenic glucosides, phenolics, salicylic and jasmonic acids, benzoxazinoids

Introduction

Higher plants synthesize an enormous number of chemicals which are of diverse structure and class which is divided into primary and secondary metabolites. The primary metabolites consist of metabolites such as sugars, fatty acids, amino and nucleic acids, as well as chemicals necessary for plants growth and development. Secondary metabolites are structurally and chemically much more diverse than the primary metabolite and are localized in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism. Plants have metabolic pathways leading to tens of thousands of secondary products capable of effectively responding to stress situations imposed by biotic and abiotic factors. Most of the secondary metabolites include terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols), phenolics (such as phenolic acids, coumarins, lignins, stilbenes, flavonoids, tannins and lignin) and nitrogen containing compounds (such as alkaloids and glucosinolates). The present review focuses on role of secondary metabolites in plant defence against biotic stress and abiotic stress.

(2) Cyanogenic glucosides

Cyanogenic glucosides are mostly derived from aromatic and branched amino acids. These compounds are found in both monocots (e.g. sorghum and barley) and dicots (e.g. cassava and clover). Tissue breakdown exposes the vacuole-located glucosides to glucosidases and hydroxynitrile lyases with subsequent hydrolysis leading to the release of cyanide which is a universal respiratory poison. The presence of cyanogenic glucosides in crop plants can cause severe problems to animals and humans. For example, Cassava a staple starch crop for most of the third world countries and to reduce exposure to cyanide preparation methods for the tubers have been developed (Rosling, 1988). Many breeding programmes have produced cassava lines with lower levels cyanogenic glucoside content but no totally acyanogenic lines have been obtained.

Cyanogenic glucosides are present in both plants and animals to detoxify cyanide through 3-cyanoalanine synthesis or the action of rhodanese which converts cyanide and thiosulphate to sulphate and thiocyanate. Bellotti and Arias (1993) indicated that the presence of cyanogenic glucosides improved the resistance of cassava tubers to the cassava root borer. The biochemistry of cyanogenic glucoside synthesis and metabolism has been extensively studied. A precursor amino acid (aromatic and branched-chain amino acids are precursors of many common cyanogenic glucosides) is N-hydroxylated via a cytochrome P450 enzyme followed by decarboxylation to produce an aldoxime. Another cytochrome P450 enzyme is involved in the C-hydroxylation of the acetonitrile intermediate prior to glycosylation to the final cyanogenic glucoside. The entire pathway is closely linked to channelled membrane-bound complex and microsomal preparations which catalyses the whole process (Halkier and Moller, 1989). Cyanogenic glucosides may be transported through the plant from the site of synthesis.

(3) Glucosinolates

The glucosinolates are sulphur- and nitrogen-containing compounds

found in plants of the order Capparales and a few other unrelated taxa. Glucosinolates are located in a separate compartment like vacuole away from a specific thioglucosidase and myrosinase, which may be sequestered in specialized 'myrosin' cells (Halkier and Gershenzon 2006). When plant tissues are disrupted by physical damage, herbivory, pathogen attack the enzyme hydrolyses the glucosinolates to produce a variety of products. Breakdown products of glucosinolates are responsible for the flavours of many food plants such as mustards, radishes and cabbages. Glucosinolates and pest/pathogen attack has focused on oilseed rape (*Brassica napus* L.) a major oil crop in Europe and North America. Breeders are working to reduce the glucosinolate content of harvested seed as the problems related with protein-rich seed meal which was obtained after oil extraction as a feed stuff for farm animals. Glucosinolates and their hydrolysis products play a role as mediators in plant-insect interactions. Several studies have reported that glucosinolates exhibit growth inhibition or feeding deterrence to a wide range of general herbivores such as birds, slugs and generalist insects (Halkier and Gershenzon 2006). To ensure the normal function of glucosinolates in defense, myrosinase should be present and therefore glucosinolate-sequestering insects contain endogenous myrosinase or rely on the myrosinase activity present in the guts of their enemies. The aphid *Brevicoryne brassicae* has been reported to possess its own myrosinase (Kazana et al., 2007) which produces isothiocyanates from sequestered glucosinolates when the aphid is damaged or killed. These isothiocyanates also serve as alarm signal to other members of the colony (Radevnikovič et al., 2008).

Several studies indicated changes in glucosinolates pattern after treatment with signal molecules providing another indication for a defense related role of glucosinolates. Previous studies showed that exogenous JA application usually elicits dramatic increase of indole glucosinolates while the aliphatic ones remain unchanged (Textor and Gershenzon, 2009) as observed in the treatment of white mustard and oilseed rape with methyl jasmonate (MeJa) accumulated indole-3-ylmethyl glucosinolate (Bodnaryk, 1992). Numerous studies have shown that glucosinolates and their hydrolysis products have an impact on herbivores and pathogens, however, many questions arise about the mechanism of glucosinolate induction, the signalling pathways involved and the plant's potential benefit from glucosinolate accumulation remain to be solved.

