INTRODUCTION

CHAPTER - I

INTRODUCTION

Disease resistance is the ability of an organism to prevent, restrict or retard disease development. Resistance and virulence are the combined result of multiple biochemical components (Horsfall and Cowling, 1980). From a genetical point of view, it is difficult to explain the basis of disease resistance in plants. Attempts to explain disease resistance in physiological or structural terms are complicated by the lack of knowledge of both passive and active resistance. Resistance and susceptibility in plants are not determined by the presence or absence of genetic information for resistance mechanisms, but rather by the speed with which the information is expressed, activity of the gene products and the magnitude of the resistance response. While resistance mechanisms can exist in a host plant before it comes into contact with a pathogen, most resistance mechanisms are induced in the host after the contact and thus involve either qualitative or quantitative changes in the host's metabolism.

Disease resistance in plants, as in animals is dependent upon coordinated multiple mechanism with different modes and sites of action (Kuć,1982a, 1987; Kuć and Tuzun 1983; Kuć and Preisig, 1984). Disease resistance can be divided into two primary components, protection and defence. Protection is a

static phenomenon and may involve either fungitoxic or fungistatic compounds in or on the host tissue or the presence of preformed structures within the tissue of the host plant. Defence is a dynamic phenomenon that does not occur until the host and the pathogen have made physiological contact, although like protection, the defence reactions may involve either structural or chemical barriers. Resistance is found far more frequently amongst plants than is susceptibility. Plants frequently show enhanced levels of resistance to disease following inoculation with pathogens or treatment with chemicals that cause local necrotic lesions or numerous scattered necrotic cells. This phenomenon is referred to as induced resistance (Matta, 1971).

Cultivars within a plant species and especially species within a genus generally show a wide variation in their levels of resistance to any given pathogen. Resistance levels vary among plant parts and tissues (Innes, 1974) and among genetically identical plants of different age (Mares, 1979). The resistance power of each plant part also varies with age. Resistance levels in stems and roots generally increase rapidly during the first two weeks of seedling or new root growth and slowly thereafter, whereas resistance levels in leaves and fruits frequently decline with age (Jones and Hayes,1971).

At the beginning of the century, Ward (1902) recognised the significance of a pathogen being checked after it had penetrated the host tissues and he also proposed that inhibition

might result from changes induced in the host by the metabolic activity. Resistance to fungi is also often expressed by the failure of the infection hyphae to penetrate into or through host cells. (Aist,1976; Ride, 1978; Heath, 1980).

RESISTANCE FACTORS

The resistance factors or the antimicrobial compounds may be preformed. Preformed resistance factors are those which are present in plants prior to their contact with pathogens. A number of preformed substances with antimicrobial properties have been implicated in resistance. These compounds are generally present at relatively high concentrations in healthy plants and in some cases are converted into more potent toxins as a result of infection. The role of preformed substances in resistance have been assessed elsewhere (Schonbeck and Schlosser 1976; Weinhold and Hancock, 1980) and the importance of secondary substances such as phenols, flavonoids, tannins, terpenes, alkaloids etc. in disease resistance have been emphasized by several researchers (King,1953; Skinner,1955; Hiller,1964; Birck, 1966; Tokin, 1967; Wain, 1969).

Infection induced resistance factors comprise of those which are either absent or present at low levels and are produced or activated upon infection. Post infectional antifungal compounds also participate in defence. In different host-parasite combinations in plants such as sweet potato, pea, broad bean, green pepper, soybean and rice, the formation or

release of antifungal compounds took place following the inoculation of fungi (Hiura,1943; Gaumann et al.,1950; Mizukami, 1953; Kuć , 1955; Uehara ,1958; Müller ,1958).

The formation of antibodies in the blood of human beings and animals in response to infection by microorganisms is well understood and forms the basis of preventive medicine. A functionally similar mechanism in plants was envisaged by Müller and Borger (1940), who found that potato tubers developed localized resistance to a virulent race of the fungus to which they were resistant. This result led to the proposal of the phytoalexin theory. One mechanism for disease resistance in plants is their ability to accumulate phytoalexins.

