Discussion

In nature plants survive in the face of attack by many microbial organisms that threaten their survival and attempt to use them as a food source by employing several layers of defense responses. In addition to specific defense responses based on so-called R-genes, against certain strains of a pathogen, plants have broad spectrum defense responses which are preformed, such as surface waxes, or that can be induced locally or systemically by biotic or abiotic agents in nature (Oostendorp *et.al*, 2001). Induced resistance in plants has been the subject of considerable research over the past two decades with the discovery that many pathogens or chemical compounds may be used to elicit host defense mechanisms leading to reduced pathogen attack. Pathogen–produced elicitors are considered to be the primary signals responsible for the induction of plant defense reactions. Chitin, the main wall component of many filamentous fungi, and chitosan, the deacetylated derivative of chitin, have been shown to be potential elicitors of several plant defense responses including lignification. Seedlings raised from pearl millet seeds (HB3) which were soaked in 0.4% chitosan for 12h showed resistance against downy mildew disease (Shivkumar, 2000).

In the present investigation, a series of experiments have been performed using twelve chemicals belonging to three separate groups, viz. eight metal salts(cupric chloride, ferric chloride, lithium sulphate, manganese sulphate, sodium molybdate, magnesium sulphate, zinc chloride and barium sulphate), three growth regulators (IAA, 2,4-D and 2,4,5-T) and one biological compound, chitosan with a view to alter disease reaction against *Sclerotium rolfsii* in susceptible soybean variety (Macs-58). Apart from this, the effect of different concentrations of the above chemicals on sclerotial germination of *S.rolfsii* was also studied. Among the tested chemicals cupric chloride and ferric chloride were found to be highly effective in reducing disease intensity. The use of nickel chloride as foliar spray to tea plants for the control of blister blight caused by *Exobasidium vexans* was demonstrated by Venkataram (1961). Host sensitization as a factor in induction of resistance in rice

(cv. Dharial) against *Drechslera oryzae* by seed treatment with phytoalexin inducers have also been reported by Sinha and Hait (1982). When rice seedlings were treated separately with sodium selenite, lithium sulphate, cycloheximide and thioglycolic acid and challenge inoculated with *D.oryzae*, disease symptoms reduced markedly.

Many plant pathogenic fungi are known to produce a range of cell wall degrading enzymes that macerate plant cell wall, including pectolytic enzymes such as pectin methylestarase, polygalacturonase and pectate lyase that may have important roles in the infection process for the development of disease symptoms (Bateman and Basham, 1976; Walton,1994;Chilosi and Magro, 1998). Pectolytic enzymes are often produced in culture and during plant infection sequentially as multiple isoenzymes and may constitute a catabolic pathway for the complete degradation of pectic polysaccharides, the process being initiated by constitutive forms (Chilosi and Magro, 1997). The importance of such enzymes in pathogenicity is supported by the ability of purified enzymes to reproduce disease symptoms (Bateman, 1968; Barash et.al., 1984; Holtz and Knox-Davies, 1985) and by the correlation of the pectolytic enzyme level with the extent of damage to the plant (Olsson, 1989; Wijesundera *et.al.,* 1989; Cleveland and Cotty, 1991; Bayen *et.al.,* 1997). Moreover there are evidences of involvement of pectolytic enzymes in pathogenic mutants (Wattad et.al., 1995), antibodies blocking enzymes (Wattad et.al., 1994), chemicals like tannic acid and rufianic acid (Martin and Grossman,1972).

Significant increases in calcium and magnesium levels in the infected tissue imply induced polygalacturonase activity. Bateman and Miller (1966) reported such changes in resistant bean plants, when infected with *Rhizoctonia solani* as a defence response. In this opinion such accumulation leads to transformation of enzyme-sensitive cell wall pectic components at and around the lesion site to less soluble, enzyme tolerant calcium and magnesium pectates and this acts as dynamic resistant factor. Similar report is also available for *Rhizoctonia solani* infection of groundnut (Reddy *et.al.*,1988). Calcium may further help in host defense by promoting biosynthesis of callose, a cell wall component (Kauss, 1987) and phytoalexin (Kurosaki *et.al*, 1987), both known to be components of plants dynamic defense.

