LITERATURE REVIEW

Plant resistance is integral to the successful management of disease in plantation crops. Host recognition of the exogenous signal-molecule initiates a cascade of reactions that culminate in the hypersensitive response (HR). The oxidative burst, phytoalexins and the various defense enzymes variously involved in the HR curtail further invasion by the pathogen. The resistance expressed in this way is the more durable qualitative resistance (Pennypacker, 2000). A better understanding of the mechanisms of plant defense against pathogens might lead to improved strategies for enhancement of disease resistance in economically important plant species (Odjakova and Hadjiivanova, 2001). Systemic Acquired Resistance (SAR) acts non-specifically throughout the plant body and reduces the severity of disease caused by all classes of pathogens. The resistance can be induced in the field by prior inoculation with avirulent pathogen. However, the definition of the quantity of the pathogen may affect the application of the results in the field and also there is always a fear of outbreak of disease under optimum conditions (Shetty, 2002). Thus, non-toxic abiotic elicitors that can induce the SAR response are the call of the day. However, it is important to define the principal biochemical manifestation of the plant defense – the so-called 'defense enzymes' and review which enzymes can be indeed used as markers of disease resistance.

(A) Defense enzymes as markers of resistance

It is well known that the ubiquitous plant response to the pathogen is the activation of some physiological and biochemical processes necessary for impeding further pathogen progress. These include initially alteration of ion fluxes across the plant cell membrane and generation of active oxygen species (AOS), changes in the phosphorylation state of regulatory proteins, and transcriptional activation of plant defense system.

Initially, upon recognition of pathogens, plants activate a battery of defense responses, including the oxidative burst, the hypersensitive response (HR), cell wall fortification and defense-related protein synthesis (Hammond-Kosack and Jones, 1996; Lamb and Dixon, 1997; Gachomo *et al*, 2003; Jones and Dangl, 2006). One of the most rapid defense reactions to pathogen attack is the oxidative burst, which leads to the transient production of large amounts of reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (·OH) (Lamb and Dixon, 1997; Kawano, 2003; Laloi *et al*, 2004). The oxidative burst occurs during the

HR in plants following the perception of pathogen avirulence signals (Hammond-Kosack and Jones, 1996; Lamb and Dixon, 1997; Grant *et al*, 2000a). The generation of AOS requires removal of the excess of such harmful radicals so as to maintain equilibrium and leave the host intact or as safe as possible. This is achieved by ROS scavengers. These are mostly enzymes like catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), superoxide dismutase (SOD) and glutathione reductase (GR) (Arora *et al*, 2002).

A substantial body of evidence indicates that AOS regulate all the important cellular processes during stress response as reviewed by Apel and Hirt (2004). In French bean (*Phaseolus vulgaris*), pea (*Pisum sativum*) and cow pea (*Vigna sinensis*), cell-wall-bound peroxidases appeared to be the main source (Kiba *et al*, 1997; Bolwell *et al*., 1998, 2002) of superoxode anion. H_2O_2 , on the other hand , is formed by the activities of peroxidase, diamine/ polyamine oxidase and/or oxalate oxidase through various mechanisms involving membrane-bound receptors as stated by Langebartels *et al* (2002). Hypohalides are formed by peroxidase when a halide reacts with hydrogen peroxide:

$$X^- + H_2O_2 \xrightarrow{POX} OX^- + H_2O$$

Peroxidase can also catalyse the formation of H_2O_2 and O_2^- :

$$NAD^+ + O_2 \longrightarrow O_2^- + NADH$$

O_2^- produced in this reaction can now be used for spontaneous dismutation with the help of SOD (Superoxide dismutse):

$$O_2^- + 2H^+ \xrightarrow{Mn^{2+}} H_2O_2 + O_2$$

The accumulation of ROS toward the apoplast is considered to originate mainly from an increased activity of apoplastic peroxidases, amine oxidases, and an NADPH-oxidase complex coupled to a decrease in cellular ROS-scavenging capacity (Bolwell, 1999; Mittler *et al*., 1999; Torres and Dangl, 2005). pH-dependent cell wall peroxidases, germin-like oxalate oxidases, and amine oxidases have been proposed as sources of H_2O_2 in the apoplast (Bolwell and Wojtaszek, 1997). Cell wall peroxidases dependent on pH are activated by alkaline pH and in the presence of a reductant, H_2O_2 is formed. Alkalinization of the apoplast upon elicitor recognition precedes the oxidative burst and the production of H_2O_2 by pH-dependent cell wall peroxidases has been proposed as an

alternative way of AOS production during biotic stress (Bolwell and Wojtaszek, 1997; Wojtaszek, 1997b). H_2O_2 formed by the oxidation of amines may be directly utilized by wall-bound peroxidases in lignification and cell wall strengthening, both during normal growth and in response to external stimuli such as wounding and pathogenesis (Allan and Fluhr, 1997; Bolwell and Wojtaszek, 1997).

Ascorbate and ascorbate peroxidase (APOX) or ascorbate oxidase act by removing H_2O_2. By reducing ascorbate, H_2O_2 is converted to H_2O. The regeneration of reduced ascorbate (monodehydroascorbate / MDHA) is achieved by monodehydrate ascorbate reductase and dehydro-ascorbate reductase (Grassmann *et al*, 2002).

$$H_2O_2 + \text{Ascorbate} \xrightarrow{\text{APOX}} H_2O + \text{MDHA}$$

Catalase is a four subunit haeme protein, catalyzing the release of oxygen from H_2O_2 according to the equation :

$$2H_2O_2 \longrightarrow 2\,H_2O + O_2$$

As well as catalyzing the destruction of peroxide, catalase is also able to effect the oxidation of substrates, notably methanol, ethanol, by its so-called peroxidatic activity. The peroxidase reaction requires acid pH for appreciable activity, in contrast to alkaline pH conditions in which most of the peroxisomal and glyoxisomal reactions occur most readily (Butt, 1980). Catalase is reported to be inhibited by salicylic acid (SA), SAR activator (Chen and Klessig, 1991; Alvarez, 2000; Kawano and Mutto, 2000). Several reports have shown that SA can change depending upon the concentration of H_2O_2 (Shakirova, 2001), the activities of catalase and peroxidase (Durner and Klessig, 1995, Guan and Scandalios, 2000; Chandra *et al*, 2001). Therefore, the antioxidant enzymes like catalase and ascorbate peroxidase are also important components in plant defense machinery.

Plant peroxidases (EC 1.11.17) are ubiquitous, heme-containing glycoproteins that catalyze oxidation of diverse organic and inorganic substances at the expense of hydrogen peroxide (H_2O_2). Higher plants have a number of peroxidase (POX) isoenzymes that are usually classified as anionic, neutral and cationic, based on their isoelectric points (Barz *et al*, 1990; Shivakumar *et al*, 2003; Maksimov *et al*, 2006). Peroxidase gene families are well described from many species, such as rice and parsley and *Arabidopsis* (Kawalleck *et al*, 1995; Chittoor *et al*, 1997; Tognolli *et al*, 2002; Passardi *et al*, 2004). The increase of peroxidases after infection or elicitation often

correlates with the appearance of new isoforms (Ludwig-Müller *et al*, 1994; Adam *et al*, 1995; Chittoor *et al*, 1997; Gogoi *et al*, 2001; Mydlars and Harvell, 2007). Anionic and neutral peroxidases are mainly cell wall-bound, and cationic forms are confined to the central vacuole (Perrey *et al*, 1989; Kawalleck *et al*, 1995), so the former are those mainly linked with defence because of their location. Some forms can also generate active oxygen species as part of the oxidative burst during incompatible interactions (Adam *et al*, 1995; Hiraga *et al*, 2001). Increase in peroxidase activity during incompatible plant–pathogen/elicitor interactions is well documented and some peroxidases have been spatially and temporally associated with inhibition of pathogen growth (Adam *et al*, 1995; Milosevic and Slusarenko, 1996; Chittoor *et al*, 1997; Shivakumar *et al*, 2003; Sundar *et al*, 2006). Results of investigations conducted by Choi *et al* (2007) indicate that the $CaPO_2$ peroxidase is involved in ROS generation, both locally and systemically, to activate cell death and *PR* gene induction during the defense response to pathogen invasion. The family of peroxidase proteins also has diverse functions in lignification, wound responses, pathogen attack and growth regulator action. While the majority of these proteins function in the removal of H_2O_2 , a subfamily of secretory peroxidases is able to produce H_2O_2 through a superoxide binding intermediate, requiring a suitable reductant as well as alkaline pH (Lamb and Dixon, 1997).

