Inducers and Elicitors of Defense Response in Plants – an Overview

Rita Som Paul

Associate Professor

Botany Department,
Ananda Chandra College, Jalpaiguri, India.

Abstract : Inducer is any compound that elicits defense response in plants. This is a very promising modern area of research. People are highly aware of the problems related to overdoze of fungicides and pesticides leading to bioaccumulation and biomagnifications. Genetically modified crops are also highly controversial. Therefore, the best possible solution is to immunise plants against the pathogens and pests. The present paper gives an overview touching the aspects of elicitos of defense response and their different types with the detais of the most promising ones and the current trends.

IndexTerms – **inducer, elicitor, defense, chitin, cell wall, hydrogen peroxide, nitric oxide, benzothiadiazide.**

I. INTRODUCTION

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.

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 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).

II. CELL WALL AND ITS DERIVATIVES AS ELICITORS

The involvement of cell wall glycoproteins as elicitors has been followed since 1975 (Albersheim and Anderson-Prouty, 1975; Callow, 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.

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; 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 elicitors (Heath, 200; Dangl & 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). 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 (Tsai et al, 2002). Chitin oligomers have been shown to induce various defense-related responses in in tomato (Baureither *et al*, 1994), wheat (Barber *et al*, 1989), 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.

III. ABIOTIC INDUCERS

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), 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).

1. Hydrogen peroxide (H_2O_2) as inducer

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;). 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). 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, 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^o 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 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.

2. Nitric Oxide (NO) as inducer

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*, 2000a; 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*, 2000a). In tobacco, NO was found to activate a MAP kinase cascade and to inhibit catalase, ascorbate peroxidase, and aconitase (Clarke *et al*, 2000b; 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.

3. BTH (Benzothiadiazole) as inducer

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).

Improvement of the host resistance by using hazard free chemical elicitors is emerging as an alternative approach in the field of plant disease management. In our present work, we have screened the efficacy and possible mechanism of abiogenic elicitors like Dipotassium hydrogen orthophosphate (K_2HPO_4), Oxalic acid (OA), Isonicotinic acid (INA), Salicylic acid (SA), Acetylsalicylate (AS), Arachidonic acid (AA) and Calcium chloride ($CaCl_2$) to stimulate innate immune responses in *Lycopersicum esculentum* Mill. (Chakraborty et al, 2016).

Benzo(1,2,3)thiadiazole-7-carboxylic acid derivatives (Kunz *et al*, 1997) 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. Zhu et al. (2016) [reported that BTH treatment enhanced the activities of defense-related enzymes, including chitinase, phenylalanine ammonia-lyase, peroxidase, and polyphenol oxidase.

IV. CONCLUSION

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-l,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). PRI (Plant Resistance Inducers) can be chemical compounds as well as microbial or plant extracts. However, they seldom lead to full pathogen control and several factors influence the success such as plant genotype, developmental stage, environment, as well as timing and way of application of the PRI . Importantly, all PRI strategies needs to be tested in an agricultural setting as many treatments have only been shown to be successful in more controlled conditions (Alexandersson et al, 2016)

With the discovery of the hidden drug-able targets in plant immune system, new synthetic immune inducers may be developed to target these hidden points. Then in turn, these new inducers can again enhance our ability to dissect plant immune system and keep this discovery cycle going on (Zhou et al, 2018).

V. REFERENCES

[1] Ahmed, A.S., Sanchez, C.P. and Candela, M.E. (2003). Effect of chitin on biological control activity of *Bacillus spp*. and *Trichoderma harzianum* against root rot disease in pepper plants. *Eur. J. Plant Pathol*. 109: 633 -637.

[2] Albersheim, P. and Anderson-Prouty, A. J. (1975). Carbohydrates, proteins, cell surfaces and the biochemistry of pathogenesis. *Annu. Rev. Plant Physiol.* 26: 31-52.

[3] Anfoka, G.H. (2000). Benzo-(1,2,3)–thiadiazole-7-carbothioic acid-S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum*. Mill cv. Vollendung) to cucumber mosaic virus. *Crop Protect.* 19: 401-405.

