REVIEW OF LITERATURE

Nature has provided plant with effective mechanism for disease resistance that have contributed to their survival under the selection pressure of evolution. In true sense disease resistance is the ability of a plant to prevent, restrict or retard disease development. On the other side virulence is the ability of a pathogen to overcome resistance. Both resistance and virulence are each the combined result of multiple biochemical component. Usually a plant respond to a pathogen by mobilizing a complex network of active defense, but very often pathogen penetrate plant defense system, established it, causing abnormality in the plant. Induction of resistance in suscept considered as one of the promising method of protecting plants against disease. Major strategies for plant disease control are based on the assumption that susceptibility is due to a lack of resistance gene and that disease can be controlled by incorporating gene for resistance or by triggering resistance in plant. Sophisticated technology is now being utilized to study the intricate mechanism of induced resistance.

Disease resistance of a plant is it's response to a pathogen by activating complex network of multicomponent defense mechanisms. The success of the plant to resist the attack of pathogen depends upon the coordination among different defense strategies and rapidity of their action. Usually plants defend themselves against pathogenic fungi by producing defense related compounds such as PR-protein, phytoalexin, phenolics, lignin and several other defense related enzymes, viz., phenylalanine ammonia lyase, peroxidase, polyphenol oxidase. A susceptible plant cannot mobilize its defense system properly. Two options are there to protect them to insert resistance gene in their genome or immunizing their own system like that of men and animals. A variety of chemicals are now known to induce systemic resistance in plant. There are also reports that plant extracts or microorganism can also induce plant defense system (Oostendorp, 2001). There are many excellent reviews in this area of induced resistance in plants (Kuc,1987; Sinha,1989; Purkayastha,1998; Hammerschmidt, 1999; Oostendorp *et.al.*,2001). Significant levels of success has been achieved by using chemicals of widely diverse nature without any direct toxic action against many plants diseases Apart from chemicals, physical agents such as X-ray,UV and biological agents (Sinha and Das, 1972, Chakraborty and Chakraborty, 1989) are also known to alter disease reaction. Numerous molecules have been implicated in mediating disease resistance. There is evidence that some products either of biotic or abiotic origin are capable of activating the host's defense reaction by accumulating secondary metabolites or "Stress" metabolites such as phytoalexin, in treated (physically or chemically) plants (Grayer, 2001). Several elicitors or phytoalexin synthesis also induce the expression of other host plant defense responses.

The present review summarizes the work on the induction of disease resistance in crop plants with a view to emphasize the importance of induced resistance as a component of integrated disease management.

Oku (1960) presented evidence indicating that resistance of rice plants to *Cochliobolus miyabeanus* could be broken down by treatment with reducing agents such as ascorbate or glutathione. The resistance of rice plants against hyphal penetration by *C. miyabeanus* could partially be attributed to fungal oxidation product, perhaps quinones, derived from host cells or membranes. The influence of gibberellic acid on the seedling blight of corn was noted by Wilcoxon and Sudia (1960). They observed that treatment of maize hybrid seed with 5, 10 and 20 ppm gibberellic acid enhanced the severity of seedling blight. Use of nickel chloride as foliar spray to tea plants (*Camellia sinensis*) for the control of blister blight caused by *Exobasidium vexans* was demonstrated by Venkataram (1961). Percentage shoot infection was lower in nickel chloride than in the cuprous oxide treatment. Hale *et al.,* (1962) reported that growth regulators (viz. Indole-3-acetic acid, napthalene acetic acid, 2,4-dichlorophenoxy acetic acid and maleic hydrazide) caused an increase in size and number of leaf spot/ plant on the susceptible inbred corn like K-44 and the resistant line K-41 when the plants were inoculated with *Helminthosporium carbonum.*

Severity of lesion development on the hypocotyls of red kidney bean increased by foliar applications of gibberellic acid when plants were grown in soil infested with *Rhizoctonia solani,* isolate Rh-5. However, when the plants were treated with gibberellic acid and grown in soil infested with two other pathogenic isolates of *R. solani*, severity of the disease was not affected. The increased virulence of Rh-5 was probably caused by root excretions resulting from the gibberellic acid treatments (Peterson *et al.,* 1963). It was speculated by Daly and Deverall (1963) that hormonal concentration in a leaf could be important in controlling the development of a pathogen. The initial establishment of the disease could be due to hormonal changes brought about by entry of pathogen. Foliar application of different concentration of IAA and GA to detached bean leaves had little effect on lesion production by *Botrytis fabae* and *Botrytis cinerea* and were ineffective in the spread of lesion by *B. cinerea* (Purkayastha and Deverall, 1965). The effect of maleic hydrazide (MH) on wheat and barley rust were studied by Joshi (1965). The solutions of maleic hydrazide were administered to wheat roots and barley seedlings at the time of emergence. Barley plants treated with 0.2% MH solution showed reduction in growth and higher susceptibility plants to *Puccinia hordii.*

Foliar or soil application of CCC [(2-chloroethyl) trimethyl ammonium chloride] reduced the infections of bean seedlings by *Sclerotium rolfsii* (Tahori *et al.*, 1965). But Crosier and Yountburg (1967) reported that CCC was ineffective against *Tilletia foetida* on winter wheat when used alone as foliar spray. Sinha and Wood (1967) have shown that IAA reduced wilt disease of tomato caused by *Verticillium alboatrum.* On the other hand, maleic hydrazide greatly retarded growth of the plant and made them susceptible. Cycocel and napthalene acetamide gave good control of disease over a range of concentrations when applied to the soil in which the plants were growing. Of the other growth regulating substances tested, 2, 4, 5-trichlorophenoxyacetic acid increased disease at some concentrations and reduced it at others.

Chalutz and Stahmann (1969) induced pisatin formation in carrot tissue by ethylene. However, production of pisatin in pea tissues in response to ethylene treatment was less than that induced by fungi. Carrot roots treated with IAA, 2,4-D and 2,4,5-T also elicited coumarin accumulation. In all cases production of isocumarin was related to the amount of ethylene produced by root tissue. Foliar spray with either GA_3 or CCC increased susceptibility of jute seedlings growing in *Macrophomina* infested soil. Maximum susceptibility observed when treated with GA_3 but minimum in case of CCC treated plants (Purkayastha *et al.*, 1972) under the influence of IAA and GA_3 some aspects of host parasite relationships were studied by Valken (1972). He reported that IAA increased the *Fusarium* wilt of tomato while the reverse result was obtained with GA_3.

Furrer and Staulfer (1972) demonstrated that by the application of cycocel in combination with nitrogen, yields of spring wheat was augmented lodging and eye spot caused by *Cercosporella herpotrichoides* was reduced. Artificially application of natural and synthetic chemicals could also induce disease resistance in plants. Sharma (1973) reported that application of DL-tryptophan and IAA induced resistance in some sorghum varieties to *Colletrotrichum graminicola.* Sad and Rashid (1973) recorded that 2-chloro ethane phosphonic acid controlled the chocolate spot disease of potato and induced the production of small size tubers and tuber crackie. On the other hand application of IAA did not influence disease but induced the production of large size tubers.

The gibberellins and tri-iodobenzoic acid decreased severity of charcoal rot disease of soybean under all experimental conditions (Oswald and Wyllie, 1973). The role of auxins in

leaf spot incidence in ragi was discussed by Vidyasekaran (1976). Young leaves of *Eleusine coracana* was resistant to blight disease caused by *Helminthosporium tetramera* while the older leaves were highly susceptible. The effect of foliar application of plant hormones on the development of anthracnose disease caused by *Colletotrichum corchorum* in two cultivars of jute (*Corchorus capsularis*) were studied by Purkayastha and Ray (1977) under identical conditions. Gibberellic acid and indole acetic acid increased disease susceptibility in both resistant and susceptible cultivars. These compounds stimulated mycelial growth of *C. corchorum* at a low concentrations. Inflorescence of grapevine sprayed with 10 ppm gibberellic acid significantly reduced *Botrytis* infection (Rivera and Mavrich, 1978). Mercuric acetate caused accumulation of rishitin and lubimin in potato tuber discs. Accumulations of these terpenoids was not directly correlated to the necrotic reaction (Cheema and Haard, 1978). When two cultivars of *P. vulgaris* showing different degrees of susceptibility were treated with $HgCl_2$, the yield of phytoalexin was similar in both the cultivars. However, the accumulation pattern differed when inoculated separately with 3 isolates of *Botrytis cinerea* differing in virulence (Cheema and Haard, 1978).

In the glass house, application of 2,4-D (40% butyl ester) and atrazine (72%) increased susceptibility of soybean to blight disease caused by *Sclerotium rolfsii.* Incidence was higher in plants with low or high sugar content, but lowest on those with normal sugar content. It was also noted that monosodium phosphate, zinc sulphate, 2,4-D and atrazine were mildly phytotoxic (Carlos, 1979).

A fungicide known as 2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid (WL 28325) has been found to activate the natural resistance of rice plants against blast disease caused by *Pyricularia oryzae.* The activity of WL-28325 is unique in that it does not itself stimulate phytoalexin production but rather increase the capacity of rice plants to synthesize more momilactones (rice phytoalexins) in response to fungal infection. The antifungal activity of rice phytoalexin may be basis for its disease production properties (Cartwright *et al.*, 1980). The effect of mercuric chloride on glyceollin synthesis or degradation of glyceollin was tested by Moesta and Grisebach (1980). They observed that $HgCl_2$ produced only a slightly effect on the biosynthetic activity but strongly inhibited glyceollin degradation.

The effect of foliar spray of bacitracin, chloramphenicol and GA on rhizosphere microflora of pea seedlings (*P. sativum* L.) infected with *V. dahliae* were studied. The antibiotics increased fungus and actinomycetes counts and reduced the bacterial populations in the rhizosphere. The GA reduced all three groups of micro-organisms while 100 ppm increased actinomycetes slightly. Foliar spray also affected the percentage occurrence of particular genera of fungi in the rhizosphere, for examples, *Trichoderma* spp. were stimulated by all treatments, the maximum being with 10 ppm GA. Foliar spray however, markedly reduced disease severity (Ramarao and Isacc, 1980).The effect of three growth substances 6-Furfuryl aminopurine (Kinetin), 6-Benzyl aminopurine (BAP) and gibberellic acid (GA_3) on the development of charcoal rot disease of soybean caused by *Macrophomina phaseolina* was studied by Chakraborty and Purkayastha (1981). Two foliar sprays with I or 10 ppm GA_3 at an interval of 3 days before inoculation of plants reduced the disease significantly. But the application of 10 ppm kinetin or BAP markedly augmented the disease.