However, the role of peroxidase, included in PR-9 family of pathogenesis-related proteins, is quite controversial. There is a conventional view according to which the high activity of peroxidase is primarily associated with resistance (Ye *et al*, 1990; Kalim *et al*, 2000;Chen *et al*., 2000; Lin and Kao, 2001). Peroxidase, is thus, accordingly, an oxido-reductive enzyme that participate in the hardening of cell wall such as oxidation of phenols, suberization, and lignification of host plant cells during the defense reaction against pathogenic agents (Ray *et al*, 1998). The resistance, therefore, is associated with the induction of peroxidase in host tissues (Van Breusegem *et al*, 2001; Lin and Kao, 2001). Besides, parallelly, and at present, in focus, the evidence has been accumulating that in some host-pathogen systems at least, peroxidase may not be associated with resistance, acquired (like SAR or ISR) or even inherent within the host's genotype (genetically modified or naturally occurring). Dalisay and Kuc (1995), in cucumber-*Colletotrichum lagenarium* system, found that the activities of peroxidase and their isoform patterns are not reliable biochemical markers for the level of induced resistance prior to the challenge inoculation with the pathogen. In their investigations, the activities

of peroxidase were high or higher in the susceptible leaves of non-induced plants at later times in the experiment than they were in the induced plants 7 days after induction, when the induced resistance was at maximum. Induction of peroxidases occurs early in the infection of *Colletotrichum gloeosporioides* on its susceptible plants of forage legume *Stylosanthes humilis,* (Harrison *et al,* 1995; Curtis *et al,* 1997). Manandhar *et al* (1999) from their findings concluded that the mRNA transcripts accumulated to a higher level in compatible than in the incompatible reaction between rice and *Pyriculariea oryzae.* According to Van Pelt-Heerschap and Smit-Bakker (1999), total peroxidase activity increased with time in all combinations of *Fusarium oxysporum – Dianthus* interactions viz. compatible and incompatible. Sulman *et al* (2001) has indicated that the peroxidase activity was associated with neighther susceptibility nor resistance. No direct correlation was found in their experiments between the amount of peroxidase extracted from mature barley kernels and the level of black point in the cultivars analysed. Genotypes that exhibited high levels of resistance to black point (Harrington and WA5034) displayed similar levels of total peroxidase activity to the genotypes with low levels of resistance to black point WI2976, Schooner, and Tallon. Similar results with respect to peroxidase profile was obtained by Som and Chakraborty (2003) in case of brown blight of tea. Recently, results by Yasupova *et al* (2006) clearly showed that the activities of peroxidase were similar in the resistant and susceptible cultivars of wheat with respect to *Septoria nodorum.* Besides, Burkhanova *et al* (2007) reported that in the seedlings of wheat cultivar susceptible to *Botrytis sorokiniana* cell wall bound peroxidase activity increased and remained at higher level relative to control throughout the experiment. On the contrary, in the resistant cultivar the level of peroxidase did not increase much relative to control.

A particular cationic peroxidase isozyme is associated with maize stress and disease resistance response (Dowd and Johnson, 2005). The *ocp3* (named due to *o*verexpression of *c*ationic *p*eroxidase) Mutant in *Arabidopsis* has enhanced resistance to necrotrophic but not to biotrophic pathogens as shown by Coego *et al* (2005). According to Maksimov *et al* (2006), cytoplasmic POX activity in normal seedlings of *Aegilops umbellata* could not be used for the prediction of its resistance to *Septoria nodorum* blotch. But, specific isozymes of peroxidase and infection-induced anionic isoforms were the markers of resistance in this case. Lagrimini *et al* (1990) reported that in both - *Nicotiana tabacum* and *Nicotiana sylvestris* transgenic plants with peroxidase activity

ten-fold than in wild cultivars, have unique phenotypes of chronic wilting through loss of turgor in leaves, which was not an effect of diminished water uptake. Thus, overproduction of peroxidase is also detrimental. Tobacco anionic peroxidase plays a pivotal role in the normal metabolism of IAA, and thus in the growth of the plant (Lagrimini, 1999). Most peroxidases are relatively unspecific for the hydrogen donors, which include phenolic substances, cytochrome c, ascorbate, IAA, amines. However, they have an almost absolute requirement for peroxide as an oxidant. Their potential role in development, besides that of lignification, is indicated by peroxidase activity in the oxidation of indole acetic acid, ethylene biosynthesis and hydroxylation of proline, and it is claimed to be a participant in wound healing and disease resistance (Butt, 1980).

As mentioned earlier, POX can also catalyze the formation of O_2^- and H_2O_2 . Therefore, it has a double role and can be called as a regulatory enzyme (Chandra *et al*, 2001). Thus, the importance of POX in disease resistance mechanism cannot be overemphasized. Being a regulatory enzyme, its levels may fluctuate heavily. Besides, presence of isozymes makes it all the more critical. Shivakumar *et al* (2003) analysed the changes in levels and isoforms of POX on induction of resistance with *Datura metel* extract in case of pearl millet against the biotrophic pathogen *Sclerospora graminicola.* According to them, susceptible seedlings of the millet failed to show early reaction against the pathogen attack and this may be the reason for establishment of the pathogen in the host. Native PAGE of peroxidase revealed a new isoform in induced susceptible seedlings. Recently, Coego *at al* (2005) have shown that overexpression of cationic peroxidase in Arabidopsis mediates resistance in case of necrotrophic pathogens *Botrytis cinerea* and *Plectosphaerella cucumerina.*

Pathogen induced biosynthesis of peroxidase in two wild species of Malvaceae, *Hibiscus trionum* and *Althea armeniaca* was studied by Golubinko *et al* (2007). After inoculation with the fungal pathogen *Verticillium dahliae*, peroxidase activity increased more rapidly in the resistant plant (*H. trionum*) than in the susceptible plant (*A. armeniaca*). Increased peroxidase activity was noted within one to five hours after inoculation of *H. trionum*, and decreased after 18 hours. After 5 days, the levels were not different from that of the mock inoculated control. In *A. armeniaca*, peroxidase activity was only marginally different from the mock inoculated control, even after 18 hours, but it was significantly higher after 5 days. Electrophoretic analysis of inoculated tissue extracts showed the occurrence of two new peroxidase isoforms in *H. trionum*. A chitin

affinity chromatography column showed that one of these was a chitin binding peroxidase isozyme; its concentration increased significantly after inoculation. The ability of the peroxidase to bind with chitin in the cell wall of the pathogen may facilitate its destruction. New isoforms also occurred in *A. armeniaca*, but they did not bind to chitin. The concentration of the two chitin binding peroxidase isozymes in *A. armeniaca* did not increase after inoculation.

In confirmation to the above views, experiments on mRNA profile analysis of different peroxidases by Liu *et al* (2005) demonstrated that induction of a particular peroxidase is associated more with susceptibility and not with resistance. In their experiment, the expression peaks of the *TmPRX1* gene after pathogen attack were concomitant with pgt (primary germ tube) penetration at 3 to 6 hpi and agt (appressorial germ tube) penetration at 12 to 24 hpi. Therefore, the expression of peroxidase in particular, needs a further verification due to multiple roles of its isozymes. Thus,the transient expression of *B. graminis* f. sp.*hordei*-inducible barley peroxidase gene *Prx7*, but not *Prx8*, results in increased susceptibility of barley epidermal cells to the powdery mildew (Kristensen *et al*, 2001).

