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Role of secondary metabolites in defense mechanisms of plants

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Abstract
In all natural habitats, plants are surrounded by an enormous number of potential enemies (biotic) and various kinds of abiotic environmental stress. Nearly all ecosystems contain a wide variety of bacteria, viruses, fungi, nematodes, mites, insects, mammals and other herbivorous animals, greatly responsible for heavy reduction in crop productivity. By their nature, plants protect themselves by producing some compounds called as secondary metabolites. Secondary metabolites, including terpenes, phenolics and nitrogen (N) and sulphur (S) containing compounds, defend plants against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses. This review presents an overview about some of the mechanisms by which plants protect themselves against herbivory, pathogenic microbes and various abiotic stresses as well as specific plant responses to pathogen attack, the genetic control of host-pathogen interactions.

Keywords: Secondary metabolites; defense mechanism; phytoalexins; terpenes; alkaloids; phenolics; cynogenic glucosides.

Abbreviations: GST, glucosinolate synthase transferase; SIR, systematic induced resistance; GSL, glucosinolates; GSH, glutathione; HCN, hydrogen cyanide.

Introduction

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 (Ballhorn *et al*., 2009). The most significant biotic and abiotic and man-made stress factors are summarized in figure 1. 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 (Agosta, 1996; Barbour *et al*., 1987; Bostock, 1999; Bostock *et al*., 2001; Hell, 1997; Thomma *et al*., 1998; Vijayan *et al*., 1998; Whittaker and Feeny, 1971; 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 signaling systems enabling the rapid detection of pathogen invasion and initiation of vigorous defensive responses (Schaller *et al*., 1996). Once infected, some plants also develop immunity to subsequent microbial attacks (Putnam and Heisey, 1983; Putnam and Tang, 1986; Bernays, 1989; Elakovich, 1987).

Plants produce a high diversity of natural products or secondary metabolites with a prominent function in the protection against predators and microbial pathogens on the basis of their toxic nature and repellence to herbivores and microbes and some of which also involved in defense against abiotic stress (e.g. UV-B exposure) and also important for the communication of the plants with other organisms (Schafer *et al*., 2009), and are insignificant for growth and developmental processes (Rosenthal *et al*., 1991). There are three major groups of secondary metabolites viz terpenes, phenolics and N and S containing compounds. Terpenes composed of 5-C isopentanoid units, are toxins and feeding deterrents to many herbivores. Phenolics synthesized primarily from products of the shikimic acid pathway, have several important defensive role in the plants. Members of the third major group i.e. N and S containing compounds are synthesized principally from common amino acids (Rosenthal *et al*., 1992; Van Etten *et al*., 2001). Recent *in vitro* experiments using plants whose secondary metabolites expression has been altered by modern molecular methods have to confirm their defensive roles (Mes *et al*., 2000; Mansfield, 2000). Although the situation is still

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unclear, it is believed that most of the 100,000 known secondary metabolites are to be involved in plant chemical defense systems, which are formed throughout the millions of years during which plants have co-existed with their attackers (Wink, 1999). Although higher concentrations of secondary metabolites might result in a more resistant plant, the production of secondary metabolites is thought to be costly and reduces plant growth and reproduction (Simms, 1992; Karban and Baldwin, 1999; Simens *et al*., 2002). The cost of defense has also been invoked to explain why plants have evolved induced defense, where concentrations generally increase only in stress situations (Harvell and Tollrian, 1999).

During the last several years, it has been discovered that hundreds of compounds that plants make have significant ecological and chemical defensive roles, opening a new area of scientific endeavour, often called ecological biochemistry (Harborne, 1988, 1989).

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 (Hartmann, 1991). 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 (Simms, 1992; Karban and Baldwin, 1997; Harvell and Tollrian, 1999; Stotz *et al*., 1999; Siemens *et al*., 2002). Therefore, defense metabolites can be divided in to constitutive substances, also called prohibitins or phytoanticipins and induced metabolites formed in response to an infection involving *de novo* enzyme synthesis, known as phytoalexins (Van Etten *et al*., 1994; Grayer and Harborne, 1994). Phytoanticipins are high energy and carbon consuming and exhibit fitness cost under natural conditions (Mauricio, 1998), but recognized as the first line of chemical defense that potential pathogens have to overcome. In contrast, phytoalexin production may take two or three days, as by definition first the enzyme system needs to be synthesized (Grayer and Harborne, 1994).

Principal groups

Plant secondary metabolites can be divided into three chemically distinct groups viz: Terpenes, Phenolics, N and S containing compounds.

(i) Terpenes

Terpenes constitute the largest class of secondary metabolites and are united by their common biosynthetic origin from acetyl-coA or glycolytic intermediates (Gerhenzon *et al*., 1991; Grayson, 1998; Fraga, 1988; Croteas, 1988; Loomis and Croteas, 1980; Robinson, 1980). A vast majority of the different terpenes structures produced by plants as secondary metabolites that are presumed to be involved in defense as toxins and feeding deterrents to a large number of plant feeding insects and mammals (Gershenzon and Croteau, 1991). Below, several examples will draw from the 5 major subclasses:

(a) *Monoterpenes (C_{10}):* Many derivatives are important agents of insect toxicity. For example, the pyrethroids (monoterpenes esters) occur in the leaves and flowers of *Chrysanthemum* species show strong insecticidal responses (neurotoxin) to insects like beetles, wasps, moths, bees, etc. and a popular ingredient in commercial insecticides because of low persistence in the environment and low mammalian toxicity (Turlings *et al*., 1995).