Eight chemicals reported to induce phytoalexin in plants were used for wet seed treatment in an attempt to develop resistance in susceptible rice seedlings to *Drechslera oryzae,* the brown spot pathogen. While all produced appreciable effects, cysteine, thioglycollic acid, cycloheximide, sodium selenite, P-chloromercuribenzoate and lithium sulphate caused marked reduction in symptoms in rice seedlings when challenge inoculation at the age of 3-4 weeks. With sodium selenite and thioglycollic acid the induced effect persisted upto 8 weeks after sowing. A second treatment in the form of foliar spray with these chemicals caused sharp increases on protection, but these disappeared 2 weeks after treatment. Leaf diffusates from 2 week old seedlings in different treatment showed considerable fungitoxicity, which declined with seedling age and become practically non-existent by the end of fourth week. Inoculation with treated plants at this age resulted in moderate to marked toxicity in their diffusates. Seed treatment was found to be more effective than foliar spray treatment (Sinha and Hait, 1982). Accumulation of phytoalexin in excise cotyledons of *P. vulgaris* was detected when treated with 10^{-4}M abscisic acid or benzylaminopurine (BAP). In case of former, cotyledons were incubated both in light and in dark but in case of latter, they were kept under light only (Stoessel and Magnalato, 1983).

Gibberellic acid (GA_3) induced momilactone synthesis in treated inoculated (with *Acrocylindrium oryzae*) leaf sheaths and coleoptiles. Since GA_3 is a degraded diterpene it may act as a precursor of gibberellin mediated enzyme (associated with momilactone

biosynthesis) which may count for the elicitation of momilactone synthesis in rice plants (Ghosal and Purkayastha, 1984). Seed treatment of wheat with dilute concentration of nickel chloride and barium sulphate significantly induced resistance to *Drechslera sorokiniana* (Chakraborty and Sinha, 1984).

Twenty out of twenty four chemicals known to induce phytoalexin production in other plants when used as seed treatment, provided effective protection to 3-week-old susceptible wheat seedlings against inoculation with *Helminthosporium sativum*. The number of lesions was very significantly reduced by most of the treatments and there was evidence for inhibition of lesions expansion in a few. Studies with twelve of the more effective chemicals showed that the protection effect persisted at significant levels even in 5 week old plants that at this stage this inhibiting effect on lesion expression was more pronounced in most of the treatments. Different treatments led to the development of a more of a moderate to high level of fungitoxicity in young wheat seedlings which markedly declined with age of the plant and disappeared in 5 week old plants. When inoculated at the age of 3 or 5 weeks, plants receiving most of the treatments developed appreciably higher fungitoxicity than the untreated plants (Hait and Sinha, 1986).

Chakraborty and Purkayastha, (1987) studied the effect of six metabolic inhibitors on the development of charcol rot disease of soybean. The effect of sodium azide (100 μg/ml) was found to the most significant among the metabolic inhibitors tested, in reducing the disease symptom. The reduction in disease was evidence by minimum loss in weight of roots and minimum root rot index. The glyceollin content of soybean roots before and after disease reactions by sodium azide treatment was estimated and compared. The production of glyceollin was maximum when plants were treated with sodium azide induced glyceollin synthesis even in inoculated soybean plants.

The effect of foliar application of growth substances on the development of charcoal rot disease of soybean caused by *Macrophomina phaseolina* was tested by Chakraborty *et al.*, (1989). Among the eight growth substances (3-indole-aceticacid, 2,4-dichlorophenoxyacetic acid 2,3,5-tri-iodobenzoic acid, 2-napthoxyacetic acid, L-naphthalene acetic acid, gibbrellic acid, 6-furfuryl amino purine and 6-benzyl aminopurine) examined, gibberellic acid was most successful in reducing the disease severity, followed by 3-indole acetic acid and 2,3,5 tri

iodobenzoic acid. Low concentrations of these compounds stimulated while high concentrations inhibited the mycelial growth of *M. phaseolina in vitro*. Glyceollin contents of host roots before and after treatments with gibberellic acid (10mg/l.) were estimated; this compound significantly increased glyceollin production in infected roots.

Spray with $AgNO_3$ and $CuCl_2$ solution on the leaves of *Brassica juncea* and *B. napus* also caused accumulation of phytoalexin and the effect of cycloheximide suggested that its accumulation was associated with induced plant metabolism. Phytoalexin was detected in *B. juncea* and *B. napus*, 6h and 18h after challenge with $CuCl_2$ a non-specific elicitor. *B. juncea* always accumulated 4 to 10 times more phytoalexin than did *B. napus* (Rouxel *et al.*, 1989).

Purkayastha and Banerjee (1990) used six antibiotics as foliar spray on a susceptible soybean cultivar (soymax) to induce resistance against anthracnose of which cloxacillin and penicillin induced maximum resistance against anthracnose. Spraying the lower surface of the first true levels of cucumber plants with 50M K_2HPO_4 induced systemic resistance to anthracnose caused by *Colletotrichum lagenarium.*

Wet seed treatment with phytoalexin inducer chemicals and related compounds protected rice plants from the attack of both brown spot and blast diseases. Such compounds were effective at dilute concentrations, mostly non-hazardous and with little or no fungitoxic effect at the concentration employed. Many of the chemicals have equally strong effective against both diseases, some are more effective against one than against the other. Sarkar and Sinha (1991) concluded that such chemicals may be mostly acting through an induction of general host resistance and also provide in the process of broad spectrum action effective simultenously against a group of pathogens. Effectiveness of 19 non-conventional chemicals in wet seed treatment in controlling wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* was demonstrated by Mandal and Sinha (1992). While most of the compounds could reduce wilt symptoms appreciably, cupric chloride, ferric chloride. zinc chloride. manganese sulphate, mercuric sulphate, L-cysteine, IAA and DL-methionine showed very strong protective effect. These reduced leaf symptoms by 52 to 71% prevented mortality completely and also limited vascular colonization by the pathogen. Most of the test compounds showed little or no *in vitro* fungitoxicity at their effective concentrations and stronger protection was often achieved at lower than higher concentration. These non-

conventional chemicals act in plant disease control toxic action but by inducing resistance in susceptible tomato plants, mediated through host tissue conditioning.

The timing of changes in the protein synthesis pattern of elicitor treated, [^{35}S] methionine-labelled parsley cells *(Petroselinum crispum)* was analyzed by two-dimensional gel elecotrophoresis by Bollamnn *et.al.* (1990). Five groups were distinguished fromn a large number of elicitor responsive as well as unresponsive proteins. Two groups were synthesized de novo either early or late after elicitor application, two other groups were strongly reduced in their rates of synthesis either early or late after elicitor application; and one group was not appreciably affected at all. The elicitor induced changes altered the total protein composition considerbly. A few selected, induced proteins were functionally identified. These included two early induced enzymes, Phenylalanine ammonia-lyase (PAL) and 4-coumarate : CoA ligase (4CI), and a late induced enzyme, a bergaptol O- methyltransferase (BMT) which is specifically envolved in the biosynthesis of furanocoumarin phytoalexins. The biological significance of the observed differential timing of changes in protein synthesis rates discused.

Pathogen attacks and treatment of many plants species by various chemicals induce the synthesis of pathogensis-related (PR) proteins. These host encoded low molecular mass proteins have been well studied by Godiard *et. al.* (1990) in tobacco reacting hypersensitively to infection by TMV. A partial cDNA clone encoding for β-1, 3 glucanase was used as a probe to study the kinetcs of accumulation of the corresponding mRNAs in tobacco leaves infiltrated with water or with compatible (K60), incompatible (GMI-1000), and aviruent (GMI-1178) isolates of a phytopathogenic bacterium, *Pseudomonas solanacearum.* A nonspecific accumulation of these transcripts, independent of the nature of the inculum, was observed. Similar results were obtained with PCHN50, a chitinase encoding cDNA clone In addition, antibodies directed against several PR proteins were used to estimate the accumulation of these proteins in tobacco leaves infiltrated for 18hrs with the same *P. solanacearum* isolates or with water : no qualitative or quantitative.

Sarhan *et. al.* (1991) demonstrated the role of endogenously induced higher level of cytokinins and exogenously applied kinetin in relation to the development of barley leaf spot caused by *Bipolaris sarokinia Shoemaker (Syn. Helminthosparium sativum Pammel,* king and Backe) was studied. Spraying barley leaves with kinetin suppressed the no. and the size of necrotic spots caused by the fungus. Inoculation of the lower leaves of berley by a spare suspension of fungus *B. sarokiana* induced resistance on the upper leaves against an subsequent challenge inoculation by the same pathogen 10 days later. An increase in the level of cytokinins was observed in these resistant leaves. Elevated levels of cytokinins may cause

a type of juvenility in leaf tissues. The juvenile state could be in a causal relationship with the suppression of necrotic spots caused by the fungus.

A procedure to detect chitinase and β-1, 3 glucanase isozymes [implicated in plant defense against fungle pathogens] and protein patterns after a single separation using native polyacrylamide gel electrophoresis (PAGE) or isoelectro focusing (IFF) was described by Pan *et.al.* (1991) After electrophoresis or isoelectropfcusing, an overlay gel containing glycol chitin as substrate for chitinase was incubated in close contact with the resolving gel chitinase isozymes were revealed by UV illumination after staining the overly gel with fluorescent brightner 28. The resolving gel was then incubated with laminarin, and β -1, 3 glucanase isozymes were detected by using 2,3, 5 triphenyltatrazolium chloride. The resolving gel with β -1, 3-glucanase bands was stained with economic Brilliant Blue R. 250 to reveol protein patterns. The isozymes were quantified by using native PAGE, and their pls were estimated by IEF.

Davis *et.al* (1992) in their article 'Enhancement of phytolexin accumulation in cultured plant cells by Oxalate" stated that oxalate (0.2.2 0mM), a compound which induces systemic resistance, can enhance secondary product synthesis 10-fold in cotton *(Gossypium hirsutum)* cell suspension cultures. To accomplish this, cells require induction with a low level (10mu-g of protein per 15mu incubation) of elicitor from *Verticilluum dahliae.* In the presence of oxalte, these minimal concentrations of elicitor required to induce phytoalexin formation did not have any necrotic effect on the growth of the cotton cultures. Even at higher concentrations of elicitor (80mμ-g of protein per 15ml inculation), oxalate was able to reduce the detrimental effect of elicitor on cell suspension growth rates indicating oxalate may have a direct effect on growth. Therefore in cotton cell suspension cultures the addition of an enhancer of elicitor-induced phytoalexin formation allows optimum stimulation of secondary metabolite formation without affecting cell mass accumulation.

Lindgren *et.al* (1992) working on Molecular analysis of plant defense responses to plant pathogens stated that a number of inducible plant response are belived to contribute to disease resistance. These responses include the hypersensitive reaction, phytoalexin synthesis, and the production of chitinase, glucanase, and hydroxyproline-rich glycoprotems Because of the coordinate induction of these responses, it has been difficult to determine whether they are functional defense response, and if they are, how they specifically contribute to disease resistance, Recent developments in molecular biology have provided experimental techniques

that will reveal the specific contribution of each response to disease resistance. In this paper, we describe a strategy to determine if the hypersensitive reaction is a functional plant defense mechanaism.