Peroxidases oxidize a broad range of substrates by reducing H_2O_2 into water, although the precise relationship between the peroxidase activity and H_2O_2 accumulation remains elusive. H_2O_2 is an electron-accepting substrate for peroxidase-dependent reactions; thus, peroxidases generally are considered to be merely ROS-detoxifying enzymes. However, on certain occasions, the extracellularly secreted cell-wall-type peroxidases actually produce H_2O_2 or other radicals (Blee *et al*, 2001; Bolwell *et al*. 2002; Kawano 2003). Bolwell *et al* (2002) proposed a three-component system for generation of the apoplastic oxidative burst based mostly on studies of the French bean peroxidase FBP1. The components of this system are the peroxidase, alkalinization of the apoplastic space, and the reductant. FBP1 provides the peroxidase activity for H_2O_2 production following alkalinization of the apoplast. Therefore, peroxidase may play different roles in defense depending upon the pathosystem and the type of isozyme (anionic, cationic, cell wall bound, ionically bound, cytoplasmic) studied.

Therefore, the role of some peroxidases in plant resistance to phytopathogens has thus beeen discussed for many years, but the contribution of each peroxidase in the formation of physiological processes that occur in plants during pathogenesis is not well-

defined (Maksimov and Yarullina, 2007). Thus, the detailed analysis of the role of different peroxidase classes in various types of pathosystems is needed.

An almost ubiquitous feature of plant responses to incompatible pathogens or to elicitors is the activation of phenylpropanoid metabolism in which phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) catalyses the first committed step of the core pathway of general phenylpropanoid metabolism. Branch pathways lead to the synthesis of compounds that have diverse functions in plants, notably in defense, such as cell wall strengthening and repair (e.g. lignin and suberin), antimicrobial activity (e.g. furanocoumarin, pterocarpan and isoflavonoid phytoalexins), and as signalling compounds such as salicylic acid (Hammerschmidt, 1999). The resulting phenolics are often converted into more reactive species by phenol oxidases and peroxidases (Mayer and Harel, 1979; Heath, 1980). PAL genes can be regulated developmentally, induced by wounding, by low temperatures, by other stress conditions and by pathogen attack (Collinge and Slusarenko, 1987; Wu and Lin, 2002). PAL induction has been linked to defence responses that involve phenylpropanoids in numerous diseases. Typically, accumulation of PAL activity and mRNA is more rapid, higher and longer lasting in incompatible plant–pathogen interactions (Cui *et al*, 1996). Inhibitors of PAL decrease resistance and associated changes in phenolic synthesis and incorporation into host cell walls, as in *Mla1* resistance of barley to powdery mildew and *Eucalyptus calophylla* to *Phytophthora cinnamomi* (Cahill and McComb, 1992; Zeyen *et al*, 1995). Marked increases in PAL synthesis and corresponding mRNA occur in response to microbial or endogenous elicitors in many plant–pathogen systems (Sharan *et al*, 1998). In particular, an elicitor derived from yeast induces PAL and the consequent accumulation of phytoalexins and other secondary metabolites in numerous plant species, including alfalfa, tobacco, *Lupinus albus*, apple, *Solanum khasianum* and soybean (Fahrendorf *et al*, 1995; Mühlenbeck *et al*, 1996; Wojtaszek *et al*, 1997; Baier *et al*, 1999; Borejsza-Wysocki *et al*, 1999).

Constitutive expression of a phenyl alanine ammonia lyase gene from *Stylosanthes humilis* in transgenic tobacco leads to enhanced diseased resistance but impaired plant growth. (Way *et al*, 2002). This study provides further evidence that PAL has a great role to play in plant defense, but the network of defense is closely interconnected with growth and development , i. e. hormonal network.

The reactions to pathogen attack culminate is local accumulation of phytoalexins and phenolics and deposition of structural polymers such as callose, lignin and suberin, as well as cell death at the site of infection (Benhamou, 1995; Benhamou and Nicole, 1999; Metraux, 2001; Hammond-Kosack and Parker, 2003). These again include oxidative enzymes such as peroxidase (POX), whose role has been discussed, and polyphenoloxidase (PPO). Other enzymes such as Tyrosine-ammonia lyase (TAL) and phenylalanine ammonia lyase (PAL) (Beaudoin-Eagan and Thorpe, 1985; Chakraborty *et al*, 2000; Jebakumar *et al*, 2001) are involved in phytoalexin or phenolic compound biosynthesis. Such enzymes have been correlated with defense against pathogens in several plants, including tobacco (Beaudoin-Eagan and Thorpe, 1985; Goy *et al*, 1992), tomato (Bashan *et al*, 1985), cucumber (Rasmussen, 1991) and rice (Rajappan *et al*, 1995) and tea (Chakraborty *et al*, 2000; Chakraborty *et al*, 2004a).

Phenolic compounds are believed to impart resistance to diseases in plants and polyphenol oxidase (catecholase and cresolase) enzyme has been reported to be responsible for *in vivo* synthesis and accumulation of these compounds. Thus, the level of polyphenol oxidase (PPO) in the early years of plant growth or in the flush of vegetative growth may provide an estimate of synthesis of phenolic compounds in the plants, which may in turn be correlated to susceptibility or resistance to floral malformation. Higher PPO activity was related to higher contents of phenolic compounds, which have been shown to provide resistance against diseases (Sharma *et al*, 1994). This group of defence proteins includes oxidative enzymes such as polyphenoloxidases (PPO), copper enzymes that catalyse, through the reduction of molecular O_2, the hydroxylation of monophenols to O-diphenols (cresolase activity, E.C.1.14.18.1) as well as the oxidation of O-diphenols to O-quinones (catecholase activity, E.C.1.10.3.2). The generated oxidized phenols contribute to the formation of defence barriers against pathogens. Although these proteins have been well investigated in herbaceous species, they have rarely been studied in woody plants. Their role in the defense mechanism in woody plants was demonstrated in grapevine after infection by *Botrytis cinerea* (Renault *et al*, 1996).

However, polyphenoloxidase activity in the soluble fractions of cocoa (*Theobroma cacao*) pods could not be correlated with the susceptibility of the different clones to *Phytophthora megakarya*. The case was different for the ionically-bound PPO. In the experiments by Omokolo *et al* (2003), there was appearance of the an ionically

bound PPO isozyme in the less susceptible clones. According to Raj *et al* (2006), the study of the PPO activities in different pearl millet genotypes showed a clear correlation between downy mildew disease reaction of pearl millet cultivars and PPO activity. The highly resistant and resistant pearl millet cultivars displayed significantly higher PPO activities than the susceptible and highly susceptible genotypes.

In addition to the above mentioned enzymes, various novel proteins known as pathogenesis related proteins (PRps) are induced (Van Loon *et al,* 1994; Van Loon and Strien, 1999; Neuhaus, 1999). Some of these proteins have been identified as β-1,3-glucanases (PR-2; EC 3. 2.1.39) and chitinases (PR-3; EC 3.2.1.14) that specifically act on the cell wall of the fungi. It has been reported that in some species, following infection, resistant genotypes accumulate these enzymes more rapidly than susceptible genotypes (Irving and Kuc, 1990; Schnöder *et al*, 1992; Tyagi *et al*, 2001; Chakraborty, 2005).

The two most important cell wall degrading enzymes produced as a result of interaction of fungus and the plant are chitinase and β-1,3- glucanase. There has been an overwhelming evidence for these two enzymes being the markers of resistance against the chitin-containing pathogens. There is compelling evidence that chitinase and β-1,3-glucanase, acting alone and particularly in combination, can help to defend against fungal infection. It has been proposed that these glucanohydrolases act in at least two different ways : directly, by degrading the cell wall of the pathogens, and indirectly, by promoting the release of cell wall-derived materials that can act as elicitors of defense reactions (Boller, 1995; Neuhaus, 1999). Plant β-1,3-glucanase can release oligosaccharides from cell walls of the pathogen, which can then act as elicitors of defense reactions. This is well-documented for interactions between soybean and the β-glucan elicitor from the pathogenic oomycete *Phytophthora megasperma f. sp. glycinea*. Following fungal attack, soybean β-1,3- glucanase releaseas β-glucan from fungal cell walls which can then induce accumulation of phytoalexin glyceollin (Boller, 1995). Antimicrobial activity of the purified basic chitinase was established by Velazhahan *et al* (2000). Antimicrobial proteins have been characterized and their association with resistance in tea plants against *Exobasidium vexans* has been established by Chakraborty *et al* (2004a).