[4] Aziz, A., Heyraud, A., Lambert, B. (2004). Oligogalacturonide signal transduction, induction of defense related responses and protection of grapevine against *Botrytis cinerea*. *Planta.* 218: 767-774.

[5] Baker, C. J. and Orlandi, E. W. (1995). Active oxygen in plant pathogenesis. *Annu. Rev. Phytopath.* 33: 299-321.

[6] Barber, M. S., Bertram, R. E. and Ride, J. P. (1989). Chitin oligosachharides elicit lignification in wounded wheat leaves. *Physiol. Mol. Plant Pathol*. 111: 125-137.

[7] Baureithel, K., Felix, G. and Boller, T.(1994). Specific, high affinity binding of chitin fragments to tomato cells and membranes. Competitive inhibition of binding by derivatives of oligosachharides and a Nod factor of *Rhizobium.J. Biol. Chem.* 269: 17931- 17938.

[8] Baysal, O, Soylu, E. M., and Sioylu, S. (2003). Induction of defence-raleted enzymes and resitance by the plant activator acibenzolar-S –methyl in tamato seedlings against bacterial canker caused by *Clavibacter michiganensis spp. Michiganensis. Plant Pathol*. 52: 747-753.

[9] Beligni, M. V. and Lamattina, L. (2001). Nitric oxide in plants: the history is just beginning. *Plant Cell Environ.* 24: 267–728.

[10] Bethke, P.C. and Jones, R.L. (2001). Cell death of barley aleurone protoplasts is
mediated by reactive oxygen species. *The Plant J*. 25: 19–29.

[11] Boller T, Gehri A, Mauch F, Vögeli U. (1983). Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function. *Planta*. 157: 23–31.

[11] Bolwell, G. P. (1999). Role of active oxygen species and NO in plant defence responses. *Curr. Opin. Plant Biol*. 2: 287-294.

[12] Borden, S. and Higgins, V. J. (2002). Hydrogen peroxide plays critical role in the defense response of tomato to *Cladosporium fulvum*. *Physiol. Mol. Plant Pathol*. 61: 227-236.

[13] Bowler, C., Van Camp, W., Van Montagu, M. and Inzé, D. (1994). Superoxide dismutase in plants. CRC *Crit. Rev. Plant Sci*. 13: 199–218.

[14] Bradley, D. J., Kjellbom, P. and Lamb, C. J. (1992). Elicitor- and wound induce oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. *Cell.* 70: 21–30.

[15] Burkhanova, G. F., Yarullina, L. G. and Maksimiv, I. V. (2007). The control of wheatdefense responses during infection with *Bipolaris sorokiniana* by chitooligosaccharides. *Russian J. Plant Pysiol*. 54(1): 119-126.

[16] Callow, J. A. (1977). Recognition resistance and the role of plant lectins in host-parasite interactions. *Adv. Bot. Res*. 4: 1-4.

[17] Chang, P. F. L., Cheah, K. T., Narasimhan, M. L., Hasegawa, P. M. and Bressen, R. A. (1995). Osmotic gene expression is controlled by elicitor synergism. *Physiol. Palntarum.* 95: 620-926.

[18] Chakraborty, N., Ghosh, S., Chandra, S., Sengupta, S., & Acharya, K. (2016). Abiotic elicitors mediated elicitation of innate immunity in tomato: an ex vivo comparison. *Physiology and molecular biology of plants : an international journal of functional plant biology*, *22*(3), 307–320. doi:10.1007/s12298-016-0373-z

[19] Charles, S. A. and Halliwell, B. (1980). Effect of hydrogen peroxide on spinach (*Spinacia oleracea*) chloroplast fructose bisphosphatase. *Biochem. J.* 189: 373-376.

[20] Clarke, A., Desikan, R., Hurst, R. D., Hancock, J. T. and Neill, S. J. (2000). NO way back: nitric oxide and programmed cell death in *Arabidopsis thaliana* suspension cultures. *The Plant J.* 24: 667-677.

[21] Dangl, J. L. and Jones, J. D. (2001). Plant pathogens and integrated defence responses to infection. *Nature*. 411: 826-833.