Benhamau *et.al.* (1992) described that Chitosan, a polyme of beta-1, 4- D-glucosamine derived nom crab-shell chitin was applied to tomato plants prior to inoculation with the root pathogen, *Fusarium oxysporum* f. sp. *radicislycopersici.* Whether chitosan was applied by leaf spraying or root coating, it was found to markedly reduce the number of root lesions caused by the fungus, and to drastically increase the formation of putative physical barriers in infected root tissues. The effect of chitosan on the induction of host cell reaction was observed at concentrations ranging from 0.5 to 2 mg ml^{-1} with an optimal effect at 2 mg ml^{-1}. The enchanced protection of tomato roots to chitosan-induced resistance is systemic. Formation of wall oppositions such a papillae and occlusion of xylem vessels with either a network of bubble-like structures or a coating material were among the most typical features of host reactions. In addition, the accumulation of amorphous deposits, probably infused with phenolics from their electron-density, was observed in most intercellular spaces and some host cells. These deposits were often found to interfere with the walls of invading hyphae causing severe alterations. The application of wheat germ aggluinnin, a lectin with N-acetylglucosamine-bindng specificity, in conjunction with gold complexed ovomucoid, to issue sections showed that the walls of severely altered hyphal cells were labelled except in the area closely appressed to host cell walls. This sugests that extracellular chitinases accumulate in the host's cell walls but are not the primary determinants of fungal damage. The possibility that toxic compounds such as phenois and chitosan- induced phytoalexins may be responsible for the obseved damage of invading hyphae is discussed.

The effects of an elicitor (CG-elicitor) from *Colletotrichum graminicola* and the host-specific pathotoxin (PC-toxin) produced by *Periconia circinata* were studied by *Ransom et.al* (1992) to determine the interaction of responses associated with resistance and susceptibility, respectively. Roots of sorghum *(Sorghum bicolor)* accumulated 3-deoxyanthocyanidium phytoalexins in response to CG-elicitor but not in response to PC-toxin over range of concentration. Elicitation of the phytoalexins prior to treatment with PC- toxin had no effect on the genotype-specific induction of electorlyte leakage or on the toxin-enhanced synthesis of a specific group of 16 kDa proteins. Similarly, prior treatment with the elicitor did not prevent infection and development of milo disease symptoms in susceptible seedlings inoculated with condia of *P. circinata.* However, treatment of roots with the CG- elicotor

enhanced the synthesis of the 16 kDa proteins in both resistant and susceptible genotypes without expression of disease symptoms. Thus, the activities of PC-toxin and CG-elicitor are separable and independnt. PC-toxin apparently does not produce disease symptoms by inducing phytoalexins, and induction of phytoalexins does not prevent pathogenesis by *P. circinata* or the determental effects of PC-toxin.

Plants have evolved a broad array of defense mechanisms involved in disease resistance. These include synthesis of phytoalexin antibiotics and proteinase inhibitors, deposition of cell wall materials, and accumulation of hydrolytic enzymes such as chitinases. Resistance appears to depend on the ability of the host to recognize the pathogen rapidly and induce these defense responses in order to limit pathogen spread. Application of molecular technologies has yielded significant new information on mechanisms involved in pathogen recognition, signal transduction, and defense-related gene activation, and is leading to novel strategies for engineering enhanced disease resistance (*Cramer et.al* (1992) had used these approcahes to analyze regulation of 3-hydroxy-3 methyl/glutaryl CoA reductase (HMGR), a key enzyme mediating the production of terpenoid defense compounds. This enzyme is encoded by four genes in tomato, hmg2 gene expression is specifically associated with responses to pathogen or defense elicitors. Transgenic plants containing DNA constructs that fuse the hmg2 promoter to reporter gene have been used to analyze both tissue specificity and patterns of defense-related experssion. Because this gene is rapidly induced in tissues directly surrounding the site of ingress by a variety of pathogens, it may serve as a valuable tool in engineering new disease resistance mechanisms.

Biotic (beta-glucan , fructosan and polygalacturonic acid fragments) and abiotic agents (ultraviolet-C radiation, gammna radiation and heat) were screened by Mercier *et.al.* (1993) for their potential to elicit the accumulation of the phytoalexin 6-methoxymellein in carrot slices. Ultraviolet radiation was the only elicitor found effective, with an optimum does of 2.20 times 10-5 erg cm0-2. At 20 degree C, the maximum level was reached in 72 hours and thereafter degradation was apparent. At lower temperatures (1 degree or 4 degree C), 6-methoxymelein accumulation was slower but reached higher levels which remained stable for up to 35 days after induction. Ultraviolet-treated slices which were stored at 1 degree or 4 degree C for two weeks to allow 6- methoxymellein accumulation were significantly more resistant to infection when challengaed with *Botrytis cinerea* or The data showed that concentrations of 6-methoxymellein above 30 mμ-g g^1 of tissue were inhibitory to *B.*

cinerea, with maximum inhibition observed around 60 mg g^1. Thus ultraviolet, treatments could have potential to enhance the resistance of carrots to storage pathogens.

Induction of phytoxlexin formation in suspension-cultured rice cells by series of N-acetylchitooligosaccharides and chitooligosaccharides was studied by Yamada *et.al* (1993), N-acetylchitooligosaccharides larger than hexaose induced the formatin of a momilactones A and B as well as oryzalexins. A,B, and D at very low concentrations like 10-9-10-6 M (N-acetylchitoheptaose). GlcNAc deacetylated chitooligosaccharides were also inactive. Strict requirement for the size and structure of GlcNAc oligomers as well as the sensitivity to them strongly indicates the presence of recognition systems specific for these compounds in rice cells. The level of momilactone A produced reached 100-500 mμ-g/g of cultured cells, which appeared to be enough to prevent the growth of pathogenic fungi such a *Pyricularia oryzae,* thus indicating the importance of this phenomenon in the defense systems of rice plants. Suspension-cultured cells obtained only form a suitable period of cultivation, mainly those from lag phase, could respond to the elicitor and produce phytoalexins.

The interaction of vesicular arbucular mycorrhizal fungus *Glomus fasciculatum* with a wilt-causing soil borne pathogen, *Fusarium oxysporum,* was studied in cowpea *(Vigna unguiculata)* by sundaresan *et. al* (1993). It was found that pre-establishment by vesicular-arbusular mycorrhizal fungus reduced the colonization of the pathogen and the severity of the disease, as determined by reduction in vescular discolouration index. In mycorrhizal plants, the production of phytoalexin compounds was always higher than in the non-mycorrhizal plants. There appeared to be a direct correlation between the concentration of the phytoalexins and the degree of mycorrhizal association. Three diferent compounds with R-f values of 0.23 (I), 0.17 (II) and 0.11 (III) were obtained from mycorrhizal plants. Similar compounds were also found to be induced by an abiotic elicitor $CuSo_4$. The first compound was identified as an isoflavonoid, daidzein and the other two remain to be identified. These compounds were checked for their antifungal acitivity in vitro. The germination of conidial spores of *Fusarium oxysporum* was strongly inhibited by the compound III than the other two. It is argued that the production of phytoalexin compounds in mycorrhizal plant cound be one of the mechanisms imparting tolerance of the plants to wilt disease.

An isoflavanoid derivative has been isolated by Seifert *et.al.* (1993) as a phytoalexin, from intact plants and cell suspension cultures of *Ornithopus sativus* after treatment with $CuSO_4$, yeast elicitor, or a spore suspension of *Collectotrichum trifolii*. Based on its physical

and spectroscopic properties the substance was identified as glabridin. After inducation the transient increase in the content of the phytoalexin was preceded by a transient increase in the activities of PAL and CHS.

A glycoprotein elicitor form the phytopathogenic fungus *Verticillium albo-atrum* induced synthesis of the phytoalexin medicarpin in *M. sativa* cv. Kabul. Treatment of seedlings with the cell-permeating cyclic AMP analogue, dibutyryl cyclic AMP, stimulated phenylalanine ammonialyase activity and induced medicarpin synthesis. Lxposure of *M. sativa* cellsuspension cultures to the fungal elicitor resulted in a significant, transient increase in intracellular cyclic AMP content, together with a pulse of adenylyl cyclase activity, both within a few minutes of the elicitor treatment and a subsequent increase in cyclic AMP phosphodiesterase activity. Incomation of a membrane fraction from the *M. sativa* cells with the fungal elicitor resulted in a does-dependent stimulation of the adenylyl cyclase activity. These observations are discussed in the context of the signal transduction mechanism of the *M. sativa* cellular defence system Cook-J *et.al.* (1994).

Fusarium moniliforme [Gibberella fujikuroi] endo-polygalacturonase, and an elicitor prepration derived from the cell-walls of *Phytophthora megasperma,* induced the accumulation of the phytoalexin, 6-methoxymellein, and induced the activity of 6-hydroxymellein 0-methyltransferase (an enzyme involved in the synthesis of 6-methoxymellein), in carrot *[Daucus carota]* cell suspension cultures. These fungal elicitors also stimulated a marked increase of phenylalanine ammony-lyase activity without affecting chalcone synthase [naringenin-chalcone synthase] activity. A constitutive caffeic acid O-methyltransferase activity was identified in the same cultured carrot cells by Marinelli-F. *et. al.* (1994).

A differential response of pathogenesis-related proteins (PRPs) to penicillin and sodium malonate was detected when these 2 phytoalexin elicitors were tested by Bera. S. *et. Purkayastha,* R.P. 91994). for the induction of resistance against sheath blight disease in a susceptible rice cv. IET-2233. Foliar sprays with penicillin (100 ug/ml) or inoculation with sheath blight fungus *Rhizoctonia solani* f.sp. *sasakii* induced at least 5 similar types of protein (Ef 0.29, 0.39, 0.49 0.42, 0.77) in rice leaf sheaths after 24h of treatment or inoculation. These PRPs were not detected in untreated/non-inoculated leaf sheaths. Leaf sheaths treated with sodium malonate (100-4M) exhibited only 1 (Ef 0.49) common PRP of the 5 previously mentioned. Penicillin 100 ug/ml) and not sodium malonate significantly reduced sheath blight

disease of rice and induced a greater number of PRPs. Based on these results it is suggested that a phytoalexin elicitor which is capable of inducing more PRPs could be a potential inducer of resistance.

Systemic acquired resistance (SAR) is an important component of plant defence against pathogen infection. Accumulation of salicylic acid (SA) is required for the induction of SAR. However SA is apparently not transducing the the translocated signal but is involved in transducing the signal in target tissues. SA accumulation is not required for production and release of the systemic signal. In addition to playing a pivotal role in SAR signal transduction, SA is important in modulating plant susceptibility to pathogen infection and genetic resistance to disease. It has been proposed that SA inhibition of catalase results in H_2O_2 accumulation and that therefore H_2O_2 serves as a secondary messenger is SAR signalling. No accumulation of H_2O_2 in tissues expressing SAR was found and it was concluded that the role of H_2O_2 in SAR signalling is questionable (Ryals *et. al.* (1995).

Phytoalexin synthesis is a defence response that is characterized by a requirement for a number of distinct elements, all of which must be present for the response to be expressed fully. These same elements : a signal, a cellular receptor, a signal transduction system and a responsive metabolic system, are also used to describe a stimulus-response system. A number of molecular species can function as signal molecules or elicitors of phytoalexin synthesis, including poly-and oligosaccharides, proteins and polypeptides, and fatty acids. Few receptors for elicitors have been identified but those that have been are proteins located on the plasma membrane of the plant. According to Smith C-J (1996). Induction of phytoalexin synthesis involves selectiv and .