It is postulated that β-1,3-glucanases and chitinases participate in the active defence response of plants to pathogens, either as direct antimicrobial molecules in the break-down of pathogen cell wall (Mauch *et al*, 1988, Tyagi *et al*, 2001; Sharma and Chakraborty, 2004; Chakraborty, 2005), or indirectly by releasing elicitors from fungal cell wall (Boller, 1987,1988). Chitinases degrade chitin, a homopolymer consisting of β-1,4-N-acetylglucosamine units and which is found in virus capsides, most bacteria and fungi (Metraux and Boller, 1986). *In vitro* growth of a number of fungi can be delayed by the addition of glucanase and chitinase in the culture media (Arlorio *et al*, 1992). Cocoa pod inoculation with *P. megakarya* in experiments conducted by Omokolo *et al* (2003), was characterised by an increase in β–1,3-glucanase activity in the less susceptible clones. These alterations could be correlated to disease tolerance through the reinforcement of the lignification of the cell wall. In fact, it is well known that β-1, 3-glucanase, in combination with chitinase release oligosa-ccharide, signal molecules that can activate a variety of plant defence events (Ryan, 1988; Krishnaveni *et al*, 1999).

The activities of β-1,3- glucanase in both leaf and root tissues of three black pepper varieties was determined in healthy and *Phytophthora casici* infected tissues. Infection generally enhanced the activities of the enzyme. SDS-PAGE study revealed the production of PR-proteins in the infected tissues. Western blotting with anti-tobacco β-1,3- glucanase antibody confirmed the presence of these isoforms in the leaf exracts. Among the three varieties tested, the *Phytophthora*-tolerant P24 expressed higher rate of these defense-related enzymes (Jebakumar *et al*, 2001). According to Rivera *et al* (2002), β-1,3- glucanase activity increased more rapidly in resistant cultivars of melon upon inoculation with *Sphaerotheca fusca* than in susceptible cultivars. A single band of β-1,3- glucanase polypeptide of 33kDa was detected by Western Blot analysis.

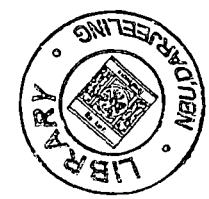
The effect of leaf rust (*Puccinia triticina*) infection on intercellular chitinase activities was studied in resistant [RL6082(Thatcher/Lr35)] and susceptible (Thatcher) near isogenic wheat lines at different growth stages by Anguelova-Merhar *et al* (2002). Resistance of plants at the flag leaf stage, during which Lr35 gene was maximally expressed, exhibited high constitutive levels of chitinase activities, compared to susceptible.

The resistance of cotton (*Gossypium hirsutum* L.) cultivars to crude toxin of *Verticillium dahliae* (VD) was correlated with the activities of β-1,3-glucanase and

chitinase in callus cells by Li *et al* (2003). The activities of the two enzymes in the callus cells treated with VD toxin were increased to higher level at earlier point in resistant cultivars than in the susceptible cultivars. Exogenous salicylic acid (SA) induced the accumulation of chitinase and β-1,3-glucanase, which resulted in resistance to VD toxin. Western blot analysis using Polyclonal Antibodies against β-1, 3-glucanase identified 28kD protein that was induced by VD toxin,SA or VD toxin plus SA. According to Jayaraj *et al* (2004), enhanced resistance in wheat against *Stagonospora nodorum* achieved by biotic agents was associated with increased level of β-1, 3-glucanase.

Contrary to the above opinion, Yi and Hwang (1996) found that accumulation of β-1,3-glucanase and chitinase was much more pronounced in compatible soyabean-*Phytophthora megasperma F. sp. Glycinea* than in the incompatible interaction. Ji and Kuc (1997) found that non-host resistance to *Colletotrichum lagenarium* in pumpkin and squash is not primarily associated with β-1,3-glucanase and chitinase activities. Disease symptoms of anthracnose were observed in cucumber, pumpkin and squash after infiltrating leaves with a conidial suspension of *Colletotrichum lagenarium*, but symptoms developed only in cucumber, when droplets of conidial suspension were applied to the leaf surface. However, penetration was reduced markedly into pumpkin and squash with or without Systemic Acquired Resistance (SAR) and into cucumber with SAR. Little β-1,3-glucanase and chitinase activities were detected in challenged pumpkin and squash leaves without symptoms even after 5 days of inoculation on leaf surfaces. However, the enzymes were detected in pumpkin and squash leaves with symptoms and activities of these enzymes were greater than in cucumber. These results suggest that β-1,3-glucanase and chitinase activities are not primarily initial defense compounds associated with non-host resistance to pumpkin and squash to *C. lagenarium*.

Ham *et al* (1997) presented evidence that fungal pathogens secrete proteins that can selectively inhibit plant β-1,3-glucanase. Fungal pathogenesis appears to involve a complex interplay between host and pathogen β-1,3-glucanase. It is now recognized that species of higher plants produce abroad range of β-glucanase differing in primary structure, cellular localization and catalytic activity. The available evidence suggests that different classes of β-1,3-glucanase have different functions in plant-microbe interactions.



Salles *et al* (2002) worked on induction of β-1,3-glucanase in *Colletotrichum trifolii – Medicago sativa* pathosystem and concluded that their results do not support the hypothesis that glucanase may function in host defense to release elicitors of the phytoalexin medicarpin.

Activities of polygalacturonase and cellulase increased in response to *Sclerospora graminicola* infection in susceptible cultivars of pearlmillet (*Peninisetum glaucum*), while these activities declined in resistant cultivars. On the other hand, β-1,3-glucanase activity increased in leaves of both resistant and susceptible cultivars but increase in activity was significantly higher in resistant ones (Wadhwa *et al*, 2001). Numerous reports on the same pathosystem (Nagarathna *et al*, 1992; Nagarathna *et al* 1993; Kini *et al*, 2000; Shivakumar *et al*, 2000) indicate that defence enzymes like lipoxygenase, phenylalanine ammonia-lyase, β-1,3- glucanase and ribonuclease recorded increased activity in highly resistant seedlings and decrease in enzyme activity in the highly susceptible seedlings. Superoxide dismutase (Babitha *et al*, 2002; Toptikov *et al*, 2002; Jung *et al*, 2005) and lipoxygenase (Babitha *et al*, 2005) are also involved positively in resistance.

Therefore, numerous plant enzymes are involved in defense reactions against plant pathogens and only a few have been discussed in the present review. Disscussion of all the defense enzymes is beyond the scope of the present work. It is obvious from the above review that the involvement and the possibility of these serving as markers of resistance is case-specific and cannot be a generalized phenomenon. Nevertheless, the level of these enzymes certainly changes on infection, but due to presence of different isoforms and the complexity of biochemical processes, they are often difficult to monitor.

Thus, a word of caution is needed while analyzing the involvement of these enzymes in pathogenesis. As observed by Maleck & Dietrich (1999) and Thomma *et al* (2001), later supported by Traw (2003), resistance traits that protect plants from biotrophic pathogens are not likely to be effective against necrotrophic pathogens. Biotrophs require living cells and can be effectively suppressed through a hypersensitive response by the plant, whereby cells near the infection experience rapid death (Sticher *et al* 1997). In contrast, necrotrophs require cell death to obtain nutrients and may actually harness the plant's own hypersensitive response to kill cells. Conversely, PR-1, PR-2 and PR-5 are pathogenesis-related proteins that are induced following infection by

biotrophic pathogens but not following attack by necrotrophic pathogens (Kunkel & Brooks, 2002). Besides, when these PRps are expressed in transgenic plants, they reduce only a limited number of diseases, depending on the nature of protein, plant species and pathogen involved (Van Loon *et al*, 2006).