Calcium did not, however, any such effect on *Fusarium solani* infection of bean (Kendra and Hadwiger, 1987) and pod rot of ground nut due to *Pythium myriotylum* and *Rhizoctonia solani* (Filonow *et.al,* 1988).

Lignification has been observed in many plant species following attempted infection by pathogenic organisms such as fungi, bacteria, viruses and nematodes. There is strong evidence that lignification is an important mechanism for disease resistance (Carver et.al., 1994; Mauchmani and Slusarenko, 1996). In the same way that lignified tissue in the host plant such as endodermis, sclerenchymatous fibres etc. seem to prevent further ingress by certain fungal pathogens (Bishop and Cooper, 1983), induced lignin deposition at or around the site of penetration would also be expected to limit the pathogen by providing a physical barrier to mechanical penetration through cell wall. Lignification of cell walls bordering lesions may play an important role in limiting lesion expansion (Stockwell and Hanchey, 1987). Ride (1980) reported that lignified papillae and haloes from wheat leaves inoculated with *Botrytis cinerea* are highly resistant to degradation *in vitro* by various fungal species. The resistance is also supported by experiments testing the release of carbohydrate by fungal culture filtrates from lignified walls. Lignified cell wall could also constitute a barrier preventing free nutrient movement and therefore help to starve a pathogen. Lignin precursors themselves might exert a toxic effect on pathogens or, by binding two fungal cell walls, make them more rigid and an impermeable, thus hindering further growth or uptake of waterband nutrients. Coniferyl alcohol, for example, is highly toxic *in vitro* for fungi at low concentrations (Hammerschmidt and Kuc, 1982). Increase levels of lignins were reported in various crop plants such as muskmelon infected with *Colletotrichum lagenarium* (Touze and Rossignol,1977), potato infected with *Phytophthora infestans* (Friend, 1981), cucumber against *Spherotheca fuliginea* (Bashan and Cohen, 1983) and *Cladosporium cucumerinum* (Hammerschmidt, 1984). Involvement of lignin and callose during systemic acquired resistance in pearl millet against downy mildew pathogen *Sclerospora graminicola* has been demonstrated by Satyan (2000). Deposition of lignin and callose were found in significant amounts in resistant and

induced resistant than in the suscepible pearl millet cultivar. It was also observed that in resistant cultivar, lignification and deposition of callose occurred earlier than the susceptible cultivar. At the region of papilla formation, pathogen development was completely arrested.

The physiological/biochemical basis of resistance of plants to fungal pathogens has been associated with both preformed and infection induced antimicrobial compounds (VanEtten *et.al.*, 1994). However, the expression of resistance (i.e. defense) in most plant-pathogen interactions cannot be explained by the presence of preformed inhibitors. Most research on resistance mechanisms has shown that the plant uses defenses that are activated after infection to stop pathogen development. Many biochemical changes occur in plants after infection, and some of these have been associated with the expression of defense because they have activity against pathogens *in vitro*. Matern and Kneusel (1988) proposed rapid synthesis of phenolics following infection to be an important first line defense in plants.

Changes in host physiology following infection or induced resistance is often associated with an activation of oxidase activity and a post infectional increase in the level of such enzyme is a common phenomenon in diseased tissue, more so in an incompatible interaction. The activation of polyphenol oxidase would seem to be important in that it can oxidise phenolics to quinones which may be more fungitoxic. The infected resistant tisssue shows in many cases a higher oxidase activity than the infected susceptible tissue as also the healthy one. Such observations have led to the speculation that stimulated polyphenoloxidase activity possibly contribute to the resistance of plant against the pathogen. Various observation on the role of polyphenoloxidase in host resistance have been summarised by Sinha (1989).Greater increase in polyphenoloxidase activity in resistant than in susceptible plant was reported for soybean infected with *Phytophthora megasperma* f.sp. *glycinea* (Lazarovits and Ward, 1982). Hait and Sinha (1987) observed that seed treatment with cystein and sodium salanite protected rice from brown spot disease was associated with greater polyphenol oxidase activity in treated plants which responded