[22] Davletova, S., Rizhsky, L., Liang, H., Shenggiang ,Z., Oliver, D.J., Coutu, J.,Shulaev, V., Schlauch, K. and Mittler, R (2005).Cytolosic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis; *Plant Cell.* 17: 268–281.

[23] Delledonne, M., Xia, Y. J., Dixon, R. A., Lamb, C. (1998). Nitric oxide functions as a signal in plant disease resistance. *Nature.* 394: 585-588.
[24] Desikan, R., Reynolds, A., Hancock, J. T. and Neill, S. J. (1998b). Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in *Arabidopsis* suspension cultures. *Biochem. J.* 330: 115-120.
[25] Durner, J. and Klessig, D. F. (1999). Nitric oxide as a signal in plants. *Curr. Opin. Plant Biol.* 2: 369-374.
[26] Edreva, A. (2004). A novel strategy for plant protection : Induced resistance. *J. Cell Mol. Biol.* 3: 61-69.
[27] Fath, A., Bethke, P., Belligni, V. and Jones, R. (2002). Active oxygen and cell death in cereal aleurone cells. *J. Exp. Bot.* 53 : 1273–1282.
[28] Felix, G., Duran, J.D., Volko, S. and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* 18: 265–276.
[29] Fidantsef, Stout, M.J., Thaler, J. S., Duffey, S.S., and Bostock, R.M. (1999). Signal interactions in pathogen and insect attack : expression of lipoxigenase, proteinase inhibitor II, and pathogenesis-related protein P4 in tomato, *Lycopersicon esculentum. Physiol. Mol. Plant Pathol*. 54: 97-114.
[30] Foissner, I., Wendehenne, D., Langebartels, C. and Durner, J. (2000). *In vivo* imaging of an elicitor-induced nitric oxide burst in tobacco. *Plant J.* 23: 817-824.
[31] Gaffney, T., Friedrich, I., Vernuij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Ryals, J. (1993). Requirements of salicylic acid for induction of systemic acquired resistance. *Science*. 261: 754-756.
[32] Gechev, T., Gadjev, I., Van Breusegem, F., Inze, D., Dukiandjiev, S., Toneva, V. and Minkov, I. (2002). Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cell Mol. Life Sci*. 59(4): 708-714.
[33] Geetha, N. P., Amrutesh, K. N. and Shetty, S. H. (2004). Chitin oligomers and polyunsaturated fatty acids act synergistically in inducing resistance in pearl millet against downy mildew disease. *J. Mycol. Plant Pathol.* 34(3) : 724-731.
[34] Geetha, H. M. and Shetty, H. S. (2002). Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide – a comparative evaluation. *Crop Protect*. 21: 601-610.
[35] Genoud, T., Buchala, A.J., Chua, N.H., and Metraux, J.P. (2002). Phytochrome signalling modulates the SA-perceptive pathway in Arabidopsis. *Plant J*. 31: 87–95.
[36] Guan, L. M. and Scandalios, J. G. (2000). Hydrogen peroxide-mediated catalase gene expression in response to wounding. *Free Rad. Biol. Med.* 28 : 1182-1190.
[37] Guleria, S. and Kumar, A. (2006). *Azadirachta indica* leaf extract induces resistance in sesame against *Alternaria* leaf spot disease . *J. Cell Mol. Biol.* 5: 81-86.
[38] Halliwell, B. and Gutteridge, J. M. C. (1989). Free radicals in biology and medicine. Clarendon Press, Oxford.
[39 Ham, K. S., Kauffmann, S., Albersheim, P. and Darvill, A. G. (1991). Host-pathogen interactions. XXXIX. A soyabean pathogenesis-related protein with □-1, 3- glucanase activity releases phytoalexin elicitor-active heat-stable fragments from fungal walls. *Mol. Plant-Microbe Interact*. 4: 545-552.
[40] Hammerschmidt, R. (1999). Induced disease resitance : how do induced plants stop pathogens? *Physiol. Mol. Plant Pathol*. 55: 77-84.
[41] Hausladen, A., and Stamler, J. S. (1998). Nitric oxide in plant immunity. *Proc. Natl. Acad. Sci.* U.S.A. 95: 10345-10347.
[42] Heath, M.C. (2000). Nonhost resistance and nonspecific plant defenses. *Curr. Opin. Plant Biol.* 3: 315–319.
[43] Imler, J-L and Hoffmann, J.A. (2001). Toll receptors in innate immunity. *Trends Cell Biol*. 11: 304–311.
[44] Jayaraj, J., Muthukrishnan, S., Liang, G. H. and Velazhahan, R. (2004). β-1,3- glucanase and thaumatin-like proteins in wheat enhance resistance against *Stagonospora nodorum. Biologia Plantarum*. 48(3): 425-430.
[45] Kaku, H., Shibuya,N., Xu, P., Aryan, A. and Fincher, G.B. (1997). N- Acetylchitooligosachharides elicit expression of a single (1-3)-glucanase gene in suspension-cultured cells from barley (*Hordeum vurgare*). *Physiol. Plant.* 100: 111-118.
[46] Keen, N. T. and Bruegger, B. (1977). Phytoalexins and chemicals that elicit their production in plants. *Am. Chem. Soc. Symp. Ser*. 62 : 1-26.
[47] Keen., N. T. and Legrand, M. (1980). Surface glycoproteins : evidence that they may function as race-specific phytoalexin elicitors of *Phytophthora megasperma f. sp. glycinea. Physiol. Plant Pathol.* 17: 175-192.
[48] Keissar, H. T., Bashar, B., Levy, Y. and Kenigsbuch, D. (2002). Stage specificity of catalase isoform activity in *Exserohilum turcicum. Physiol. Mol. Plant Pathol*. 60: 163-168.
[49] Khanam, N. N., Ueno, M., Kihara, J., Honda, Y. and Arase, S. (2005). Suppression of red light-induced resistance in broad beans to *Botrytis cinerea* by salicylic acid. *Physiol Mol. Plant Pathol*. 66: 20-29.
[50] Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D. (2000). Nitric oxide and salicylic acid signaling in plant defense. *Proc. Natl. Acad. Sci*. USA. 97: 8849–8855.
[51] Kotchoni, O. S. (2004). Molecular and Physiological characterization of transgenic Arabidopsis plants expressing different aldehyde dehydrogenase (ALDH) genes . Ph. D thesis, University of Bonn, Germany.
[52] Kotchoni, S. O. and Gachomo, E, W. (2006). The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants; *J. Biosci*. 31: 389-404.
[53] Kumar, D., Klessig, D. F. (2000). Differential induction of tobacco MAP kinases by the defense signals nitric oxide, salicylic acid, ethylene, and jasmonic acid. *Mol. Plant Microbe Interact.* 13: 347–351.
[54] Lafontaine, P. J. (1996). Chitosan Treatment: An Emerging Strategy for Enhancing Resistance of Greenhouse Tomato Plants to Infection by *Fusarium oxysporum f.sp. radicis-lycopersici.* Biocontrol Science and Technology. 6(1) : 111-124.
[55] Lamb, C. and Dixon, R., A. (1997). The oxidative burst in plant disease resistance. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. 48: 251-257.
[56] Lawton, M. A. and Lamb, C. J. (1987). Transcriptional activation of plant defence genes by fungal elicitor, wounding and infection. *Mol. Cell Biol*. 1 : 335-341.
[57] Lee, S., Choi, H., Suh, S.J., Doo, I-S., Oh, K-Y., Choi, E.J., Schroender Taylor, A.T.,Low, P.S. and Lee, Y. (1999). Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and *Commelina communis. Plant Physiol*. 121(1):

