LITERATURE REVIEW

Disease resistance in plants depends on multiple defence mechanisms which include preformed defence barriers such as the cuticle, the cell wall or constitutive antimicrobial compounds as well as defenses triggerred by the invader. Resistance to disease can also be described on several levels such as nonhost resistance, parasite- and race-specific resistance, plant age- and organ-specific resistance, and acquired resistance. To fully understand each type of resistance, we need to determine what physical and biochemical factors are needed to stop the pathogen from developing in the tissue after infection. The physiological/biochemical basis of resistance of plants to fungal and bacterial pathogens has been associated with both preformed and infection induced antimicrobial compounds. In fact, if one considers the multitude of microorganisms to which plants are being continuously exposed in nature, the significance of specificity becomes more apparent (Chakraborty, 1988). The success or failure of infection is determined by dynamic competition and the final outcome is determined by the sum of favourable and unfavourable conditions for both the pathogen and host cells. Purpose of this review is to present briefly the observation of previous workers in concord with the present line of investigation on two major aspect i.e. (a) Phenolic compounds and their role in plant disease resistance and (b) Serological cross reactivity between host and parasite.

Phenolic compounds and their role in Plant Disease Resistance

Phenols have been found in plants investigated to date. Some occur constitutively and are thought to function as preformed inhibitors associated with nonhost resistance (Millar and Higgins, 1970; Schonbeck and Schlosser, 1976; Mansfield 1983; Stoessal, 1983). Others which are formed in response to ingress of pathogens and their appearance is considered as part of an active defence response. Since the phenolic intermediates have a role in the active expression of resistance, an underlying problem in ascertaining that such secondary metabolites are of primary (rather than secondary) importance has been the localization and timing of the host response (Nicholson and Hammerschmidt, 1992).

Phenols are significant components of the host response following infection. Paschenko (1978) demonstrated the role of phenols in resistance of *Nicotiana didebta* to

Peronospora tabacina. From a study of the effects of pyrogallol, pyrocatechol and hydroquinone and aqueous extracts from leaf tissues of *N. didebta* and leaf washing on conidial growth, no direct relationship was found between their quantity and the resistance of mature plants to *P. tabacina.* Pyrocatechol and hydroquinone showed extremely high fungitoxicity in relation to *P. tabacina.* Spore growth was more strongly inhibited by extracts from tissue of receptive cultivars. Pyrogallol somewhat stimulated conidial growth. Polyphenoloxidase of resistance cultivars were highly activated during infection. In potato tubers chlorogenic acid was reported to accumulate slower following inoculation with *P. infestants* than in non-inoculated controls, regardless of cultivar resistance (Gans, 1978). In contrast, in some susceptible cultivars chlorogenic acid accumulates at an accelerated rate after inoculation (Henderson and Friend, 1979). The differentiation of the responses of plants to pathogens based on host and non-host interactions also has been argued by Health (1980).

Chlorogenic acid act as a reservoir for the caffeoyl moiety that, as an activated phenylpropanoid, could be shunted to the synthesis of other phenolics possibly involved in containment of the pathogen (Friend,1981). The accumulation of chlorogenic acid may represent a general rise in phenolic biosynthesis. Such synthesis can ultimately result in the accumulation of compounds with sufficient toxicity to be involved in resistance. When carrot root slice is infected with *Botrytis cinerea*, the infection leads to the production of inhibitors such as 6-methoxymellein, p-hydroxybenzoic acid and falcarinol (Harding and Heale,1981). Oat produces nitrogen containing phenolic phytoalexins, the avenalumins, and these compounds accumulated only in incompatible host pathogen interactions (Mayama et at. 1981)

Mayama and Tani (1982) took advantage of the UV-absorbance and autofluorescence spectra of the avenalumins and used microspectrophotometry to reveal the presence of intense fluorescence only in cells immediately associated with the infection site. Rapid accumulation of phenols may result in the effective isolation of the pathogen (or non-pathogen) at the original site of ingress (Legrand, 1983; Ride,1983). For most plants it is low molecular weight phenols, especially the phenyl propanoid, that are involved in the initial response to stress. In potato, phenols accumulate as an initial response to infection (Hammerschmidt,1984; Hachler and Hohl, 1984). The accumulation

of polymerized phenols also occurs as a rapid response to infection. Hydroxycinnamic acids and their derivatives are thought to contribute to the discoloration and autofluorescence of host tissues at the site of infection (Farmer, 1985; Bolwell *et al.*, 1985).

Change in phenolics in maize leaves after inoculation with *Bipolaris zeicola* and their antifungal activity was demonstrated by Werdes and Kern (1985). Maize inbreds Prl (resistant) and Pr (susceptible) to *B. zeicola* race 1 were inoculated and phenolic material was extracted from maize leaf tissue. The components were than analyzed and resistance was studied with respect to phenol metabolism and accumulation of fungitoxic compounds. Host responses could be differentiated by changes in content of phenolic compounds. The pattern of changes of total phenolic content (hydrolyzed and unhydrolyzed ethylacetate soluble phenols) of resistant and susceptible inbreds did not differ much between 0 hr. and 96 hr. after inoculation. However, phenolics content in the resistant inbred increased between 96 and 120 hr. after inoculation to a level two to three times higher than that of susceptible and non-infected control in breds. They isolated four antifungal compounds, A, B, C and D from hydrolyzed maize leaf extracts. All four compounds were fungitoxic to *B. zeicola* in spore germination and chromatographic bioassays. Compounds A and B were inhibitory to *B zeicola* only in high concentrations. The investigators suggested a role of the phenol metabolism in the resistance of maize to *B. zeicola* based on different content of total phenolics in resistance and susceptible inbreds. The compounds C and D were supposed to play a role in the resistance mechanism as fungitoxic component.

Change in phenolics of two each of resistant and susceptible varieties of wheat leaves in response to *Puccinia recondita* causing brown rust were evaluated by Saxena et al (1986). They found that resistant varieties exhibited higher concentration of phenolics than the susceptible one. Esterification of phenols to cell-wall materials has been considered as primary theme in the expression of resistance (Fry, 1986; 1987). Biochemical analysis of pea varieties resistant and susceptible to *Erysiphe polygoni* causing powdery mildew disease revealed that the quantity of total phenol and orthodihydroxyphenol was high in stem and leaves of resistant varieties as compared to susceptible ones which decreased as the age of plant increased in all the varieties (Parashar and Sindhan, 1987).