PHYTOALEXINS

Phytoalexins are defined as low molecular weight antimicrobial compounds that are synthesised by and get accumulated in plants as a result of microbial attack. Most of the known phytoalexins are lipophilic substances that are products of a plant's secondary metabolism and they often are accumulated at infection sites in concentrations enough to inhibit the development of fungi and bacteria. The role of phytoalexins as factors contributing to disease resistance has been studied for more than four decades by many workers. (Deverall ,1976; Kuć, 1976; 1985; Stöessl et al.,1977, Albershein,1977, Keen,1981). The fact that phytoalexins are absent in most of the healthy plants and are accumulated at

the site of microbial infection also indicates a defence function of phytoalexins. Phytoalexins are induced by fungi, bacteria, viruses, nematodes, toxic chemicals and physical treatments. (Bell 1967; Hadwiger and Martin 1971; Rich et al. 1977; Misaghi, 1982). Phytoalexins are reported from more than 100 species of plants belonging to 21 families. The ability of higher plants to accumulate phytoalexins is widespread if not ubiquitous, being present in monocots as well as in dicots.

In addition to being antimicrobial, some phytoalexins are also toxic to nematodes (Kalpan et al.,1980) plants (Shiraishi et al.,1975; Skipp et al.,1978; Glazner & Venetten, 1978) and animals (Van Etten and Batemen 1971; Oku et al., 1976a). However phytoalexins are most effective against fungi and some are considered to be capable of limiting fungal colonization of plant tissues (Johnson et al.,1976; Skipp and Bailey,1976; Deverall, 1977; Smith,1978; Yoshikawa et al.,1978). Studies on phytoalexin production by fungi have generally been confined to saprophytes and nonbiotrophic parasites. However some biotrophic fungi are also known to elicit phytoalexin production (Oku et al., 1976b; Shiraishi et al.,1977). Phytoalexin synthesis occurs in living cells by means of organized and regulated pathways, however it is associated with plant necrosis or metabolic insult and accumulation often occurs in damaged or dead tissues. The phytoalexins accumulated in and around the site of infection and the speed and magnitude with which they are produced and accumulated appear to determine the disease reaction in some plant interactions with fungi and

bacteria (Kuć, 1972; 1976). Phytoalexin accumulation as a factor for disease resistance is not determined by the presence or absence of genetic information for the requisite biosynthetic pathways, but rather by information specifying their expression.

THE PHYTOALEXIN THEORY

Muller and Borger (1939, 1940) and Müller et al.,(1939) studied symptom responses following the inoculation of cut tuber surfaces of potato varieties with virulent and avirulent strains of phytophthora infestans. The following conclusions drawn from their studies form the basic postulates of what is known as the phytoalexin theory.

1) A principle, designated as "phytoalexin", inhibits the development of the fungus in hypersensitive tissue and is formed or activated only when the host cells come into contact with the parasite.
2) The defensive reactions occur only in living cells.
3) The inhibitory material is a chemical substance and may be regarded as the product of necrobiosis of the host cell.
4) This phytoalexin is non-specific in its toxicity towards fungi, however, fungal species may be differentially sensitive to it.
5) The basic response that occurs in resistant and susceptible hosts is similar. The basis of differentiation between resistant and susceptible host is the speed of formation of the phytoalexin.

6) The defense reaction is confined to the tissue colonized by the fungus and its immediate neighbourhood.

7) The resistant state is not inherited. It is developed after the fungus has attempted infection. The sensitivity of the host cell that determines the speed of the host reaction is specific and genotypically determined.