Treatment of *Allium cepa* L. cell-suspension cultures with a biotic elicitor derived from the fungus *Botrytis cinerea,* resulted in phytoalexin synthesis. Two phytoalexins, 5-octyl-cyclopenta-1, 3 dione and 5-hexyl-cyclopenta-1,3- dione, were accumulated in cultured onion cells, Removal of extracellular Ca^{2+} by the calcium chelator ethylene glycol bis)b-aminoethyl ether) N, N'-tetraacetic acid abolishd the elicitor-mediated phytoalexin synthesis. The calcium channesl blockers, verapamil and 8-N,N-(dimethylamino) octyl-3. 4. 5-trimethoxybenzoate caused similar effects. whereas the addition of the Ca^{2+} ionophore A23187 enhanced the accumulation of phytoalexins in the absence of the elicitor. Increase in the cytoplasmic Ca^{2+} concentration elicitor-treated onion cells was obsrved as monitored by the fluorescence calcium indicator indo-1. These observations of Dmitriev - Alexander *et. al.*

(1996) suggest that Ca^{2+} acts as a second messenger in the regulation of phytoalexin synthesis in cultured onion cells.

Systemic acquired resistance (SAR) is an inducible plant defence response in which a prior foliar pathogen infection activates resistance in non-infected foliar tissues. Salicylic acid (SA) accumulation is essential for the establishment of SAR. While SA is probably not the long-distance systemic signal in non-infected tissues. Although SAR was first described as a response to necrogenic pathogen infection, synthetic chemicals have been identified that effectively activate SAR. Elucidation of SAR signal transduction has been facilitated by the identification and characterization of *Arabidopsis* mutants. Disease lesion miniemutants exhibit constitutive SAR as well as spontaneous lesion formation similar to pathogen.

Systemic resistance induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression, a novel resistance mechanism were described by Pieterse *et.al* (1996).

Hoffland *et. al* (1996), analysed the spectrum of protection achieved by the plant growth-promoting rhizobacterium (PGPR) *P. fluorescens* strain WCS417-mediated induced resistance and to compare its effectiveness with pathogen-mediated systemic acquired resistance, which is associated with necrosis and induction of pathogenesis-related (PR) synthesis. It was demonstrated that pre-treatment with *P. fluorescens* protects radish through induction of systemic resistance not only against *Fusarium oxysporum* f. sp. *raphani,* but also against the avirulent bacterial leaf pathogen *P. syrinage* pv. tomato and the fungal leaf pathogens *Alternaria brassicicola* and *F. oxysporum.* It was demonstrated, for the first time, that one PGPR stram can induce resistance against multiple pathogens. The level of protection was at least as high as that achieved by the necrotizing, PR- inducing *P. syringae* pv. tomato, and the spectrum was even broader indicating that necrosis is not a prerequisite for effective, biological induction of resistance and that the absence of PR after induction by *P. fluorescens* does not lower the level of protection.

Gorlach *et. al* (1996) discussed that systemic acquired resistance is an important component of the disease resistance repertoire of plants. In this study, a novel synthetic chemical, benzo (1 2,3) thi-adiazole-7 carbothioic acid S-methylester (BTH), was shown to induce acquired resistance in. BTH protected wheat systemically against powdery mildew *(Erysiphe graminis* f. sp. tritici) infection by affecting multiple steps in the life cycle of the

pathogen. The onset of resistance was accompanied by the induction of a number of newly described wheat chemically induced (WCI) genes, including genes encoding a lipoxygenase and a sulfur-rich protein. With respect to both timing and effectiveness, a tight correlation existed between the onset of resistance and the induction of the WCI genes. Compared with other plant activators, such as 2,6 bichloroisonicotinic acid and salicylic and, BTH was the most potent inducer of both resistance and gene induction BTH is being developed commercially as a novel type of plant protection compound that works by inducing the plant's inherent disease resistance mechanisms.

Pathogen recognition at the plant cell surface typically results in the initiation of a multicomponent defense response. Transient influx of Ca^{2+} across the plasma membrane is postulated to be part of the signaling chain leading to pathogen resistance by Zimmermann *et. al* (1997). Patch - clamp analysis of parsely protoplasts revealed a novelCa^{2+} -permeable, La-3^{+}-sensitive plasma membraneion channel of large conductance (309 ps in 240 mM $CaCl_2$). At an extracellular Ca^{2+} -concentraion of 1mM, which is representative of the plant cell apoplast, unitary channel conductance was determined to be 80 pS. This ion channel (LEAC, for large conductance elicitor-activatd ion channel) is reversibly activated upon treatment of parsley protop asts with an oligopeptide elicitor derived from a cell wall protein of *Phytophthora sojae.* Structural features of the elicitor found previously to be essential for receptor binding, induction of defense-related gene expression, and phytoalexin formation are identical to those required for activation of LEAC. Thus, receptor-mediated stimulation of this channel appears to be causally involved in the signaling cascade triggering pathogen defense in parsley.

Plants respond to infection by accumulating many compounds some of which may function in disease resistance. These include : phytoalexins, antifungal proteins, chitinases, glucanases, esterases, protease, phospholipases, lipoxygenases, ribonucleases, peroxidases, phenoloxidases, lignin, callose, hydroxyproline and glycine-rich glycoproteins, phenolic cross-linked polysaccharides, melanin-like pigments, salicylic acid, jasmonic acid, ethylene, peptides, oligosaccharides, hydrogen peroxide and active oxygen species. Though specific avirulence genes, elicitors and elicitor receptors have been reported, the production of defense-related compounds is nonspecific and can be elicited by pathogens, pathogen products and many organics and inorganics. The molecular implications of this specificity/nonspecificity and their significance to disease resistance and practical disease control will be discussed.

The ways in which plants acquire resistance to various insect and pathogenic invaders through induced systemic acquired resistance (SAR) are reviewed. The mechanisms involved in SAR significantion and other structural barriers, pathogenesis-related proteins and conditioning) are discussed by Sticher *et. al.* (1997). Evidence is presented for the signalling function of salicylic acid, jasmonates, systemin, ethylene and electrical activity, as well as other organic, inorganic and synthetic compounds.

Davis *et. al.* (1997). analysed the cell cultures of slash pine *(Pinus elliottii* var. elliottii genotype 52-56) treated with the general elicitor chitosar to induce expression of the Pschi4 gene, which codes for the defence enzyme chitinase, whose mRNA accumulates after treatment of the pine cells with chitosan. The gene acts as a reporter gene for the induction of defence response. The study described here tested whether the pine chitinase gene still perceives chitosan after removal (as a 7 kb genomic fragment containing both the coding sequence for Pschi4 and flanking sequences) from the pine tissue and introduction into tobacco plants via the *Agrobacterium binary* vector pCIB10. Nine transgenic tobacco plants were randomly selected and tested for chitosan induced expression of Pschi4 using RNA gel blot analysis. Results showed that the pine gene was still transcribed in leaf sections from transgenic tobacco plants in response to chitosan treatment, thus supporting the hypothesis that aggressor preception mechanisms mediated through signal transduction pathway are similar in gymnosperms and angiosperms. Given that these paths are similar in trees and higher plants, the paper also describes genetic variants within the species *Arabidopsis thaliana* that serve to illuminate the range of genetic variation that could be observed among tree genotypes will respect to these pathways.

After root pretreatment with 2, 6-dichloroisonicotinic acid (DCIA or INA), hypocotyls of etiolated cucumber seedlings acquired resistance to infection by *Colletorichum lagenarium [C. orbiculare]* caused by the failur of the fungus to penetrate epidermal cell walls. The hypocotyls contained only low levels of class III chitinase and its mRNA prior to infection. The pathogenesis-related (PR) gene was expressed strongly upon infection but only in resistant hypocotyls and soon after germination of the fungal spores. Chitinase was also induced early by an albino mutant strain of *C. lagenarium* that can barely penetrate the epidermis. Thus, early recognition of the fungus implies signal compounds able to pass, or being generated in, the hydrophobic eqidermal surface. As the apoplastic chitinase accumulates timely at the site of a subsequent attack, it may contribute to disease resistance. The mechanism behind the enhanced responsiveness of eqidermal cells was studied by gently

abrading the cuticle of susceptible hypocotyls to allow permeation of a watersoluble polymeric fungal elicitor. Induction of chitinase occurred only when the elicitor was applied simultaneously with a resistance inducer such as DCIA, salicylic acid or a benzothiadiazole. In addition, long-term root pretreatment with DICA conditioned the hypocotyls for enhanced elicitor responses. These results Obtained by Kastner - B *et. al.* (1998) demonstrate that the above inducers of acquired resistance can affect expression of the cucumter chitinase gene not only as direct inducers. They can also actsynergmically with fungal elicitor and, in addition, condition the hypocotyls in a development manner for potentiated elicitation.The possible exploitation of localised defence responses and systemic acquired resistance in plant protection of fruit are discussed by Gues *et. al.* (1998).

Siegrist *et. al.* (1997) described that, with the help of the chemical agent benzo [1,2,3] thiadiazole-7-carbothioie acid-s-methyleste (BTH), the active ingredient of the plant activator Bion R. induction of systemic acquired resistance (SAR) was achieved in green bean *(Phaseolus vulgoris cv. Dufrix)* against different fungi and one bacterial pathogen of agricultural importance. To induced SAR, bean leaves were either sprayed directly with BTH or as a new developed from of application, bean seeds were allowed to germinate in the inducer solution A minimal period of 4 days was necessary to obtain resistance against the bictrophic rust fungus *Uromyces appendiculatus*, the perthotrophic soil borne pathogen *Rhizoctonia solani,* and the causal agent of anthrocnose *Colletotrichum lindemuthianum.*

Treatment of cell-suspension cultures of *Platonts acerifolia* with a crude elicitor preparation from *Ceratocystic fimbriate* f. sp. *platani* germlings induced the synthesis of the hydroxycoumarin phytoalexins, scopoletin and umbelliferone, and their accumulation in the growth medium. Only the protein-containing fraction of the culture filtrate was involved in cell response By ultrafiltration of this last fraction, a major eliciting glycoprotein with the ability to induce 80% coumarin synthesis was isolated. The glycoprotein was substituted by N-glycan(s) containing terminally linked mannose as revealed by lectin immunoblotting. The molecular mass of the eliciting compound was 66KD (SDS-PAGE) and the native conformation was necessary for elicitor recognition by *P. acerifolia* cells and thereby phytoalexin synthesis. The possible involvement of the GP 66 elicitor from the canker stain agent of *P. acerifolia* in the activation of plant phenolic metabolism is discussed by Alamr *et. al.* (1998).

Phytoalexin induction inplants as a basis for crop protection were analysed by Jeandet *et. al.* (1998). A metallic salt, hexahydrated aluminium chloride (A1C13), was shown to act as a potent inducer of phytoalexin synthesis in grapevines. The development of new approaches to grapevine disease control through the chemical stimulation of phytoalexin synthesis is reviewed with reference to the litreature concerning phytoalexins from the Vitaceae, the chemical stimulation of phytoalexin (resveratorl) synthesis in grapevines and in other plants, and the induction mechanism of resveratrol synthesis in grapevine cells. The results indicate that stimulation of grapevine defence mechanism. i.e. phytoalexin biosynthesis, can increase the resistance of the plant to *Botrytis cenerea* infection in the vineyard. The use of elicitors of natural defence responses offers novel opportunities for biological control and crop protection (Jeandet et. al. (1998).