(4) Plant phenolics

Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Phenolics and polyphenols arises biogenetically from the shikimate- phenylpropanoids- flavonoids pathways which produces monomeric and polymeric phenols and polyphenols (Massad et al., 2012) (Figure 1). Each class of phenolic compounds has distinctive absorption characteristics and their spectra varied phenols and phenolic acids (250-290 nm), cinnamic acid derivatives (290-330 nm), flavones and flavonols (250 and 350 nm), chalcones and aurones (350 nm), anthocyanins and betacyanins (475-560 nm and 535-545 nm, respectively) (Harborne, 1991).

(a) UV-Screens

Plants are exposed to ambient solar ultraviolet-B (UV-B) radiation (280–320 nm) under natural conditions which is harmful for plants. Phenolic compounds are synthesized to protect against UV-B radiations and pest attack too. Phenolics act as a screen inside the epidermal cell layer and also by adjusting the antioxidant systems at both cell and whole organism level (Singh et al., 2015).

(b) Phenolics as defence

Plant phenolics acts in defence response against herbivory. Wheat cultivars having high intrinsic concentrations of soluble and cell wall-bound phenolics are much less attractive to the cereal aphid (*Rhopalosiphum padi*) than cultivars with low phenolic concentrations (Luczynski et al., 1985). When tissue is damaged phenolic glycosides such as salicin, salicetin and fragilin derivatives of salicylic acid are formed through hydrolyses to releases against plant defence which delays development of the mites as phenolics bind to mite digestive enzymes and inactivate them (Tahvaneinen et al., 1985). Plants inducing high levels of phenolics are less palatable to herbivores.

(5) Plant Terpenes, Sesquiterpenoids and sterols

The largest group of plant secondary metabolites are plant terpenes and till date ten thousand of terpenes are isolated and purified and their structures are validated (Degenhardt et al., 2009). Terpenes are hydrocarbons derived from isoprene (isopentane) C_5 units. They act as plant growth regulators, defense molecules against herbivores and pathogens, attracting compounds for pollinators and increases thermotolerance (Berli et al., 2010; Mazid et al., 2011). Leaves fumigated with monoterpenes lead to increase in thermotolerance of the oak (*Quercus ilex*). Terpene play important role in attracting pollinators and defence against herbivores. Within plant tissue storage of terpenes are required for interaction with insects. Sterols are terpene derivatives which is an essential components of the cell membrane. There are more examples of plant terpenes involved in resistance to insects than against microorganisms. The insecticidal activity of the terpenes is either due to their action as antifeedants (or deterrents), toxins or as modifiers of insect development (Harborne, 1991). In desert plants a number of terpenoids and sesquiterpenoids have been found to be good insect deterrents (Rodriguez, 1983). The red, orange and yellow carotenoids are tetraterpenes which function as accessory pigments in photosynthesis and protect photosynthetic tissues from photooxidation. Carotenoids have two major roles in photosynthetic organisms and first role is to act as light-harvesting pigments, extending the range of the light spectrum available for use in the photosynthetic process. The hormone abscisic acid is a C_{15} terpene which is produced by degradation of a carotenoid precursor (Mazid et al., 2011).

(6). Benzoxazinoids

Benzoxazinoids are secondary metabolites and represent protective and allelopathic metabolites and mostly found in Poaceae (Frey et al., 2009) (Gramineae) (Figure 2) maize, wheat and rye. Apart from Poales, benzoxazinoids are detected in Ranunculales and Lamiales. DIBOA [2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one] and its C-7-methoxy derivative DIMBOA (Fig. 1) are the major representatives of benzoxazinoids in plants (Niemeyer, 1988). The end product of the benzoxazinoid biosynthesis is the glucoside that has a reduced toxicity compared to the aglucon. The glucoside is stored in the vacuole or in the plastid. The toxic aglucon is produced upon disintegration of the cell due to pathogen or pest attack. The reactivity of DIBOA and DIMBOA with e.g. NH_2 and SH nucleophilic groups in biomolecules (Sicker et al., 2000) confers protection against a wide range of herbivores, pathogenic fungi and bacteria DIMBOA functions as a defense regulatory signal in maize and acts as an activity of a biocidal defense metabolite (Dick et al., 2012) (7) Salicylic acid and methyl jasmonate