to inoculation with *Helminthosporium oryzae* with more pronounced increases in response to inoculation. Protection of bean plants from chocolate spot disease treating with wyerone was correlated with increased level of polyphenoloxidase and peroxidase activity (Tarrad *et.al.*, 1993).The effect of two resistance inducing chemicals, viz., sodium selenite (10^{-5}M) and zinc sulphate (10^{-4}M) on phenylalanine ammonialyase (PAL), peroxidase (PO) and polyphenol oxidase(PPO) activities in rice plant infected with *Rhizoctonia solani* was studied (Bhattacharyya and Roy,2000).

In the present investigation, it has been clearly established that wet seed treatment with some metal salts can induce strong protective effects in soybean plants against *Sclerotium rolfsii* infection. Such induced effects are mostly correlated with an increased biosynthesis of phenolics and stimulated oxidase activity at and around the site of host-pathogen interaction. It is well known that PAL is the first enzyme of the phenyl propanoid pathway and considered as the key enzyme in the regulation of the flux of the phenyl propanoid compounds such as lignin and their derivatives and also appeared to be associated with hypersensitive reaction. In the present study, PAL, PO and PPO activities were more in infected plants than in healthy plants, and the treated soybean plants had a higher activity in comparison to susceptible check. Though all of the treatments recorded higher post infection levels of PO activity, the three more effective treatments recorded much higher levels than the lesser effective treatments like lithium sulphate and sodium molybdate. Increased PO activity is presumed to be associated with enhanced lignification, an important mechanism for limiting the spread of the pathogen. Though a decreased trend of PAL activity was recorded as time progressed, the treated plants showed significantly higher activity even after seven days of inoculation.The plants treated with cupric chloride, the most effective treatment, elicited the maximum increase in PAL activity following inoculation and also led to the highest post-infection level. There is a strong correlation between activation of PAL and the production of both lignin and a range of phenolic compounds in resistant interactions of soybean plants with *Phytophthora* species (Mohr and Cahill, 2001).

One type of biochemical response that is strongly associated with defense is the accumulation of phytoalexins (Daniel and Purkayastha, 1995). In the present investigation, at the onset, pathogenicity test of *Sclerotium rolfsii* was carried out on six soybean varieties. Among the tested varieties, Macs-58 was found to be highly susceptible while PK-262 and Bragg were moderately susceptible. As soybean varieties showed differential responses towards *S.rolfsii*, it was considered worthwhile to detect the level of phytoalexin (glyceollin) accumulation in those varieties in response to fungal infection. It appears from the experimental results that PK-262 and Bragg contained more glyceollin (377-392 μg/g fresh wt. tissue) than Macs-58 (189 μg/g fresh wt. tissue).

Disease resistance of several crop plants have been correlated with the rate of production of phytolaexin (Hammerschmidt, 1999). The majority of studies of resistance and susceptibility in soybeans to *Phytophthora megasperma* f.sp. *glycinea* have dealt with infection of root and hypocotyl region. This system has attracted attention because it has provided a model system for study of the production and role of a phytoalexin (glyceollin) in a gene-for-gene system (Bhattacharyya and Ward,1987). In another study it has been confirmed that resistant and susceptible responses can also be differentiated in leaves. However, responses of leaves were found to be greatly influenced by age and stage of development. All leaves became resistant as they aged, and very young unfolding trifoliate leaves were susceptible even in resistant cultivars (Ward, 1989). Evidence that glyceollin, the pterocarpan phytoalexin from soybean, occur in four isomeric forms (glyceollin I-IV) was established by Burden and Bailey (1982). Of these, glyceollin IV has been isolated in minor amounts only, from cotyledons treated with $CuCl_2$ and no evidence that it play a significant role in the resistance response has been provided. Glyceollin I-III are all inhibitory to mycelial growth and zoospore germination of *P. megasperma* f.sp.*glycinea* and have been demonstrated to accumulate in significant amount in soybean tissues (Bhattacharyya and Ward, 1985). The proportions of the three isomers reported by different authors have varied considerably. Glyceollin-I predominated in roots treated with $CuSO_4$ or hypocotyl treated with $AgNO_3$. In cotyledons, glyceollin I and III have been reported to occur in roughly equal amounts