Though in the light of recent experiments (Müller, 1956; Müller, 1958), the phytoalexin theory is restated (Müller, 1961), no major changes of theoretical importance is made till today. It was Müller (1958) who first reported the changes occurring in infection droplets (containing spores of Monilinia fructicola) in the seed cavities of opened bean pods. A chemical inhibitor was found to be present in the infection droplets. Muller reported a chemical entity formed during a hypersensitive reaction which had the features of a phytoalexin. Cruickshank and Perrin (1960) worked with the pea system and found that the inhibitor in pea behaved as a single substance during chromatography. Pisatin, the antifungal compound was crystallized and subsequently characterized as a pterocarpan (Perrin and Bottomley, 1962). A little later the entity in infection droplets in bean was isolated and characterized as a closely related pterocarpan called phaseollin (Cruickshank and Perrin 1963; Perrin, 1964).

PHYTOALEXINS FROM DIFFERENT FAMILIES

Phytoalexins appear to be more common in angiosperms than in gymnosperms. Several distinct but chemically related

phytoalexins are produced by different plants. Moreover various combinations of classes of secondary products are produced in response to specific pathogens in a single plant (Keen, 1975; Price et al., 1976; Vanetten and Pueppke, 1976; Rich et al., 1977). Multiplicity of some of these phytoalexins is sometimes attributed to the chemical modifications of parent molecules by microbial activities (Ward and Stoessl, 1977).

The chemical nature of phytoalexins is mostly decided by the biosynthetic pathways operative within the host plant. The plants from the Fabales commonly produce isoflavonoid phytoalexins while members of the Solanaceae and Asteraceae produce sesquiterpenoids and/or polyacetylenes. Of the 102 phytoalexins reported from the Fabales, 84 are isoflavonoid derivatives, 2 are chromones and 1 is a flavonoid (Ingham, 1982). Similarly in the Solonaceae, of the 43 phytoalexins reported, 4 are terpenoid derivatives, 6 are phenylpropanoid phenols and 3 are polyacetylenes (Kuć, 1982b). Some of the phytoalexins are distributed among many families for eg. Caffeic acid derivatives accumulate in potato, carrot and sweet potato. None of the plants belonging to the Fabales have been reported to produce sesquiterpenoid phytoalexins and none of the solanaceae have produced isoflavonoid phytoalexins. Till today most of the research or phytoalexins happened to be conducted in plants belonging to the Fabales and Solanaceae. The chemical variety of phytoalexins and their sources are presented in Table I.

Table I : PHYTOALEXINS FROM DIFFERENT PLANT FAMILIES

	Family	Plant name	Phytoalexin	Chemical nature	References
1.	Solanaceae	*Solanum tuberosum*	Rishitin	Sesquiterpene	Chalova *et al.*, 1971; Metlitskii *et al.*, 1970
2.	"	*Lycopersicon esculentum*	Rishitin	"	Sato et al., 1968; De Wit and Flach, 1979; Hutson and Smith, 1980.
3.	"	*Solanum melongena*	Lubmin Aubergenone	"	Ward *et al.*, 1975
4.	"	*Nicotiana tabacum*	Capsidol	"	Bailey *et al.*, 1975; Cruickshank, *et al.*, 1976.
			Phytuberin	"	Hammerschimdt and Kuc, 1979.
			Rishitin	"	Budde and Helgeson, 1981.
			Solavetivone	"	Uegaki *et al.*, 1981.
5.	"	*Datura stramonium*	Lubmin	"	Ward *et al.*, 1976.
			Capsidol	"	Birnbaum *et al.*, 1976.
6.	"	*Nicotiana debneyi*	Debneyol	"	Burden *et al.*, 1985.
7.	Fabaceae	*Cajanus cajan*	Cajanin	Isoflavone	Ingham, 1976.
8.	"	*Centrosperma pubescena*	Cajanin	"	Markham and Ingham, 1980.