There has been considerable cross talk about roles of salicylic acid and methyl jasmonate of the secondary metabolites in plants as a primary or secondary metabolites. Apart from their potential roles as plant signals but also their production and biosynthetic regulation. Both salicylic acid and methyl jasmonate modulate and induce many plant genes. They have recently been found to regulate the biosynthesis of other secondary metabolites. Salicylic acid (SA) can be synthesized in plants via two pathways, but in both cases the

main precursor is phenylalanine. The first step is the conversion of phenylalanine to trans-cinnamic acid catalyzed by PAL (Gao et al., 2015) (Figure 2). SA can be formed from cinnamate via *o*-coumarate or benzoate depending on whether the hydroxylation of the aromatic ring takes place before or after the chain-shortening reactions. Klambt (1962) reported that isotope feeding showed that SA was formed from benzoate in sunflower, potato and pea plants, which is synthesized by cinnamate chain shortening reactions most likely through a β -oxidation process analogous to fatty acid β -oxidation. While in *Primula acaulis* and *Gaultheria procumbens* leaf segments feeding of ^{14}C -labeled phenylalanine and cinnamate showed that SA was formed via *o*-coumarate (Truman and Glazebrook). Likewise, in young tomato seedlings, SA appeared to be formed mostly from cinnamate via benzoate but after infection with *Agrobacterium tumefaciens*, 2-hydroxylation of cinnamate to *o*-coumarate was favoured (Chen et al., 2009). The role of SA as a systemic signal for the induction of pathogenesis-related proteins (PR proteins) and resistance to pathogens has been demonstrated by numerous groups (Shah and Zeier, 2013; Truman and Glazebrook, 2012). It has also reported that SA induced glucosinolates in oilseed rape (*Brassica napus*) when applied as a spray or as a soil drench treatment (Kiddle et al., 1994) led to a selective induction of phenyl ethyl glucosinolate (gluconasturtin) 7 d after SA treatment. Salicylic acid has been shown to be an important signal in plant resistance to pathogens but there are still many unanswered questions regarding its transport and sites of action within the plant.

Methyl jasmonate (MJ) and jasmonic acids have been found in a large number of plant species (Anderson, 1989). They are synthesized in plants from the fatty acid linolenic acid. The first step in the pathway is catalysed by a lipoxygenase to form a hydroperoxide (Anderson, 1989). Methyl jasmonate and jasmonic acids are known to regulate a number of physiological processes in plants including the induction of senescence, vegetative storage proteins, proteinase inhibitors (herbivore antifeedants), meristematic growth (Farmer and Ryan, 1990; Koda, 1992). They also have a role in signal transduction especially in relation to defence gene induction (Gundlach et al., 1992). Responses that are regulated or induced by methyl jasmonates provide evidence for a key role in plant development and defence, especially the induction of responses directly involved in resistance, i.e. glucosinolates, PAL and lipoxygenases. Still the regulation of pathways is coordinated by salicylic acid and methyl jasmonates and other signals in healthy and infected plants (susceptible or resistant) needs to be determined.

Conclusions

The present review dealt with small segment of plant metabolites reported till date and their potential role in plant defence. But still there are more metabolites with a vary varied distribution. It was also found that few secondary metabolites like glucosinolates and cyanogenic glucosides are species-specific, while few are universal secondary metabolites have varied role to play against pest and pathogen defence these compound may be involved in storage, protection from UV, attracting pollinators, allelopathic interactions with other plants. These interactions are complex and many factors are involved which need to be studied in details. There are many cross talks between secondary metabolites or as signalling molecules. So in the present scenario, we need exploit in detail mechanism of secondary metabolites formation and their role in plant defence or as signalling molecules.

Acknowledgements



Figure 1: Pathway of synthesis of phenolic compounds (adapted from Ewané et al., 2012)

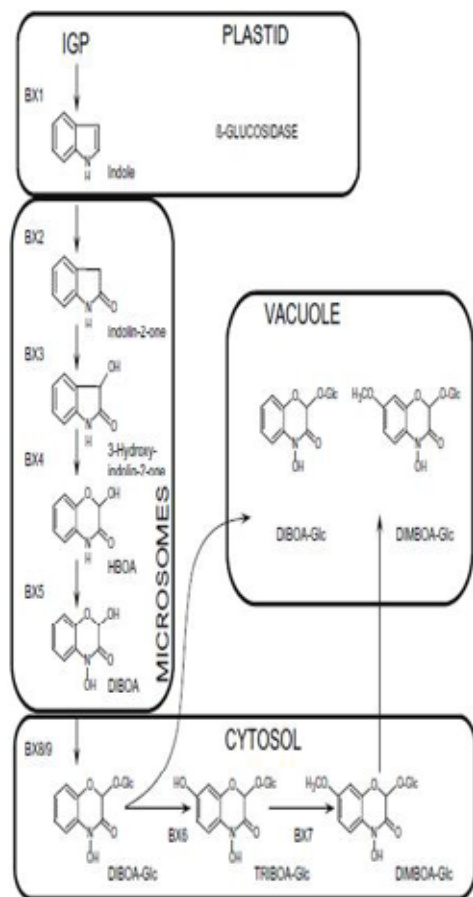


Figure 2: Biosynthetic pathway of Benzoxazinoid in plants (Adapted from Frey et al., 2009)

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