In Gymnosperms (conifers) like Pine and Fir, monoterpenes accumulate in resin ducts found in the needles, twings and trunks mainly as α-pinene, β-pinene, limonene and myrecene, all are toxic to numerous insects including bark beetles, serious pest of conifer species throughout the world (Turlings *et al*., 1995).

(b) *Sesquiterpenes (C_{15}):* A number of sesquiterpenes have been till now reported for their role in plant defense such as costunolides are antiherbivore agents of family composite characterized by a five membered lactone ring (a cyclic ester) and have strong feeding repellence to many herbivorous insects and mammals (Picman, 1986).

ABA is also a sesquiterpene plays primarily regulatory roles in the initiation and maintenance of seed and bud dormancy and plants response to water stress by modifying the membrane properties (Van-steveninck, 1983) and act as a transcriptional activator (McCarty *et al*., 1991; Giraudat *et al*., 1992). In addition, it increases the cytosolic calcium concentration and causes alkalinisation of the cytosol (Irving *et al*., 1992; Blatt and

Armstrong, 1993; Thiel *et al*., 1992). The level of UV-B absorbing flavonols, quercetin and kaempferol were significantly increased when ABA was applied. The concentration of two hydroxy-cinnamic acids, caeffic and ferulic acids were also increased by ABA. All of above changes in the protective compounds, anti-oxidant enzymatic activities and sterols were correlated with lessened membrane harm by UV-B. Thus, defense system of plants against UV-B is activated in which ABA acts downstream in the signaling pathway (Berli *et al*., 2010).

(c) *Diterpenes (C_{20}):* Abietic acid is a diterpene found in pines and leguminous trees. It is present in or along with resins in resin canals of the tree trunk. When these canals are pierced by feeding insects, the outflow of resin may physically block feeding and serve as a chemical deterrent to continued predation (Bardley *et al*., 1992). Another compound phorbol (diterpene ester), found in plants of Euphorbiaceae and work as skin irritants and internal toxins to mammals**.** Moreover, phytol a highly hydrophobic 20-C alcohol found in chlorophyll as a side chain help to anchor certain molecules in membranes and therefore increase the efficiency of chlorophyll during the photosynthesis (Knaff, 1991), a strategy for maximum CO_2 fixation and biomass production (Jagendorf, 1967). Furthermore, gibberellins, a group of plant hormones are also diterpenes, that play various detrimental roles in numerous plant developmental processes such as seed germination, leaf expansion, flower and fruit set (Davies, 1995), dry mass and bio mass production (Gupta *et al*., 2001), stomatal conductance (Bishnoi *et al*., 1992), CO_2 fixation, phloem loading, assimilate translocation (Ouzounidou *et al*., 2005) and also known to exert their numerous physiological effects via specific enzymes, the synthesis of which they induce by influencing the basic process of translocation and transcription (Hutty *et al*., 1995).

(d) *Triterpenes (C_{30}):* Several steroid alcohols (sterols) are important component of plant cell membranes, especially in the plasma membrane as regulatory channels and maintain permeability to small molecules by decreasing the motion of the fatty acid chains. The milkweeds produce several better tasting glucosides (sterols) that protect them against herbivory by most insects and even cattle (Lewis and Elvin-Lewis, 1977). Phytoecdysones have some defensive role against insects by disrupting moulting and other developmental and physiological processes with lethal consequences (Heftmann, 1975; Slama, 1979, 1980). Another triterpene, limnoid, a group of bitter substances in citrus fruits and act as antiherbivore compounds in members of family Rutaceae and some other families also. For example, Azadirechtin, a complex limnoid from *Azadirachta indica*, acts as a feeding deterrent to some insects and exerts various toxic effects (Mordue and Blackwell, 1993).

(e) *Polyterpenes ($(C_5)_n$):* Several high molecular weight polyterpenes occur in plants. Larger terpenes include the tetraterpenes and the polyterpenes. The principal tetraterpenes are carotenoids family of pigments. Other one is rubber, a polymer containing 1500-15000 isopentenyl units, in which nearly all the C-C double bonds have a cis (Z) configuration while in gutta rubber has its double bond in trans (E) configuration. Rubber found in long vessels called laticifers, provide protection as a mechanism for wound healing and as a defense against herbivores (Eisner *et al*., 1995; Klein, 1987).

(ii) Phenolic compounds

Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group on an aromatic ring called Phenol, a chemically heterogeneous group also. They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes (Wuyts *et al*., 2006). Elevated ozone (mean 32.4ppb) increased the total phenolic content of leaves and had minor effects on the concentration of individual compounds (Saviranta *et al*., 2010).

(a) *Coumarin:* They are simple phenolic compounds, widespread in vascular plants and appear to function in different capacities in various plant defense mechanisms against insect herbivores and fungi. They derived from the shikimic acid pathway (Murray *et al*., 1982), 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 possessing a range of improved antifungal activity (figure 2).

Halogenated coumarin derivatives work very effectively *in vitro* to inhibit fungal growth. For example, 7-hydroxylated simple coumarins may play a defensive role against parasitism of *Orobanche cernua*, by preventing successful germination, penetration and connection to the host vascular system (Serghini *et al*., 2001). Some coumarin derivatives have higher anti-fungal activity against a range of soil borne plant pathogenic fungi and exhibit more stability as compared to the original coumarin compounds alone (Brooker *et al*., 2008).