The temporal and spatial differences in the accumulation of phenylalanine ammonia-lyase (PAL) mRNA occurred as a response to infection which was rapidly elevated in interactions involving an incompatible race of fungus, where as a significantly different profile of mRNA accumulation occurred in interactions involving a compatible race (Cuypers *et al*, 1988). The kinds of phenolic compounds that accumulate prior to the active defence response as well as their origin has been addressed by Matern *et al* (1988) using parsley leaves with *P. megasperma f. sp. glycinea* (Pmg) or treatment of parsley cell suspensions with a Pmg elicitor results in the accumulation of substantial concentrations of coumarin phytoalexins as well as esterification of phenylpropanoids, in particular ferulic acid, to cell walls. Treatment of parsley cells with the Pmg. elicitor cause the synthesis of the coumarin phytoalexins isopimpinellin, psoralen, bergapten, xanthotoxin and graveolone. The healthy leaves of *Morinda tomentosa* contained the two methoxyflavonols 4'-OMe Kaempferol and 3', 4'- di OMe quercentin, and the four phenolic acids-vanillic, syringic, gentisic and ferulic. The *Colletotrichum gloeosporoides* infected leaves contained the hydroxyflavonols kaempferol and quercetin along with four phenolic acids found in healthy leaves. The diffusates of both the pathogen and non-pathogen (*F. solani*) treated leaves contained quercetin and kaempferol (Abraham and Daniel, 1988).

Matern and Kneusel (1988) have proposed that the defensive strategy of plants exists in two stages. The first is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow (or even halt) the growth of the pathogen and to allow for the activation of "secondary" strategies that would more thoroughly restrict the pathogen. Secondary responses would involve the activation of specific defences such as the de-novo synthesis of phytoalexins or other stress-related substances. They argue that the initial defense response must occur so rapidly that it is unlikely to involve de novo transcription and translation of genes, which would be characterstic of the second level of defence. The sequence of events in a defence response can be thought to include-host cell death and necrosis, accumulation of toxic phenols, modification of cell walls by phenolic substituents or physical barriers such as appositions or papillae, and, finally, the synthesis of specific antibiotics such as phytoalexins.

Prasada *et al* (1988) reported that after infection total phenol increased in green and ripe tomato fruits in course of rotting due to *Sclerotium rolfsii*. There is often a greater

increase in phenolic biosynthesis in resistant host species than in susceptible host and it is sometimes postulated that the inscrease in phenolic compounds is part of the resistance mechanism. Some of these compounds are toxic to pathogenic and nonpathogenic fungi and have been considered to play an important role in disease resistance (Vidyasekharan, 1988). Changes in phenol contents was also demonstrated by Oke (1988) in young, matured, healthy and *Cassicola corynospora* and *Colletotrichum nicotianae* infected leaves of tobacco. After infection the quantity of total pehnols and orthodihydroxyphenol increased in both stem and leaves of susceptible and resistant varieties.

Tore and Tossi (1989) investigated the changes in phenolic and nitrogen metabolism in healthy and infected with *(Thielaviopsis basicola)* tobacco roots and leaves. The chlorogenic acid content increased in infected root and leaves compared with the control beginning on the 8th day after inoculation. Polyphenol content in sweet cherry bark was drastically changed after infection by *Cercospora personii* (Bayer, 1989). Infected tissue and closely neighbouring areas were characterised by the appearance of phenolic aglycons which inhibited growth of both the pathogen. Mechanically wounded bark tissue showed different phenolic patterns than infected ones.

Etenbarian (1989) detected quantitative changes in phenolic compound at different time intervals on barley varieties incoulated with *Puccinia hordei.* Luthra (1989) determined the levels of total phenol in sorghum leaves, resistant and suceptible to *Ramulispora sorghicola* at 15 days interval after 25 days of sowing. Resistant varieties exhibited high phenol content in comparison to susceptible ones at all stages of growth.

Phenolic compounds inhibitory to the germination of spores of *Colletotrichum graminicola* were shown to leach from necrotic lesions on corn leaves caused by the fungus. Primary components of the phenolic mixture were identified as esters and glycosides of p-coumaric and ferulic acids as well as the free compound themselves. Spores of *C. graminicola* produced in acervuli of infected leaves were shown to be surrounded by a mucilaginous matrix as in the case when the fungus is cultured *in vitro.* It is suggested that the mucilage protects spores from the inhibitory effects of the phenols by the presence of proline rich proteins that have been shown to have a high binding affinity for a variety of phenols (Nicholson et al., 1989).The relatively non-specific disruptive effects on cells that result from wounding lead almost immediately to a variety of physiological changes, including oxidation of secondary metabolites. The

accumulation of these esters preceded the onset of visible necrosis of infection sites, the concentration of the compounds fell substantially after the onset of necrosis both of which strengthen the argument for their involvement in the browing response (Bostock and Stermer, 1989). Toxic phenylpropanoids, such as ferulic acid, can form rapidly without the involvement of the traditionally accepted route of phenylpropnoid synthesis and conversion to CoA esters (Hahlbrock and Scheel, 1989). It has long been recognized that responses are characterized by the early accumulation of phenolic compounds at the infection site and that limited development of the pathogen occurs as a result of rapid (hypersensitive) cell death (Fernandez and Heath, 1989). Baker *et. al* (1989) examined specific race interaction with clones of resistant and susceptible genotypes and they found greater accumulation of phenolic compounds in resistant reaction than in susceptible reaction. They suggested that accumulation of phenolics may play a role in natural and induced interaction involving *Colletotrichum trifolii* and *Medicago sativa.*

Kumar *et al.* (1990) analysed certain biochemical changes in the pearl millet shoots infected with downy mildew pathogen *(Sclerospora graminicola).* The estimation revealed that the total phenol and free amino acids content were found to be low both in diseased shoot and roots of pearl millet *(Pennisetum glaucum).* In maize there is a marked accumulation of two caffeic acid esters after inoculation with *Glomerella graminicola* or *C. heterostrophus* in both compatible and incompatible combination (Lyons et al., 1990). One compound was identified as caffeoyl glucose, whereas the other was a caffeoyl ester of an unknown organic acid moiety. Although neither compound was fungitoxic, a pattern of rapid accumulation followed by a sharp decrease in the amount of both compounds in the tissue suggested that they may serve as a pool of phenols required for diversion to other products. Mansfield (1990) has proposed that cell death results from irreversible membrane damage that may occur in response to pathogen recognition or as a result of activated host response.