following treatment with $CuCl_2$. In leaves infiltrated with sodium iodoacetate, glyceollin-III was reported to be the main constituent. Although these reports suggest that there are major differences in the ability of soybean organs to synthesize three isomers, the possibility remains that the differences result from a combination of different experimental conditions and the use of different eliciting agents. None of these studies has examined the relationship of the accumulation of three isomers to resistance and susceptibility in the host-pathogen interaction.

The aforesaid statements indicate that phytoalexin has a role in disease resistance in plants. The differential response of soybean varieties towards *Sclerotium rolfsii* could probably be attributed to their capacity to accumulate more glyceollin. Induced changes in disease reactions by the application of diverse group of non-conventinal chemicals capable of inducing phytoalexin production have been elucidated by several research workers (Sinha, 1984; Chakraborty and Purkayastha, 1987; Rouxel *et.al.*, 1989; Purkayastha, 1994). It was reported by Keen *et.al.*,(1981) that sodium iodoacetate acts as an abiotic elicitor of glyceollin in primary leaves of cv. Harosoy soybeans and that it is associated with the resistance expression. Copper sulphate, sodium nitrate and chloram-phenicol were found to be effective in inducing capsidiol production in *Capsicum annum* (Watson and Brooks, 1984). Accumulation of increased levels of glyceollin following treatment with sodium azide was reported by Chakraborty and Purkayastha (1987). Rouxel *et.al.*,(1989) reported the accumulation of phytoalexin in $CuCl_2$ and $AgNO_3$ treated leaves of *Brassica juncea.* It seemed highly interesting, therefore, to induce changes in disease reactions in susceptible soybean variety (Macs-58) by the application of selected non-conventional chemicals.

Reduction in disease symptoms in susceptible soybean variety (Macs58) after the treatment with $CuCl_2$ and/or $FeCl_2$ may be correlated with the higher accumulation of glyceollin in treated plants. Results revealed that treatment with $CuCl_2$ and $FeCl_2$ induced a high level of glyceollin (485µg/g fresh wt and 332µg/g fresh wt) after challenge inoculation with the pathogen (*S.rolfsii*) in comparison to the untreated inoculated plants(190µg/g fresh wt.). The results of the present study as well as all the above reports, therefore, point to the ability of certain chemicals to induce

protection, which in some cases, could be due to direct fungitoxic effects of these chemicals on the pathogen, or it could be due to the activation of certain metabolic process within the host cells leading to the production of greater amounts of antifungal compounds (phytoalexins).

The genetic information contained in nucleic acid is expressed in the cell via protein synthesis.Several proteins function as enzymes in the metabolic pathways which synthesize or break down cellular components. When plants are infected by pathogens, the protein in the penetrated plant cells are changed chemically and physically. Some enzymatic proteins are also produced in penetrated cells by pathogens themselves. Thus, qualitative and quantitative changes of proteins are related to both plants and pathogens (Uritani, 1971).

In the present investigation, it was noticed that protein content increased in the infected roots of susceptible cultivars (Macs-58, J-80). Increased protein level was also detected after infection of susceptible bean leaves by *Uromyces phaseoli.* Similar findings were reported by other workers (Tomiyama,1966; Daly,1972; Ouchi *et.al.*,1974).They suggested that protein configuration changes in the host may induced the host's accessibility to the pathogen which is related by susceptibility. The great accumulation of protein in susceptible host after infection could be attributed to the total proteins of both host and parasite. However , it is difficult to separate the relative contribution of host and parasite to the total protein content.Sometimes protein content of the host after inoculation remains unchanged but their isozymes pattern may change. Changes in protein patterns in barley leaves after inoculation *with Erysiphe graminis* f. sp. *hordei* could be detected by polyacrylamide gel electrophoresis (PAGE) study but there is no change in protein content of mildew infected barley leaves in comparison with healthy leaves (Johnson *et. al.*, 1976).