(b) *Furano-coumarins:* Also a type of coumarin with special interest of phyto-toxicity, abundant in members of the family Umbelliferae including celery parsnip and parsley. Normally, these compounds are not toxic, until they are activated by light (UV-A), causes some furano-coumarins to become activated to a high energy electronic state, which can insert themselves into the double helix of DNA and bind to the pyramidine bases and thus blocking transcription and repair and eventually leading to cell death (Rice, 1987). Psoralin, a basic linear furacoumarin, known for its use in the treatment of fungal defense and found very rarely in SO_2 treated plants (Ali *et al*., 2008) (figure 3).

(c) *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 lignin. The reactive proportions of the three monomeric units in lignin vary among species, plant organs and even layers of a single cell wall (Lewis and Yamamoto, 1990). Its physical toughness deters feeding by herbivorous animals and its chemical durability makes it relatively indigestible to herbivores and insects pathogens (Mader and Amberg-Fisher, 1982). Lignifications block the growth of pathogens and are a frequent response to infection or wounding (Gould, 1983).

(d) *Flavonoids:* One of the largest classes of plant phenolic, perform very different functions in plant system including pigmentation and defense (Kondo *et al*., 1992). 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). In addition, exposure of plants to increased UV-B light has been demonstrated to increase the synthesis of flavones and flavonols suggesting that flavonoids may offer a measure of protection by screening out harmful UV-B radiation (Caldwell *et al*., 1983; Saviranta *et al*., 2010).

(e) *Isoflavonoids:* Isoflavonoids are derived from a flavonone intermediate, naringenin, ubiquitously present in plants and play a critical role in plant developmental and defense response. They secreted by the legumes and play an important role in promoting the formation of nitrogen-fixing nodules by symbiotic rhizobia (Sreevidya *et al*., 2006). Moreover, it seems that synthesis of these flavonoids is an effective strategy against reactive oxygen species (ROS) (Posmyk *et al*., 2009). The analysis of activity of antioxidant enzymes like SOD, CAT, POX, APX, GPX and GR suggested that peroxidases were the most active enzymes in red cabbage seedlings exposed to Cu^{2+} stress. It could result from the fact that phenolic compounds (Phc), which could be also substrate for different peroxidases were the first line of defense against various environmental stress like metal stress (Posmyk *et al*., 2009; Novak *et al*., 2009) (figure 3).

(f) *Tanins:* It included under the second category of plant phenolic polymers with defensive properties. Most tannins have molecular masses between 600 and 3000. Tannins are general toxins that significantly reduce the growth and survivorship of many herbivores and also act as feeding repellents to a great diversity of animals. In mammalian herbivores, they cause a sharp, astringent sensation in the mouth as a result of their binding of salivary proteins. Mammals such as cattle, deer and apes, characteristically avoid plant with high tannin contents (Oates *et al*., 1980). 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 (Vickery, 1981; Butt and Lamb, 1981; Mayer, 1987). It is thought by some that chlorogenic acid and certain other related compounds can be readily formed and oxidised into potent fungistatic quinones by certain disease resistant cultivars but less readily so by susceptible ones.

(iii) Sulphur containing secondary metabolites

They include GSH, GSL, phytoalexins, thionins, defensins and allinin which have

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been linked directly or indirectly with the defense of plants against microbial pathogens (Hell, 1997; Crawford *et al*., 2000; Leustek *et al*., 2000; Saito, 2004; Grubb and Abel, 2006; Halkier and Gershenzon, 2006), and a number of them thought to be involved in the SIR (ElkeBloem *et al*., 2005).

(a) *GSH:* It is one of the major forms of organic S in the soluble fraction of plants and has an important role as a mobile pool of reduced S in the regulation of plant growth and development, and as an cellular anti-oxidant in stress responses (Kang *et al*., 2007; Noctor *et al*., 1998), reported as a signal of plant S sufficiency that down regulates S-assimilation and S-uptake by roots (Lappartient and Touraine, 1997; Lappartient *et al*., 1999). Specialized cells such as Trichomes exhibit high activities of enzymes for synthesis of GSH and other phytochelatins necessary for detoxification of heavy metals (Gutierez-Alcala *et al*., 2000; Choi *et al*., 2001). The GSH content varies between 3 to 10 mM and is present in the major cellular compartments of the plant (Leustek and Saito, 1999). To mitigate oxidative stress, GSH functions as a direct anti oxidant and also as a reducing agent also for other anti oxidants such as ascorbic acid (Nocito *et al*., 2002) as well as an integral weapon in the defense against ROS generated by O_3 (Pasqualini *et al*., 2002; Conklin *et al*., 2004) or as a reaction to biotic and abiotic stress.

Additionally, GSH is also involved in the detoxification of xenobiotics and cytotoxins by targeting them in to vacuole (Rea *et al*., 1998). GSH is rapidly accumulated after fungal attack, may act as systemic messenger carrying information concerning the attack to non-infested tissues (Foyer and Rennenberg, 2000; Edwards *et al*., 1991).

