CHAPTER 1: INTRODUCTION

Tomato (*Lycopersicum esculentum*) is a globally important cultivated vegetable which is extensively attacked by *Pseudomonas syringae* pv. *tomato* [Barone *et al*., 2008]. Chemical methods to control bacterial and fungal pathogens are environmentally unacceptable [Luna *et al*., 2012]. Therefore, there is an explicit need to develop alternate environmentally safe methods of pest and disease control. Though many biological control methods have been developed but most of them have limitations. However, induction of Systemic Acquired Resistance (SAR) is most potent in crop plants [Vallad and Goodman, 2004]. Plant defense responses primed by SAR are followed by the enhanced expression of varied defense related proteins which provide broad-spectrum resistance against a large number of pathogens. Age is the determinant in the expression of defense related proteins [Dat *et al*., 2000; Levy and Lapidot, 2008] as well. However, the correlation between plant age and SAR induction is not well understood. Therefore, an understanding of implication of age in induction of SAR by elicitors (biological or chemical) would help in elucidating the molecular aspects of defense response due to induced SAR. Such understanding would help in development of an effective biocide capable of inducing defense response through SAR.

1.1. Age related resistance (ARR):

The ability of a plant to protect itself from any infection depends upon a number of factors including environmental conditions, nature of the infected tissue and the genotypic combination of the host species and the pathogen. Plant developmental stage is just as important, but is far less frequently taken into account. Thus the influence of plant development and its age on disease resistance is a crucial break in our understanding of plant–pathogen interactions. In most of the plant-pathogen interactions, the developmental stage of the plant plays a vital role in the expression of resistance against the invading pathogens [Develey-Rivière and Galiana, 2007]. This may reflect an increase in resistance over time, with plants already resistant to a pathogen, increasing their ability to control infection and colonization at a precise growth phase. This increase or acquisition of resistance to pathogenic infections as a function of plant development has been given several names: ‘ontogenic resistance’, ‘developmental resistance’, ‘mature seedling resistance’, ‘adult seedling resistance’ and ‘age-related resistance (ARR)’ [Whalen, 2005]. However, many studies have also reported the increase in susceptibility i.e decrease in

resistance of the plant against a pathogen as it matures [Jacome and Scuh, 1992] or absence of role of growth stage in development of resistance [Visker *et al*., 2003].

Age-related resistance may be effective against several pathogens, a particular pathovar or a given strain or race of pathogen. In cases of race-specific resistance, the expression of resistance is generally associated with the functional regulation of plant resistance (*R*) genes. The involvement of plant hormones in many aspects of plant development and plant–pathogen interactions [Raskin, 1992; Johnson and Ecker, 1998; Mayda *et al*., 2000; Mauch-Mani and Mauch, 2005] also play important part in conferring ARR to the host plant. Salicylic Acid (SA), jasmonic acid (JA), ethylene and abscisic acid (ABA) have been studied in detail [Develey-Rivière and Galiana, 2007] to determine their effects on the expression of resistance. The variation in the capacity of foliar antioxidants (largely responsible for defense in plants) with the physiological age of leaf was emphasized by Dat *et al*. (2000). Kus *et al.* (2002) enumerated the role of Salicylic Acid in the establishment of ARR in Arabidopsis.

Many studies have been published on this phenomenon within the plant kingdom in order to testify its extent. ARR may be instrumental in providing protection against a specific pathogen or has broad-spectrum activity. This resistance is thus of clear agronomic interest, but remains little explored.

1.2. Biological induction of SAR:

Indiscriminate use of agrochemicals has not only resulted in environmental pollution but also been instrumental in altering plant-microbe interaction. Therefore, new strategies of biological control of plant diseases are being developed which can either control pathogens through direct antibiosis or indirectly through the induction of systemic acquired resistance [Stangarlin *et al*., 2011]. Prophylactic treatment of plants with culture filtrates from non pathogenic bacteria and fungi could protect the plant from diseases [Kowalewski and Herger, 1992]. Daayf *et al*. (1995) reported that aqueous plant extracts could induce resistance in plants against bacterial, viral and fungal pathogens. Aqueous extracts of *Artemisia camphorata* (camphor) when sprayed prior to pathogen inoculation could induce systemic resistance in wheat against *Bipolaris sorokiniana* [Franzener *et al*., 2003]. The aqueous extract of *Eucalyptus citriodora* could induce local resistance in cucumber against *Colletotrichum lagenarium* [Bonaldo *et al.*, 2004]. Application of ginger mass to soil near base of lettuce plants reduced disease incidence due to enhanced

peroxidase activity [Rodrigues *et al*., 2007]. Enhanced chitinase activity induced by aqueous extracts of basil could protect cucumber against *Colletotrichum lagenarium* [Colpas *et al*., 2009]. Franzener *et al*. (2007) reported that flower extracts of *T. patula* could protect tomato plants against *M. incognita*. Extracts of *Cymbopogon citratus*, *Curcuma longa* and *Rosmarinus officinalis* could effectively control *Septoria glycines, Cercospora kikuchii* and *Microsphaera diffusa* respectively on soybean under field conditions [Becker *et al*., 2004]. 43.5 % reduction in disease incidence in chickpea was observed after the application of aqueous leaf extract of *Azadirachta indica* [Ghazanfar *et al*., 2010].