In the present study, protein patterns of healthy and *S.rolfsii* infected roots, collar region and leaves of susceptible soybean variety (Macs-58) as well as mycelia of *S.rolfsii* were evaluated by SDS-PAGE. Protein preparation from collar region of susceptible soybean variety (Macs-58) inoculated with *S. rolfsii* exhibited 2-3

additional protein bands, in relation to their healthy control. This is in conformity with the work of Uritani and Stahmann (1961) who reported that sweet potato infected by *Ceratocystis fimbriata* developed new proteins both in resistant and susceptible varieties. Five new isozyme bands such as acetyl esterase, acid phosphatase, malate dehydrogenase, succinate dehydrogenase and peroxidase were detected in the susceptible line of barley after inoculation with *Erysiphe graminis* f. sp. *hordei* (Sako and Stahmann,1972). Differential changes in soluble leaf protein of tomato after inoculation with virulent and avirulent races of *Cladosporium fulvum* were determined by Dewit and Bakker (1980).

The pesence of cross-reactive antigen (CRA) between plant host 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 workers (De Vay *et.al.*,1972; DeVay and Adler, 1976; Kalyansundaram, 1978; Chakraborty, 1988; Purkayastha, 1989; Purkayastha *et. al.*,1991). In the present study root antigens of 6 soybean varieties (Macs-58, PK-262, Bragg, NRC-7, Pusa-16, J-80) and 6 isolates of *S. rolfsii* were cross reacted seperately with antisera of *S.rolfsii.* Reciprocal cross reaction was also caried out with antisera of host (Macs-58). Antigens from two non-pathogens of soybean, viz. *Fomes lamaoensis, Sphaerostilbe repens* were also considered for serological comparisons. It is significant to note that in immunodiffusion test susceptible soybean varieties shared the common antigens with the different isolates of *S. rolfsii* tested. Antigens of non-pathogens failed to develop any precipitin band. Immunoelectrophoretic analysis with antigen and antisera preparation from soybean roots and *S. rolfsii* also substantiated the results of immunodiffusion tests.

Several earlier studies have also implicated the importance of common antigens in host-pathogen compatibility. Presence of CRA has been demonstrated in various host-parasite combinations such as flax and *Melamspora lini* (Doubly et.al.,1960), cotton and *Verticilium albo-atrum* (Charudattan and DeVay, 1972), cotton and *Fusarium oxysporum* f. Sp. *vasinfectum* (Charudattan and DeVay,1970; Kalyansundaram *et.al.*,1975), sweet potato and *Ceratocystis fimbriata* (DeVay

et.al.,1967), potato and *Phytophthora infestance* (Palmerley and Callow, 1978, Alba and DeVay, 1985), soybean and *Macrophomina phaseolina* (Chakraborty and Purkaystha, 1983), soybean and *Colletotrichum dematum* var. *truncata* (Purkayastha and Banerjee, 1986), soybean and *Myrothecium roridum* (Ghosh and Purkayastha, 1990), jute and *Colletotrichum corchori* (Bhattacharyya and Purkayastha, 1985). Coffee and *Hemilea vastatrix* (Alba et.al.,1983), ground nut and *Macrophomina phaseolina* (Purkayastha and Ghosal, 1987), Carrot and *Pythium violae* and *Pythium sulcatum* (Lyons and White, 1992), ground nut and *Sclerotium rolfsii* (Purkayastha and Pradhan, 1994), tea and *Bipolaris carbonum* (Chakraborty and Saha, 1994), tea and *Pestalotiopsis theae* (Chakraborty et.al., 1995), soybean and *Fusarium oxysporum* (Chakraborty et.al.,1997).