(b) *GSL:* A group of low molecular mass N and S containing plant glucosides that produced by higher plants in order to increase their resistance against the unfavourable effects of predators, competitors and parasites because their break down products are release as volatiles defensive substances exhibiting toxic or repellent effects (Mithen, 1992; Wallsgrove *et al*., 1999; De Vos *et al*., 2009), for example, mustard oil glucosides in cruciferae and allyl cys sulfoxides in allium (Leustek, 2002). The smelling volatiles from GSL catalyzed by myrosinase, cleave glucose from its bond with the S atom. The resulting aglycon rearranges with loss of the sulphate to give pungent and chemically reactive products, including isothiocyanates and nitriles, function in defense as herbivorous toxins and feeding repellent (Renwick, 1992; Grubb and Abel, 2006; Halkier and Gershenzon, 2006; Talalay and Fahey, 2001). This became obvious in 1986 when the switch from single low to double low oil seed rape varieties led to increasing infestation of oil seed rape with fungal diseases such as light leaf spot (*Pyrenopeziza brassicae*), sclerotina stem rot (*Sclerotinia sclerotiorum*) and Alterneria (*Alternaria brassicae*). This phenomenon was attributed to the drastically reduced glucosinolate content in double low varieties (Dornberger *et al*., 1975; Koch, 1989; Mithen *et al*., 1986; Pedras and Sorensen, 1998). The potency of GSL arises when the plant tissue is damaged and GSL come in to contact with the plant enzyme myrosinase removes the β-glucose moiety leading to formation of an unstable intermediates i.e. isothiocyanates (R-N=C=S) and nitriles function in defense as herbivore toxins and feeding repellents (Geu-Flores *et al*., 2009; Poultron and Moller, 1993; Ratzka *et al*., 2002; Zukalova and Vask, 2002). They are metabolized and absorbed as isothiocyanates that can affect the activity of enzymes involved both in the antioxidant defense system and in the detoxification from xenobiotics and significantly affect GST activity and cell protection against DNA damage (Lipka *et al*., 2010; Porrini, 2008) whereas toxicity of glucosinolatic products is well documented but their mode of action has not yet been elucidated and results from experiments with *Brassica* plants modified in GSL content generated doubts about their contribution to plant defense. Studies of Mithen and Magrath (1992) were able to show that the level of alkenyl GSL within the leaves of a *Brassica* line that was resistant to *Leptosphearia maculans* was always consistently higher than that of a susceptible line but they could not find a correlation between the level of alkenyl GSL and disease resistance. Lazzeri *et al*. (1993) demonstrated the action of several isothiocyanates against the nematode *Heterodera schachti*. Pedras and Sorensen (1998) discovered that the germination and radial growth of spores of a virulent *Phoma lingam* pathotype was inhibited by higher concentrations of different isothiocyanates. In studies of crosses of *Brassica* lines with different glucosinolate level resistance to fungal attacks failed to correlate with high and low glucosinolate level (Mithen and Magrath, 1992; Giamoustaris and Mithen, 1995; Wretblad and Dixelius, 2000).

The presence of GSL may help explain the characteristic patchy distribution of most cruciferous plants. This result challenges one end of the continuum of the long-standing plant apparency hypothesis, which essentially states the opposite causation, that low molecular weight toxins like GSL are evolutionary responses of patchy distribution and correlated life history traits necessary for better productivity (Siemens *et al*., 2009).

(c) *Phytoalexins:* Phytoalexins are synthesized in response to bacterial or fungal infection or other forms of stress that help in limiting the spread of the invading pathogens by accumulating around the site of infection, appears to be a common mechanism of resistance to pathogenic microbes in a wide range of plants (Van Etten *et al*., 1994; Grayer and Harborne, 1994; Bailey and Mansfield, 1982; Darvill and Albersheim, 1984). Many of these changes are linked to a rapid apoptotic response, resulting in the death of one or a few invaded plant cells, known as the hypersensitive response (HR) (Hammond-Kosack and Jones, 1996). Most plant families produce organic phytoalexins of diverse chemistry; these groups are often associated with a family, for example sesquiterpenoids of Solanaceae, isoflavonoids of Leguminosae, while phytoalexins from *Brassica* have an indole or related ring system and one S atom as common structural features. Crucifereae appears to be the only plant family producing these S metabolites (Gross, 1993; Pedras *et al*., 1997, 1998), which are clearly different from the other well-known GSL (Harborne, 1999). Cruciferous crops are cultivated worldwide because they are extremely valuable and for the last decades, various research groups have investigated cruciferous phytoalexins (Harborne, 1999; Gross *et al*., 1994; Monde *et al*., 2000) as well as their biological activity (Mehta *et al*., 1995). Typically, there are multiple responses involving several related derivatives such as up to nine wyerone (Furano-acetylenic derivatives) forms in *Vicia fava* and several forms of phaseollin in *Phaseolus vulgaris* and glyceollins in *Glycine max*, pistin in *Pisum sativum* pods, ipomeamarone in sweet potato, orchinol in orchid tubers, trifolirhizin in red clover (Keen and Kennedy, 1974).

(d) *Defensins, thionins and lectins:* All these are S-rich non-storage plant proteins synthesize and accumulate after microbial attack and such related situations (Van Loon *et al*., 1994). All of which inhibits the growth of a broad range of fungi (Thomma *et al*., 2002). Some defensins are antifungal or occasionally anti-bacterial activity (Thomma *et al*., 2002). Additionally defensins genes are partly pathogen-inducible (Chiang and Hadwiger, 1991; Gu *et al*., 1992) and others that are involved in resistance can be expressed constitutively (Terras *et al*., 1995; Parashina *et al*., 2000). The components seem to be involved in the natural defense system of plants as they can be highly toxic to microrganisms, insects and mammals. Accumulation of thionins in the cell wall of infected wheat spikes of resistant wheat cultivars indicating that the accumulation of thionins may be involved in defense responses to infections and in spreading of *Fusarium culmorum* (Kang and Buchenauer, 2003). Some plant species produce lectins as defensive proteins that bind to carbohydrate or carbohydrate containing proteins. After being ingested by herbivores, lectins bind to epithelial cell lining of the digestive tracts and interfere with nutrient absorption (Peumans and Van Damme, 1995) (figure 2).