Synthesis of phytoalexins, dianthalexin and methoxydinathramide S, was observed in carnation treated with the culture filtrates of *Fusarium oxysporum* f. sp. *dianthi* and *Phytophthora parasitics pPhytophthora nicotianae* var. *parasitica].* Complex fractions inducing phytoalexin synthesis were isolated from culture filtrates of both fungi by anion-exchange chromatography. The DEAE-cellulose non-binding fraction of *F. oxysporum* f. sp. *dianthi* and the binding fraction of *P. nicotianae* var. *parasitica* contained elicitor-act ve neutral polysaccharides and proteinaceous compounds present in the culteriltrates of both fungi. Further fractionation of the extracellular polysaccharides of *F. oxysporum* f. sp. *dianthi* led to the isolation of an active neutral polysaccharide fraction with molecular mass of approx. 10 kDa. The active extracullular proteinaceous compounds of *P. nicotianae* var. *parasitica* were purified by PAGE and identified as a glycoprotein of 30 kDa. It is concluded that the elicitors of phytoalexins in carnation are not of high molecular mass. In addition, these active fungal compounds contain the same neutral sugars, glucose, galactose and mannose. It is suggested that these sugars may play a fundamental role in the recognition of the 2 fungi by carnation (Gandon *et. al.* 1998).

Kaestner *et. al.* (1998), reported after root pretreatment with 2, 6-dichloroisonicotinic acid (DCIA or INA) hypocotys of etiolated cucumber seedling acquired resistance to infection by *Colletorichum lagenarium* caused by the failure of the fungus to penetrate epidermal cells walls. The hypocotyls contained only low levels of class III chitinase and its mRNA prior to infection. This pathogenesis-related (PR) gene was expressed strongly upon infection but only in resistant hypocotyls and soon after germination of the fungal spores. Chitinase was also induced early by an albino mutant strains of *C. lagenarium* that can barely penetrate the epiderms. Thus, early recognition of the fungus implies signal compound able

to pass, or being generated in, the hydrophobic epidermal surface. As the apoplastic chitinase accumulates timely at the site of a subsequent attack, it may contribute to disease resistance. The mechanism behind the enhanced responsiveness of epidermal cells was studied by gently abrading the cuticle of susceptible hypocotyls to allow permeation of a water soluble polymeric fungal elicitor. Induction of chitinase occurred only when the elicitor was applied simultanoeousl with resistance inducer such a DICA salicylic acid (SA) or a bengothiadiazole (BTH). In addition, long term root.pathegenesis-related proteins (PRs) were detected inleaves of 2 tomato varieties with different degrees of susceptibility to *R. solant* by Fernadez *el. al.* (1998). Using Western Blot analysis and antibodies raised against PRs isolated from tobacco, chitinases and beta-1, 3-glucanases were identified. The intensity of protein patterns was higher in the reisstant variety compared with the susceptibility variety. Three chitinases with approximate molecular masses of 31.5,33 and 35.5 KDa were only observed in the resistant variety. These results suggest that chitinases constitute biochemical defense mechanisms in tomato plants against *R. solani.*

The experiment of differential elicitation of defense responses by pectic fragments in bean seedlings was performed by Boudart *et. al.* (1998). The cell walls of two near-isogenic lines of bean *(Phaseolus vulgaris)* seedlings. susceptible or resistant to the bean anthracnose pathogen *Colletoirichum lindemuthianum* were digested with the pure endopolygolacturoriase (endo PG, EC 3.2.1.15) isolated from the fungus. The solubilized pectic fragments were separated according to their charge and size. Analysis of their uronic acid contents showed that their elution patterns were quite dissimilar, depending on whether they originated from the resistant or the susceptible host plant. Their sugar compositions revealed that neutral sugars were more abundant in the fragments released from the resistant plant that from the suceptible one, while the reverse was true for acidic residues. The fragments solubilized from the resistant plant induced an increase of pathogenesis-related (PR) proteins when challenged on resistant or susceptible bean seedlings, both at the transcript and enzyme-activity levels. On the other hand, pectic fragments released from susceptible bean cell walls exhibited either no significant activity or only a weak elicitor effect on the defence of susceptible or resistant bean seedlings. The differential elicitor effect observed between pectic fragments was inversely correlated with their acidity. Thus, endoPG-released pectic fragments from bean cell walls exhibited the same ablity as the endoPG itself to elicit defence responses in a cultivar-specific manner.

Fluorescent *Pseudomonads* and non-pathogenic *F. oxysporum* have been shown to suppress Fusarium wilts by Duijff *et. al.* (1998). Suppression has been related to both microbial antagonism and induced resistance. The relative importance of systemic induced resistance in the suppression of *Fusarium* wilt of tomato (F. O. fsp. lycopersici) in commercial-like conditions assessed by a reference strain of each type of microorganism *(P. fluorescens* WC5417r and non-pathogenic *F. oxysporum Fo47).* The accumulation of pathogenesis related (PR) proteins in tomato plants inoculated with WC5417r or with Fo7 was also determined. Suppression of Fusarium wilt by *P. fluorescence*WC517r was ascribed to systemic induced resistance without any detection of the PR-proteins tesed (PR-1 and chitinases). In contrast the suppression achieved by non-pathogenic *F. oxysporum* Fo7 appeared to the mainly ascribed to microbial antagonism but also to a lesser extent to systemic induced resistance. The induced resistance could be related to the accumulation of PR-1 and chitinases. The possible relationship between the ability of Fo7 to suppress Fusarium wilt more efficiently than WCS 417C and its ability to show both mechanisms is discussed.

Chamnongpol *et. al* (1998) showed that defense activation and enhanced pathogen tolerance is induced by H_20_2 in transgenic tobacco. Transgenic tobacco deficient in the H_20_2-removing enzyme catalase (cat ISA) was used as an inducible and non-invasive system to study the role of H_20_2 as an activator of pathogenesis-related (PR) proteins in plants. Excess H_20_2 in cat IAA plants was generated by simply increasing light intensities. Sustained exposure of Catl AS plants to excess H_20_2 provoked tissue damage, stanulated salicylic acid and ethylene production and induced the expression of acidic and basic PR proteins with a timing and magnitude similar to the hypersensitive response against pathogens. Salicylic acid production was biphasic, and the first peak of salicylic acid as well as the peak of ethylene occurred within the first hours of high light, which is long before the development of tissue necrosis. Under these conditions, accumulation of acidic PR proteins was also seen in response leaves that were not exposed to high light, indicating systemic induction of expression. Short exposure of Cat IAS plants to excess H_20_2 did not cause damage, induced local expression of acidic and bacis PR proteins, and enhanced pathogen tolerance. However, the timing and magnitude of PR protein induction was in this case more similar to that in upper unifected leaves than to that in hypersensitive-response leaves of pathogen-infected plants. Together, these data demonsirate that sublethal levels of H_20_2 activate expression of acidic and basic PR proteins and lead to enhanced pathogen tolerance. However, rapid and strong activation of PR protein expression, as seen during the hypersensitive response, ocures

only when excess H_2O_2 is accompanied by leaf necrosis.The role of salicylic acid as a plant growth regulator is discussed including its biosynthesis, mode of action and role in systemic acquired resistance by Gehlot *et. al.* (1998).

The practical application of systemic acquired resistance (SAR), the use of infectious agents and chemical compounds to induce resistance to pathogens in plants, the use of immunization to control plans disease in the field advantages and disadvantages of SAR. and possible directions for the future are reviwed by Fodar *et. al.* (1998). Systemic acquired resistance (SAR) was studied by Hevesi *et. al.* (1998) in transgenic tobacco plants which could not accumulate salicylic acid (SA) and in non-transformed tobacco plants. The plants ever inoculated with *E. c.* subsp. *carotovora* to evaluate symptom development. In 40-and 60- day old tobacco plants the putative induction of SAR was ineffective against the multiplication fo *E. c* subsp. *caratovora* and necrosis caused by a bacterial and viral pathogen *(Pseudomonas syringae* pv. *syringae* and tobacco mosaic tobamovirus respectively), in both the transgent and non-transgenic plants.

Systemic acquired resistance (SAR) is a widely distributed plant defence system that confers broad-spectrum disease resistance and is accompanied by coordinate expression of the so-celled SAR genes. This type of resistance and SAR gene expression can be mimicked with chemical inducers of resistance. Chemical inducers of resistance are active in maize. It is demonstrated by Morris *et. al.* (1998). Chemical induction increases resistance to downy mildew *[Peronosclerospora sorghil]* and activates expression of the maize PR-1 and PR-5 genes. These genes are also coordinately activated by pathogen infection and function as indicators of the defence reaction. Specifically, after pathogen infection, the PR-1 and PR-5 genes are induced more rapidly and more strongly in an incompatible that in a compatible interaction In addition, it was shown that monocot lesion mimic plants also express these defence-related genes and that they have increasd levels of salicylic acid after lesions develop, simlar to pathogen-infected maize plants. The existence of chemically inducible disease resistance and PR-1 and PR-5 gene expression in maize suggests that maize is similar to dicots in many aspects of induced resistance. The reinforces the notion of an ancient plant-inducible defence pathway against pathogen attack that is shared between monocots and dicots.

Nonpathogenic rhizobacteria can induce a systemic resistance in plants that is phenotypically similar to pathogen-induced systemic acquired resistance (SAR)

Rhizobacteria-mediated induced systemic resistance (ISR) has been demonstrated by Loon *et. al* (1998) against fungi, bacteria and viruses in Arabidopsis, bean carnation, cucumber, radisn, tobacco and tomato under conditions in which the inducing bacteria and the challenging pathogen remained spatially separated Bacterial strains differ in their abilty to induce resistance in different plant species, and plants show variation in the expression of ISR upon induction by specific bacterial strains. Bacterial determinants of ISR include lipopolysaccharides, siderophores, and salicylic acid (SA). Whereas some of the rhizobacteria induce resistance through the SA-dependent SAR pathway other siegrist (1988) explained cultured parsley cells, a model system for the raid testing of abiotic and natural substances as inducers of systemic (SAR) in various plant species were used to condition cultured parsley *(Petroselinum crispum)* cells for an enhanced response to low doses of an elicitor from *Phytophinora sojae* cell walls. Except for probenazole, a number of agents which have been described as effective inducers of disease resistance-in plants were able to activate cells for enhanced elicitor-mediated furanocoumar in accumulation. Of the compounds tested, benzo (1,2,3) thiodiazole-7-carbothioic acid-S-methyl ester the active ingredient of the commercially availabe, SAR activator Bion R, had the highest potential for conditioning parsley cells. Compounds which are know to be inactive as inducers of disease resistance did not enhance the elicitor response. It is suggested that cultured parsley cells provide a suitable system for the identification of SAR-inducting agents.