9.	Fabaceae	Dolichos biflorus	Genistein	Isoflavone	Keen and Ingham, 1980
10.	"	Lupinus albus	Luteone	"	Ingham and Dewick, 1980b.
11.	"	Phaseolus vulgaris	Daidzein	"	Woodward, 1980
12.	"	Glycine max	Diadzein	"	Keen and Kennedy, 1974
13.	"	Vicia faba	Wyerone epoxide	Furano-acetylene	Hargreaves et al., 1976
14.	"	Phosphocarpus tetragonolobus	Phaseollidin	pterocarpan	Ingham, 1978
15.	"	Astragalus cicer	Astraciceran	Isoflavan	Ingham, and Dewick, 1980a
16.	"	Pisum sativum	Pisatin	Pterocarpan	Robeson, 1978 Sutherland et.al., 1980 Bailey, 1973 Robeson and Harborne, 1980
17.	"	Dalbergia sericea	Vestitol	Isoflavan	Ingham, 1979
18.	"	Lablab niger	Isovestitol	Isoflavan	Ingham, 1977
19.	Linaceae	Linum usitatissimum	Coniferyl alcohol	phenyl propanoid	Keen and Littlefield, 1979
20.	Malvaceae	Gossypium hirsutum	Isohemigossypol	Terpene	Sadykov et al., 1974
			Gossyvertin	"	Karimdzhanov et al., 1976
			Gossypol	"	Bell, 1967
20.	Poaceae	Oryza sativa	Momilactone A	Diterpene	Cartwright, et al., 1977
			Momilactone B	"	

21.	Euphorbiaceae	*Ricinus communis*	Casbene	Diterpene	Sitton and West, 1975
22.	Convolulaceae	*Ipomea batatus*	Ipomeamarone	Sesquit erpene	Kubota and Matsuura, 1953
			Ipomeamaronol	"	Kato *et al.*, 1971
23.	Chenopodiceae	*Beta vulgaris*	Betavulgarin	flavonoid	Geigert *et al.*, 1973.

ELICITORS OF PHYTOALEXINS

Plant tissues accumulate phytoalexins when challenged by microorganisms and also do so in response to various agents termed elicitors. (Keen and Bruegger,1977). Elicitors of phytoalexins include biotic and abiotic substances. Biotic elicitors refer to complex carbohydrates from fungal and plant cell walls and microbial enzymes. Abiotic elicitors range from physical factors such as ultraviolet irradiation, point-freezing and mechanical injury to treatment of plant tissues with many chemicals such as polyamines, antibiotics, DNA intercalating agents and salts of heavy metals (for example, mercuric chloride, cupric chloride and silver nitrate). Respiratory inhibitors, eg. sodium idoacetate, sodium fluoride, potassium cyanide and 2,4-dinitrophenol are also effective.

Moniliolin A, was the first biotic elicitor to be isolated. It was obtained from the mycelia of Monilinia fructicola a fungal pathogen of fruit trees. (Cruickshank and Perrin,1968). Monilicolin A was reported to be a small peptide that elicited phytoalexins in the seed pods of true bean (Phaseolus vulgaris c.v. Redkidney). The physiological role of monilicolin A is not elucidated and it is considered unlikely that it has played a part in disease resistance because it has never shown to elicit phytoalexins in another host of M. fructicola. Moreover bean pod is the only tissue reported to be sensitive to this elicitor. Plants other than bean that are resistant to M. fructicola do

not accumulate phytoalexins in response to monilicolin A. Elicitors of biotic origin may be involved in the interaction of plants and potential pathogens whereas abiotic elicitors are not involved in normal host pathogen interactions. Besides biotic and abiotic elicitors, a further category of elicitors has been proposed to describe those present and active in the plant at all times. These were first described as 'constitutive elicitors' (Hargreves and Bailey,1978). Later, essentially similar materials, probably not present in healthy untreated tissue, were termed as endogenous elicitors.(Hahn et al.,1981). Release of endogenous plant elicitors has shown phytoalexin formation in bean tissues infected with viruses (Bailey and Ingham, 1971). Depending on the spurce and purity of the elicitor preparation, its capacity to induce phytoalexin accumulation can be equal to or even higher than that of the organisms from which it is isolated. For example, an elicitor from Cladosporium fulvum induced 10-50 times as much rishitin in tomato fruit tissue as a live conidial suspension of this fungus containing 5×10^6 conidia per millimeter (De wit and Roseboom, 1980).