(iv) Nitrogen containing secondary metabolites

They include alkaloids, cyanogenic glucosides, and non-protein amino acids. Most of them are biosynthesized from common amino acids. All are of considerable interest because of their role in the anti herbivore defense and toxicity to humans.

(a) *Alkaloids:* A large family of N containing secondary metabolites found in approximately 20% of the species of vascular plants (Hegnauer *et al*., 1988), most frequently in the herbaceous dicot and relatively a few in monocots and gymnosperms. Generally, most of them, including the pyrrolizidine alkaloids (PAs) are toxic to some degree and appear to serve primarily in defense against microbial infection and herbivoral attack. They are usually synthesized from one of the few common amino acids, in particular, aspartic acid, lysine, tyrosine and tryptophan (Pearce *et al*., 1991).

Now most alkaloids are believed to function as defensive elements against predators, especially mammals because of their general toxicity and deterrence capability (Robinson, 1980; Harborne, 1988; Hartmann *et al*., 1991). Large number of livestocks death is caused by the ingestion of alkaloids containing plants. In US, a significant % of all grazing livestock are poisoned each year by consumption of large quantities of alkaloid containing plants such as lupines (*Lupinus*) and larkspur (*Delphinium*) (Keeler, 1975). On a cellular level, the mode of action of alkaloids in animals is quite variable. Some interfere with components of the nervous system, especially the chemical transmitters, other affect

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membrane transport, protein synthesis and miscellaneous enzyme activities (Creelman and Mullet, 1997) (figure 2).

(b) *Cyanogenic glucosides:* 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 H_2S when the plant is crushed; their presence deters feeding by insects and other herbivores such as snails and slugs (Taize and Zeiger, 1995).

Amygdalin, the common cyanogenic glucoside found in the seeds of almonds, apricot, cherries and peaches while Dhurrin, found in *Sorghum bicolar*. Normally, both are not broken down in the intact plant because the glucosides and degradative enzymes are separated in different compartments (Poulton, 1990). Under ordinary conditions, this compartmentalization prevents decomposition, however, on damaging as during herbivore feeding, the cell contents of different tissues mix and form HCN, a toxin of cellular respiration by binding to the Fe-containing heme group of cytochrome oxidase and other respiratory enzymes. Similarly, the presence of cyanogenic glucosides in cassava, make it suitable for long time storage without being attacked by pests (Pearce *et al*., 1991). Lima bean (*Phaseolus lunatus* L.) is a model plant for studies of inducible indirect anti herbivore defences including the production of volatile organic compounds (VOCs) (Ballhorn *et al*., 2009). The cyanogenic potential (HCNp; concentration of cyanogenic glucosides) as a crucial parameter determining Lima bean cyanogenesis and quantitative variability of cyanogenesis in natural population of wild Lima bean in Mexico, was significantly correlated with missing leaf area and therefore, cyanogenesis has to be considered as an important direct defensive trait affecting Lima beans' overall defence in nature (Ballhorn *et al*., 2009).

(c) *Non-protein amino acids:* Many plants also contain unusual amino acids called non-protein amino acids that incorporated into proteins but are present as free forms and act as protective defensive substances (Johnson *et al*., 1989). For examples, canavanine and azetidine-2-carboxylic acid are close analogs of arginine and proline respectively. They exert their toxicity in various ways. Some block the synthesis of or uptake of protein amino acid while others can be mistakenly incorporated in to proteins. After ingestion, canavanine is recognized by herbivore enzyme that normally binds arginine to the arginine transfer RNA molecule and so become incorporated in to proteins in place of arginine. The usual result is a non-functional proteins because either its tertiary structure or it catalytic site is disrupted (Rosenthal, 1991). Plants that synthesize non-protein amino acids are not susceptible to the toxicity of these compounds but gain defense to herbivorous animals, insects and pathogenic microbes. Also, a number of plants including Arabidopsis uses Arginine as a storage and transport form of N and proline as a compatible solute in the defense against abiotic stresses causing water deprivation (Funck *et al*., 2009) (figure 3).

Recognition of some pathogenic substances necessary to initiate defense responses

Within a species, individual plants often differ greatly in their resistance to microbial pathogens. These differences often lay in the speed and intensity of plants reactions (Shulaev *et al*., 1997; Ryals *et al*., 1996). Resistant plants respond to pathogens than susceptible plants. Hence, it is important to learn how plants sense the presence of pathogens and initiate defense.