1.3. Systemic Acquired Resistance (SAR):

Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are the two forms of induced resistance. In both SAR and ISR, plant defenses are preconditioned either by prior infection or treatment with elicitors that results in elevated resistance (or tolerance) against subsequent challenge by a pathogen [Vallad and Goodman, 2004]. SAR and ISR (Figure 1.1) can be differentiated on the basis of the elicitor and the pathways involved [Knoester *et al*., 1999; Maleck *et al*., 2000; Yan *et al*., 2002]. According to Mauch-Mani and Metraux (1998), SAR is a salicylic acid (SA) dependent process. According to Pieterse and Van Loon (1999), Induced Systemic Resistance (ISR) is mediated by a jasmonate/ethylene sensitive pathway. Metraux (2001) stated that the terms SAR and ISR are synonymous.

Harman *et al*. (2004) demonstrated that exogenous application of certain inducers also elicit similar responses in plants. Thus, SAR is a phenomenon in which prior application of biological or chemical inducers activates the defense system of the plant against subsequent attack of bacterial, fungal or viral pathogen [Percival, 2001]. This activation of defense system is characterized by accumulation of SA, enhanced expression of pathogenesis-related (*PR*) genes, accumulation of their expression products and activation of phenylpropanoid pathway, leading to the synthesis of higher phenolic compounds toxic to microbial pathogens [Durrant and Dong, 2004]. "Pathogenesis-related (PR) proteins" is a term which collectively encompasses all microbe-induced proteins and their homologues which are generally present constitutively and increased only during most infections.

The term "inducible defense-related proteins" was used by Van Loon *et al.* (2006) to coin both the known PR-protein families and non-classified proteins meeting the criteria mentioned above.

Peroxidase (POX), Polyphenol Oxidase (PPO), Lipoxygenase (LOX) and Lysozyme (Lys) are the key enzymes which are induced by the pathogen attack or elicitors and are involved in the defense of plants [Porta and Rocha-Sosa, 2002; Wang *et al*., 2005; Bhuvaneshwari and Paul, 2012].

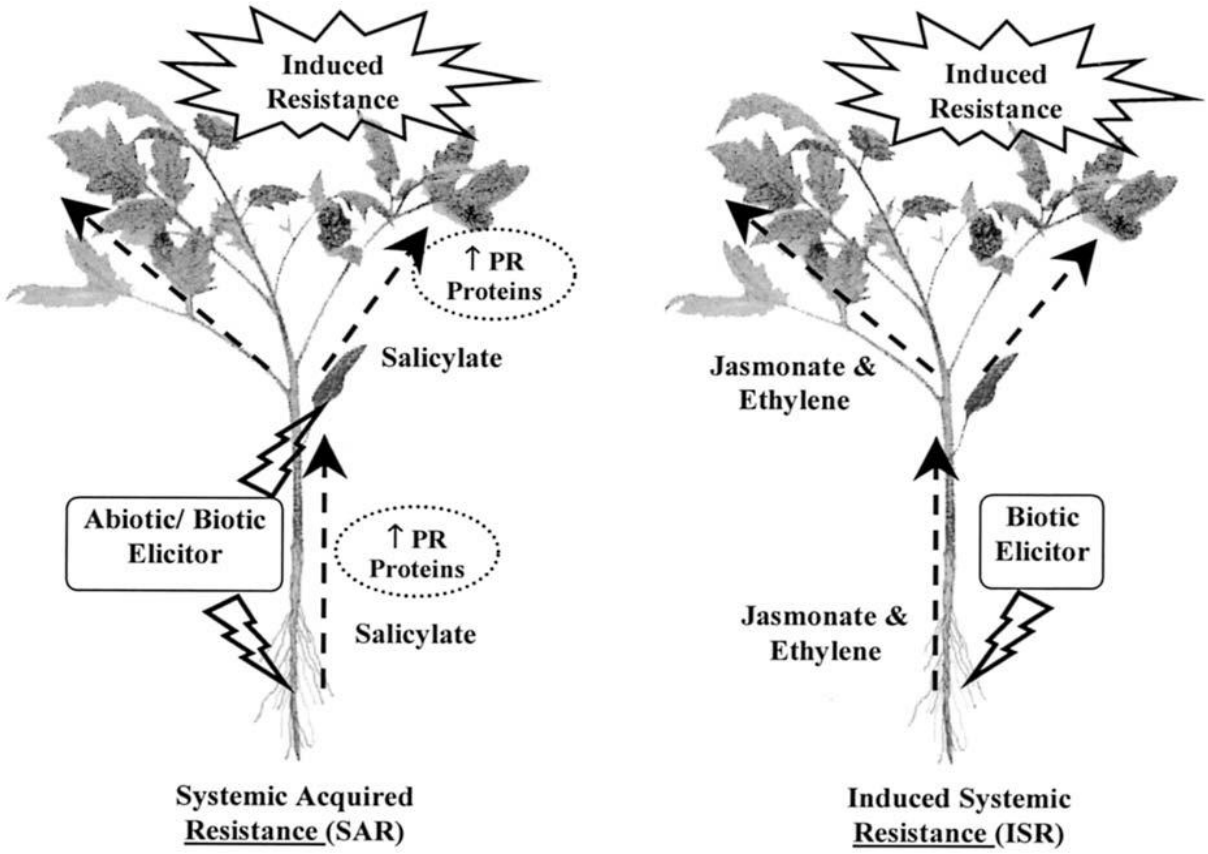

Figure 1.1. A pictorial comparison of SAR and ISR.