Low molecular weight phenols, such as benzoic acids and the phenylpropanoids, are formed in the initial response to infection (Niemann et al., 1991). Early after infection, low molecular weight phenols accumulate in both incompatible (resistant) and compatible (susceptible) interactions. Whether these compounds, are significant in the

ultimate host response presents a perplexing problem. Bruzzese and Hasan (1991) demonstrated that accumulation of phenols at the infection site occured as early as 3 hr. after inoculation, indicating an association of phenols with the initial stages of the response. The contents of phenols, O-dihydroxyphenols and peroxidase activity in healthy and *Curvularia andrepogonis* infected leaves of *Java citronella (Cymbopogan winterianus)* were determined by Alam et al. (1991). As a result of infection the content of phenols and peroxidase increased two and fourfold, respectively in necrotic lesions compared to healthy leaves. . It has been suggested by Permulla and Heath (1991) that the accumulation of phenolics as an initial response to infection may reflect a general increase in host metabolism as well as an accumulation of relatively non-toxic secondary metabolites, which could ultimately serve as precursors for compounds essential to expression of resistance. In the interaction of potato tubers with *Verticillium dahliae*, hypersensitive browning and suberization are characteristic of the initial events in resistance rather than production and accumulation of phytoalexins (Vaughn and Lulai 1991).

The *Fusarium* sp infected leaves of *Trianthema portulacastrum* contained 6,7, dimethoxy-3, 5, 4'- trihydroxy flavone, vanillic acid, p-hydroxybenzoic acid, quercetin and ferulic acid. By using drop diffusate technique it was found that the pathogen induces the formation of quercetin and ferulic acid (Darshika and Daniel, 1992). Changes in carbohydrates, amino acid and phenolic contents in jute plant on inoculation with Macrophomina phaseolina, *Colletotrichum corchori* and *Lasiodiplodia theobromae* were studied by Sahabuddin and Anwar (1992). Total sugars, non-reducing sugars, starch and total free amino acids were found to decrease on inoculation with all the three test pathogens of jute, while reducing sugars, total phenols and orthodihydric phenols increased.

Among fourteen varieties of tea tested separately against *Glomerella cingulata, Pestalotiopsis theae* and *Bipolaris carbonum,* TV-18 and TV-26 were highly susceptible and resistant respectively to *G. cingulata* and *B. carbonum.* While TV-23 and CP-1 were found to be highly susceptible and resistant to *P. theae.* Twelve separate phenolics were detected on thin layer chromatograms after extraction from healthy tea leaves and some were identified as gallic acid catechol, caffeic acid and p-coumaric acid. Total phenol

level decrease by 4.5, 1.2 and 8.5% in the susceptible varieties TV-18, TV-9 and TV-17 respectively after inoculation with *B.carbonum*, whereas in case of resistant varieties TV-26, TV-25 and TV-16 total phenol level increased by 11.1, 5.7 and 12.2% respectively after inoculation. Similar pattern was observed for O-dihydroxy phenol content in healthy and inoculated leaves of resistant and susceptible varieties (Chakraborty et al., 1994b).

The healthy leaves of *Tectona grandis* contained two flavones : 4'-O Me-apigenin and luteolin. The phenolic acids present were syringic, sinapic, vanillic, melilotic and gentisic acid. The other constituents of the leaves were quinones (lepachol and tectaquinon), proanthocyanidins, iridoids, alkoloids and tannins. The infected leaves did not contain any flovone but a flovonol 3', 4'- dimethoxyquercetin instead and phenolic acid such as ferulic, vanillic, melilotic and gentisic acids. They contained the same quinones as at healthy leaves as well as proanthocyanidins, iridoids, alkoloids and tannins. There was no significant chemical differences between the diffusate of control and treated leaves when the healthy leaves were treated with the spore suspension of *Curvularia clavata.* But when the leaves were treated with a non-pathogen *Fusarium solani,* the diffusate contained p-hydroxybenzoic acid. Mycelial growth, spore germination and germ tube growth of *F solani* and *C. clavata* is strongly inhibited by P-hydroxybenzoic acid (Daniel, 1995).

Two antifungal compounds isolated from healthy and *Bipolaris carbonum* infected tea leaves exhibited clear inhibition zones at Rf 0.8 and 0.65, respectively in a chromatographic bioassay. On the basis of their colour reaction on TLC and UV-spectra these were identified to be catechin and pyrocatechol, respectively. Resistant varieties accumulated 439-510 ug/g fresh weight tissue of catechol in comparison to 187-212 ug/g fresh weight tissue in susceptible varieties after inoculation with *B. carbonum.* Low concentration of this compound was also detected in healthy leaf tissues (Chakraborty and Saha, 1994, 1995). Phenolic contents in pea genotypes in relation to powdery mildew disease was studied by Sharma *et.al* (1998) . Guleria *et.al* (2001) demonstrated increased levels of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and phenols in salicyclic acid sprayed leaves in comparison to untreated control plants.

In some host parasite interactions phenolics have been associated with phytoalexin accumulation (Mansfield et al 1974, Langcake and Pryce, 1976, Langcake and Macarthy, 1979, Holliday et al., 1981, Pierce and Ersenberg, 1987, Baker et al., 1989). Phytoalexin

accumulation is believed to be an important early defence response in several plant pathogen interactions. A lot of work has been done and several comprehensive reviews have appeared on phytoalexins and their role in disease resistance (Cruickshank, 1963, 1978, 1980, Kuc, 1966, 1972, 1976; Deverall, 1972, 1976; Ingham, 1972, 1973, 1982; Purkayastha, 1973, 1976, 1985, 1986; Van Etten and Pueppke, 1976; Keen and Brueggar, 1977; Harborne and Ingham 1978 ; Keen 1981, 1982, 1990; Van Etten et al., 1982, 1989; Wood 1982; Bailey and Deverall, 1983; Ward, 1986; Paxton 1988; Ebel and Grisebach, 1988; Daniel, 1995; Purkayastha, 1995; Chakraborty *et al.*, 1995; Hammerschmidt, 1999; Greyer and Kokubun, 2001).