Until in the last few years, researchers have isolated numerous plant resistance genes, recognised as R genes, function in defense against fungi, bacteria and nematodes. Most of the R-genes are thought to encode receptors that recognise and bind specific molecules originating from pathogens and alert the plant to the pathogens presence. The specific pathogen molecules recognised are referred to as elicitors include proteins, peptides, lipids etc. arising from the pathogen wall, the outer membrane or a secretion process (Bollar, 1995) can induce phytoalexins production and activate other defense reactions (Ebel, 1986; Boller, 1989). Some are polysaccharides produced when pathogenic fungi and bacteria attack on the plant cell wall (Templeton and lams, 1988; Bollers, 1989; Stone, 1989) while others are oligosaccharides produced by degradation of fungal cell wall by plant enzymes that the fungus causes the plants to secrete. Apparently, such exogenous elicitors are recognized by certain proteins in membrane, which then signal increases transcription of mRNA molecules that code for enzymes that synthesize the phytoalexins (figure 4). For example, in case of glyceollin production by infected soya bean roots, the signal may be calcium. The hypersensitive response (HR) of cell death and pathogenesis related (PR) gene expression of these gene induced by various elicitors which induced HR and activation of the genes. Some protein elicitors are boehmerin, harpin or INF1 (Zhang *et al*., 2009). All R-gene products themselves are nearly proteins with a leucine rich domain that is repeated in exactly several times in the amino acids sequence (Hammond-Kosack and Jones, 1997). Similarly, β amino-butyric acid can induce disease resistance in *Arabidopsis* against the fungal pathogen *Hyaloperonospora arabidopsis* (Vander Ent *et al*., 2009) and bacterial pathogen (*Pseudomonas syringae*).

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 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 SIR 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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Figures follow……….

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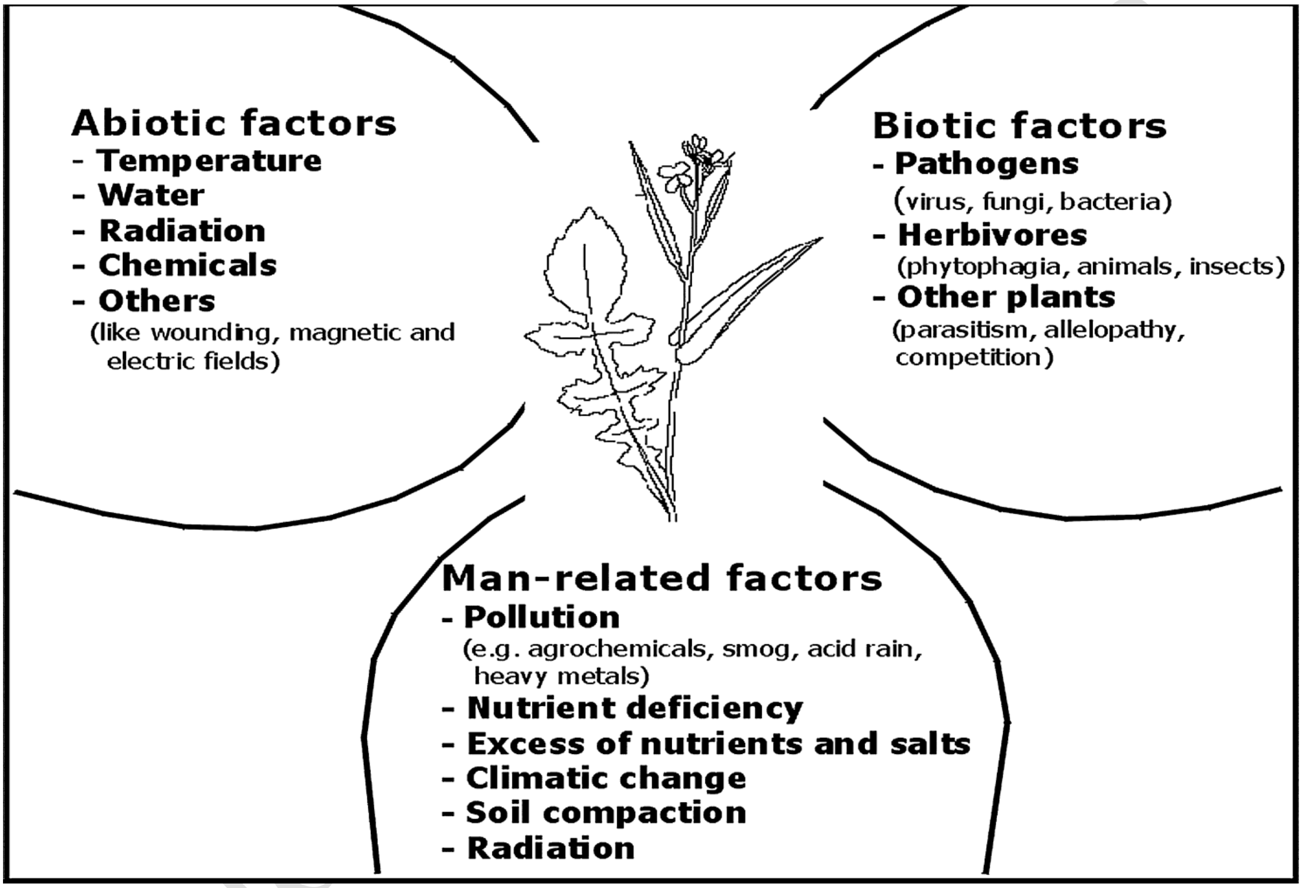

Figure 1. Biotic, abiotic and man related factors that can induce stress related reactions in terrestrial plants (Lichtenthler, 1996; Reigosa et al, 1999, 2002; Bloem, 2005).