[Source: Vallad and Goodman, 2004]

1.3.1. Priming during SAR:

Systemic acquired resistance can be induced by non-pathogenic microbes, plant extracts or synthetic compounds such as salicylic acid or β-aminobutyric acid (BABA). Induced resistance is often associated with an enhanced capacity to mobilize infection-induced cellular defense responses–a process called 'priming' [Conrath *et al*., 2002]. *NPR1* (also known as *NIM1* or *SAI1*) is a key regulator of priming that has a major effect on the regulation of cellular plant defense responses [Shi *et al.*, 2012]. Activation of local and systemic plant defenses in response to pathogen attack involves dramatic cellular reprogramming. Benhamou and Nicole (1999)

could demonstrate that SAR response to restrict pathogen entry involves changes in cell biochemistry and physiology along with structural modifications including the formation of callose-enriched wall appositions and the infiltration of phenolic compounds at sites of pathogen penetration including activation of the phenylpropanoid pathway. Priming leads to more robust induction of defense responses and resistance, which may include improved perception and/or amplification of the defense response-inducing signal from the pathogen. It is associated with increased accumulation and/or posttranslational modification of inactive cellular signaling proteins that play an important role in signal amplification. Subsequent exposure to stress could activate, or modulate these ‘‘dormant’’ signaling proteins, thereby initiating the signal amplification leading to faster and/or stronger activation of defense responses and SAR [Conrath *et al*., 2006]**.** Priming in plants involves enhanced expression and *de novo* synthesis and accumulation of pathogenesis-related proteins in uninfected tissues, thereby protecting them against any future pathogen attack [Ramos Solano *et al*., 2008]. Conrath *et al*. (2006) therefore rightly stated that “Priming: Getting ready for battle”. Though the phenomenon of priming is known for long, yet the associated molecular mechanisms still need to be elucidated [Tonelli *et al*., 2011].

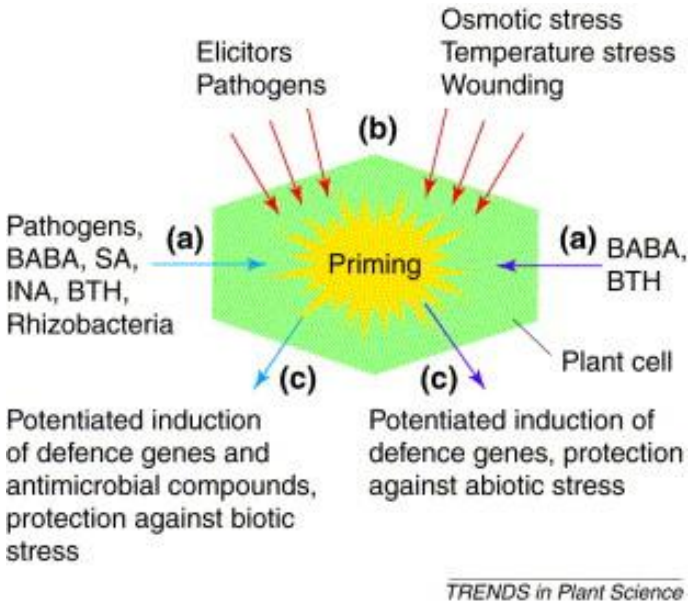

Figure 1.2. “Priming” induced by different elicitors.

[Source: Conrath *et al*., 2002]

1.4. Pathogenesis-related (PR) proteins:

Pathogenesis-related proteins (PRs) are defined as plant proteins that are induced in pathological or related situations [Van Loon *et al.*, 1994]. The term pathological condition is used here to indicate direct pathogen attack of different origins including fungi, bacteria, viruses, insects and herbivores. Related situations include:

(i) the application of chemicals that mimic the effect of pathogen attack [e.g. the plant hormones ethylene (ET), jasmonate (JA) and salicylic acid (SA)], and

(ii) wound-induced responses that give rise to proteins which also accumulate during infections [Sels *et al*., 2008].

The use of this term was specified to indicate proteins induced by various types of pathogens as well as stress conditions such as those provoked by pathogens, and those induced by the application of chemicals that mimic the effect of pathogen infection or induce similar stresses [Van Loon *et al.*, 1994]. The PRs are known to be the products of SAR genes and their accumulation is a hallmark of pathogen-induced systemic acquired resistance. Upon infection with various types of pathogens, defense-related genes are coordinately activated and may be expressed in both infected and non-infected tissues concomitant with the development of SAR [Van Loon *et al*., 2006]. ROS (reactive oxygen species) mediated synthesis of PRs and their induction by plant cell wall fragments has also been noted [Edreva, 2005]. The PRs are regulated by the defense regulatory SAR and ISR-mediating hormones such as Salicylic acid (SA), Jasmonic acid (JA) and Ethylene (ET) which therefore suggests that they play an important role in alleviating the effects of attack by pathogens [Van Loon *et al*., 2006]. Toxicity of PRs towards the pathogens is generally attributed to their hydrolytic, proteinase-inhibitory and membrane-permeabilizing ability [Edreva, 2005]. Based on the sequence similarities, serologic or immunologic relationships, and enzymatic properties, the PRs from different plants have been classified into seventeen families (Table 1.1) [Van Loon *et al*., 1994; Van Loon and Van Strien, 1999; Ebrahim *et al*., 2011].