The primary mechanism of action of biotic and abiotic elicitors has been claimed to be distinctly different (Yoshikawa, 1978). The biotic elicitors stimulate synthesis of phytoalexins but have no effect on synthetic activity but strongly inhibit degradation. Since the steady state concentration of a given phytoalexin is determined by its rates of

synthesis and degradation, the final effect would be the accumulation of phytoalexin with either type of elicitor.

Biotic elicitors have been easily obtained from fungal cells killed by heat or partially purified cell walls and they have also been found to be as effective as the living organism (Albersheim and Valent, 1978; Cruickshank,1980). These compounds (biotic elicitors) are of three types, peptides, glycopeptides and polysaccharides. The physiological effects of these compounds have not been thoroughly studied. It has been suggested that these elicitors act on cellular metabolism by initiating or regulating phytoalexin synthesis (Cruickshank, 1980) or possibly by modifying cellular DNA (Hadwiger and Beckman,1980). Comparisons of the effects of biotic and abiotic elicitors (Dixon and Lamb 1979; Moesta and Grisebach, 1980) have also led to the conclusion that elicitation occurs through a common primary response.

It has also been reported that biotic elicitors cause damage to plant cell membranes. The glycoprotein from Cladosporium fulvum caused ion leakage and death of tomato cells (Lazarovits and Higgins, 1979; Dow and Callow, 1979). The glucan from phytophthora megasperma inhibited growth of suspension cells (Albersheim and Valent,1978) and the cell wall elicitor from P. infestans was toxic to leaf cells of several plant species (Doke et al.,1979).

Fungi contain specific elicitors which are known to be race/cultivar specific. Specific elicitors have been isolated from Phytophthora megasperma f.sp. glycinea which function as glyceollin elicitors in soybeans (Keen 1975, 1978; Keen and Bruegger, 1977). Non specific elicitors are those elicitors which are not specific to any host plant and are present in both compatible and incompatible races of plant pathogens. Non-specific elicitors have been isolated from Phytophthora infestans (Chalova et al.,1976; Henfling et al.,1980), Fusarium solani (Daniels and Hadwiger, 1976) and Phythophthora megesperma f.sp.glycinea (Ayers et al.,1976 a,b). Non-specific elicitors have been found to be efficient inducers of phytoalexins in the tissues of both the susceptible and resistant cultivars of soybean (Keen et al.,1975; Albersheim et al.,1977; Valent and Albersheim, 1977) and potato (Wade et al.,1977). Some of the elicitors isolated are presented in Table II.

Studies on elicitors seem to hold promise for unravelling unknown features of plant-pathogen interactions. Their role in plant disease protection has been predicted. Studies on phytoalexins have also been reviewed by a few research workers (Albersheim and Anderson prouty,1975; Keen 1981; Darvill and Albersheim, 1984).