Fungicide action is generally assumed to be dependent on an antibiotic effect on a traget pathogen, although a role for plant defence mechanisms as mediators of fungicide actionhas not been excluded. Evidence is presented by Molina *et. al.* (1998) to show that in Arabidopsis, the innate plant defence mechanism contributes to the effectiveness of fungicides. In NoRG and nim1 (for non-inducible immunity) Arabidopsis plants, which normally exhibit increased susseptibility to pathogens, the fungicides metaiaxyl fosetyl, and Cu(OH)2 are much less active and fail to control *Peronospora parasitica.*

Zhang *et. al.* (1998) demonstrated the compost and compost water extract induced systemic acquired resistance in Cucumber and Arabidopsis A biocoutrol agentfortified comost mix, suppressive to several cisease caused by soilborne plant pathogens, induced systemic acquired resistance (SAR) in cucumber against anthrocnose casued by *Colletotrichum orbiculare* and in Arabidopsis against bacterial speck caused by *Pseudomonas syringae* pv. moculicola KD4326. A peat mix conducive to soilborne disease did not induce SAR. The population size of *P. syringae* pv. maculicola KD4326 was significantly lower in leaves of Arabidopsis plants grown in the compost mix compared with those grown in the peat mix. Autoclaving destroyed the SAR-inducing effect of the compost

mix, and inoculation of the autoclaved mix with nonautocled compost mix or pantoea agglomerans 27BA restored the effect, suggesting the SAR-inducing activity of the compost mix was biological in nature. The peat mix water extract applied as spray did not control bacterial speck on plants grown in either mix. Topical sprays with salicylic acid (SA) reduced the severity of bacterial speak on plants in the peat mix but did not further reduce the severity of symptoms on plants in the compost mix. The activity of the compost water extract was heat-stable and passed through.

The mechanism and pathways of plant systemic acquired resistance are reviewed, Cia *et. al.* (1999). After a plant is inoculated with a necrotizing pathogen or treated with some chemicals, the uninoculated or untreated parts of the plant demonstrated resistance to the infection of a second pathogen. This is plant systemic acquired resistance (SAR). SAR is systemic, long lasting resistance to a wide range of pathogens. The inoculated or treated parts immediately produce a systemic signal and induce the expression of SAR genes. Salicylic acid (SA) is one of the signal molecules inducing SAR. They react more rapidly and more efficiently to a challenge infection with a virulent pathogen. SA in the sensitized tissue activates the pathogen-dependent fine control mechanism, which potentiates the nonspecific pathogen signal, and activates nonspecific defense response mechanisms. The signal transduction pathway of SAR is complicated. A series of SAR mutants were selected, several genes encoding components of SAR signal transduction pathway were cloned, and their functions were analysised. Cole (1999) showed that Acibenzolar-S-methyl (ABM), a benzethadiazole, is a novel plant protection product that mimics the pathogen host interaction and results in systemic acquired resistance in plants.

Two signalling pathways, one involving salicylic acid and another involving jasmonic acid, participate in the expression of plant resistance to pathogens and insect herbivores. In this study, it was shown by Thaler *et. al.* (1999) that stimulation of systemic acquired resistance in field-grown tomato plants with the salicylate mimic, benzothiodiazole : (1) attenuated the jasmonate-induced expression of the antiherbivore defence-related enzyme polyphenol oxidase and (2) compromised host-plant resistance to larvae of the beet armywarm, *Spodoptera exigua.* Conversely, treatment of plants with jasmonic acid at concentrations that induce resistance to insects reduced pathogenesis-related protein gene expression induced by benzothiadiazole, and partially reversed the protective effect of benzothiadiazole against bacterial speak disease caused by *Pseudomonas syringae* pv. tomato it was concluded that the effective utilization of induced plant resistance to the multiple pests

typically encountered in agriculture will require understanding potential signalling conflicts in plant defence responses.

Since the application of salicylic acid (SA) to induce systemic acquired resistance (SAR) in plants is currently discussed as an alternative for copper against downy mildew *(Plasmopara viticola),* as sensitive HPLC method with UV/Vis-DAD-detection was developed by Nikfardjam *et. al.* (1999) to determine SA in must and wine. The rate of recovery was 92% at a level of 0. 15 mg/litre with a detection limit of 0.003 mg/litre. Several must and wines were analysed from field experiments with SA application and then SA concentrations were compared with 23 commercially available German wines Nearly all samples contained small amounts SA. The mean concentration in white and red wines was 0. 05 mg/litre (0. 11mg/ litre max.) and 0.16 mg/litre (0.43 mg/ litre max.), respectively. Application of SA downy mildew control did not increase the amounts of SA in must or wine.

Thomson *et. al.* (1999) reported that Bion (benzothiodiazole) was evaluated in fields tests to control fire blight *(Erwinia amylovora)* of pear and apple in Utah, USA, Hamilto, New Zealand and Ageners, France. The active ingredirent benzo [1,2,3] thiadiaxole-7-carbothioic acid-5 methyl ester or benzothiadiazole (BTH) is known to elicit systemic acquired resistance (SAR) against fungal diseases of several plants including tobacco and cucumber BTH alone provided significant protection against fire blight but was not as effective as streptomycin along under test conditions. BTH plus streptomycin provided up to 2 times better control than either BTH or streptomycin alone in the apple trials, BTH was more effective in apples than in pear.

An excellent multicomponent coordinated defence response of rice plants (cv. IET-2233) to fungal attack has been demonstrated and a plausible relationship among them has been proposed by Bera, S and Purkayastha, PP (1999). Some selected defence components such as momilactone 'A' (a rice phytoalexin), beta-1, 3-glucanases and exo chitinases (both pathogenesis related (PR)-proteins) and an enzyme phenylalanine ammonia lyase (PAL) were employed as biochemical parameters for evaluating the degree of response of rice plant to *Rhizocionia solani* Kuhn, a fungus causing sheath blight disease. A systemic fungicide kitazin which reduced disease significanty also concomitantly activated biosyathesis of momilactone A, induced PR- proteins and increased PAL activity in rice. Treatment of rice leaf sheaths with a PR- protein inhibitor (kinetin + NAA) increased disease markedly but inhibited beta-1, 3 glucanass and exo-chitinase activities in treated plants. Similarly, amino

oxyacetic acid (AOA), a PAL inhibitor also enhanced disease intensity and inhibited PAL activity in treated, inoculated plants, Results confirm the coordinated function of various defence components in rice following infection by *Rhizoctonisa* and also after abiotic induction of resistance.

A metallic salt, hexahydrated aluminium chloride ($AICI_3$) was shown to act as a potent inducer of phytoalexin synthesis in grapevines *(Vits vinifera* cv. *Pinot Noir* clone 113 and *V. rupestris* cv. *Rupestris du Lot clone* T 110). AICI is the constituent of a new fungicide manufactured by laboratories Goemar and sold under the Synermix trademark. The efficacy of Synermix was tested for the control of grey mould, caused by *Botrytis cinerea,* in the vineyard, alone, or used in association with iprodione. It has been shown by *Jeandet-P et. al.* (1999) that Synermix increased the efficancy of anti - *B. cinerea* treatments. Results strongly suppor the hypothesis the Synermix can act by stimulating host defence mechanisms in grapevines.

Jasmonic acid and a glycoprolein elicitor produced by *Ceratocystis fimbriate* f. sp. *Platani,* the canker stain agent, were tested for induction of coumarin phytoalexin accumulation in detached leaves of resistant *(Platanus occidentalis)* and susceptible plane trees *(Platanus acerifolia* and *P. occidentalis)*. It was shown by *Clarivet - Alain et. al.* (1999) that leaves responded by different levels of phytoalexin accumulation after fungal elicitor treatment. The phytoalexins were excreted from elicited leaf tissues and accumulated in elicitor-containing droplets on the leaf surface. The highest level was found in leves of resistant tree. Furthermore, pretreatment of leaves by jasmonic acid, sprayed before elicitor application, induced a further enhancement in phyloalexin accumulation which was higher in leaves of susceptible trees without any change in the ratio of the three phytoalexins, scopoletin, umbelliferone and exnthoarnol. However, jasmonic acid did not mimic biotic strees by itself. It only increased the response of leaf cells to fungal elicitor leading to an activation of coumarin metabolism.

Zeller *et. al.* (1999) described the control of fireblight with the plants activator BION-R. The plant activator Bion (Acibenzolar-5-methyl) has a systemic.

Kuwabara *et. al.* (1999) showed that in suspension-culture cells of winter wheat cv. chinokukomugh the accumulation of soluble secretory *proteins* in the culture medium was promoted by ABA treatment in cornparison with non-treated cells. The total amount of

secretory proteins in ABA-treated cells was 1-7 fold higher than that in non-treated cells. The analysis of two-dimensional electrophoresis revealed that at least twelve secretory proteins were induced by ABA, and these were named WAS (wheat ABA-induced secretory) proteins 1-12, N-terminal amino acid sequence analysis of WAS proteins sevealed the sequences of WAS-2 and WAS-3. Homology searches showed that WAS-2 had 55% identity with the N-terminus of the wheat chemically induced gene (WCI-5 gene) product WAS-3 was also shown to have 93% identity with the N-terminus of the barley protein R, a typical member of thaumatin-like proteins (TLPS) Immunobot analysis also suggested that WAS-3 was related to protein R. These results suggest that exogenous ABA induces some basic secretory proteins that are related to the plant defence system in wheat.

A multicomponent coordinated defence response of rice plants (cv. IET-2233) to fungal atack was demonstrated by Bera and Purkayastha (1999). Some selected defence components such as momilactone A (a rice phytoalexin), beta-1, 3-glucanases and exo-chitinases (both pathogenesis related (PR) proteins) and phenylalanine ammonia lyase (PAL) were employed as biochemical parameters for evaluating the degree of resistance of rice plant to *R. solani* the causal agent of sheath blight disease. A systemic fungicide (iprobenfos, as Kitaxin) which reduced disease significancly also concomitantly activated biosynthesis of momilactone A, induced PR-proteins and increased PAL activity in rice. Treatment of rice leaf sheaths with a PR-protein inhibitor (kinetinex N-AA) increased disease but inhibited beta-1, 3-glucanases and exo-chitinase activites in treated plants. Similarly, amino oxyacetic acid (a PAL inhibitor) enchanced disease intensity and inhibited PAL activity in plants treated with amino oxyacetic acid and inoculated with R. solani.

Induction of defence- and stress-related genes by the plant activator, benzothiadiazole (BTH), was investigated in barely (cv. Golden promise) seedlings by Tokunaga *et. al.* (1999). All the genes except 1 encoding a type-1 pathogenesis-related (PR) protein, Bpr1, were clearly up-regulated following BTH treatment. These included defence-and stress-related genes encoding proteins that are secreted out of the cell (leaf-specific thionin, a putative proteinase inhibitor Bsil and another PR-1 protein, HvPR1a) and genes encoding enzymes involved in regulating H_2O_2 levels and generation of reactiv oxygen species (oxalate oxidase, peroxidase and lipoxygenase). The pattern of gene expression observed indicated that BTH may act by imposing an oxidative stress on the plant, as well as being a functional analogue of salicylic acid (SA) in the signal transduction pathway leading to systemic acquired

resistance (SAR). Plants treated with BTh and inoculated with powdery mildew *(Erysiphe graminis* f. sp. *hordei)* showed reduced symptom development.