Family	Type	Properties	Gene symbols
PR-1	tobacco PR-1a, tomato PR-1b1, PR-1b2, P4, P6, P14,	antifungal	Ypr 1

	P14a, P14b, P14c, C2, C4		
PR-2	tobacco PR-2, tomato Cel1 EGase, P3, P5, C3, C5, P31, P36, Q'a, Q'b	class I, II, and III endo-beta-1,3-glucanases	Ypr 2, [Gns2 ('Glb')]
PR-3	tobacco P, Q tomato C6, C7, P26, P30, P31, P32, P34	class I, II, IV, V, VI, and VII endochitinases	Ypr 3, Chia
PR-4	tobacco R tomato P2	antifungal, *win*-like proteins, endochitinase activity, similar to prohevein C-terminal domain	Ypr 4, Chid
PR-5	tobacco S, tomato AP24, NP24, P23	antifungal, thaumatin-like proteins, osmotins, zeamatins, permeatins, similar to alpha-amylase/trypsin inhibitors	Ypr 5
PR-6	tomato inhibitor I	protease inhibitors	Ypr 6, Pis ('Pin')
PR-7	tomato P_{69}, P_{70}, Rcr3	endoproteases	Ypr 7
PR-8	cucumber chitinase	Class III chitinases, chitinase/lysozyme	Ypr 8, Chib
PR-9	lignin-forming peroxidase Cevi-1, TPX1, TPX2	peroxidases, peroxidase-like proteins	Ypr 9
PR-10	parsley PR-1, tomato STH-2	ribonucleases, Bet v 1-related proteins	Ypr 10, Prx
PR-11	tobacco class V chitinase	Class 1 endochitinase activity	Ypr 11, Chic

PR-12	radish Ps-AFP3, tomato tgas118	plant defensins	Ypr 12
PR-13	*Arabidopsis* THI2.1, Tomato Thi2.1	thionins	Ypr 13, Thi
PR-14	barley LTP4, tomato LpLtp1, LpLtp2, LpLtp3	nonspecific lipid transfer proteins (ns-LTPs)	Ypr 14, Ltp
PR-15	barley OxOa (germin)	oxalate oxidase	Ypr 15
PR-16	barley OxOLP	oxalate-oxidase-like proteins	Ypr 16
PR-17	tobacco PRp27	unknown	Ypr 17

Table 1.1. Classification of Pathogenesis related (PR) proteins.

[Source: Van Loon *et al*, 2006; Ebrahim *et al*, 2011]

1.4.1. Peroxidase (POX):

Peroxidases (POXs) are haem-containing glycoproteins found in animal and plant tissues, as well as in microorganisms. The class III family of plant peroxidases (POX, EC 1.11.1.7) encoded by a large multigene family comprises of a number of peroxidase isoenzymes [Hiraga *et al*., 2001]. Peroxidases generally oxidize a wide variety of compounds in the presence of hydrogen peroxide (H_2O_2).

$$2RH + H_2O_2 \longrightarrow 2R^{\circ} + 2H_2O$$

Gaspar *et al*. (1991) reported that peroxidases are involved in auxin and ethylene metabolism, redox reactions in plasma membranes, cell wall modifications (lignification and suberinization) as well as in developmental and defense processes (Figure 1.3). Studies revealed that, inspite of low substrate specificity, peroxidases are sensitive and accurate stress markers [Lepedus *et al*., 2004]. Peroxidases, found both in the cell wall and in the cytoplasm can catalyse the oxidation of diverse hydrogen donors. POX activity is implicated in a broad range of physiological processes throughout the life of the plant, probably due to its large number of enzymatic isoforms (isoenzymes) and to the versatility of their enzyme-catalysed reactions [Passardi *et al.*, 2005]. Detoxification of activated O_2 forms, an important function of POX is very important in the formation of metabolic response of plants to different stress factors [Bybordi *et al*., 2010].

The plant peroxidases (class III) are targeted to the outside of the plant cell or to the vacuole via the endoplasmic reticulum (ER) [Welinder *et al*., 2002]. POXs can also generate active oxygen species as part of the oxidative burst during incompatible interactions [Gomez-Vasquez *et al.*, 2004]. POXs are involved in the production of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical (OH^-) and H_2O_2 as one of the earliest cellular responses following successful pathogen recognition. The production of ROS has also been related to hypersensitive response and induction of SAR in the host plant [El-Khallal *et al*., 2007; Liu *et al*., 2010].

