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Role of Secondary Metabolite in Plant Defense

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1.1 Introduction:

Plants face an enormous number of enemies and therefore have evolved stacks of defense mechanisms by which they are able to tackle various kinds of abiotic and biotic stress like fungi, bacteria, viruses, parasites, weeds, and insects. To overcome the biotic and abiotic stresses they have evolved multiple mechanisms of defense. These stresses result in abnormal physiology of the plant which impairs the plant's normal growth, structure, and functions. For accommodating these changes in their fluctuating growth conditions and for better functional flexibility under the influence of various abiotic factors without impacting various physiological and biochemical processes plants have developed numerous mechanisms by producing a repertoire of metabolites in response to changing environment that plays a variety of roles (Yang *et. al.*, 2018). Metabolites are the intermediates and end products of various metabolic processes. The plant produces a vast and diverse assortment of organic molecules, which are classified into two major groups: primary and secondary metabolites. Primary metabolites are intermediate or end products of all living systems, essential for the growth and life of plants. Secondary metabolites are products that do not significantly participate in the direct growth and development of the plant. Their absence does not result in the death of the plants, unlike primary metabolites. These bioactive compounds are used in pharmaceuticals, flavorings, and agrochemical industries.

Humans have been using these metabolites as a source of medicines and drugs for the treatment of many human diseases. Studies on bioactive metabolites have shown their nutritional value in food and flavoring industries. The natural products of plants have shown prominent antimicrobial activity in many in-vitro studies. They provide protection against pests, pathogens, and herbivores some of them also are involved in defense against abiotic stresses (e.g. UV radiations) (Schafer *et al.,* 2009). Plant are able of synthesizing secondary metabolites, like phenols, terpenes, alkaloids, cyanogenic glycosides, tannins, and flavonoids (Cowan, 1999). Phenolics, like thymol, eugenol and carvacrol are highly bioactive compounds against pathogenic microbes.

These compounds show defensive properties against pathogenic microorganisms due to their toxicity and repellency. Allicin is a volatile antimicrobial compound found in garlic and its accumulation increases rapidly when the garlic tissues are damaged by biotic factors. Allicin was found effective to control many seed-borne pathogens like *Alternaria* spp. in carrot, leaf blight of tomato caused by *Phytophthora*, *Magnapor the* on rice, downy mildew of *Arabidopsis thaliana,* and tuber blight of potato by *Alternaria solani* (Slusarenko *et. al.,* 2008). Various metabolites are also effective to control post-harvest diseases. Phytochemicals also have antiviral properties. Anti-viral proteins (AVP) are found in extracts of *Prosopis chilinesis* and *Bougainvillea spectabilis* were effective to manage the sunflower necrosis virus (SFNV) of sunflower and cowpea (Lavanya *et. al.,* 2009). The current focus is to search for biological properties of new phytochemicals or natural bioactive products for antibiotics, medicines, insecticides, herbicides, and fungicides production.

1.2 Plant Secondary Metabolites:

Secondary metabolites can be broadly divided into four major classes: Terpenes, Phenolics, Alkaloids, and Glycosides.

1.2.1 Terpenes:

The terpenes comprise a large class of secondary natural products. All terpenes are derived from the union of five carbon elements that have the branched carbon skeleton of isopentane. The plant produces a wide range of different terpenes structures as a secondary metabolite that are believed to be involved in defense against plant-feeding herbivores, insects, and mammals as toxins and feeding deterrents (Gershenzon,1991). The mode of defense can be either directly or indirectly. Accumulation of phytochemicals that have antimicrobial activities can be included in the direct mode of defense. In response to various biotic stress i.e., fungal or bacterial infection antimicrobial compounds such as phytoalexins are synthesized as part of plant defense. Phytoalexins are a low-molecular-weight chemical compound that accumulates at the site of infection that help in limiting the spread of the pathogen. Few plants produce phytoalexins such as diterpenes and sesquiterpenes e.g. In *Oryza sativa* 14 different diterpenes phytoalexins have been investigated which can be grouped into four types- oryzalexin A-F (Peters, 2006), monilactones A and B and oryzalexin S (Tamongani and Mitani, 1993). In papaya fruit, a phytoalexin Danielone is found against a pathogenic fungus *Colletotrichum gloesporioides*, which exhibits high antifungal activity.

The indirect mode of defense is indicated by their ability to defend plants against herbivores by enhancing the effectiveness of natural enemies of the herbivores. Certain monoterpenes and sesquiterpenes are produced by corn, wild tobacco, cotton, and other species, released only after tissues damaged due to insect feeding. Terpenes play an important defensive role in plants due to their toxicity and deterrent nature toward plant-feeding insects and mammals (Gershenzon, 1991). Like, Pyrethroids a monoterpenoid ester, found in the flowers and leaves of Chrysanthemum species have insecticidal properties. Both natural and synthetic pyrethroids have negligible toxicity to mammals and very low persistence in the environment so they are highly demanding ingredients in commercial insecticides.