Phytoalexins constitute a chemically heterogeneous group of substances belonging to various classes of natural products which include isoflavonoids, sesquiterpenoids, polyacetylenes and stilbenoids. Many phytoalexins are absent in healthy, unchallenged plants. It was originally believed that phytoalexins were host specific. With the evidences accumulated so far, concerning the wide spread occurrence, isolation and characterization of phytoalexins during the past 50 years, it is now clear that more than one phytoalexin could occur in a single host species of which one may be dominated (Purkayastha, 1995). Again , similar phytoalexins may also occur in different host species. Plant organs including roots, stem, leaves and fruits have been shown to respond to infection with the formation of phytoalexins. Among plant pathogens, fungi, some bacterial and viruses are capable of including phytoalexin production in plants, but involvement of the last two groups of organisms seems to be quite negligible in comparison with the large group of fungi. During incompatible host-parasite interaction, phytoalexin is synthesized rapidly and accumulates at the infection site (Akazawa and Wada, 1961 Cruickshank and Perrin, 1968, Partridge and Keen, 1976; Purkayastha et al., 1983). In contrast in the compatible host parasite interaction the plant also synthesis the phytoalexin but relatively slowly and in reduced concentration.

The degree of stimulation of phytoalexin biosynthesis depends on several factors such as quantity of elicitors, presence or absence of receptors in the host cell membrane, if present, strong or weak response of receptor, duration of treatment, and environmental conditions. Some selected observations in this line of research have been incorporated in the following paragraphs.

A glucan was isolated from the cell wall extracts of *Fusarium oxysporum* f sp *lycopersici* (Anderson, 1980) and a polypeptide (monilicolin A), from mycelia of *Monilina fructicola* (Cruckshank and Perrin, 1968). Both compounds elicited phaseollin production. An elicitor of phaseollin was isolated from the mycelial walls and culture filtrates of *Colletotrichum lindomuthianum*, which was identified as a polysaccharide. The molecular weight varied between 1 million and 5 million Da, and consisted predominantly of 3-and 4- linked glycosyl residue (Anderson and Albersheim, 1975). An amount equivalent to 100 ng of glucose elicited a similar response in the bean tissue.

The isolates of *Fusarium solani* which differed in their pathogenicity also should differential pisatin-eliciting potential. It was confirmed when their culture filtrates were tested on pea (Daniels and Hadwiger, 1976). There was a difference in the concentration of elicitor in the culture filtrates of isolates. The elicitor was fairly heat-stable and also stable in freezing, but eliciting activity was reduced significantly by pronase digestion. This strongly suggests that some of the activities were due to proteinaceous components.

An elicitor of glyceollin was isolated from the mycelial wall of *Phytophthora megasperma* var. Sojae by Ebel et al., (1976). This elicitor stimulated the activity of phenylalanine ammonialyase and also induced glyceollin production in soybean cell cultures. They concluded that the action of elicitors is not species or variety specific but is a part of the general defence response of plants. Shiraishi et al., (1978) detected both elicitor and suppressor of pisatin in the pycnospore germination fluid of *Mycosphaerella pinoides.*

The regulation system of phaseollin synthesis in cell suspension cultures of dwarf french bean *(Phaseolus vulgaris)* was studied by Dixon and Christopher (1979). Considerable amount of phaseollin accumulated when french bean was treated with an elicitor from the cell wall of *C. lindemuthianum.* But the elicitors isolated from the cell walls of *P. sojae* and *Botrytis cinerea* were less effective.

Elicitors extracted from the cell walls of *Saccharomyces crevisiae* were identified as structural glucans. These are able to stimulate glyceollin accumulation in soybean. Specific elicitors of glyceollin were also detected in the cellular envelops of incompatible races of *Pseudomonas syringe pv. glycinea.* However, elicitor activity could not be detected in lipopolysaccharide preparation of exopolysaccharide fraction, or the culture fluids of various races of *P glycinea.* elicitors were solubilized with sodium dodecyl

sulfate and then preparations from five bacterial races expecting one had similar specificity for elicitation of glyceollin in cotyledons of two soybean *(Glycine max)* cultivars (Bruegger and Keen, 1979). These observations suggests that elicitors are not always race specific. Glycoproteins were extracted from isolated cell walls of *Phytophthora sojae* with 0.1 N NaOH at 0°C and elicited glyceollin in soybean hypocotyls with the same specificity as the fungus races from which they were obtained (Keen and Legrand, 1980). Fraction of the crude extracts on DEAE Bio-Gel and Bio-Gel A-5 m columns showed that specific elicitor activity was associated with the presence of high molecular weight glycoproteins detected by SDS gel electrophoresis. The glycoproteins appeared to contain only glucose and mannose as neutral sugar. The elicitor activity of the glycoproteins was not diminished by boiling at 100°C or pronase treatment, but was destroyed by periodate, thus indicating that the carbohydrate portions are important for activity. The glycoproteins were the only concanavalin A reactive species detected in the crude cell wall extracts, and fluorescein labelled concanavalin A was hapten-specifically bound to living hyphae of the fungus.

Purkayastha and Ghosh (1983) reported elicitor activity of fresh mycelial wall extract of *Myrothecium roridum.* Spores were suspended in mycelial wall extract, drops placed on leaf surfaces of soybean and incubated for 48 hr. The results of bioassay test revealed that the spores suspended in mycelial wall extract were more inhibitory than the spores suspended in mycelial wall extract were more inhibitory than the spores suspended in sterile distilled water and incubated on leaf surfaces for a similar period. Mycelial wall extract induced greater production of glyceollin in soybean leaves.

Yomoto et. al (1986) demonstrated that pisatin could be induced in pea leaves by elicitors from *Mycosphaerella pinoides, M. melonis* and *M lingulicola*. Accumulation of pisatin increased after removal of epidermis and application of elicitors from germination fluid of the fungus.

A carbohydrate rich extracellular component from a race of *C. lindemuthianum* showed a high level of phytoalexin activity on a resistant cultivar "Dark Red" of kidney bean but not on the susceptible cultivar "Great Northern." Other extracellular components were also recognised as elicitors by both cultivars. It is noteworthy that the two cultivars of *Pheseolus vulgaris* displayed a differential response to extracellular components.

These observations support the hypothesis that both general and specific mechanisms exist in race cultivar interaction (Tepper and Anderson 1986).

Metabolites and viable cells of *Pseudomonas corrugata* from liquid culture medium elicited biosynthesis of the phytoalexin medicarpin in ladino white clover *(Trifolium repens)* leaflets and callus. The biologically active elicitors components were soluble in 80% ethanol. They were partially purified by removing components greater then 3,500 Da by dialysis and fractionating by preparative reversed phase HPLC. None of the four fractions separated by HPLC elicited appreciable quantities of medicarpin in callus, but fraction 1 combined with fraction 4 elicited high concentrations of medicarpin. Any combination of fractions 2, 3 and 4 synergistically elicited medicarpin in callus. Elicitor activity was concentration - dependent. The active fractions were acidic in solution, but their elicitor activity was not dependent on low pH. Fraction 1 contained primarily uncharacterized reducing carbohydrate and phosphate. Fractions 2 and 3 were composed primarily of two related, unidentified fluorescent compounds and fraction 4 contained another unidentified fluorescent compound (Gusine et al., 1990).