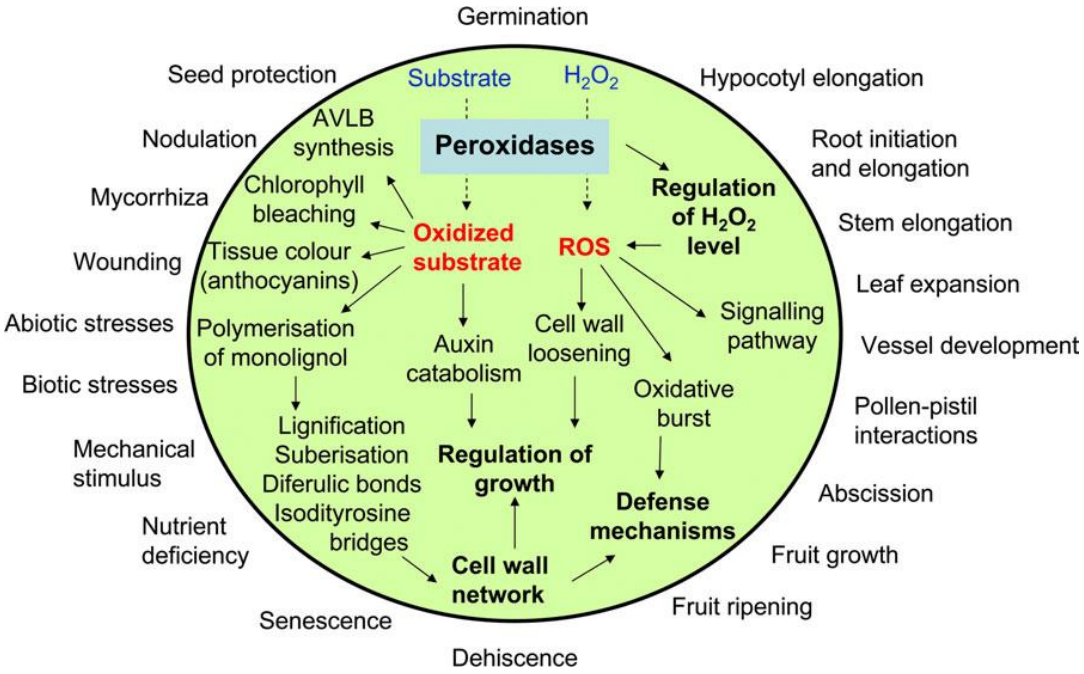

Figure 1.3. Overview of the specific roles of plant Peroxidases in defense reactions.

[Source: Cosio and Dunand, 2009]

Four possible mechanisms have been proposed to explain how ROS are produced in host plant cells:

i. one is located at the level of the external face of the plasma membrane, and is mediated by NADPH oxidases,

ii. and three are located at the level of the cell wall matrix, and which would involve the action of POXs, Poly(di)amine oxidases and Oxalate oxidases.

Unlike Poly(di)amine oxidases and Oxalate oxidases, which directly generate H_2O_2, both NADPH oxidases and POXs catalyse the initial formation of $O_2^{\cdot -}$, which later dismutates to

H_2O_2 [Almagro *et al*., 2009]. Plant POXs are able to catalyse the synthesis of bioactive plant products [Ros Barcelo´ and Pomar, 2002], and therefore a role in plant defense through their involvement in the synthesis of phytoalexins has been proposed for these enzymes. O'brien *et al*. (2012) and Daudi *et al*. (2012) also enumerated the role of POX generated ROS in the orchestration of pattern-triggered immunity in tissue culture cells of *Arabidopsis*. Plant immunity can be suppressed by inhibition of host Peroxidase activity [Hemetsberger *et al*., 2012].

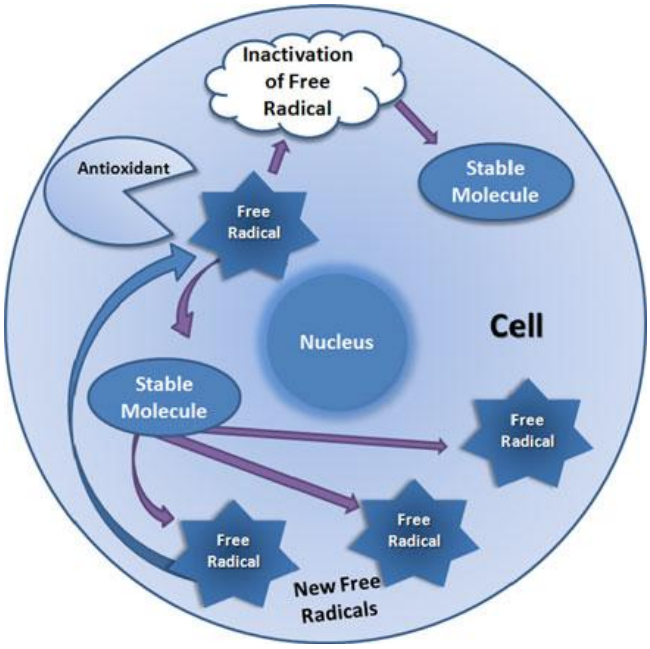

Figure 1.4. Antioxidant enzymes confiscating the free radical chain reaction.
[Source: Bhaduri and Fulekar, 2012]

1.4.2. Polyphenol Oxidase (PPO):

Polyphenol oxidases (PPOs) [EC 1.14.18.1 or EC 1.10.3.2] are ubiquitously present nuclear-encoded copper-containing enzymes which catalyze the O_2-dependent oxidation of mono and *o*-diphenols to *o*-diquinones, highly reactive intermediates whose secondary reactions are believed to be responsible for the oxidative browning which accompanies plant senescence, wounding, and responses to pathogens [Thipyapong *et al.*, 2004]. They can be found in almost all living organisms including animals, plants, fungi and bacteria.

PPO has generated interest since its first description in 1895, and several hypotheses regarding its functions during growth and development and in response to stresses have been proposed. The defensive roles of PPO against disease and insect pests have been clearly established

[Newman *et al*., 2011]. PPO involvement in aurone biosynthesis and the phenylpropanoid pathway has been proposed earllier [Vaughan and Duke, 1984; Purwar *et al*., 2012]. Pathogen-induced PPO activity continues to be reported for a variety of plant taxa, including monocots and dicots. Direct evidence about involvement of PPO in inhibiting pathogen ingress or growth has come from transgenic tomato plants with enhanced or suppressed PPO levels. When challenged by the bacterial pathogen *Pseudomonas syringae* pv. *tomato*, PPO-overexpressing tomato plants showed reduced bacterial growth, whereas PPO suppressed lines had higher disease incidence [Constabel and Barbehenn, 2008]. PPOs are also involved in the generation of reactive oxygen species, quinones, or hydroperoxides in plant cells and in the polymerization of polyphenolics, carbohydrates, and proteins in plant cell walls [Cippolini and Redman, 1999].