Tuzun et. al. (1999), discussed the role of hydroytic enzymes in the induction and maintenance of plant disease resistance. The interactions between plants and associative rhizosphere microorganisins, which lead to the induction of plant growth and/or disease resistance responses, are discussed, as well as the possibility that these mutualistic interactions may have co-evolved from originally antagonistic ones. Mechanisms by which organisms (plant growth promoting rhizobacteria, or PGPR, in particular) may elicit multigenic plant defence responses are presented. Plant defence responses are multi-component, meaning there are multiple and apparently complimentary defence responses involved (e.g, production of phytoalexins, the formation or strengthening of physical barriers, or the production of pathogenesis-related (PR) proteins). The pattern of expression of hydrolytic enzymes and other PR proteins has been correlated with the ability of a variety of plants to mount an effective response against disease.

Influence of different biotic an abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida* were analysed by *Pitta et. al.* (2000). Hairy root cultures of *B. candida* produce the tropane alkaloids scopolamine and hyoscyamine. In the attempt to increase productivity, several biotic and abiotic elicitors were tested. Salicylic acid increased significantly the release of both alkaloids (2-to 12-fold) and it also acted positively on specific production without altering the production profile. $AgNO_3$ increased significantly scopolamine release (3-fold) and the accumulation of both alkaloids (5-to3-fold) in the roots, thus favouring the production of scopolamine (up to 2-fold). The inhibiting effects of $AgNO_3$ and salicylic acid on ethylene could be partly responsible for these responses. Yeast extract incremented the intracellular content of both alkaloids (ca. 3-fold), but particularly increased the release on scopolamine (7-fold). $CaCl_2$ had little effect on accumulation or release of either alkaloid. $CDCl_2$ had little effect on accumulationor release of either alkaloid. $CdCl_2$ acted positively on the release of both alkaloids (3-to 24-fold). but was highly determental to growth, Hairy roots of *B. candida* are therefore susceptible to elicitation by biotic and abiotic elicitor. with variations in the kinetics of induction and the extent of release of each metabolite. thereby also exerting different effects on the alkaloid profile.

Disease response of plants in terms of induced browing and phytoalexin (induced secondary metabolites) production were recored by Bi-Fatim and *Igbal-Seem* (2000) in the tissues of *Cicer arietinum* (Chick pea) treated with the High Molecular Weight Crude elicitor Preparations, HMWCEP "Physaccharides" of IHypnea musciformis (red algae), IPadina tetrastrom (brown algae) and *Ulva lactulus* (green algae). A UV-visible spectrophotometric method ha been developed for the quantification of induced secondary metabolites with time.

When epicotyl tissues of pea were treated with a diacylglycerol (DAG) kinase inhibitor R59022), enhanced induction of the phytoalexin accumulation occurred which was induced by fungal elicitor *(Mycospharella pinodes* germination fluid). The marked induction was associated with a sustained accumulation of phenylalanine ammonia-lyase (PAL)-mRNA and the consequent increase in PAL activity. These results obtained by *Tyoda et. al.* (2000) suggest that inhibition of DAG breakdown leads to increased induction for phyatoalexin accumulation and support the hypothesis that DAG kinase negatively regulates the signal transduction.

Salicylic acid (SA) plays a critical signaling role in the activation of plant defense responses after pathogen attack. *Klessig et. al.* (2000), identified several potential components of the SA signaling pathway, including (i) the H_20_2-scavenging enzymes catalase and ascorbate peroxidase, (ii) a high affinity SA-binding protein (SABP2), (iii) a SA-inducible protein kinase (SIPK), (iv) NPR1, an ankyrin repeat-containing protein that exhibits limited homology to Ik appaBalpha and is required for SA signaling, and (v) members of the TGA/OBD family of bZIP transcription factors. These bZIP factors physically interact with NPR1 and bind the SA-responsive elementin promoters.

The role of rivoflavin as an elicitor of systemic resistance and an activator of a novel signaling process in plants was demonstrated by Dong-H and Beer (2000). Following treatment with riboflavin, *Arabidopsis thaliana* developed systemic resistance to *Peronospora parasitica* and Pseudomonas syringae pv. Tomato, and tobacco developed systemic resistance to Tobacco mosaic virus (TMV) and *Alternaria alternata* F. boflavin, at concentrations necessary for resistance induction, did not cause cell death in plants or directly affect growth of the culturable pathogens Riboflavin induced experssion of pathogenesis-related (PR) genes in the plants, suggesting its ability to trigger a signal transduction pathway that leads to systemic resistance. Both the protein kinase inhibitor K252a and mutation in the NIM2/NPR1 gene which controls transcription of defense genes imparied responsiveness to

riboflavin. In contrast, riboflavin induced resistance and PR gene expression in NaHG plants, which fail to accumulate salicylic acid (SA). Thus riboflavin-induced resistance requres protein kinase signaling mechanisms and a functional NIM1/NPR1 gene, but not accumulation of SA. Riboflavin is an elicitor of systemic resistance, and it triggers resistance signal transduction is a distinct manner.

Selected strain of rhizosphere bacteria reduce disease by activiting resistance mechanism in the plant named rhizobacteria-mediated induced systemic resistance (ISR) were studied by *Pieterse et. al.* (2000). Rhizobacteria mediated ISR resembles pathogen-induced systemic acquired resistance (SAR) in that both types of induced resistance render uninfected plant parts more resistant towards a broad spectrum of plant pathogens. Some rhizobacteria trigger the salicylic acid (SA)-dependent SAR pathway by producing SA at the root surface. In other cases, rhizobacteria trigger a different signalling pathway that does not require SA. The existence of a SA-independent ISR pathway has been demonstrated in *Arabidopsis thaliana.* In contrast to pathogen-induced SAR, ISR induced by *Pseudomonas fluorescens* WCS417r is independent of SA accumulation and pathogenesis-related (PR) gene activation but, instead, requires responsiveness to the plant hormones jasmonic acid (JA) and ethylene. Mutant analyses showed that ISR follows a novel signalling pathway in which components from the JA and ethylene response.

Treatment of the seedlings of *Lotus japonicus,* a model legume for molecular genetic studies, with reduced glutathione (GSH) resulted in the accumulation of an isoflavan phytoalexin, vestitol Shimada *et. al.* (2000). Using PCR strategies based on he conserved amino acid sequences, full length P450 cDNAs were obtained from GSH-treated seedling roots. When the clones, LJCYP-1 (CYP93C family) and LJCYP-2 (CYP31E family), were heterologously expressed in veast, the proteins exhibited 2-hydroxyisoflavanone synthase (IFS) and isoflavone 2'-hydroxylase (12'H) activites, respectively. The transcription levels of LJCYP-1, LJCYP-2 and isoflavone reductase, which are all involved in vestitol biosynthesis. coordinately increased upon elicitation. Genomic Southern blot analysis indicated that the IFS gene forms a small gene family and a single copy of the 12'H gene is present in the *L. janonicus* genome. Molecular biological aspects of P45s involved in the isoflavonoid pathway and the genomic approach to flavonoid metabolism in this unique plants are discussed. e

The *Pseudomonas putida* isolate BTP1 and its sid-muntant M3 were recently reported to protect cucumber against *Pythium aphanidermatuni* root root. This protection was mainly

associated with an accumulationof antifungal phenolics in the treated roots. In this study of Ongena *et. al.* (2000), split-root experments showed that 80-88% of *Pythium aphenidermatum* infecte control plants died, whilst only 44-45 and 33-54% mortality was recorded in BTPI and M3 treatements inoculated with *Pythium aphanidermatum,* respectively. Analyses of root extracts from these experments showed that phytoalexins were produced systemically. Several antifungal molecules accumulated similarly in both treated and non-treated root parts of plants protected against *P. aphanidermatum* with BTP1 or M3. In addition, analyses of leaf samples also revealed increased amounts of fungitoxic molecules in *Pseudmonas putiuda*-treated plants, although the nature of these molecules appeared to be different from those detected in roots. The antifungal compounds isolated both from roots and leaves were mainly detected in acid-hydrolyzed extracts containing aglycones. Investigations into possible systemic plant colonozation by *Pseudomonas putida* BTP1 using a rifampicin-resistant mutant (R75 3) of the strain, never detected R 75 3 in leaf extracts of root-treated cucumber plants. These results suggested that PGPR can elicit phytoalexins systemically in cucumber and that the overall defence response is not based on a single phytoalexin but is chemically complex and organ-specific.

Chowpea (*Vigna unguiculata* (L) walp.) seedlings, raised from seeds treated with acibenzolar-S-methyl (benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester : BTH), were inoculated at 7 days old with *Colletotrichum destructivum.* Tissue penetration was reduced markedly and intracellular infection vesicles were invariably restricted to the initially-infected epidermal cells of treated hypocotyls and leaves. The destructive necrotrophic phase of disease development was effectively blocked by a hypersensitive response in these cells, thereby protecting seedlings against damping-off. The enhanced resistance of BTH-treated tissues was associated with rapid, transient increases in the activities of two key enzyms of the phenylpropanoid/flvonoid pathway, phenylalanine ammonia lyase (PAL) and chalcone isomerase (CH1). Subsequently, there was an early, accelerated accumulation of the isoflavonoid phytoalexins kievitone and phaseollidin in treated hypocotyls. In addition, several protein bands, in the low-molecular weight range. developed in these treated challenged tissues. These responses occurred following inoculation of a normaly susceptible cultivar (TT82E-60) with the pathogen and were not observed in induced, uninoculated tissues. These results suggest that BTH protects cowpea seedlings by potentiating an early defence response rather than by altering the constitutive resistance of tissues- Latunde-Dada-Akinwunmi-O *et. al.* (2001).

The effects of an elicitor prepared from liquid cultures of an isolate of *Verticillium albo-atrun,* which is nonpathogenic to *M. sativa,* on accumulation of H_2O_2, medicarpin, deposition of phenolic polymer and phenylalanine ammomalyase (PAL) activity in cultured cells of *M. sativa* cv. Kabul (lucerne) are reported by *Tang and Smith (2001)* PAL activity and phytoalexins were assayed spectrophotometrically and by HPLC, respectively. The scopoletin fluorescence-quenching and thioglycolic acid methods were used to measure H_2O_2 and phenolic polymer deposition, respectively. Studies with inhibitors. suggested that an NAD (P) H oxidase and a peroxidase were involved in the elicitor stimulated accumulation of H_20_2 and that an increase in cytosolic Ca^2, but not H_2O_2, was part of a signalling pathway leading to the induction of defence responses. Both the influx of Ca^{2+} and release of Ca^{2+} from intracellular stores from part of the signalling pathway leading from precption of elicitor to induction of defence responses. Although H_2O_2 is not part of the pathway, evidence is presented that O_2-is part of the signal transduction chain.