147–152.
[58] Leon, J. Lawton, M. A. and Raskin, I. (1995). Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. *Plant Physiol*. 108 : 1673-1678.
[59] Lopez, A. M., and Lucas, J. A. (2002) Effects of plant defence activators on anthracnose disease of cashew. *European Journal of Plant Pathology*. 108:409-420.
[60] Lotan, T. and Fluhr, R. (1990). Xylanases, a novel elicitor of pathogenesis-related proteins in tobacco, uses a nonethylene pathway for induction. *Plant Physiol*. 93 : 811-817.
[61] Lu, H., and Higgins, V. J. (1999) . The effect of hydrogen peroxide on the viability of tomato cells and of the fungal pathogen *Cladosporium fulvum*. *Physiol. Mol. Plant. Path*ol. 54: 131 – 143.
[62] Lyon, G. Newton, A. and Walters, D. (2007). Induced resistance in crop protection : the future, drivers and carriers. In : *Induced resistance for Plant Defence* (Eds. : Walters, D., Newton, A. and Lyon, G.).
[63] Maksimov, I. V. and Yarullina, L. G. (2007). Salicylic acid and local resistance to pathogens. In : *Salicylic acid – A Plant Hormone* ; Hayat, S. and Ahmad, A. (Eds.). 323-334.
[64] Malolepsza, U. and Rozalska, S. (2005). Nitric oxide and hydrogen peroxide: nitric oxide modulates hydrogen peroxide level in o- hydroxyethylorutin - induced resistance to *Botrytis cinerea* in tomato. *Plant. Physiol. Biochem.* 43: 623-635.
[65] Medzhitov R and Janeway CA (1997) Innate immunity: the virtues of a nonclonal system of recognition. *Cell*. 91: 295–298.
[66] Mellersh, D. G., Foulds, I. V., Higgins, V. J., Heath, M. C. (2002). H_2O_2 plays different roles in determining penetration failure in three diverse plant fungal interactions. *Plant J*. 29: 257-268.