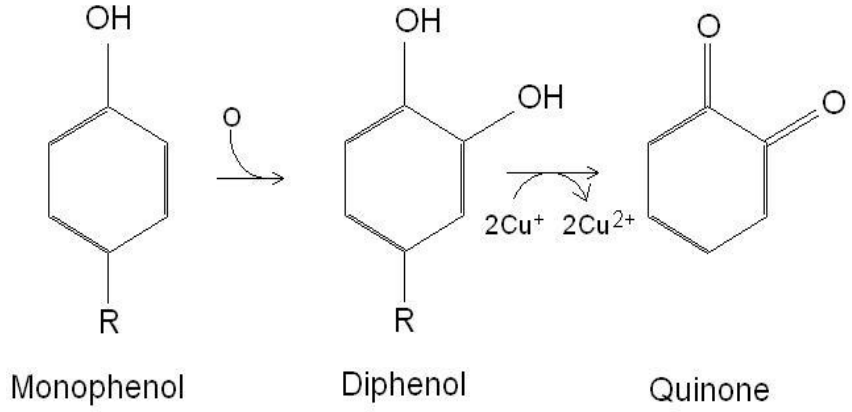

Figure 1.5. Reactions catalyzed by PPO. [Source: Queiroz *et al*., 2008]

Native-PAGE analysis showed increase in the number of PPO isoforms after inoculation with different inducers and the intensity of banding was more in resistant cultivars, proving the defense associated role of PPO [Lavanya *et al*., 2012]. Increased expression of PPO during the management of tomato root rot by a chitin-fortified *Trichoderma/Hypocrea* formulation has been reported [Solanki *et al*., 2011]. He *et al*. (2011) suggested the involvement of PPO in aphid resistance in *Chrysanthemum* as evident by its increased activity in the inoculated plants. PPO has been observed to play a vital role in imparting resistance to potato soft rot infection [Ngadze *et al*., 2012]. Inspite of many studies, the differential role of PPO in plant development and defense is poorly understood.

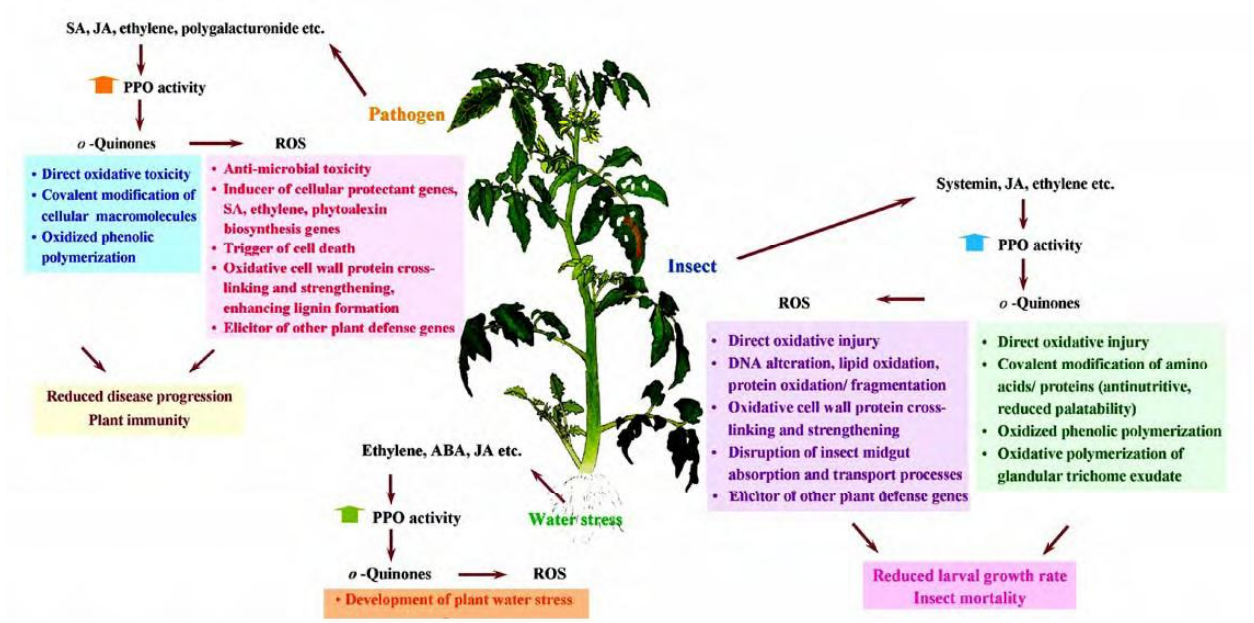

Figure 1.6. Hypothetical mechanisms of action of tomato PPO in response to pathogen infection, insect infestation and water stress. [Source: Thipyapong *et al*., 2007]