Present result also support the findings of previous workers. Purkayastha and his co-workers have examined various host-pathogen / non-pathogen combinations including cultivars of soybean, rice, jute, pegion pea, bean, groundnut to find out their serological relationship with some fungal pathogens as well as non-pathogens following agar-gel double diffusion and immunoelectrophoretic tests. More than 50% combinations exibited cross-reactive antigen (CRA) between host and pathogens. No such CRA could be detected between resistant host and their respective pathogens. However, at a very low concentration CRA was detected between resistant host (soybean) and *Colletotrichum dematium* following enzyme linked immunosorbent assay (Purkayastha and Banerjee, 1990).

Conventional serological techniques have sometimes failed to detect cross reactive antigens in some host-parasite interactions such as wheat and *Puccinia graminis* var. *tritici* (Johnson, 1962), alfalfa and *Corynebacterium insidosum* (Caroll *et.al.*,1972). The preparation and treatment of antigens are most important because most antigens are labile and easily denatured. The selection of test animals and amount of antigen for immunization purpose are also important since too much material may reduce antibody formation. Moreover, a number of factors such as age of plant tissue, culture of microbes and methods of extraction of antigen have profound influence on the yield of antigenic substance and this may account for the failure to detect common antigens as suggested by DeVay and Adler (1976). Alba

et.al.,(1983) showed that uredinispores of *Hemilea vastatrix* shared common antigenic determinent with coffee plant, in contrast to their previous conclusions made with the same host-parasite system (Alba *et.al.*, 1973). This agreement was attributed to the low concentrations of antigenic preparations used in the earlier investigation.

Enzyme linked immuno sorbent assay has proved to be one of the most sensitive serological technique in detecting CRA at very low concentration (Alba and DeVay, 1985; Mohan, 1988; Chakraborty and Saha, 1994).In the present study the presence of CRA among *S.rolfsii* and soybean cultivars was evident in indirect ELISA using antigen and antisera of host and parasite and goat antirabbit IgG conjugate. Indirect ELISA readily detected CRA in semipurified mycelial preparation at concentrations ranging from 10-25 µg protein/ml with antiserum dilution of 1/125 and 1/250. Antigenic preparation (10µg/ml) from *S.rolfsii* exhibited higher absorbance value at 405 nm with antiserum of susceptible soybean cultivar (Macs-58). Higher absorbance value was also noticed in the reciprocal cross-reactions involving antiserum of the *S.rolfsii* and antigenic preparation of cv.Macs-58 (40 µg/ml). Since the indirect ELISA test made under the same condition and with at least three replications of each combination, it appears that this observed antignic disparities as reflected in their OD values developed as a result of antigen antibody reaction have some significance in the basic compatibility of host (*Glycine max*) and pathogen (*S. rolfsii*).

CRA was also detected in crude preparations and purified preparations from mycelia of *Phytophthora infestans* (races 4 and 1,2,3,4,7) with antisera of potato cvs. King Edward and Pentland Dell in concentrations lower than 50 µg protein/ml (Alba and DeVay, 1985) using indirect ELISA. Antiserum raised against *Phytophthora fragariae* detected homologous soluble antigen at protein concentrations as low as 2 µg/ml (Mohan, 1988). Indirect ELISA could also readily detected CRA in semipurified mycelial preparation of *B. carbonum* (isolate BC-1) higher absorbance value was deteced than the reaction with antiserum of susceptible tea variety (TV-18)

with antigenic preparation from *B. carbonum* (isolate BC-1) higher absorbance value was detected than the reaction with resistant variety (TV-26) of tea (Chakraborty and Sinha, 1994). Based on these findings it can be assumed that indirect ELISA may serve as an important technique to detect cross-reactive antigens, to determine their properties and to investigate their properties and to investigate their possible role in host-parasite interactions, even in those interactions where conventional serological techniques have failed to detect (Johnson, 1962; Caroll *et.al.*, 1972).