The phytopathogenic fungi *Phytophthora* subspecies elicit hypersensitive-like necrosis on their nonhost tobacco *(Nicotina tabacum)*, with the exception of the tobacco pathogen *Phytophthora nicotianae*. In culture, these fungi except *P nicotianae* secrete proteins, called elicitins, that cause these remote leaf necrosis and are responsible for the incompatible reaction. These proteins protect tobacco against invasion by the agent of the tobacco black shank, *P nicotianae*, which is unable to produce such an elicitor. Cryptogein secreted by *P cryptogea*, has been purified, sequenced and characterized by terce-Laforgue (1992) as an elicitin, a novel family of 10k da holoproteins. The secretion of cryptogein began later than its synthesis and stopped earlier, simultaneously with mycelium growth, when the nitrogen source in the culture medium was nearly exhausted. Electrophoretic patterns of total protein from mycelium extracts and N-terminal sequence analysis showed that cryptogein accumulated in the mycelium in its natural form. Cryptogein was synthesized as a preprotein.

Fifteen isolates of *Phytophthora parasitica*, nine from tobacco (causing black shank disease) and six from other host plants were compared by root inoculation with regard to their pathogenicity to young tobacco plants. A progressive invasion of the aerial parts over 1 week was observed only with the black shank isolates, while the non-tobacco

isolates induced leaf necrosis within 2 days. Similar necrosis occured when the roots of tobacco plants were dipped in diluted culture filtrates from non-tobacco isolates, but not in those from tobacco isolates. The necrosis inducing filtrates were shown contain a c 10K Da protein band which was not present in the other filtrates. This protein (named parasiticein) was purified by ion exchange chromatography to homogeneity in SDS - PAGE and reverse phase HPLC. Parasiticein was serologically related to cryptogein, a member of the elicitin family of proteinaceous elicitors. Like the other elicitins, parasiticein induced necrosis in tobacco plants and protected them against black shank. It most closely resembled little leaf necrosis. Ricci et al., (1992) suggested that the absence of parasiticein production by the black shank isolates might be a factor involved in their specific pathogenicity to tobacco.

A glycoprotein elicitor of phytoalexin accumulation in leaves of *Phaseolus vulgaris* produced well before lysis in the medium of cultures of *Colletotrichum lindemuthianum* was purified to homogeneity by Coleman et al., (1992). The glycoprotein was a monomer of M.W.28k Da with a pI of 4.25. The glycosyl side chains which accounted for 43% of the weight of the holoprotein, were composed principally of galactose, mannose and rhamnose exhibited a minimum degree of polymerization of eight and were apparently O-linked to abundant serine and / or threonine residues of the peptide backbone. In a *P. vulgaris* leaf infection bioassay the purified glycoprotein had activity easily detectable at nanomolar concentrations and inducing browning of the treated tissue and the accumulation of both phenylalanine ammonia-lyase and the isoflavanoid phytoalexins phaseollinisoflavin. For these three linked defence responses, sub optimal concentrations of the glycoprotein induced respectively 4.2,7.6 and 9.7 fold more activity in the cultivar resistant to race delta (cv. Kievit) than in a cultivar susceptible to that race (cv. Pinto). Protein integrity was not required for elicitor activity and glycosyl side-chains isolated from the protein were shown to be active elicitor. The effects of an elicitor (CG-elicitor) from *Colletotrichum graminicola* was studied by Ransom et al., (1992). Roots of sorghum *(Sorghum bicolor)* accumulated 3-deoxyanthocyanidin phytoalexins in response to CG elicitor. Elicitation of the phytoalexins prior to treatment with the elicitor did not prevent infection and development of milo disease symptoms in susceptible seedlings inoculated with conidia of *Periconia circinata.* However, treatment of roots with the CG

elicitor enhanced the synthesis of 16k Da proteins in both resistant and susceptible genotypes without expression of disease symptoms.

Effects of the elicitor and the suppressor from a pea pathogen, *Mycospharella pinodes,* on polyphosphoinositide metabolism in pea plasma membranes were examined *in vitro* by Toyoda et al., (1992). Lipid phosphorylation in the isolated pea plasma membrane was drastically stimulated by the elicitor, but markedly inhibited by the suppressor. A similar inhibitory effect was observed by the treatment with orthovanadate or K-252a that blocked pisatin production induced by the elicitor. Neomycin, an aminoglycoside antibiotic that interacts with the polyphosphoinositide metabolism, also affected the lipid phosphorylation *in vitro* and blocked the elicitor induced accumulation of pisatin *in vivo*. Rapid changes of poly phosphoinositide metobolism in pea plasma membranes in one of indispensable process during the elicitation of defence responses. Cell walls of germ tubes from wheat stem rust *(Puccinia graminis f. sp. tritici)* contain a glycoprotein with a molecular mass of about 67 KD referred to as the Pgt elicitor. This glycoprotein induces a hypersensitive-like response in wheat leaves. In elicitor active intercellular washing fluid (IWF) from compatible wheat stem rust interactions, several elicitor-active glycoproteins were detected by Beissmann et al., (1992). One of these glycoproteins had an electrophoretic mobility identical to the Pgt elicitor. This IWF glycoprotein exhibited elicitor activity upon elution from SDS gels. It was recognised by anti Pgt elicitor antiserum suggesting partial structural identity between Pgt and IWF elicitors. As with Pgt elicitors, the elicitor activity of the IWF glycoprotein residues in the carbohydrate moiety because periodate, but not trypsin or pronase destroyed activity. These results suggest that the Pgt elicitor is released from hyphal cell walls into the wheat apoplast during stem rust infection.