1.4.3. Lipoxygenase (LOX):

Lipoxygenases (LOXs) (linoleate: oxygen oxidoreductase, EC 1.13.11.12) are a family of enzymes found ubiquitously in plants and mammals, but have also been detected in corals, moss algae, fungi, yeast and a number of bacteria. Plant LOXs are a class of non-heme, iron-containing, monomeric proteins of about 95 to 100 kDa made of two domains. The amino-terminal domain of about 25 to 30 kDa is a beta-barrel domain (domain I). The carboxyl-terminal domain of about 55 to 65 kDa consists primarily of alpha-helices (domain II) and harbours the catalytic site of the enzyme. Plant LOXs are encoded by multigene families. LOXs can be found in all parts of the plants. LOXs catalyze the oxygenation of polyunsaturated fatty acids (PUFAs) containing *cis*,*cis*-1, 4 pentadiene moiety such as linoleic acid and linolenic acid [Hu *et al*., 2011] to convert them into hydroxyperoxides (Figure 1.7). These hydroxyperoxides are then converted into JA by the Lipoxygenase pathway (Figure 1.8), which in turn is responsible for signaling and defense response in plants [Vardar and Unal, 2011; Yang *et al*., 2011].

LOXs in response to wounding or pathogen interaction have been also described in a number of non-legume plants, like tomato, wheat, *N. attenuate*, maize and others. LOXs have also been implicated in water and drought stresses [Yang *et al*., 2011]; mobilization of storage lipids

during germination and as storage proteins during vegetative growth [Porta and Rocha-Sosa, 2002].

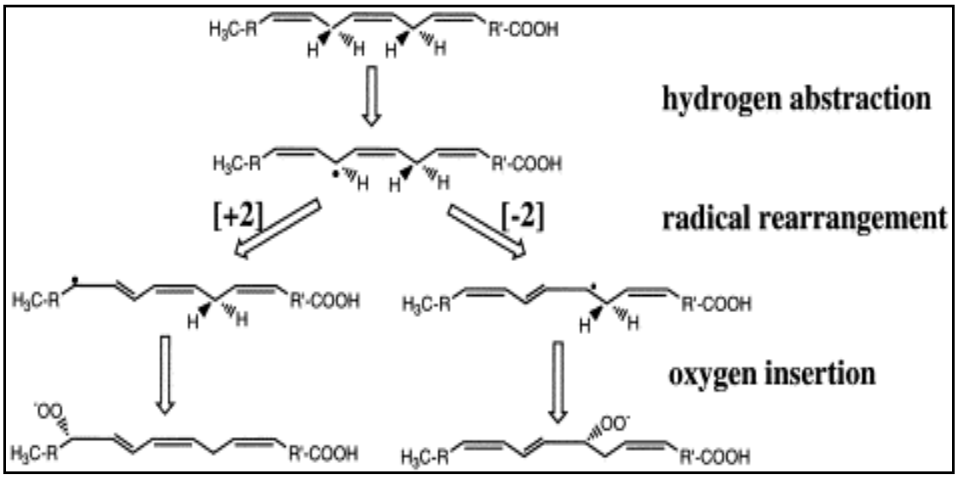

Figure 1.7. Radical mechanism of the lipoxygenase reaction.
[Adapted from: http://edoc.hu-berlin.de/dissertationen/pattabhiraman-shankaranarayanan-2003-11-03/HTML/chapter1.html]

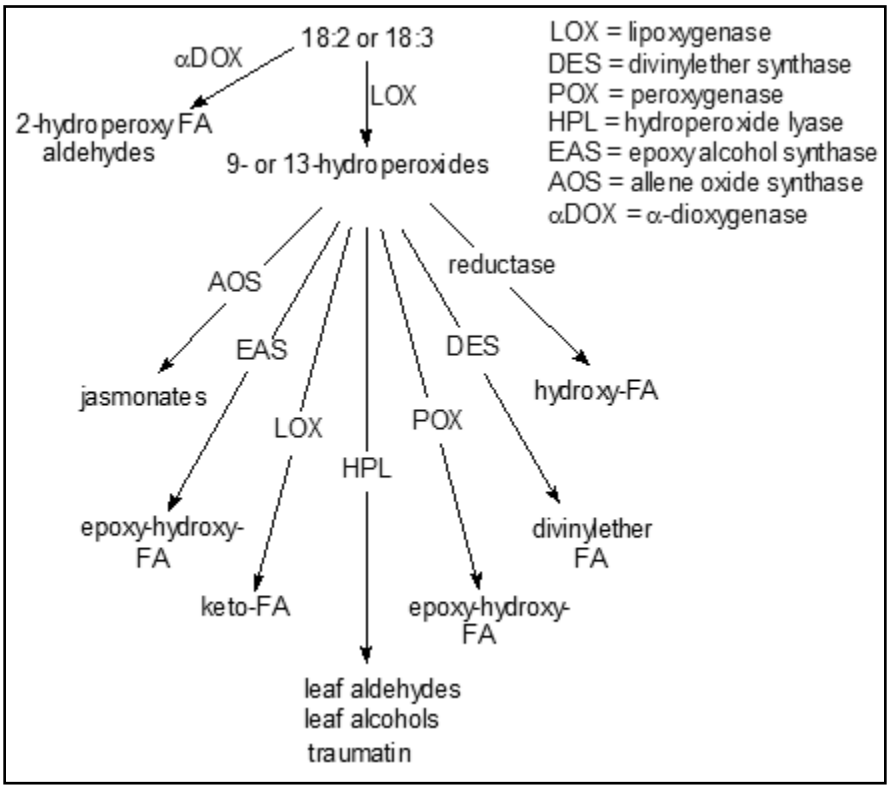

Figure 1.8. The Lipoxygenase pathway.