Table - II : SOURCES AND CHEMICAL NATURE OF PURIFIED ELICITORS

	Source	Chemical nature	Phytoalexin assay system	References
1	*Phytophthora megasperma* f.sp.*glycinea* Culture filtrate and cell wall	Branched β -glycan predominantly 3 and 3,6 linked glucosyl residues	Glyceollins in *Glycine max*	Albersheim and Valent (1978)
2	*Colletotrichum lindemuthianum* Culture filtrate and cell wall	Glucan with predominantly 3 and 4-linked glycosyl residues	Phaseollin in *Phaseolus vulgaris*	Abderson-Prouty and Albersheim(1975) Anderson (1978)
3	*Phytophthora infestans* Cell wall and mycelia	Glucan	Rishitin in *Solanum*	Lisker and Kuc (1977)
	P. infestans	Eicosapentanoic acid arachidonic acid	Rishitin,lubminin in *Solanum tuberosum*	Bostock *et al.*(1981)
4.	*Rhizopus stolonifer*	Polygalacturonase	Casbene synthetase in *Ricinus communis*	Stekoll and West (1978) Lee and West (1981 a,b)
5	*Cladosporium fulvum* Culture filtrate, mycelia cell walls	Glycoprotein	Rishitin in *Solanum lycopersicum*	De Wit and Kodde (1981)
6	*Fusarium solani* f.sp. phaseoli Cell walls	Chitosan	Pisatin in *pisum sativum*	Hadwiger and Beckmann (1980)

TOXICITY OF PHYTOALEXINS

To demonstrate the antifungal activity of phytoalexins different types of bioassays have been used. The bioassays assess the effects of the compounds on spore germination (Tomiyama et al.,1968; McCance and Drysdale,1975), germ tube growth (Duczek and Higgins, 1976; Rossel and Mansfield, 1978; Higgins, 1978), radial growth of mycelium on solid medium (Pierre and Bateman, 1967; Ward et al.,1974) and growth of mycelium on liquid medium (Bailey and Deverall,1971; Mineo, 1976; Smith, 1976).

The Bioassay conditions are rarely standardized among researchers and therefore it is difficult to come to a general conclusion on the antifungal activity of the compounds. Many procedural variables may effect in vitro estimates of phytoalexin toxicity and any one bioassay represents but a model system providing only limited information. Moreover the assays never give a complete explanation of the actual role of phytoalexins in vivo as they do not take into account the very nature of the host-parasite interactions. For a complete evaluation of the results, the timing of growth measurements and the changes occuring in bioassays should also be monitored (Smith, 1978; Bailey and Skipp, 1978).

The fungitoxicity of phytoalexins is apparent from the inhibition they cause to germ tube elongation, radial mycelial growth and dry weight accumulation. Such superficial

observations can be better understood by examination of individually affected cells by both light and electron microscopy. A variety of cytological effects have been noted including rapid cessation of cytoplasmic streaming, granulation of the cytoplasm, disorganisation of cell contents and breakdown of the cell membrane. Although damage may often prove to be total to individual fungal cells, cell or colony death is not an inevitable consequence of phytoalexin treatment.

Cytological studies indicate that blockage of phytoalexin could also lead to tolerance. Physiological consequences of phytoalexin treatment represent either direct or indirect manifestations of the toxicity of these compounds. For example indirect activity might occur by inactivation of cell wall degrading enzymes which are important to certain plant pathogens.

The antibacterial properties of phytoalexins have not been widely studied as their antifungal activities. The various bioassays leave no doubt that phytoalexins exert antibacterial activity. The effects may either be bacteriostatic or bactericidal. Differential sensitivity to phytoalexins amongst bacteria have been reported (Cruickshank,1962) and it has also been found that Gram-negative bacteria are usually less sensitive to phytoalexins than Gram-positive bacteria.

MODE OF ACTION OF PHYTOALEXINS

Though a number of attempts have been made to determine the mode of action of phytoalexins (Harris and Dennis, 1976,1977;

Skipp and Bailey, 1976, 1977) the data obtained are fragmentary.

The available evidence suggests two principal features of activity ;

(1) Phytoalexins probably represent multi-site toxicants.
(2) Phytoalexins cause dysfunction of membrane systems, particularly the plasmalemma, which is instrumental in their toxicity. Multi-site toxicants could be capable of affecting other reactions, resulting in interference with respiration, substantial loss of dry weight, leakage, swelling and bursting of affected hyphae; all testify to membrane damage, particularly to plasmalemma. Since the plasmalemma would be the first membrane system to be encountered by an external chemical, plasmalemma dysfunction may reflect a primary mode of action of phytoalexins. For e.g when 200 spores of Phytophthora infestans (Mont) Dby. were placed into antifungal diffusates obtained from bean pods in response to germinating spores, the spores swelled and burst within 60 seconds (Muller, 1956, 1958).