The elicitor induced incorporation of phenylpropanoid derivatives into the cell wall and the secretion of soluble coumarin derivatives (phytoalexins) by parsley (*Petroselinum crispum* L.) suspension cultures can be potentiated by pretreatment of the cultures with 2, 6-di chloroisonicotinic acid or derivatives of salicylic acid. The cell walls and an extra cellular soluble polymer were isolated by Kauss et al., (1993) from control cells or cells treated with an elicitor from *Phytophthora megasperma f. sp. glycinea.* After alkaline hydrolysis, both fractions from elicited cells showed a greatly increased content of 4-coumaric, ferulic, and 4- hydorxybenzoic acid, as well as 4-hydroxybenzaldehyde and

vanillin. Two minor peaks were identified as tyrosol and methoxy tyrosol. The pretreatment effect is most pronounced at a low elicitor concentration. Its specificity was elaborate for coumarin secretion. When the parsley suspension cultures were preincubated for 1 day, with 2, 6-dichloroisonicotinic, 4-or 5- chlorosalicylic, or 3, 5-dichlorosalicylic acid, the cells exhibited greatly increased elicitor response. Pretreatment with isonicotinic, salicylic, acetylsalicylic, or 2, 6-dihydroxybenzoic acid was less efficient in enhancing the response, and some other isomers were inactive This increase in elicitor response was also observed for the above mentioned monomeric phenolics, which were liberated from cell walls upon alkaline hydrolysis and for "lignin-like" cell wall polymers determined by the thioglycolic acid method. It was shown for 5-chlorosalicylic acid that conditioning most likely improves the signal transduction leading to the activation of genes encoding phenylalanine ammonia lyase and 4-coumarate: coenzyme A ligase. The conditioning thus sensitizes the parsley suspension cells to respond lower elicitor concentration. If a similar mechanism were to apply to whole plants treated with 2, 6-dichloroisonicotinic acid, a known inducer of systemic acquired resistance, one can hypothesize that fungal pathogens might be recognised more readily and effectively.

The elicitor molecules that function *in vivo* for phytoalexin elicitation in soybean *(Glycine max)* infected with *Phytophthora megasperma f. sp. glycinea* have been identified as B-1, 6-and B-1, 3-linked glucans that are released from fungal cell walls by B-1, 3- endoglucanase (EC. 3. 2.1.39) contained in host tissue. Yoshikawa and Sugimoto (1993) identified the putative receptor like target sites for glucanase-released elicitor in soybean membranes. The binding was dependent on the pH of the incubation chamber, as well as on the duration and temperature of the incubation. The binding of the glucanase released elicitor to membranes was abolished by both heat and proteolytic enzymes. Therefore, the binding site was probably composed of proteinaceous molecules.

Resistance or virulence are modelled by multiple biochemical components of two living organisms. *Costus speciosus* a major sapogenin bearing medicinal plant was severely affected by *Drechslera rostrata* causing leaf blight disease. An interesting interaction phenomenon was noticed by Kumar et al., (1995). The HPLC analysis indicated the accumulation of glyceollin II and III as potent phytoalexins by *C. speciosus* in response of nonpathogenic *D. longirostrata.* Further the presence of a polysaccharide

elicitor, or mycelial wall component seems to be detrimental cause of phytoalexin accumulation. The same elicitor was also present in mycelial wall of pathogenic *D. rostrata* but in much lower concentration. Additionally it was associated with another polysaccharide component with different identity. The bioassay method of elicitor preparation was expressed in terms of antimicrobial activity mediated through glyceollins. It was determined to be 88.6% in incompatible which was considerably low (13.7%) in pathogenic reaction. During the pathogenesis of *D. rostrata* the susceptibility was not only exercised with low concentration of elicitor but also being mediated with the association of additional carbohydrate component of mycelial wall hence expressing the involvement of multiple biochemical components to regulate susceptibility.

The nonspecific elicitors (which include proteins, glycoproteins, various types of oligosaccharides, and unsaturated fatty acids) are more difficult to assign a role in the induction of phytoalexin production by pathogens (Hahn,1996). A race specific elicitor has been isolated from *Uromyces vigna*. This elicitor can induce phytoalexin production in cowpea resistant to this race of the pathogen based on hypersensitive response (HR)-like symptoms induced by treatment of resistant cowpea leaves with the elicitor (D'Silva and Heath, 1997). The presence of phenolic acids in cell walls- esterified p-coumaric acid and ferulic acids bound to cell wall polysaccharides are widespread in gramineae. Cell wall bound phenolics in resistance to rice blast disease was demonstrated by Kumar *et.al* (1997). The relative roles of glyceollin, lignin and the hypersensitive response (HR) in pathogen containment and restriction were investigated in soybean cultivars that were inoculated with *Phytophthora sojae*. Incompatible interactions in leaves and hypocotyls were characterized by HR, phenolic and lignin deposition and glyceollin accumulation. The uncoupling of glyceollin synthesis from the HR and phenolic and lignin deposition by ABA treatment showed that glyceollin is a major factor in restriction of the pathogen during these interactions (Mohr and Cahill, 2001).

Several comphrensive reviews pertaining to elicitors of phytoalexins have also been published (Albersheim and Anderson-Prouty, 1975; Callow, 1977; Keen and Bruegger, 1977; Purkayastha, 1986; Yoshikawa et al., 1993; Smith et al., 1995; Yoshikawa 1995; Paxton, 1995; Hahn, 1996;_Hammerschmidt, 1999; Grayer and Kokubun, 2001).

Serological cross reactivity between host and parasite

One of the most difficult and intriguing aspects in the study of biology is an understanding of the significant events of the interaction between plants and micro-organism at the cellular and subcellular level. The success or failure of infection is determined by dynamic competition and the final outcome is determined by the sum of favourable and unfavourable conditions for both pathogen and host cells. It is generally accepted that the cells recognize one another through pairs of complimentary structures on their surfaces. There is evidence that host-parasite compatibility is related to their antigenic similarly (Devay and Adler, 1976). The presence of cross-reactive antigens (CRA) between plant hosts and their parasites and the concept that these antigens might be involved in determining the degree of compatibility in such interactions have been demonstrated by several authors (Chakraborty, 1988; Purkayastha, 1994). Besides, recent trends in detection of plant pathogens include the development of more rapid diagnostic techniques with high specificity for the target organism. These techniques can be used to detect fungi , bacteria, and viruses present in low amounts in and on plant tissue and, therefore, in many cases the pathogen can be detected at an earlier stage of disease development than was previously possible. Some of these rapid, sensitive techniques are enzyme linked immunosorbent assay (ELISA), immunofluorescence (IF) and the polymerase chain reaction (PCR). Although most plant pathogenic fungi can be detected by microscopy or other conventional means, serological techniques have advantage where (a) the fungus in question is not readily identified by morphological characteristics, (b) species identification is important and difficult by conventional means, (c) detection of root pathogens prior to development of foliar symptoms is necessary, (d) large numbers of samples must be processed for a particular disease for which conventional methods are time-consuming, (e) rapid, on-site detection is necessary for making disease management decisions for high-value crops, (f) regulations governing the use of pesticides require demonstration of the presence of a particular pathogen, (g) the fungus causes disease at low, difficult-to-detect populations in plant tissue, or (h) plant material is subject to quarantine regulation.