(B) Inducers or elicitors of defence response

The use of abiotic inducers or elicitors of defence response instead of the harmful fungicides and pesticides that contribute to biomagnification and bioaccumulation is being popularized due to their broad range of protection. Most plant protection methods currently applied use toxic chemicals noxious to the environment. Induced resistance exploiting natural defense machinery of plants could be proposed as an alternative, non-conventional and ecologically friendly approach for plant protection. Induced resistance can be defined as an increased expression of natural defense mechanisms of plants against different pathogens provoked by external factors of various types and manifested upon subsequent inoculation. Hence, low specificity is an inherent character of induced resistance (Edreva, 2004).

The involvement of cell wall glycoproteins as elicitors has been followed since 1975 (Albersheim and Anderson-Prouty, 1975; Callow, 1977; Keen and Bruegger, 1977; Keen and Legrand, 1980). The fungal elicitor used by Lawton and Lamb (1987) was the high-molecular weight fraction released by heat treatment of isolated mycelial cell walls. Treatment of suspension-cultured bean (*Phaseolus vulgaris* L.) cells by this elicitor caused marked transient stimulation of transcription of genes encoding apoproteins of cell wall hydroxyproline-rich glycoproteins (HRGP) and the phenylpropanoid biosynthetic enzymes PAL (phenyl alanine ammonia lyase) and CHS (chalcone synthase) concomitant with the onset of rapid accumulation of the respective mRNAs. Induction and transcription of PAL, CHS and HRGP genes was also observed in wounded hypocotyls and infected hypocotyls during race-cultivar-specific interaction, with the fungus *Colletotrichum lindemuthianum*, the causal agent of anthracnose. Transcriptional activation occured not only in directly infected tissues but also in distant, hitherlo uninfected tissue, indicating intercellular transmission of an endogenous signal for defense gene activation. It is concluded that transcriptional activation of defense genes characteristically underlies induction of the corresponding defense responses and expression of disease resistance.

Elicitors have thus been initially defined as signal molecules which elicit the defense mechanism in host plants (Vidyasekharan, 1997). Elicitors of both pathogen and host origin are involved in the induction of defense genes and synergistic action of these elicitors has also been reported (Tepper and Anderson, 1990) These elicitors are derived from complex polymers by the action of enzymes like cellulases (Chang *et al*, 1995), xylanases (Lotan and Fluhr, 1990), β-1,3- glucanases (Ham *et al*, 1991) and chitinases (Boller *et al*, 1983), which are induced during interaction between pathogen and the host as reviewed earlier; and these enzymes may be of pathogen or host origin, or both. Rapid increase on these elicitor-releasing enzymes may release more amounts of these elicitors, which in turn may induce synthesis of more defense chemicals which will confer disease resistance (Ham *et al*, 1991).

The potential of chitosan, a non-toxic and biodegradable polymer of β-1,4-glucosamine, for controlling fusarium crown and root rot of greenhouse-grown tomato caused by *Fusarium oxysporum f.sp. radicis-lycopersici* (FORL) was investigated by Lafontaine (1996). The amendment of plant growth substratum with chitosan at concentrations of 12.5 or 37.5 mg l^{-1} significantly reduced plant mortality, root rot symptoms and yield loss attributed to FORL. Maximum disease control was achieved with chitosan at 37.5 mg l^{-1}, when plant mortality was reduced by more than 90% and fruit yield was comparable with that of non-infected plants. In the absence of FORL, chitosan did not adversely affect plant growth and fruit yield. Cytological observations on root samples from FORL-inoculated plants revealed that the beneficial effect of chitosan in reducing disease was associated with increased plant resistance to fungal colonization. In chitosan-treated plants, fungal growth was restricted to the epidermis and the cortex. Invading hyphae showed marked cellular disorganization, characterized by increased vacuolation and even complete loss of the protoplast. The main host reactions included the formation of structural barriers at sites of attempted fungal penetration, the deposition of an opaque material (probably enriched with phenolics according to its electron density) in intercellular spaces and the occlusion of xylem vessels with tyloses, polymorphic bubbles and osmiophilic substances. Although chitosan may also have antifungal properties, the ultrastructural observations provide evidence that chitosan sensitizes tomato plants to respond more rapidly and efficiently to FORL attack. Chitosan has the potential to become a useful agent for controlling greenhouse diseases caused by soil-borne pathogens.

Chitin, a linear polysaccharide composed of (1→4)-linked 2-acetamido-2-deoxy-β -D-glucopyranose (GlcNAc) residues, and chitosan, the fully or partially *N*-acetylated, water-soluble derivative of chitin composed of (1→4)-linked GlcNAc and 2-amino-2-deoxy-β-D-glucopyranose (GlcN), have been proposed as elicitors of defense reactions in higher plants. Vander *et al* (1998) tested and compared the ability of purified oligomers of GlcNAc (tetramer to decamer) and of GlcN (pentamer and heptamer) and partially *N*-acetylated chitosans with different degrees of acetylation to elicit phenylalanine ammonia-lyase (PAL) and peroxidase (POX) activities, lignin deposition, and microscopically and macroscopically visible necroses when injected into the intercellular spaces of healthy, nonwounded wheat (*Triticum aestivum* L.) leaves. Purified oligomers of (1→4)-linked GlcNAc with a degree of polymerization ≥ 7 strongly elicited POX activities but not PAL activities. Partially *N*-acetylated, polymeric chitosans elicited both PAL and POX activities, and maximum elicitation was observed with chitosans of intermediate decrees of acetylation. All chitosans but not the chitin oligomers induced the deposition of lignin, the appearance of necrotic cells exhibiting yellow autofluorescence under ultraviolet light, and macroscopically visible necroses; those with intermediate DAs were most active. These results suggest that different mechanisms are involved in the elicitation of POX activities by GlcNAc oligomers, and of PAL and POX activities by partially *N*-acetylated chitosan polymers and that both enzymes have to be activated for lignin biosynthesis and ensuing necrosis to occur.

Stomatal opening provides access to inner leaf tissues for many plant pathogens, so narrowing stomatal apertures may be advantageous for plant defense. Lee *et al* (1999) investigated how guard cells respond to elicitors that can be generated from cell walls of plants or pathogens during pathogen infection. The effect of oligogalacturonic acid (OGA), a degradation product of the plant cell wall, and chitosan (β-1,4-linked glucosamine), a component of the fungal cell wall, on stomatal movements were examined in leaf epidermis of tomato (*Lycopersicon esculentum* L.) and *Commelina communis* L. These elicitors reduced the size of the stomatal aperture. OGA not only inhibited light-induced stomatal opening, but also accelerated stomatal closing in both species; chitosan inhibited light-induced stomatal opening in tomato epidermis. The effects of OGA and chitosan were suppressed when EGTA, catalase, or ascorbic acid was present in the medium, suggesting that Ca^{2+} and H_2O_2 mediate the elicitor-induced decrease of stomatal apertures. They showed that the H_2O_2 that is involved in this

process is produced by guard cells in response to elicitors. Their results suggest that guard cells infected by pathogens may close their stomata via a pathway involving H_2O_2 production, thus interfering with the continuous invasion of pathogens through the stomatal pores.