In the case of many phytoalexins, there appeared no single site of action, but a range of targets. Selective inhibition of one particular intracellular process such as protein synthesis, respiration or nucleic acid transcription is unlikely since a considerable time is taken before secondary effects are noted and ultrastructural damages are evident only after a few minutes of treatment. However, site-specific

action of phytoalexins have also been reported (Lyr,1977; Misato and Kakiki, 1977).

Phaseollin was reported to cause leakage of metabolites, loss of dry weight, reduction of nutrients and alterations in respiration in treated mycelium of Rhizoctonia soloni (Van Etten and Bateman, 1971). Rupture of the tonoplast was also observed with phaseollin (Hergreaves, 1980). Glyceollin is also known to inhibit oxygen uptake by Meloidogyne incognita (Kalpan et al., 1980). It was also shown that glyceollin was a potent inhibitor of oxygen uptake by isolated mitochondria from soybeans and table beets and it did not function as a uncoupler of oxidative phosphorylation but rather as a inhibitor of the electron transport system at a point after the site of succinate dehydrogenase.

ROLE OF PHYTOALEXINS IN DISEASE RESISTANCE

Many research workers have attempted to implicate phytoalexins in disease resistance due to their antimicrobial properties in vitro. Such studies have sought to establish that in the resistant plant, the concentration, the site of accumulation and the timing are consistent with the observed cessation of growth of the parasite. Biochemical and microscopical studies were conducted to investigate the role of phytoalexins in disease resistance (Higgins and Miller, 1968; Jones et al., 1975; Macfoy and Smith 1979; Dewit and Flach 1979).

The rapid accumulation of low molecular weight compounds i.e. the phytoalexins, at the site of infection is considered as one of the inducible defense mechanisms which are effective against diseases caused by fungi, bacteria and viruses (Kuc and Rush, 1985). The majority of data supporting a role for phytoalexin accumulation as the cause of the cessation of fungal growth in resistant plants come from interactions in which resistance is expressed following penetration and is associated with the necrosis of plant cells. Nearly all species in the Fabaceae, Malvaceae and Solanaceae synthesize phytoalexins in association with the necrogenic resistance response to pathogens.

The following evidences strongly support the involvement of phytoalexins in disease resistance:

(1) Evidences have been provided in a number of studies that phytoalexins not only accumulate at the site of infection but do so following penetration by the microbe quickly enough and in sufficiently high concentrations to inhibit the growth of both fungi (Sato et al.,1971, Bailey, 1974) and bacteria (Lyon and Wood 1975). Cessation of fungal growth of Botrytis fabae in broad bean leaves follows accumulation of phytoalexins (Rosall et al., 1980). Accumulation of phytoalexins caused the restriction of fungal growth of Colletotrichum lindemuthianum in beans.

(2) In the soybean Phytophthora megasparma f.sp.glycinea system, a normally compatible interaction was rendered

incompatible by the application of purified phytoalexins to the infection site (Chamberlain and Paxton, 1968), while removal of phytoalexins led to increased incompatibility (Klarman and Gerdemann, 1963). Co-inoculation of soybean plants with compatible races of Phytophthora sp. showed accumulation of phytoalexins and restriction of the growth of fungi (Paxton and Chamberlain, 1967). Preinoculation treatments have also shown reduced level of phytoalexin accumulation and thus altering a normal resistant reaction to a susceptible one (Bell and Presley, 1969; Murch and Paxton, 1977). Treatment of susceptible soybean plants with ultraviolet light, elicited glyceollin production and rendered the plants resistant to subsequent inoculation with P. megasperma f.sp. glycinea (Bridge and Klarman, 1973).