Biol

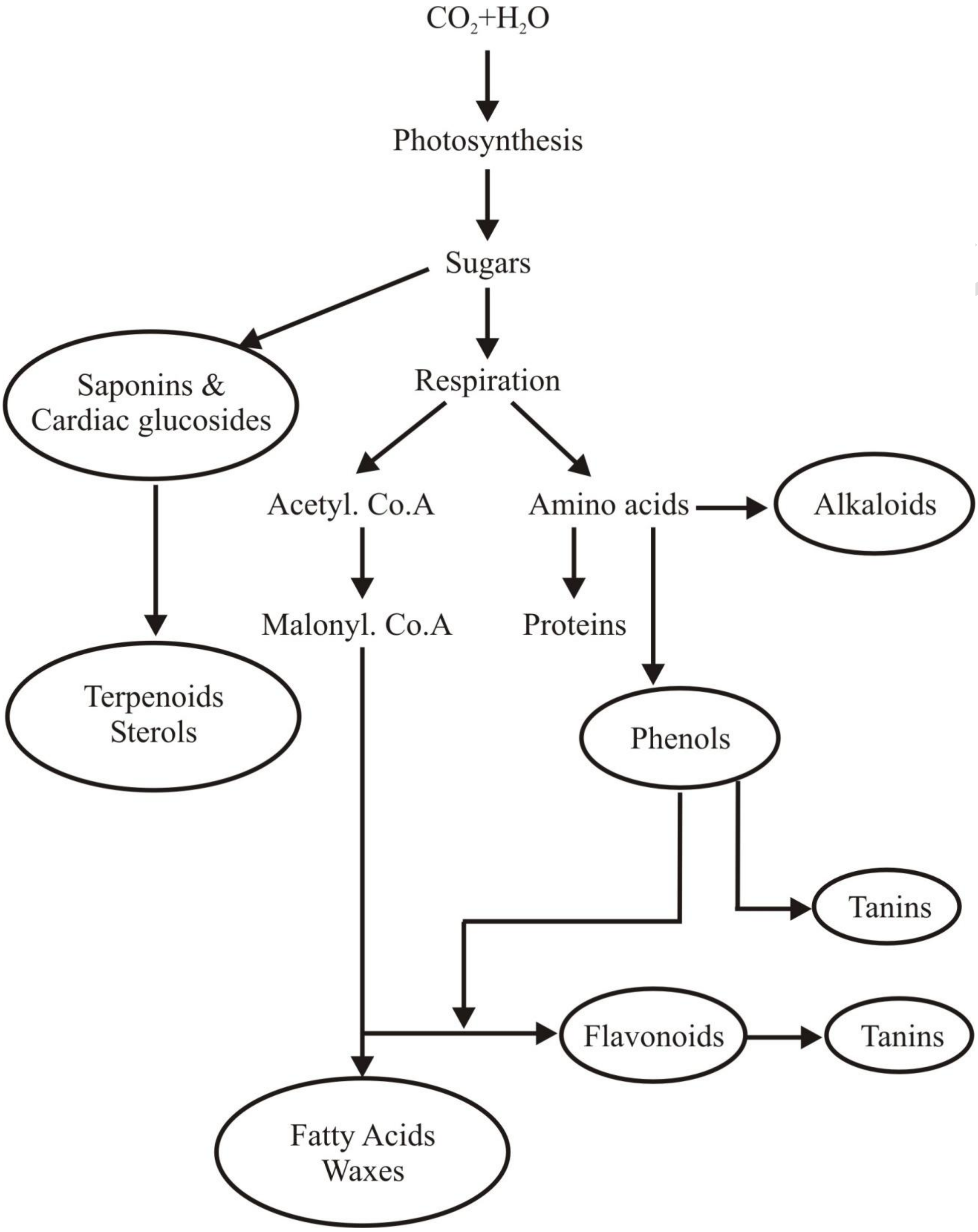

Figure 2. Biosynthetic relationship among some primary and secondary metabolites. The principal group of secondary metabolites are circled.