[67] Minami, E., Midoh, N., Ohtsuki, Y. and Shibuya, N. (1996). Two novel genes rapidly and transiently activated in suspension-cultured cells by treatment with N-acetylchitoleptose, a biotic elicitor for phytoalexin production. *Plant Cell Physiol.* 37: 563-567.
[68] Mittler, R., Hallak, Herr E, Orvar, B. L, Van Camp, W., Willekens, H., Inzé D., Ellis, B. E. (1999). Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. *Proc. Natl. Acad. Sci.*, USA. 96: 14165–14170.
[69] Morita, S., Kaminaka, H., Masumura, T., Tanaka, K. (1999). Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress, the involvement of hydrogen peroxide in oxidative stress signalling. *Plant Cell Physiol*. 40: 417–422.
[70] Mur, L. A. J., Carver, T. L. W., and Prats, E. (2006). NO way to live; the various roles of nitric oxide in plant–pathogen interactions . *J. Exp. Bot*. 57(3): 489-505.
[71] Mur, L. A. J., Kenton, P. and Draper, J. (2005a). *In planta* measurements of oxidative bursts elicited by avirulent and virulent bacterial pathogens suggests that H_2O_2 is insufficient to elicit cell death in tobacco. *Plant Cell Environ.* 28 : 548-561.
[72] Navarre, D. A., Wendehenne, D., Durner, J., Noad, R., Klessig, D. F. (2000). Nitric oxide modulates the activity of tobacco aconitase. *Plant Physiol.*, 122: 573–582.
[73] Neill, S. J., Desikan, R., Clarke, A., Roger, D., Hurst, R. D., and Hancock, J. T. (2002). Hydrogen peroxide and nitric oxide as signalling molecules in plants. *J. Exp. Bot*. 53(372): 1237-1247.
[74] Neill, S. J., Desikan, R., and Hancock, J. T. (2003). Nitric oxide signalling in plants. *New Phytol.* 159: 11–35.
[75] Nita-Lazar, M., Heyraud, A., Gey, C., Braccini, I. and Lienart, Y. (2004). Novel oligosaccharides isolated fron Fusaruim oxysporum L. rapidly induce PAL activity i9n Rubus cells. *Acta Biochim. Pol*. 51(3) : 625-647.
[76] Odjakova , M. and Hajiivanova, C. 2001. The complexity of pathogen defense in plants. *Belg J. Plant Physiol.* 27 (1-2) : 101-109.