Pea (*Pisum sativum* cv. *Alcan)* endocarp tissue challenged with an incompatible fungal pathogen, *Fusarium solani* f. asp. *phaseoli* of fungal elicitors results in the induction of pathogenesis- related (PR) genes and the accumulation of pisatin, a phytoalexin. Essentially the same response occurs in pea tissue exposed to DNA-specific agents that crosslink or intercalate DNA. In this study, the effects of DNA-damaging agents were assessed relative to the inducible expression of several pea PR gene : phenylalanine ammonia lyase, chalcone synthese, and DRR206. Mitomycin C. and actinomycin D [dactinomycin] mimicked the biotic elicitors in enhancing the expression of all three PR genes. The activities of these PR gene promoters, isolated from different plants, were evaluated heterologously in trangenic tobacco. It is remarkable that beta-glucuronidase expression was induced when plants containing the hetrologous phenylalanine ammonia lyase, [naringenin] chalcone synthase, and DRR206 promoterbeta-glucuronidse chimaeric reporter genes were treated by DNA-damaging agents. Finally, cytological analyses indicated that many of these agents caused nuclear distortion and collapse of the treated pea cells. Yet we observed that cell death is not necessary for the induction of the PR gene promoter assessed in this study. Based on these observations and previously published results. Choi-JJ *et. al.* (2001) propose that DNA damage or the associated alteration of chromatrion can signal the transacriptional activation of plant defense genes.

The effect of a novel synthetic signal molecule acibenzolar-S-methyl (CGA-245704: benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester), in inducing resistance in sugaarcane against red rot disease caused by the fungus *Colletotrichum falcatum* were studied by *Sundar et. al.* (2001). Application of CGA-245704 as acid drench or along with marcotting rooting mixture induced resistance in sugarcane to challenge inoculation with *C. falcatum.* When the pathogen was inoculated by the plug method, it caused discoloration in the untreated control stalk tissues; however, in the stalk tissues pretreated with acibenzolar-S-methyl, pathogen colonization was considerably reduced. When the pathogen was inoculated by nodal swabbing, its penetration was arrested in the sensitized stalk tissue. An induced systemic resistance effect was found to persist up to 30 days in the preneated cut canes. Increased phenolic content and accumulation of pathogenesis-related (PR) proteins, viz, chitinase beta-1,3 glucanase and thaumatin-like protein (PR-5), were observed is sugarcane plants treated with acibenzolar-S-methyl.

Jeun and Buchenauer (2001), demonstrated that, systemic acquired resistance (SAR) in tomato plants (*Lycopersicon esculentium*) agisnt late blight caused by *Phytophthora infestans* was induced by pre-treatment with a chemical inducer, DL-3 aminobutyric acid (3-ABA) or by pre-inoculation with a biotic inducer, Tobacco necrosis virus (TNV). Ultrastructural studies revealed tha haustoria of the late blight fungus in tomato leves expressing SAR were morphologically changed and some of them were severely damaged. Both inducers eventually caused a significant inhibition of haustorium development and hyphal growth. To investigate the possible role of pathogenesis-related proteins (PR-proteins) in the defence of leves of tomato plants expressing SAR, the accumulation and localization of the tomato PR-protein AP24 were studied by immunocytochemical methods. Immunofluorescence labelling investigations demonstrated as systemic accumulation of AP24 in leaves of tomato plants either pre-treated with 3- ABA or pre-inoculated with TNV. In the corresponding leaves of control plants without SAR induction this protein was accumulated only after inoculation with *P. infestans* Furthrmore, AP24 became detectable by immunogold labelling in strach granules of chloroplasts of untreated upper leaves expressing SAR. After invasion of the late blight fungus, AP24 was also detected in fungal cell walls and the space formed between fungal cell walls and the invaginated plasma membrane. A more dense accumulation of AP24 was observed in fungal cell walls as well as in cell wall appositions (papillae) in leaves expressing SAR. These findings indicate that pre-treatment with the chemical inducers 3-ABA and pre-inoculation with the biotic inducer TNV activated a similar accumulation patten of AP24 in leaves of tomato plants exhibiting SAR.

Ziadi *et. al.* (2001) studied that, acibenzolar-S-methyl (ASM), a member of the benzothiadiazole (BTH), on cauliflower *(Brassica oleracea* var. *botrytis)* seedlings, induce resistance against downy mildew (*Peronospora parasitica).* Seven-day-old seedlings of the susceptible cultivar Billanbong sprayed with solutions containing 0.015 or 0.075 mg a.1.mL.-11 ASM solution, inoculated 4 days later and harvested 0-7 days later, were analysed for pathogenesis-related (PR) proteins, chitinase and beta-1,3 glucanase. ASM significantly induced beta-1,3 gluanase activity which increased with time in inoculated seedlings, as confirmed by the presence of PR-2 Chitinase activity was not significantly induced by ASM, and the treated seedlings also did not accumulate the basic PR-3C and the acidic Pr-3Q which both exhibit chitinase activity. Analysis of three other acidic (PR-1C, Pr-55, Pr-8) and one basic (PR-6) Pr proteins in the ASM-treated seedlings showed that only PR-1 and PR-5 were slightly and slowly induced (4-5 days after treatement), but this induction was more pronounced after inoculation with *P. parasitica* .

An inducible S-adenosyl-L-methionine; naringenin 7-0- methyltransferase (NOMT) catalysing the methylation of naringenin to sakuranetin; a major rice phytoalexin, was purified approximately 985-fold from ultraviolet (uv)- irradiated rice leaves by Rakwal *et. al.* (2000). The enzyme is not found in healthy tissues and was purified to a nearlyhomogeneous preparation n one step using adenosine-agrose affinity chromato graphy, with 1g rice leaves (uv-irradiated) as starting material. Get filtration chromatography resulted in a almost pure enzyme, as evidenced by a major band migorating to a position corresponding to a molecular mass of approximately 41 KDa by SDS-PAGE. The purified NOMT was strongly inhibited by Mn^{2+}, Nl^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+} and Cd^{2+}, and to a low degree by Co^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+} and ethylenediamine tetraacetate acid. The amino acid sequence of NOMT cyanogen bromide (CNBR)-eleavage peptide was highly homologous to that of a caffeic acid 3-0 methyltrausferase from maize, peptides generated by CNBr and/or formic acid hydrolysis. NOMT was also shown to be induced in a time-dependent manner, and purified from rice leaves treated with jasmonic acid and copper chloride.

Nandakurmar *et. al.* (2001) selected two *Psendomonas fluroscence* strain viz PF1 and PF7 which inhibited the mycelial growth of sheath blight fungus *Rhizoctonia solani* and increased the seedling vigour of rice plants in vitro were for assesing induced systemic resistance (ISR) against *R. solani* is rice. The *Psendemonas* application as a bacterial suspension or a talk-based formulation through seed . root soil and folia application either alone or in combination effectively reduced sheath blight disease incidence, promoted plant growth and ultimately increased yield under glasshouse or field conditions. Efficacy of

Pseudomonas strains against *R. solani* was comporable to that of the fungicide carbendazim. which is normally used in the field to manage the disease. *Psenodmons* treatment of rice cv. 1R 50 lead to induction of systemic resistance against *R. solani,* as a result of increase in chitinase and peroxidase activity. However, the extent of increase varied between treatment, *Psendomenas* strains used and their duration. Though two chitineare iso forms (35 and 28 KDa) and five peroxidase isoenzyme (PD1-PD5) were found to be associated with ISR, 35 KDa chitinase and three peroxidase isoenzymes (PD3-PD5) were established as the major determinants of ISR. Although a single application of *Pseudomonas* strain resulted in ISR, the combined application through all of the four sed, root, sail and foliar) methods increased the durability of ISR in rice plants. In addition, the *Pseudomonas strains* produced chitinase in the culture medium. If is presumed that the induced chitinase, peroxidase and bacterial chitinase may be either directly or indirectly involved in the reduction of sheath blight disease development in rice.

Genetic transformation has been attempted by Datta *et. al.* (2001) for management of rice sheath blight disease, caused by *Rhizoctonia solani.* They introduced a PR-3 rice chitinase gene (R C7), isolated from *R. solani* infected rice plants, into indica rice cultivars IR72, IR64, IR68899B, MH63, and chitinase Boro II by the biolistic and polyethylene geycol-mediated transformation system inheritance was studied up to the T_2 generation by southern blot analysis. Western blot analysis of transgenic plants with polyclonal antibody revealed the presence of chitinase protein with a molecular weight of 35 KDa that reacts with chitinase antibody. The transformants synthesized different levels of chitinase proteins constitutively and progeny from the plants containing the chitinare gene showed different levels of enhanced resistance when challenged with the sheath blight pathogen *R. solani.*

Kamalakanna *et. al.* (2001) screened leaf extracts from 20 plants species for their inhibitory effect against the rice blast pathogen *Pyricularia grisea Magnaportha grisea. Prsopis juliflora* followed by *Ziziphus jujuba* and *Abutilon indicum* significantly inhibited the mycelial growth, biomass as well as toxin production and spore germination under laboratory considitions. The plant extracts were thermolabile and lost inhibitory effects upon storage and solvent extraction.

The mode of aetion of probenazol which activates the plant disease defence system has been described by Iwata-M (2001) which is related to control of *Magnaporthe grisea* on rice. Probenazole activates the phenyl propanoid pathway and causes accumulation of fungicidal substances.

Oostendorp *et. al.* (2001) worked out that plants can be induced locally and systematically to become more resistant to desease through various biotic or abiotic stresses. The biological inducers include necrotising pathogens, non-pathogens or root colonizing bacteria. Though a network of signal pathways, they induce resistance speetra and marker proteins that are characteristic for the different plant species and activation systems. The best characterised signal pathway for systematically induced resistance is systemic aquired resistance (SAR) that is activated by localized infection with necrotizing pathogens. It is characterised by protection against a broad range of pathogens, by as set of induced proteins and by its dependence on salicylic acid (SA). Various Chemicals have been discovered that seem to act at varous points in these defence activating networks and mimic all or parts of the biological activation of resistance of these, only few have reached commercialization. The best-studied resistance activationis acibenzolar-S-methyl (BION). At low rates it activates resistance in many crops against a broad speetrum disease, including fungi, bacteria and viruses. In monocots, activated resistance by BION typically is very long lasting, while lasting effect is less pronounced in dicots BION is translocated systemically in plants and can take the place of SA in the natural SAR signal pathway, inducing the same spectrum of resistance and the same set of molecular markers. Probenazole (ORYZEMATE) is used mainly on rice against rice blast and bacterial leaf blight. Its mode of actionis not well understood partly because biological systems of systematically induced resistance are not well difined in Rice treated plants clearly respond faster and in a resistant manner to infection by the two pathogens. Other compouns like beta-aminobutyric acid as well as extracts from plants and micro organisms have also been discribed as resistance inducers. For most of these, neither the mode of action nor reliable pre-challenge markers are knwon and still either pathway for resistance activati on are suspected. Resistance inducing chemicals that are able to induce broad disease resistance offer on additional option for the farmer to complement genetic disease resistance and the use of fungicids. It integrated properly in plant health management programms, they can prolong the useful life of both the resistance genes and the fungicids presently used.