The involvement of CRA in host-parasite compatibility has been discussed by several authors (DeVay *et.al.*, 1967; DeVay and Adler,1976; Chakraborty, 1988; Lyons and White, 1992) and is strongly supported by results of the present investigation. These results are also in conformity with those of previous workers (Palmerly and Callow,1978; Alba *et.al.*, 1983; Alba and DeVay, 1985) who suggested that not all CRA contribute towards host-parasite compatibility but rather that only certain key CRA are important.

Recent trends in detection of plant pathogenic fungi include the development of more rapid diagnostic techniques with high specificity for the target organisms. The technique can be used to detect fungi present in low amount in or on plant tissue and therefore, in many cases, the pathogen can be detected at an earlier stage of disease development than was previously possible. Results of both pathogenicity tests and cross reactivity tests between *S.rolfsii* and soybean varieties revealed the differential responses of the different varieties towards the pathogen. Following this, the ability of the antisera raised against mycelial antigens of *S.rolfsii* to detect infection in artificially inoculated soybean roots were tested using DAC-ELISA formats. The antisera could detect infections in all varieties irrespective of their susceptibility or resistance in other tests. A number of previous workers have also successfully detected pathogenic fungi within host tissue (Hansen and Wick, 1993).

The cellular location of the CRA is also important in determining the nature of host-pathogen interaction. In order to determine the cellular location of CRA, fluorescence studies were conducted with cross sections of soybean roots as well as

mycelia and sclerotia of *S.rolfsii*. Bright fluorescence were observed on the epidermal and cortical tissues of the soybean roots as well as on the young hyphal tips and the sclerotia. The major CRA shared by cotton and *F.oxysporum* f.sp.*vasinfectum* was determined by De Vay *et.al* (1981). Cellular location of major CRA shared between tea and foliar fungal pathogens have also been described [Chakraborty and Saha, 1994 ; Chakraborty *et.al.* 1995].

In the present investigation the changes in antigenic patterns were also detected in susceptible soybean variety (Macs-58) after chemical induction of resistance. One common antigenic substance was found to be missing from the uninoculated cupric chloride treated soybean roots. This change increased the antigenic disparity between treated roots and the parasite and consequently the resistance of soybean to *S.rolfsii* increased to a considerable extent. In this situation cupric chloride appeared to act as a "conditioner" of the plant cells which responded to infection by producing glyceollin at a greater rate than they would normally. Selection of parasite by host or vice versa may be controlled to some degree by fortuitous homologies of their genomes. Where there is a similarity for synthesis of cortical cell component, a compatible relationship would result, whereas lack of homology would either repress metabolic processes or trigger the formation of metabolites which are toxic to cells of both host and parasite. If CRA have a functional role, other than in recognition phenomena, it probably will be found in the infective process and be subject to the over-riding effects of substances such as phytoalexins or other inhibitory substances already present in host tissues or induced by parasitic microorganisms.

Research on chemically induced disease resistance with the commercially available activators, and a large number of studies with various biological systems and experimental agents has led to a dramatic increase in our knowledge about the various defense signaling pathways in plants. Of these pathways, the salicylic acid dependent SAR pathway seems to be the most robust to exploited for practical crop protection. With this knowledge and with the pathway mutant sets available in Arabidopsis, it will be much easier in the future to determine quickly whether novel

disease control chemicals with suspected inducing activity do in fact have primary targets in the plant. This will also help the optimal utilization of the complex interactions between the various signaling pathways for practical crop protection. The experience with the chemical plant activators available, so far, suggests that some basic inducible broad spectrum defense responses are preserved across the plant kingdom. Chemical activation of disease resistance in plants represent an additional option for growers to protect their crops from losses due to plant diseases.

A synthetic resistance activator must fit the same stringent set of criteria concerning environmental and toxicological safety and reliability under practical conditions and it must be commercially interesting for agrochemical producers, farmer and supplier. Integration into existing crop management schemes or development of new crop management programmes may be possible with this novel tool of induced plant defense.