[77] Pedroso, M. C., Magalhaes, J. R., Durzan, D. (2000). A nitric oxide burst precedes apoptosis in angiosperm and gymnosperm callus cells and foliar tissues. *J. Exp. Bot*. 51: 1027–1036.
[78] Peng, M., Kuc, J. (1992). Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. *Phytopatol*. 82: 2494–2498.
[79] Pennypacker, B. W. (2000). Differential impactof carbon assimilation on the expression of quantitative and qualitative resistance in alfalfa (*Medicago sativa*). *Physiol. Mol. Plant Pathol*. 57 : 87-93.

[80] Pnueili, L., Hongjian, L., and Mittler, R. (2003). Growth suppression, abnormal guard cell response, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1)–deficient Arabidopsis plants. *Plant J.* 34: 187 – 203.
[81] Rairdan, G. J. and Delaney, T. P. (2002). Role of salicylic acid and NIM1/NPR1 in race-specific resistance in Arabidopsis. *Genetics.* 161: 803–811.
[82] Rate, D. N. and Greenberg, J. T. (2001). The Arabidopsis *aberrant growth and death2* mutant shows resistance to *Pseudomonas syringae* and reveals a role for NPR1 in suppressing hypersensitive cell death. *Plant J.* 27: 203–211.
[83] Reglinsky, T., Whitaker, G., Cooney, J. M., Taylor, J. T., Poole, P. R., Roberts, P. B. and Kim, K. K. (2001). Systemic acquired resistance to *Sclerotinia sclerotiorum* in kiwifruit vines. *Physiol. Mol. Plant Pathol*. 58: 111-118.
[84] Rizhsky, L., Davletova, S., Liang, H. and Mittler, R. (2004). The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in Arabidopsis . *J. Biol. Chem.* 297: 11736 – 11743.
[85] Romero-Puertas, M. C., Perazzolli, M., Zago, E. D., and Delledonne, M. (2004). Nitric oxide signaling functions in plant-pathogen interactions. *Cellular Micribiol.* 6(9): 795 -803.

[86] Serrano, A. R., Castillo, J. L., Novo, J. J., Ocaña, A. F; Rodríguez, M. G. (2007). Chitinase and peroxidase activities in sunflower hypocotyls: Effects of BTH and inoculation with *Plasmopara halstedii* .51(1) : 149-152.
[87] Shetty, H.S. (2002). Cross-talk during host-pathogen interaction and strategies for plant defense management. *J. Mycol. Plant Pathol*. 32(3) : 305-316.

[88] Slaymaker, D.H., Navarre, D.A., Clark, D., del Pozo, O., Martin, G.B., and Klessig, D.F. (2002). The tobacco salicylic acid-binding protein 3(SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. *Proc. Natl. Acad. Sci.* USA. 99: 11640–11645.
[89] Stamler, J. S., Lamas, S., and Fang, F. C. (2001). Nitrosylation: the prototypic redox-based signaling mechanism. *Cell.* 106: 675–683.
[90] Staskawicz, B.J. (2001). Genetics of plant-pathogen interactions specifying plant disease resistance. *Plant Physiol*. 125: 73–76.
[91] Strange, R. N. (2003). *Introduction to Plant Pathology*, Wiley Publishers, Chichester, England, pp. 295-297.

[92] Tenhaken, R., Levine, A., Brisson, L. F., Dixon, R. A., Lamb, C. (1995). Function of the oxidative buirst in hypersensitive disease resistance. *Proc. Nat. Acad. Sci*. USA. 92: 8179-83.]
[93] Tepper, C. and Anderson, A.J. (1990). Interactions between pectic fragments and extra cellular components from the fungal pathogen *Colletotrichum lindemuthianum. Physiol. Mol. Plant Pathol.* 36:147-158.
[94] Tiedemann, A. (1997). Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. *Phyisol. Mol. Plant Pathol*. 50: 151-166.
[95] Tsai, G-J., Su, W-H., Chen, H-C. and Pan, C. (2002). Antimicrobial activity of shrimp chitin and chotisan from different treatments and applications of fish preservation. *Fisheries Sci*. 68 : 170-177.
[96] Underhill DM and Ozinsky A (2002) Toll-like receptors: key mediators of microbe detection. *Curr Opin Immunol*. 14: 103–110.
[97] Vallad, G.E. and Goodman, R.M. (2004). Systemic Acquired Resistance and Induced Systemic Resistance in Conventional Agriculture. *Crop Sci*. 44:1920–1934.
[98] Vander, P., K.M. Varum, A. Domard, N.E. El-Gueddari, and B.M. Moerschbacher. (1998). Comparison of the ability of partially n-acetylated chitosans and chitooligosaccharides to elicit resistance reactions in wheat leaves. *Plant Physiol*. 118: 1353-1359.
[99] Vasyukova, N.I. and Ozeretskovskaya, O.L. (2007). Induced plant resistance and salicylic resistance : A review. *Appl. Biochem. Microbiol*. 43(4) : 405-411.
[100] Verberne, M.C., Verpoorte, R., Bol, J.F., Mercado-Blanco, J., and Linthorst, H.J. (2000). Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen resistance. *Nat. Biotech*. 18: 779–783.
[101] Vidyasekharan, P. (1997). Fungal pathogenesis in plants and crops : Molecular biology and host defence mechanisms. Marcel Dekker, New York, pp. 553.