Immunological techniques used for detection of fungal pathogens in soil, water and plant tissues and immunoassays for disease diagnosis have been reviewed by Hansen and Wick (1993) and Werres and Steffens (1994).

Root antigen from four cotton varieties and isolates of *Fusarium* and *Verticillium* species exhibited common antigen relationship in immunodiffusion test. Five to eight precipitin bands were observed in the homologous reaction, of these only one or two bands were common in heterologous reactions. The common antigenic determinant shared by cotton and fungal isolates did not appear to be related to the severity of wilt symptoms, but it may have affected host-pathogen compatibility during the process of root infection (Charudattan and DeVay,1972). Using fluorescein isothiocyanate (FITC), DeVay et al., (1981) demonstrated indirect immunofluorescence in cross-sections of cotton roots. CRA was concentrated mainly on epidermal cells, cortical tissue, endodermis and around xylem elements. Treatment of conidia and mycelia of *Fusarium oxysporum* with antiserum of cotton followed by labelling with FITC indicated that CRA was mainly present in hyphal tips and in patch-like areas on conidia.

Rabbit antisera were raised against the antigens of *Macrophomina phaseolina* (isolate MPI) and roots of soybean cultivars viz. Soymax and UPSM-19, susceptible and resistant to charcoal rot disease respectively. These antisera were used in immunodiffusion and immuno-electrophoretic tests for the presence of common antigens between isolates of *M phaseolina* and soybean cultivars. Four antigenic substances were found common between the susceptible soybean cultivars and isolates of *M phaseolina* but no common antigens were detected between resistant cultivars and the fungus (Chakraborty and Purkayastha, 1983). CRA were also detected between *Phytophthora infestans* and potato cultivars (King Edward and Pentland Dell) using ELISA (Alba and Devay, 1985).

Immunodiffusion, immuno-electrophoretic and crossed immunoelectrophoretic analysis of rice antigens using polyclonal antisera raised against *Acrocylindrium oryzae* was done by Purkayastha and Ghoshal (1985). When the antigen preparation of *A. oryzae* was cross-reacted with its own antiserum or against the antisera of four susceptible rice

cultivars, one precipitin band was detected. However, no precipitin band was detected when antiserum of the resistant cultivar was cross reacted with antigen preparations of three isolates of *A oryzae.* Purkayastha and Ghoshal (1987) also compared the antigenic preparations from two isolates of *Macrophomina phaseolina* (causal agent of root rot of groundnut), four non-pathogens of groundnut (viz. *Corticium sasakii*, *Colletotrichum lindemuthianum, C corchori* and *Botrytis alii*), and five cultivars of groundnut using immunodiffusion, immunoelectrophoresis and crossed immunoelectrophoresis in order to detect CRA. Common antigens were found among the susceptible cultivars of groundnut and two isolates of *M.phaseolina* but not between nonpathogenic and groundnut cultivars. No antigenic similarity was found between nonpathogenic and *M. phaseolina* isolates.

Changes in the antigenic patterns after induction of resistance by sodium azide in susceptible soybean cultivar (Soymax) to *Macrophomina phaseolina,* was demonstrated by Chakraborty and Purkayastha (1987). Similar results were also obtained by Ghosh and Purkayastha (1987) in susceptible rice cultivar (Jaya) and *Sarocladium oryzae* after altering disease reaction by the application of gibberellic acid and sodium azide. Purkayastha and Banerjee (1990) used six antibiotics as foliar spray on a susceptible soybean cultivar (Soymax) to induce resistance against anthracnose. In addition, common antigenic relationship between seven soybean cultivars, their pathogens and non-pathogen were also studied using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Among the six antibiotics tested, cloxacillin and penicillin induced maximum resistance against anthracnose, and altered the antigenic pattern of treated leaves. They detected CRA between susceptible soybean cultivars and the virulent strain of *Colletotrichum dematium* var *truncata* but no CRA were detected between soybean cultivars and avirulent pathogen (*C dematium*) or non-pathogen (*C .corchori*)

Rabbit antisera were raised against three strains of *Myrothecium roridum* (M-1, ITCC 1143, ITCC-1409) two susceptible cultivars (DS-74-24-2 and PK-327) and one resistant cultivars (UPSM-19) of soybean for analysis of CRA shared between host and parasite. Results of immunodiffusion revealed that common antigens were present only between the virulent strain and susceptible host- cultivars. But no CRA was detceted in case of resistant cultivars (UPSM-19 and DS-73-16). Immunoelectrophoretic analysis

showed that one common antigen was shared by susceptible hosts and the virulent strains (Ghosh and Purkayastha, 1990).

Serological relationship between *Sclerotium rolfsii* and groundnut cultivars were studied by Purkayastha and Pradhan (1994). Among three strains of *S. rolfsii* (266, 23, M) 266 was most virulent and exhibited antigenic relationship with susceptible cultivars (Gangapuri, J-11 and AK-12-24) of groundunt. The strain 23 also exhibited common antigenic relationship with CV AK-12-24. Resistant cultivar JL-24 and ICGS-26, however, showed no antigenic relationship with fungal strains. A systemic fungicide kitazin EC 48% (500ug/ml) altered the antigenic pattern of roots of a susceptible cultivar AK-12-24 and also reduced disease markedly.

CRA among susceptible tea varieties (TV-17 & 18) and isolates of *Bipolaris carbonum* (BC-1, 2, 3, and 4) were detected by Chakraborty and Saha (1994). Such antigens were not detected between isolates of *B. carbonum* and resistant varieties (TV-16, 25 and 26), non pathogens and tea varieties, as well as non-pathogens and isolates of *B. carbonum.* Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of tea leaves (TV-18), the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and conidia of *B. carbonum* with antisera to leaves of (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia.

Polyclonal antisera were also raised against mycelial suspension of *P theae* (isolate-pt-2) causal agent of grey blight disease and leaf antigens of Teen-Ali-17/1/54 and CP-1 and immunological tests were performed in order to detect CRA shared by the host and parasite. CRA were found among the susceptible varieties and isolates of *P. theae* (pt-1, 2 and 3). Such antigens were not detected between isolates of *P theae* and resistant varieties, *B tetramera* and tea varieties or isolates of *P theae.* Indirect staining of antibodies using FITC also indicated the presence of CRA in the epidermal cells and mesophyll tissue of tea leaves. CRA was evident in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae* (Chakraborty *et, al.*1995).