Recognized PAMPs (Pathogen-Associated Molecular Patterns) that trigger innate immune responses in plants include bacterial lipopolysaccharide (LPS), lipoproteins and flagellin, in addition to fungal cell wall-derived carbohydrates and proteins (Medzhitov and Janeway, 1997; Aderem and Ulevitch, 2000; Imler and Hoffmann, 2001; Underhill and Ozinsky, 2002). Plants also possess non-self recognition systems (receptors) for numerous microbe-derived molecules which mediate activation of plant defense responses in a non-cultivar-specific manner and have been described as 'general eicitors Heath, 2000; Cohn *et al*, 2001; Dangl and Jones, 2001). These include - heptaglucan structures from oomycete cell walls, fungal cell wall chitin fragments and an N-terminal fragment of bacterial flagellin, flg22 (Felix *et al*, 1999; Nürnberger and Scheel, 2001). Thus, several glycosidic components from the cell wall of plant pathogenic fungi have been implicated in inducing plant defense response in many host-pathogen systems. Chitin oligomers (oligichitin, N-acetylchito-oligosaccharides), which can be generated from fungal cell walls by endochitinase, induce defense-related cellular responses in many plants (Nurnberger, 1999 ; Tsai *et al*, 2002). Chitin oligomers have been shown to induce various defense-related responses in tomato (Baureither *et al*, 1994), wheat (Barber *et al*, 1989), melon (Roby *et al*, 1987), barley (Kaku *et al*, 1997), pepper (Ahmed *et al*, 2003) and mango (Vivekanandhan *et al*, 2004). Typical defense-related genes such as PAL and chitinase are induced on treatment with purified chitin fragments (Minami *et al*, 1996). When applied to seeds of pearl millet in greenhouse experiments, chitin oligomers alone induce 64% protection against downy mildew pathogen, *Sclerospora graminicola* (Geetha *et al*, 2004). Thus, the chitin oligomers act as immuno-modulators.

Results of Nita-Lazar *et al* (2004) indicate that a disaccharide fraction isolated from *Fusarium oxysporum L.*, promotes rapid and transient phenylalanine ammonia lyase activity in *Rubus fructicosus* cells at nanomolar concentration. The disaccharides were isolated by size-exclusion chromatography directly from extracts obtained by alkaline treatment of *F. oxysporum* mycelium. Their structure was determined by 500-MHz-1H-NMR spectroscopy combined with methylation analysis and fast atom

bombardment mass spectrometry. Wolski *et al* (2005) isolated and characterized a cell wall α-glucan from binucleate *Rhizoctonia* isolate, an effective biocontrol factor, which induces β-1,3 glucanase activities in potato sprouts, the primary site of infection by *R. solani*. According to Burkhanova *et al* (2007), treatment of susceptible wheat plants with the low-molecular weight water -soluble derivatives of chitin prevented pathogen-induced drop in cytokinin level, thus stimulating resistance response, which are characteristic of resistant plants.

However, it is physically difficult to use cell wall fragments or other glucans for field application. The cost of production and also the specificity of the fragments to the fungus from which it is produced may limit its applicability potentials. Therefore, potentiality of other abiotic inducers are being realized. ROS (reactive Oxygen Species), as defined earlier, are the radicals produced due to stress response of the plants. There are two candidate signal molecules among the ROS involved during HR (Hypersensitive Reaction) and PCD (programmed Cell Death). These are hydrogen peroxide (H_2O_2) and NO (Nitric Oxide). ROS as mentioned earlier are known to play a dual role depending on their accumulation levels. The levels of ROS need to be tightly regulated to avoid cell damage (Neill *et al* 2002a ; Kotchoni, 2004; Mittler *et al* 2004).

H_2O_2 is moderately reactive and is a relatively long-lived molecule (half-life of 1 ms) that can diffuse some distances from its production site. H_2O_2 may inactivate enzymes by oxidizing their thiol groups. For example, enzymes of the Calvin cycle, copper/zinc superoxide dismutase and iron superoxide dismutase are inactivated by H_2O_2 (Charles and Halliwell, 1980; Bowler *et al.*, 1994). The most reactive of all AOS is the hydroxyl radical that is formed from H_2O_2 by the so-called Haber–Weiss or Fenton reactions by using metal catalysts (Halliwell and Gutteridge, 1989). Hydrogen peroxide thus when produced might conceivably add to and reinforce ROS already produced during the oxidative burst after an infection and trigger an array of local defense responses. (Baker and Orlandi, 1995; Tenhaken *et al*, 1995; Dangl *et al*, 1996). Mellersh *et al* (2002) demonstrated that localized generation of H_2O_2 is one of the earliest cytologically detectable defense responses to penetration of plant cell walls by various fungal pathogens. On the other hand, transgenic plants expressing H_2O_2 –generating enzymes have been reported to display increased protection against bacterial and fungal pathogens (Wu *et al*, 1995; Schwezer *et al*, 1999). Different members of gene families involved in the protective mechanism against pathogen attacks were up-regulated in

plants under exogenous application of ROS (H_2O_2) (Rizhsky *et al*, 2004). Exogenous application of H_2O_2 was found to be essential to activate different pathogenesis-related proteins and to provide adequate protection against plant pathogenic fungus *Diplocarpon rosae* causing black spot disease of rose leaves (Kotchoni and Gachomo, 2006). The knock-out (KO) plants deficient in ROS-scavenging proteins maintain a high steady state level of H_2O_2 in cells and activate ROS defence mechanisms when grown under control conditions (Pnueili *et al*, 2003 ; Rizhsky *et al*, 2004; Davletova *et al*, 2005) .

Therefore, a well-established role for H_2O_2 is as a signal molecule during the HR (Lamb and Dixon, 1997, Mittler *et al*, 1999; Grant and Loake, 2000) and PCD (Bethke and Jones, 2001; Fath *et al*, 2002). Infiltration of tobacco leaves with H_2O_2 activated benzoic acid 2-hydroxylase, an enzyme forming SA from benzoic acid, resulting in subsequent SA accumulation (Leon *et al*, 1995). H_2O_2 generated following pathogen challenge mediates cross-linking of cell wall proteins (Bradley *et al*, 1992) and plant cell wall-bound phenolics (Grant and Loake, 2000), and, although this is still somewhat controversial, may also have microbicidal function (Peng and Kuc, 1992, Wu *et al*, 1995). Lu and Higgins (1999) had studied tomato- *Cladosporium fulvum* and suggested that the amount of H_2O_2 accumulating during an elicitor-induced response in leaves may be sufficient to affect fungal colonization but not to affect viability of host cells unless the Fe^{2+} status in the apoplast is in some way altered by the elicitor to facilitate OH° production via the Fenton reaction (Haber-Weiss reaction).

Studies using transgenic catalase/ peroxidase-deficient tobacco (i.e. in which endogenous H_2O_2 will not be readily catabolized) showed that such plants were hyperresponsive to pathogen challenge, thus providing direct evidence for a role for H_2O_2 in HR cell death (Mittler *et al*, 1999). H_2O_2 can induce the expression of genes potentially involved in its synthesis, such as NADPH oxidase (Desikan *et al*, 1998*b*), and also of those encoding proteins involved in its degradation, implying a complex mechanism for cellular regulation of oxidative status. H_2O_2 induced the expression of genes encoding ascorbate peroxidase in germinating rice embryos (Morita *et al*, 1999) and in *Arabidopsis* leaves (Karpinski *et al*, 1999), and wounding induced the expression of gene encoding a catalase via H_2O_2 in embryos and leaves of maize (Guan and Scandalios, 2000).

Hydrogen peroxide generated in tomato as a result of interaction with tomato anthracnose fungus (*Colletotrichum coccodes*) was necessary and sufficient to account

for fungal penetration failure (Mellersh *et al*, 2002). In tobacco, moderate doses of H2O2 enhanced the antioxidant status and induced stress tolerance, while higher concentrations caused oxidative stress and symptoms resembling a hypersensitive response (Gechev *et al*, 2002).

Tolerance against oxidative stress generated by high light intensities or the catalase inhibitor aminotriazole, was induced in intact tobacco plants by spraying them with H_2O_2 by Gechev *et al* (2002). Stress tolerance was indicated by higher activity of catalase, ascorbate peroxidase, glutathione peroxidase and guaiacol peroxidase. Moderate dozes of H_2O_2 enhanced the antioxidant status and induced stress tolerance. Higher concentration caused symptoms resembling HR (Hypersensitive Reaction). Stress resistance was monitored by measuring levels of malondialdehyde, an indicator of lipid peroxidation. Thus, activation of plant antioxidant system by H_2O_2 plays an important role in induced tolerance against oxidative stress. Similarly, a rapid and transient generation of H_2O_2 increased grapevine defense responses required for protection against *Botrytis cinerea* (Aziz *et al*, 2004).