1.2.2 Phenolic Compounds:

Phenolics are chemical compounds that contain a phenol group or hydroxyl substituents on an aromatic ring. They probably constitute the most abundant and largest group of secondary metabolites and are usually found as esters or glycosides rather than as free compounds. Phenolics play a variety of roles in plants. They are involved in the defense against UV-radiation. They contribute significantly as defense compounds against herbivores and pathogens. Phenolic compounds are primarily synthesized from the product of the Shikimic Acid Pathway. Most of the phenolic compounds in plant-derived from the deamination of phenylalanine to cinnamic acid. This conversion is catalyzed by phenylalanine ammonia-lyase (PAL).

A. Lignin:

It is a branched polymer of phenylpropanoid groups. After cellulose, it is second most abundant substance present in secondary cell walls of xylem tracheary elements. It provides physical toughness and chemical durability rigidity to plants and acts as a physical barrier to herbivores and rigid to pathogens and insects. It has been reported that lignification blocks the invasion and growth of pathogens and is a defense response by plants to infecting or wounding pests. Monolignol biosynthesis in wheat plays an important role in cell wall apposition (CWA)-mediated defense against penetration of powdery mildew fungus into wheat (Bhuiyan et.al. 2009). In wheat monolignol genes led to the high susceptibility of wheat leaf tissues to an appropriate pathogen, Blumeria graminis f. sptritici, and compromised penetration resistance to a non-appropriate pathogen, B. graminis f. sp. hordei.

B. Flavonoids:

More than 5000 different flavonoids are known and they constitute a diverse class of natural phytochemicals present in all plant species. A flavanone Sakuranetin is found manily in Polymnia fruticosa and Oryza sativa, where it acts as a phytoalexin to inhibit the spore germination of Pyricularia oryzae (Kodama et. al., 1992). A Study by Wuyts (2006) shows that flavonoids can inhibit egg hatching of plant parasitic nematodes in the egg stages kaempferol, a flavonoid that inhibited the egg hatching of burrowing nematode Radopholus similis. For plant parasitic juveniles, flavonoids (1) Induces quiescence by Slowing down their movement resulting in periods of reversible inactivity (2) Repel them by modifying their migration (3) sometimes kill

Them. At micromolar concentrations the flavonols kaempferol, quercetin, and myricetin repelled and slowed the movement of root-knot nematode *M. incognita* juveniles. At various duration of exposure and concentration Patuletin, patulitrin, quercetin, and rutin killed the juveniles of cyst nematode *Heterodera zeae* (Faizi *et. al.*, 2011).

1.2.3 Tanins:

They are second category of phenolic polymers, besides lignins which provide toughness and rigidity to plants. It has been reported that pigmented red onions are resistant to fungus *Colletotrichum circinans* cause of onion smudge due to presence of catechol and protocatechuic acid in significant amount while white onions do not contain significant quantities of these phenolic compounds so are susceptible to onion smudge. Catechol and protocatechuic acid show defensive properties in onion against smudge disease (Walker, 1923).

1.3 Nitrogen Containing Metabolite:

This category includes alkaloids and cyanogenic glycosides.

1.3.1 Alkaloids:

Approximately 20% species of vascular plants synthesizes nitrogen containing secondary metabolites. They are one of the diverse and largest metabolites (Hegnauer *et al.*, 1988). Due to their high toxicity, they are efficient against pathogens and predators and acts as defense compound in plants. Poulton (1990) found that dhurrin, a nitrogenous alkaloid, present in sorghum is highly effective against soilborne pathogens. It has been reported berberine induces crop resistance in the greenhouse conditions against TMV, *Blumeria graminis, Botrytis cinerea, and Phytophthora nicotianae*. It induces immune responses, including an increase in defense enzymes, accumulation of H2O2, hypersenstivity response, upregulation of salicylic acid (SA) biosynthesis, and high accumulation of pathogenesis-related (PR) protein

1.3.2 Glycoside:

A glycoside is a molecule in which a carbohydrate (sugar) is bound to a non-carbohydrate moiety containing a hydroxyl group by a glycosidic bond. Three most important glycoside are saponins, cyanogenic glycosides and Glucosinolates. Cyanogenic glycosides are a particularly toxic class of nitrogenous compound. They are bioactive plant product have protective function in plants. These are natural source of HCN. On enzymatic hydrolysis cyanogen glycosides yield the aglycone (that is an alpha-hydroxynitrile) and the sugar moiety.

The plant produces cynogenic glycosides and also produces enzymes that convert these compounds into hydrogen cyanide, including glycosidases and hydroxyl nitrile lyases. The breakdown of cyanogenic glycosides releases HCN, which inhibits the activity of iron-containing cytochrome oxidase. It has been reported in rice HCN inhibits hyphal growth of *Magnaporthe grisea*, a blast fungus of rice to protect against fungal attacks.