[102] Vivekananthan, R., Ravi, D., Saravankumar, N., Kumar, V. Prakashan and Samiyappan, R. (2004). Microbially induced defense related proteins against post-harvest anthracnose infection in mango. *Crop. Prot*. 23 : 1061-1067.
[103] Wang, J. and Higgins, V.J. (2006). Nitric oxide modulates hydrogen peroxide mediated defenses in the *Colletotrichum coccodes*-tomato interaction. *Physiol. Mol. Plant Pathol.* 67: 131-137.
[104] Wang, X., Kobiler, I., Lichter, A., Leikin-Frenkel, A., and Prusky, D. (2004). Enhanced expression of avfae1 encoding a long-chain fatty acid elongase during the induction of antifungal diene in avocado fruits. *Physiol. Mol. Plant. Pathol*. 65: 171-180.
[105] Wendehenne, D., Pugin, A., Klessig, D. F., and Durner, J. (2001). Nitric oxide:comparative synthesis and signaling in animal and plant cells. *Trends Plant Sci.* 6: 177–183.
[106] Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature.*
414: 562–565.
[107] Wolski, E. A., Lima, C., Agusti, R., Daleo, G. R., Andreu, A. B. and de Lederkremer, R. M. (2005). An □-glucan elicitor from the cell wall of a biocontrol binucleate *Rhizoctonia* isolate. *Carbohydrate Research.* 340(4) : 619-627.
[108] Wu, G., Shortt, B. J., Lawrence, E. B., Fitzsimmons, K. C., Shah, D. M. (1995). Disease resistance conferred by expression of a gene encoding H_2O_2-generating glucose oxidase in transgenic potato plants. *Plant Cell.* 7: 1357–1368.
109] Wu, J.Y. and Lin L. D. (2002). Ultrasound-induced stress responses of *Panax ginseng* cells: Enzymatic browning and phenolics production. *Biotechnology Progress.* 18: 862–866.
[110] Yamamoto, T. and Bing, R. J. (2000). Nitric oxide donors, *P.S.E.B.M*. 225 : 200-206.
[111] Zhao, H., Zhao, H., Wang, B. and Wang, J. (2005). Effect of local stress induction on resistance-related enzymes in cucumber seeding. Colloids and surfaces B : Biointerfaces. 43(1): 37-42.

[112] Zhou, M., & Wang, W. (2018). Recent Advances in Synthetic Chemical Inducers of Plant Immunity. *Frontiers in plant science*, *9*, 1613. doi:10.3389/fpls.2018.01613
[113] Zhu, Shijiang., and Baocheng, M. A. (2007). Benzothiadiazole- or methyl jasmonate-induced resistance to *Colletotrichum musae* in harvested banana fruit is related to elevated defense enzyme activities. *J. Hort. Sc. Biotech.* 82(4): 500-506.
[114] Zhu X, Lin H, Si Z, Xia Y, Chen W, Li X (2016) Benzothiadiazole-Mediated Induced Resistance to *Colletotrichum musae*and Delayed Ripening of Harvested Banana Fruit J. Agric. Food Chem. 64 (7), pp 1494–1502. DOI: 10.1021/acs.jafc.5b05655