Lignification is well known to contribute to resistance by increasing the resistance of cell walls to enzymes and setting up impermeable barriers. In interaction ROS (mostly H_2O_2) seems to play a critical role in limiting colonization by the pathogen either affecting it directly or playing a significant role (Borden and Higgins, 2002). Precursors, such as coniferyl alcohols, free radicals (especially H_2O_2), and peroxidase, form lignin by the process of polymerization (Strange, 2003).

According to Shetty (2002), activation of proton ATPase acts as a first line of defense along with activation of NADPH oxidase, as a result this phenomenon of oxidative burst occurs, which acts as a central component of integrated signaling system. H_2O_2, a component of oxidative burst, acts as an initiator molecule for subsequent defense responses including oxidative cross-linking and activation of peroxidases. Hydrogen peroxide has shown 59% protection against downy mildew of pearl millet (Geetha and Shetty, 2002). Seed treatment with H_2O_2 enhanced seed germination and vigour index to the maximum level as compared to the other two inducers used in the study. Besides, it was more efficient in inducing the vegetative growth significantly. According to Whang *et al* (2004), infiltration of whole unripe avocado (*Persea americana*) fruits with H_2O_2 one day before inoculation with *Colletotrichum gloeosporioides,* induced higher levels of AFD- a major antifungal compound - *cis,cis-*

1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene and thereby increased resistance of Avocado fruits to the fruit rot fungus. They have proposed a model, according to which the quiescence of *C.gloeosporioides,* in unripe avocado is induced by the production of ROS (Reactive Oxygen Species) by both pathogen and the host. During this process ROS would directly induce fatty acid precursors of AFD, higher AFD levels and inhibition of fungal development. This model is supported by their findings that ethylene, cold stress and fungal inoculation, which induce AFD synthesis in avocado, were shown to stimulate production of H_2O_2 in other systems. Thus, this H_2O_2 is a signal molecule with a central role in the induction of resistance.

The short H_2O_2 pulse sufficient to induce PCD in leaves had a lipoxygenase-dependent oxylipin signature similar to that induced by a pathogenic elicitor (cryptogein). In contrast, the continuous H_2O_2 accumulation generated by long-term high light exposure or H_2O_2 feeding led to necrosis and ROS-mediated lipid peroxidation (Montillet *et al,* 2005).

On the contrary, *Escerohilum turcicum* is a necrophilic pathogen of wheat surviving in tissues with raised levels of H_2O_2 and localized cell death. Unlike other pathogens, this fungus germinates and survives in high H_2O_2 concentrations (Keissar *et al,* 2002). In another necrotrophic interaction, red light induced resistance in broad leaves to *Botrytis cinerea* was significantly inhibited by exogenous application of H_2O_2 (Khanam *et al,* 2005). Thus H_2O_2 plays a dual role and is a likely candidate as a signal and/or regulatory molecule in signal transduction system occurring during host-pathogen interaction.

NO (Nitric Oxide) has been demonstrated to be a signal in plant defense responses (Bolwell, 1999; Durner and Klessig, 1999; Hausladen and Stamler, 1998; Klessig *et al,* 2000; Wendehenne *et al,* 2001). NO burst has been observed in *Arabidopsis,* tobacco and soybean plant tissues, suspension-cultured cells treated with avirulent bacterial pathogens or elicitors, and apoptosis of plant cells (Delledonne *et al,* 1998; Clarke *et al,* 2000 ; Foissner *et al,* 2000; Pedroso *et al,* 2000). Also, NO may be involved in the initiation of programmed cell death, activation of pathogenesis-related (PR) gene expression, and production of phytoalexins (Noritake *et al,* 1996; Huang and Knopp, 1998; Delledonne *et al,* 1998; Durner *et al,* 1998; Clarke *et al,* 2000). In tobacco, NO was found to activate a MAP kinase cascade and to inhibit catalase, ascorbate peroxidase, and aconitase (Clark *et al,* 2000 ; Kumar and Klessig, 2000;

Navarre *et al,* 2000). NO and its exchangeable redox-activated forms are now recognized as intra- and intercellular signaling molecules (Durner *et al,* 1998; Hausladen and Stamler, 1998; Bolwell, 1999; Durner and Klessig, 1999; Klessig *et al,* 2000; Wendehenne *et al,* 2001; Mur *et al,* 2005; Mur *et al,* 2006).

Nitric oxide, according to Romero-Puertas *et al* (2004), is a free radical that can either gain or lose an electron to energetically more favourable structure, namely, the nitrosonium cation (NO^+) and the nitroxyl radical (NO^-). Because of its unique chemistry, which permits both its stability and reactivity. The free radical of NO has a half-life of few seconds and rapidly reacts with O_2 to form nitrogen dioxide (NO_2) that degrades to nitrite and nitrate in aqueous solution (Neill *et al,* 2003). However, this gaseous free radical rapidly diffuses across biological membranes and can play a part in cell-to-cell signaling in brief periods of time (Belingi and Lamattina , 2001). In addition, NO can react with free radical superoxide (O2-) to form the reactive molecule peroxynitrite (ONOO-). Moreover, NO also reacts rapidly with proteins, especially reactive amino acids such as cystein and tyrosine, as well as with various receptors and transcription factors (Stalmer *et al,* 2001). The emission of NO from plants occurs under stress situations as well as under normal growth conditions and is linked to the accumulation of NO_2 (Klepper, 1990). Based on these observations, Romero – Puertas *et al* (2004) hypothesized that ONOO– is continuously formed in healthy cells. Consequently, plant cells may have developed specific mechanisms to overcome the toxicity of ONOO–, and may have adopted different, still unknown, NO/ROS signals for triggering cell death during the HR.

However, NO cannot be applied directly and NO donor should be used, that has been excellently reviewed by Yamamoto and Bing (2000). The elevated NO concentration in tomato leaves strongly decreased H_2O_2, which coincided with quick and severe infection development in NO-supplied leaves. This speaks for the direct NO-H_2O_2 interaction (Malolepza and Rozalska, 2005). Studies on *Colletotrichum coccodes* – tomato interaction by Wang and Higgins (2005, 2006) support this view.Thus, NO-mediated resistance and its effect on various defence-related enzymes needs further investigation, since direct induction of SAR by these signal molecules, is still lacking.

Systemic acquired resistance acts non-specifically throughout the plants and reduces severity of disease caused by all classes of pathogen and it is certainly produced following expression of hypersensitive response (HR). As excellently reviewed by

Hammerschmidt (1999), localized treatment of plants with certain biotic or abiotic chemicals can result in LAR (Local Acquired Resistance) or SAR (Systemic Acquired Resistance in the treated plants to the subsequent pathogen attack. Further, SAR can be induced by exogenous application of Salicylic Acid (SA) or a benzothiazole derivative known as BTH. Systemic acquired resistance is a broad-spectrum resistance that can be induced in plants following a localized infection with a necrotizing pathogen or treatment with chemical elicitors. SAR development is mediated by a mobile signal that originates at a primary infection or treatment site and is thought to be translocated systemically in the phloem (Reglinsky *et al*, 2001).