A cyanogenic glycoside Amygdalin is found in excessive amounts in seeds and kernels of almonds, cherries, peaches, apples, and apricot (Nahrstedt, 1972; Jones, 1957) and is also abundant in seeds of plums. In sorghum Dhurrin a glycoside accumulates in vacuoles of epidermal cells, in response to attack or damage by insects, and herbivores. It has been reported transgenic that sorghum was severely attacked by herbivores and insects, since it cannot produce dhurrin while in wild-type varieties of sorghum dhurrin accumulate at the site of damage (Krothapalli *et al.,* 2013).

Glucosinolates are immensely found in the Brassicaceae family also known as mustard oil glycosides and break down to release defensive substances. In unfavorable environmental stimuli and other biotic stresses, they rapidly breakdown to release defensive substances isothiocyanates by enzyme beta-thioglucoside glucohydrolases (TGGs), also known as myrosinases (Andreasson et.al. 2001; Husebye et.al. 2002). Due to serve infection of white rust and club roots in and Indian mustard roots and Broccoli there is an accumulation of glucosinolates in excessive amounts (Islam, 2009). In *Arabidopsis thaliana* non-host resistance against bitrophic fungi is enhanced by glucosinolates derived from tryptophan. (Sanchez *et al.,* 2010). Also, *Arabidopsis thaliana* establish disease resistance to *Phytophthora brassicae* in response to this pathogen plant induces genes which encode for enzymes involved in biosynthesis glucosinolates biosynthesis. Therefore, tryptophan-derived secondary metabolites establish disease resistance in Arabidopsis. The active metabolic products released in soil by degradation of Brassicaceous tissues is widely used to tentatively control soil-borne pests (Angus e*t al.,* 1994; Brown *et al.,* 1997).

Saponin are one of diverse groups of secondary metabolite widely found in plant kingdom to provide protection against pathogen and herbivores. Structurally saponins consists of steroid aglycone to which one or more sugar is attached. The aglycone part called sapogenin can be either a triterpene (C30) or steroid (C27), which is widely found in monocots and dicots. Their main role in plants is to dispense protection against attack by pest and pathogen. (Price *et al.,* 1987; Morrissey and Osbourn, 1999). A class of saponin triterpenoid have been reported from oats species. Oats contain four the aglycone part called sapogenin can be either a triterpene (C30) or steroid (C27), which is widely found in monocots and dicots.

Their main role in plants is to dispense protection against attack by pest and pathogen. Unique saponins, Avenacins A-1, B-1, A-2, and B-2 which are structurally related to each other (Crombie *et al.,* 1986). Among the four in extracts from roots of young oat plant Avenacin A-1 is present in plenteous amount constituting around 70% of the total avenacin content (Crombie and Crombie, 1984).

Saponins act on pathogen by the formation of micelles like complex with sterols in fungal membranes resulting in membrane perturbation and loss of membrane integrity. It has been reported oat confer resistance against “take-all disease” which is caused by *Gaeumannomyces graminis var. tritici,* a root-infecting fungus due to the presence of Avenacin (triterpenoid saponin). Even though the fungus *G. g. var. tritici* causes extreme yield losses in barley and wheat, which is unable to infect oats, and unlike the oat-attacking variety of *G. graminis, G. g.* var. *avenae,* it is relatively sensitive to avenacins. Therefore, resistance conferred by oats to *G. g. var. tritici* is attributed to presence of these saponins in oat roots (Turner, 1953).

1.4 Mode of Action of Secondary Metabolites:

They form covalent bond with free amino groups or sulfide group which results in loss of function or change in structure in three-dimensional structure of protein (Wink, 2008). Most of the secondary metabolites act on pathogens by inhibiting their enzymes, reproductive system and DNA alkylation, etc and also affect the physiological activities of the pathogen.

Phenolic compound results deformation of structure and function of membrane proteins which changes cell permeability of microbes which disturbs pH gradient, membrane bonded enzymes, ATP production, and substrate utilization for ATP production. The multitarget mechanisms of action of alkaloids are disruption of the outer membrane or cytoplasmic membrane and leakage of cell components, respiratory inhibition in aerobic pathogens, the Z-ring perturbation, and inhibition of cell division (Cushnie *et al.*, 2014).

Table 1.1: Mode of Action Different Secondary Metabolite

Sr. No	Secondary Metabolite	Mode Of Action
1	TERPENIODS AND ESSENTIALOIL	Membrane disruption
2	TANNINS	Bind to proteins, Inhibition of enzymes, substratedeprivation
3	PHENOLICS	Membrane disruption, substrate deprivation
4	PHELOLICS ACID	Bind to adhesins, complex with the cell wall, inactivate enzymes
5	FLAVANOIDS	Bind to adhesins, complex with cell wall, Inactivate enzymes
6	ALKALOIDS	Intercalate into cell wall, Z-ring perturbation, Respiratory inhibition
7	SAPONIN	Cell membrane disruption and cell component leakage

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