(3) The involvement of phytoalexin in resistance of vascular wilts was seen in cotton stem inoculated with spores of Verticillium albotrum. Accumulation of phytoalexins such as gossypol and other related compounds was found to be greater in resistant than in susceptible varieties during the first few days after inoculation (Bell and presley, 1969 a,b).

(4) Rishitin was found to be the primary cause of inhibition of hyphae of Phytophthora infestans in potato tuber tissue (Sato et al.,1971; Ishisaka et al.,1969).

(5) Accumulation of considerable amounts of phytoalexins in susceptible cultivars during the onset of necrosis was found

to take place as a result of resistance to bacterial disease in bean (Gnanmanickam and Patel, 1977, Webster and Sequeira 1977), cowpeas (Patridge and Keen, 1976), cotton (Essenberg et al., 1979), and fungal wilt diseases of tomato (Stromberg & Corden, 1977) and alfafa (Khan and Milton, 1978). Accumulation of phytoalexins in toxic levels causing confinement of the pathogen is also reported (Smith et al.,1975).

(6) Inhibition of phenylalanine ammonia-lyase in soybeans by 1,2-amino,3-phenyl propionic acid leads to loss of resistance to Phytophthora megasperma f.sp. glycinea, the fungal pathogen that causes root and stem rot in its host plant, soybean. Inhibition of this enzyme leads to a decrease in phytoalexin content as well as loss of resistance to the pathogen (Moesta and Grisbach, 1982).

Failure to produce phytoalexins also is found to cause disease. The situation of a resistant reaction in a host arises when the concentration of phytoalexins produced is above the required quantity needed to inhibit the fungus. Susceptibility may also be due to the capacity to be tolerant to the level of phytoalexins produced. It is envisaged that the quantitative level of phytoalexin concentration and the differential esnsitivity of the fungus species or strain are the two primary factors responsible for a disease reaction. It is also recognised that physiological factors associated with either or both of these primary factors in vivo may modify the

disease reaction on the basis of chemical analysis for phytoalexin and its biological assay *in vitro* (Cruickshank, 1963).

The inheritance of disease reaction in many host-parasite interaction is well understood. A gene to gene relationship has been postulated (Flor, 1956; Person *et al.*, 1962). Host specificity of phytoalexins has been found to occur between genera but not within a species or even taxonomically related species. Fungal species show differential sensitivity to phytoalexins. It is thus postulated in conformity for the gene for gene relationship that the host genotype determines the phytoalexin characteristic of the host and the fungus genotype determines the sensitivity of the fungus to the phytoalexin formed, and the multiplicity of disease reaction types can be explained on a quantitative basis within a species or genus and on a qualitative basis between genera.

PRESENT WORK

Since most of the work on phytoalexins has been done on food crops and only a few efforts were made to study the forest crops, an attempt has been made here to study the economically important forest crops. The present work incorporates studies on the phytoalexins and other post-infectional compounds of eight trees of which (i) *Tectona*

grandis Linn. (2) Cassia fistula Linn. (3) Morinda tomentosa Heyne (4) Madhuca indica Gmel and (5) Anogeissus latifolia Wall. are seen in the wild state in the forests and the remaining i.e. (6) Mangifera indica Linn. (7) Eucalyptus globulus Labill and (8) Syzygium cumini (L.) Skeels are cultivated. The work has been confined only to the leaves since only the leaf spot disease was found to occur on all these trees. The principal objectives of the present work were as follows:

(1) To isolate, culture and identify the pathogenic fungus from the diseased leaves of all these trees.

(2) To test the pathogenicity of the fungi isolated from the leaves of plants and to study the disease symptoms.

(3) To study the pre-infectional antifungal compounds of some of the trees.

(4) To compare the healthy and infected leaves of the trees for locating the post-infectional chemical changes.

(5) To isolate and identify the post-infectional compounds.

(6) To induce the phytoalexin production in leaves using the pathogenic as well as non-pathogenic fungus and compare their performances.

(7) To prove the antifungal activity of phytoalexins.