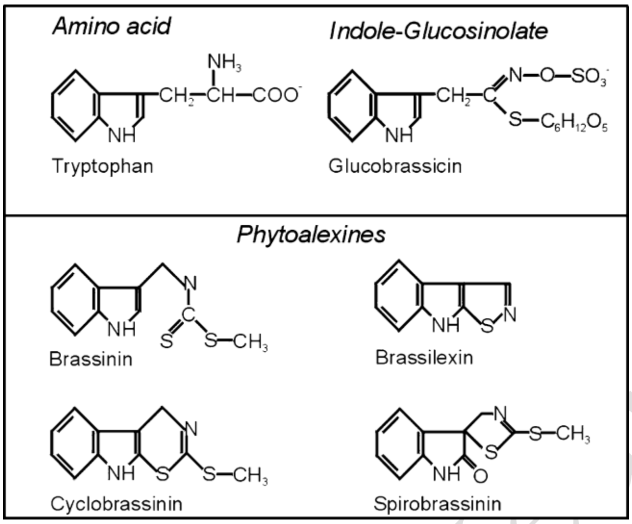

Figure 3a

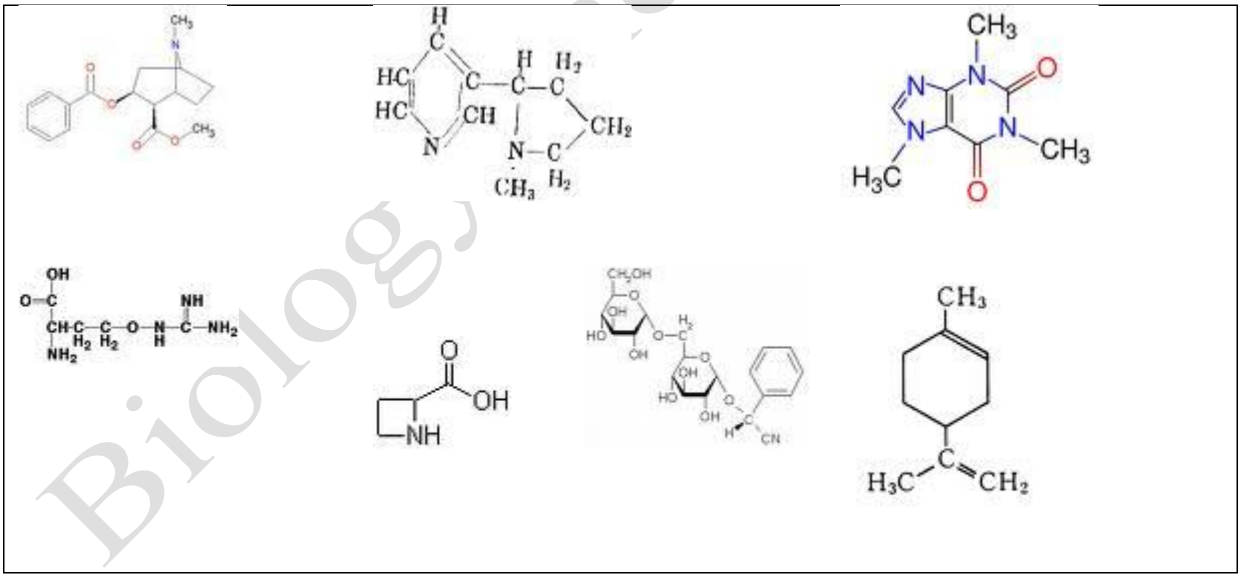

Figure 3b

Figure 3. Structure of N and S containing secondary metabolites and their precursor amino acids. (a) Structure of some typical cruciferous phytoalexins (Monde and Takasugi, 1992) (b) Structures of cocaine, nicotine, caffeine, canavanine, azetidine-2-carboxlicac, amygdalin, limonene.

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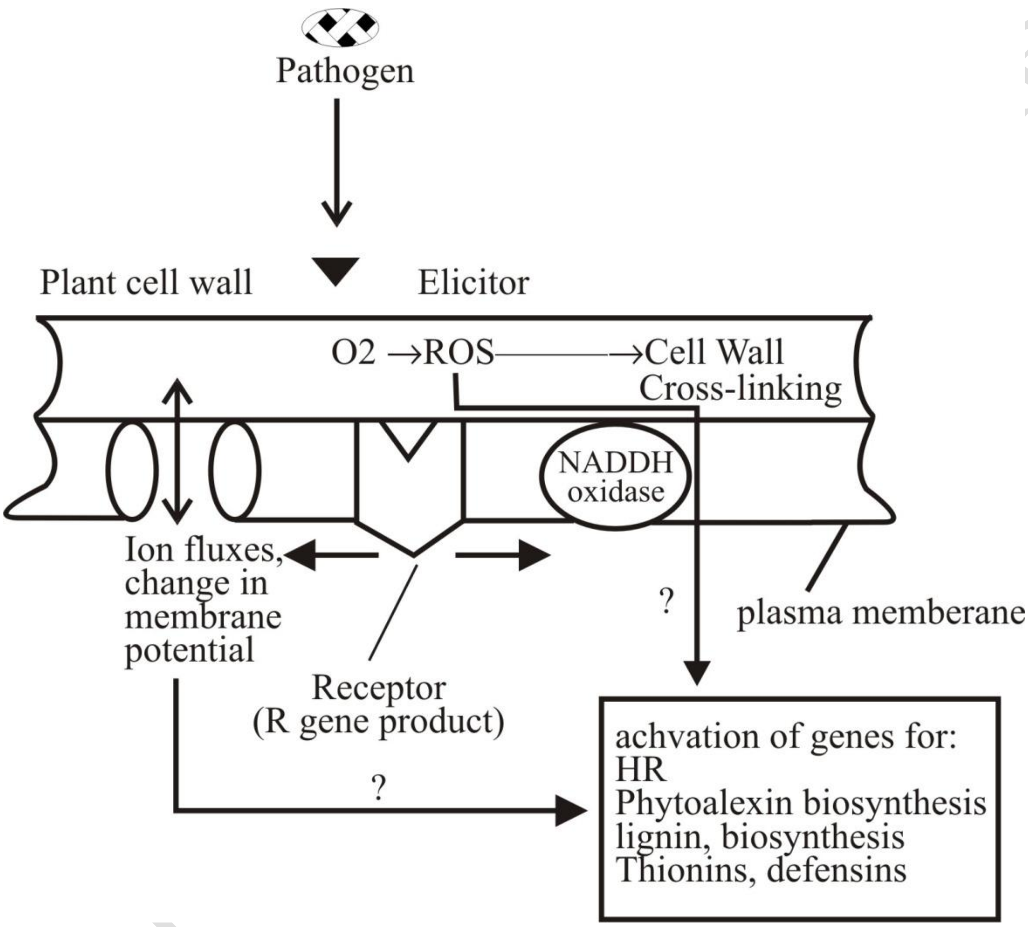

Figure 4. Many modes of anti-pathogenic defence are induced by infection. Fragments of pathogen molecules called elicitors initiate a complex signalling pathway leading to the activation of defence responses.