The small phenolic compound salicylic acid (SA) plays a central role in disease resistance in higher plants. Its synthesis is induced in response to many types of pathogens (Ryals *et al*, 1996). SA is both necessary and sufficient for general resistance to many pathogens. Plants carrying a *nahG* transgene whose product catabolizes SA or plants harboring a mutation in the SA biosynthetic pathway or signaling are more susceptible to many pathogens (Gaffney *et al*, 1993; Delaney *et al*, 1994; Wildermuth *et al*, 2001). Conversely, plants engineered to produce high SA levels constitutively and plants treated exogenously with SA or an SA agonist such as benzothiazole (BTH) have enhanced disease resistance (Friedrich *et al*, 1996; Verberne *et al*, 2000). SA plays multiple roles in the regulation of plant defenses. It is required for the induction of broad-spectrum disease resistance in the systemic tissue of plants previously infected with anecrotizing pathogen (a phenomenon termed systemic acquired resistance) (Gaffney *et al*, 1993). Some plants also require SA to mount a strong resistance response during so called gene-for gene resistance. In this response, plants have a resistance (*R*) gene allele that confers the ability to recognize specific pathogen proteins encoded by *avr* genes (Staskawicz, 2001). In some *R-avr*– mediated interactions, SA is required for the *R* gene– dependent host programmed cell death (called the hypersensitive response [HR]) and/or for disease resistance (Delaney *et al*, 1994; Brading *et al*, 2000; McDowell *et al*, 2000; Rate and Greenberg, 2001; Rairdan and Delaney, 2002). However, SA on its own is not sufficient to activate an HR and some defenses when produced at high levels in plants, suggesting that SA acts as a coactivator with another signal(s) to induce these responses (Rate *et al*, 1999). One such coactivator appears to be light, because the light receptors called phytochromes are important for some SA responses (Genoud *et al*, 2002). The molecular basis of SA perception remains unclear, although several SA

binding proteins have been identified (Chen *et al*, 1993; Klessig *et al*, 2000; Slaymaker *et al*, 2002). JA and SA induce accumulation of SAR- associated priteins in wheat (Jayaraj *et al*, 2004). SA induced resistance against *E. vexans* has been reported by Sharma and Chakraborty (2005). Thus, till date, the importance of SA is considered to be immense (Vasyukova and Ozeretskovskaya, 2006; Maksimov and Yarullina, 2007).

Benzo(1,2,3)thiadiazole-7-carboxylic acid derivatives (BTH in brief), have been developed as a novel class of crop protecting agents which do not themselves have antimicrobial properties, but instead increase crop resistance to disease by activating SAR signal transduction pathway (Lawton *et al*, 1996; Cole, 1999; Godar *et al*, 1999; Anfoka, 2000; Lopez and Lucas, 2002).Tiedeman *et al* (1997) raised cowpea seedlings from seeds treated with BTH and then inoculated with *Colletotrichum destructivum*. Tissue penetration was reduced markedly and intracellular infection vescicles 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 HR. The enhanced resistance of BTH-treated tissues was associated with rapid transient increases in the activities of two key enzymes of the phenylpropanoid/flavonoid pathway, PAL and CHI (chalcone isomerase). Microscopic examination of cleared and stained inoculated leaves and hypocotyls revealed no differences in pre-penetration development of the pathogen on the surfaces of tissues in the control and treated categories. Conidial germination, appressorium formation and the melanization of appressoria occurred at comparable frequencies in both the cases. In control seedlings, host cuticle was penetrated directly by 36h after inoculation and fungal development progressed rapidly through the formation of large infection vesicls, development of narrow, secondary hyhae and finally acervuli by 120hpi. In BTH-treated seedling, fungal development was restricted. Where penetration occurred, smaller multilobed vesicles were formed but secondary invasive hyphae did not develop. Instead, pathogen remained confined to the first penetrated cell.The extractable activities of PAL and CHI underwent approx. 4-fold increase in BTH-treated seedlings. These transient patterns of increased activity were followed by marked decreases in activity, with PAL showing a more pronounced decline.

BTH, the salicylate mimic, strongly induces PR-protein accumulation and SAR in tobacco (Fidantsev *et al*, 1999). Maximum protection of pearl millet from downy mildew (78%) has been achieved by Geetha *et al* (2002) with BTH as compared to the

other two inducers (hydrogen peroxide and calcium chloride). On the other hand, 76.3% protection was achieved with BTH against bacterial canker of tomato (Baysal *et al*, 2003). BTH and INA are by far, the best studied chemical elicitors available (Vallad and Goodman, 2004) and found efficient in numerous crops. Both are considered functional analogs of SA, and elicit systemic form of induced resistance across a broad range of plant-pathogen interactions.

Treatment of mustard [*Brassica juncea* (L.) Czern. & Coss.] cv. Varuna with benzothiadiazole (BTH) induced changes in the qualitative profile of total soluble phenols and acid soluble extra cellular proteins. There was temporal increase in the level of total soluble phenolics after BTH treatment and maximum content was observed 72 h after treatment. Thin layer chromatography of an aqueous methanol extract of BTH treated leaves of mustard revealed presence of new phenolic compounds which were not present in control. Twelve acid soluble proteins with apparent molecular masses ranging from 13.2 – 69.5 kDa accumulated in BTH treated leaves of mustard plants. Proteins P13.8, P33.7 and P34.5 were present in traces in control. The most prominent proteins 24 h after BTH treatment were with apparent molecular mass of 33.0 and 33.7 kDa indicating towards their early induction, whereas, P33.0 was the most prominent protein 48 h after treatment with BTH. It is suggested that changes in specific phenols and proteins as a result of BTH treatment might be the useful markers of induced resistance in mustard (Guleria and Kumar, 2006).Hypocotyls from susceptible and resistant BTH-treated sunflower seedlings showed increased peroxidase and chitinase activities. Inoculation with *Plasmopara halstedii* increased chitinase and peroxidase activities in inoculated hypocotyls from susceptible but not from resistant sunflower seedlings (Serrano, 2007).

The ability of benzothiadiazole (BTH) or methyl jasmonate (MeJA) to induce disease resistance in harvested banana fruits was investigated in relation to the activities of several defense-related enzymes by Zhu and Baocheng (2007). Harvested banana fruit were sprayed with BTH or MeJA solution before being stored at 22°C. Disease development and the activities of six defense-related enzymes were monitored during storage. Compared with untreated fruits, BTH or MeJA treatment significantly reduced the severity of disease in non-inoculated bananas, and lesion diameters and the incidence of disease in bananas inoculated with *Colletotrichum musae*. The activities of the defense-related enzymes peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO),

phenylalanine ammonia lyase (PAL), β-1,3-glucanase, and chitinase were all enhanced in BTH- and in MeJA-treated banana fruit whether inoculated with the pathogen or not. The results suggest that post-harvest decay in bananas can be controlled by BTH or MeJA, and involves activation of the disease defense system. In non-inoculated bananas, MeJA and BTH had similar effects on the three defense enzymes (CAT, PPO and PAL), but different effects on the three PR proteins (POX, β-1,3-glucanase and chitinase). In inoculated bananas, MeJA and BTH had similar effects on all six enzyme.

Therefore, the number of SAR-inducing compounds is increasing by the day. As reported by Shetty (2002), a few resistance-inducing compounds were tested for their efficacy in inducing resistance in pearl millet. Of the various chemical compounds tested i.e. salicylic acid, acetyl salicylic acid, amino butyric acid, benzothiadiaazole, calcium chloride, hydrogen peroxide, sodium triphosphate, methionine, polyacrylic acid, jasmonic acid, iso nicotinic acid showed promising results in inducing resistance and giving a range of protection from 54-73%. In order to build up resistance, a minimum time gap of 2-4 days was required between inducer treatment and challenge inoculation. Seed treatment with inducers not only protected pearl millet from downy mildew pathogen, but also enhanced vegetative and reproductive growth parameters .

According to Zhao *et al* (2005), the stress stimulus can act as a kind of elicitor, which can efficiently induce the resistance of cucumber against fungal pathogen. After the treatment of the stress stimulus on leaves, the activities of resistance-related enzymes were increased significantly. Such as phenylamine ammonia lyase (PAL), peroxidase (POX) and polyphenoloxidase (PPO), which are strongly associated with the plant disease resistance. Also the expression of pathogenesis-related protein (PR protein) were activated by stress stimulus, with the results that the activities of chitinase and β-1,3-glucanase were increased obviously. The data showed that one of the mechanisms of stress stimulus - induction plant resistance, may act via eliciting the metabolism related to disease resistance within plant, which can produce many suppressing and antimicrobial compounds to against pathogens infection efficiently.

Thus, the signal compounds that give rise to broad range protection, should now be used as inducers of resistance. The potential of induced resistance is immense and all attempts are being made to exploit it in agriculture (Lyon *et al*, 2007). Its importance needs to be realized in woody plants, especially in an important cash crop like tea.