[Adapted from: http://lipidlibrary.aocs.org/lipids/eicplant/index.htm]

Other proposed physiological roles of LOX include membrane degradation during hypersensitive resistance responses, production of fatty acid-derived anti-microbial molecules and the synthesis of Abscisic acid (ABA) [Saravitz and Siedow, 1995]. Lipoxygenases are also known for breaking down the lipid component of membranes. The activity of these enzymes increases following inoculation with pathogens or treatment with elicitors [Peever and Higgins, 1989].

1.4.4. Lysozyme:

Lysozyme (muramidase, EC3.2.1.17) is a ubiquitous enzyme existing in numerous phylogenetically diverse organisms such as bacteria, bacteriophages, fungi, plants and animals. It was serendipitously discovered by Fleming in 1922 in human nasal mucous [Wang *et al*., 2011]. Subsequently, he reported the presence of lysozyme in many vegetable tissues, especially flowers. Lysozyme catalyzes the hydrolysis of the β-1,4-glycosidic linkage between *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) alternating sugar residues in the peptidoglycan layer present in the bacterial cell wall. The bactericidal activity of lysozyme has been hypothesized to reside in its muramidase activity leading to degradation of the murein layer and the reduction of mechanical strength of bacterial cell wall [Wang *et al*., 2005]. This cleavage reaction is believed to be the first step in breakdown of the bacterial cells walls.

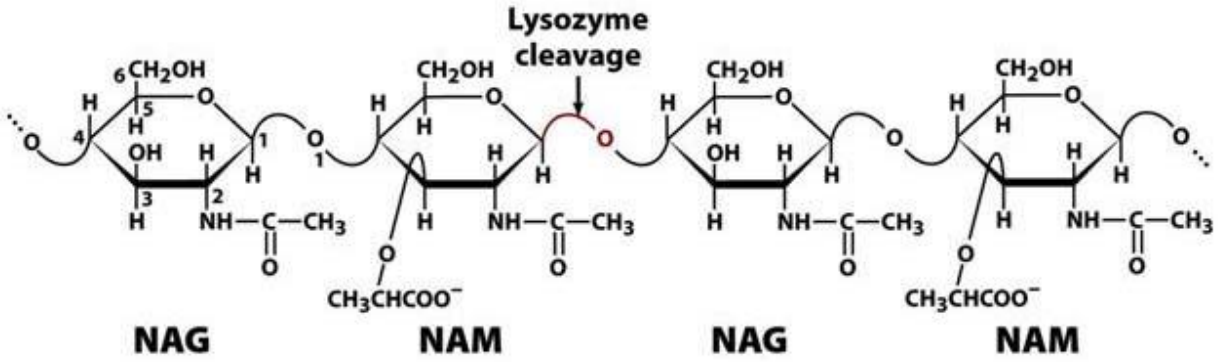

Figure 1.9. Mechanism of action of Lysozyme. [Source: Voet and Voet, 2011]

Lysozyme activity in plants was first reported in crude proteolytic enzyme preparations from papaya and fig latex [Sakthivel *et al*., 2010]. It has been widely accepted that lysozyme functions as a crucial biodefense effector in innate immunity. Recent studies have demonstrated that lysozymes could augment the activity of antibacterial peptides (AMPs) through a synergistic mechanism. In addition to the enzymatic bacteriolytic activity, some lysozymes have been demonstrated to contain independent non-enzymatic bactericidal domains [Zhao *et al*., 2007].

Van Loon *et al*. (2006) stated that lysozyme activity has been observed in members of the *PR-8* family which may be directed against bacteria.

Based on their differences in structural, catalytic and immunological characters, lysozymes have been traditionally categorized into six types [Zhao *et al*., 2007]:

i. chicken-type (c-type) lysozyme
ii. goose-type (g-type) lysozyme
iii. invertebrate-type (i-type) lysozyme
iv. phage lysozyme
v. bacterial lysozyme
vi. plant lysozyme

The plant lysozymes are shown to have both chitinase and lysozyme activities but higher in chitinase and lower in lysozyme. Almost all of the enzymes with lysozyme function have been classified as chitinases [Düring, 1993]. Beintema and Van Scheltinga (1996) had drawn a similar conclusion, "All plant lysozymes are chitinase, but only a limited number of plant chitinase are also lysozymes". The plant lysozymes can be divided into three categories based on their structural similarity to chitinase [Beintema and Van Scheltinga, 1996]:

i. hevamine-type (h-type or family 18 chitinase),
ii. barley-type (b-type or family 19 chitinase), and
iii. those without clear structural relationship to other enzymes.

They have also been categorized in chitinases Class I, II, III and IV [Beintema and Van Scheltinga, 1996]. Because of wide spectrum of substrate specificity and high lytic activity, lysozyme might have potential to protect plants from both bacterial and fungal diseases [Nakajima *et al*., 1994].

1.5. Objectives

The objectives of the present work were:

- To study the effect of fruit extracts of *Azadirachta indica* on activity of SAR gene products: Peroxidase, Polyphenol Oxidase, Lipoxygenase and Lysozyme.
- To study the induced isoenzyme profiles of Peroxidase, Polyphenol Oxidase, and Lysozyme.
- To study the changes in the expression of *CEVI1* (Peroxidase), *PPOA* (Polyphenol Oxidase) and *TomLoxD* (Lipoxygenase) genes.