Another serological experiment was performed by Chakraborty *et. al.*, (1996) by raising polyclonal antisera against leaf antigens to tea varieties (TV-18, Teen Ali 17/1/54 and CP-1) and mycelial antigens of *G. cingulata* (isolate GC-1) separately in white rabbits. CRA were found among the susceptible varieties and *G. cingulata* isolates. Such

antigens were not detected between *G cingulata* and resistant varieties of tea, non pathogens and tea varieties as well as *G cingulata* and non-pathogens. In cross section of tea leaves (TV-18), the CRA was found to be concentrated in epidermal cells, mesophyll tissue and vascular elements.

Cross reactive antigens shared by *Fusarium oxysporum* and *Glycine max* were also detected using indirect immunofluorescence test by Chakraborty et al., (1997). For this, polyclonal antisera were raised against the mycelial suspension of *F. oxysporum* and root antigen of the susceptible soybean cultivar (UPSM-19). The immunoglobulin (IgG) fraction of those antisera were purified by ammonium sulfate precipitation and DEAE-Sephadex column chromatography. Antigens of susceptible cultivars showed higher absorbance values than resistant cultivars when tested against the purified anti *F. oxysporum* antiserum. Indirect fluorescence tests using FITC indicated that in cross-sections of roots of susceptible cultivars (UPSM-19) CRA were concentrated around xylem elements, endodermis and epidermal cells while in resistant varieties fluorescence was concentrated around epidermal cells.

Immunodetection of teliospores of *Telletia indica,* causal agent of Karnal bunt(KB) of wheat using fluorescent staining test were done by Gupta *et. al.* (2000). Polyclonal antibodies were raised against teliospores in Newzealand white rabbits. The indirect immunofluorescence (IIF) test was developed using anti-teliospores serum and binding was monitored by goat-rabbit antibody conjugated to FITC. The standarization of IIF test was carried out by optimization of dilutions of anti-teliospores antibodies, fluorescent probe and exposure time. The teliospores of *T. indica* showed bright green, patchy and ring shaped fluorescence around the teliospore. The spore exhibited uniform distribution in discrete regions of spore pobably in spore episporium. Similar fluorescence pattern in the teliospores of KB isolated from infected wheat seeds of cultivars HD 23328,UP 2338, PBW 393, WH 542, as well as RR 21 (susceptible cultivars) respectively, is an indication of the presence of similar antigenic configuration of teliospores. Again, they did not exhibit varition in the expression of teliospore associated molecular pattern duing previous and subsequent years of infection.

Polyclonal antiserum raised against *T. indica* also reacted strongly in agglutination reaction with intact teliospores of pantnagar isolate. The wheat grains with different grades of infection could be readily detected by Seed Immunoblot Binding Assay

(SIBA). The teliospores of Karnal bunt ine\fected wheat seeds when kept for vigour testing on nitrocellulose paper, formed a coloured imprint after the paper was assayed. The SIBA developed should not only be a better indication of teliospores load on seed but also quality of seed in terms of vigour. The developed immuno detection method appearantly proves to be useful in routine monitoring of wheat lots for the presence of Karnal bunt pathogen (Kumar *et.al.*, 2000)

Enzyme linked immunosorbent assay using PAb raised against *Colletotrichum falcatum* was performed in order to detect pathogen well before the symptom development. When 20 different sugarcane varieties were subjected to ELISA test after pathogen inoculation, it showed a clear variation in disease resistance among them as in field testing. (Viswanathan *et.al* , 2000).

Immunological detection of *Sphaerostilbe repens*, *Trichoderma viride* and *Trichoderma harzianum* using DAC-ELISA formats have been demonstrated by Chakraborty *et.al* (2000) in order to develop strategies for management of violet root rot of tea. Polyclonal antibody based immunoassay for detecting *Fomes lamaoensis* , causing brown root rot disease of tea has also been developed (Chakraborty *et.al* 2001a). Eight blood samples were collected and IgG were purified using DEAE cellulose. Immunodiffusion tests were performed in order to check the effectiveness of mycelal antigen preparations of *F.lamaoensis* for raising PAbs. Optimization of PAbs were done using indirct ELISA. Increased activity of PAbs against *F.lamaoensis* could be noticed from second bleedings, which continued upto fourth bledding. Root antigens prepared from healthy and artificially inoculated (with *F.lamaoensis*) tea palnts (Teen Ali – 17/1/54, TV-18, TV-22, TV-26, TV-27, TV-28, TV-30, S-449, BSS-2) were analysed following DAC-ELISA format. Such format was also used to detect the pathogen in infested soil. Young mycelia of *F.lamaoensis* gave bright fluorescence in indirect immunofluorescence tests using PAbs and FITC-conjugates of goat specific for rabbit globulin. Such immunological assays developed for detection of *F.lamaoensis* in rhizosphere of tea plantation can enable disease prevention at an early stage.

Immunodiagnostic kits were developed for detection of *Ustulina zonata,* causing charcoal stump rot disease, in the soil and tea root tissues . PAbs were raised separately against mycelial and cell wall antigens prepared from 10-day-old culture of *U.zonata.* Optimization of PAbs were done using indirect ELISA. Two different ELISA formats

such as direct antigen coated (DAC) and double antibody sandwich (DAS) were tested to detect the pathogen in soil and artificially inoculated tea root tissues. Indirect immunofluorescence using PAbs and FITC-conjugates of goat specific for rabbit globulin were assessed for their potential to detect mycelia and spores in soil (Chakraborty et. al, 2001b)

Serological cross reactivity between *Glomerella cingulata* and *Camellia sinensis* were studied by Chakraborty *et.al* (2002 b). PAbs were raised against antigen preparations from mycelia and cell wall of *G.cingulata* (isolate Gc-1), causal agent of brown blight of tea, mycelia of *Fusarium oxysporum* (non pathogen of tea) and leaf antigens of TV-18 and CP-1. CRA were found among the susceptible varieties of tea and isolates of *G.cingulata* (Gc-1,2 and 3). Such antigens were not detected between resistant varieties of tea and isolates of *G.cingulata* (Gc-1,2 and 3); non-pathogen (*F.oxysporum*) and tea varieties; isolates of *G.cingulata* and *F.oxysporum* and between non-host (*Glycine max*, *Cicer arietinum* and *Camellia japonicum*) and *G.cingulata.* Antisera raised against cell wall preparations gave better recognition than that against mycelial preparations as observed in ELISA test with antigens of tea leaves of different ages.