CHAPTER 2: REVIEW OF LITERATURE

2.1. Age related resistance:

The induction of resistance to pests and pathogens during the life span of a plant occurs naturally. Resistance occurs at different stages of growth of plant [Millett *et al.*, 2009], alters with plant's age, and can be specific or broad spectrum, depending on various factors including host-pathogen interactions [Develey-Rivière and Galiana, 2007]. The term 'age-related resistance' (ARR) was one of the first to be proposed [Lazarovits *et al*., 1981; Kus *et al*., 2002], and is age-specific. However, the term can be used to cover all forms of resistance positively correlated with host plant development, inspite of molecular and phenotypic variations. The term differentiates developmental resistances from all other forms of resistance. The development of ARR has been reported for a large number of plants. In most of the cases, once induced this ARR persists for the rest of the life of the plant.

The relationship between plant age and disease resistance has been investigated in many plant-pathogen systems [Bateman and Lumsden, 1965; Griffey and Leach, 1965; Hunter *et al*., 1977; Ward *et al*., 1981; Chase and Jones, 1986; Chang *et al*., 1992; Heath, 1993; Rupe and Gbur, 1995].

Some plants become more susceptible to pathogens as they mature [Miller, 1983]. The susceptibility of rice was observed to be lowest at younger stages and it increased with age of plants [Padmanabhan and Ganguly, 1954]. In the intermediate stages of growth, tomato lines were observed to be slightly more resistant than the mature stages against *Phytophthora infestans* Race O [Dowley *et al*., 1975]. Dolar (1997) reported that the young Chickpea leaves were more resistant to *Ascochyta rabiei* than the mature leaves. Casas and Kaiser (1992) concluded from their studies that although the development of disease in mature plants was slower as compared to younger plants, the intensity of disease didn't vary with age. The findings of Newcombe (1998) indicated higher resistance in poplar leaves during young stages. In a study carried out at different ages of sesame plants, it was observed that four weeks old plants were least susceptible to leaf spot pathogen while plants aged six weeks or more were more susceptible [Ojiambo *et al*., 1999]. Vloutoglou and Kalogerakis (2000) reported that although tomato plants are susceptible to *Alternaria solani* at all stages of growth, yet the susceptibility increased with age of the plants. Roach (2001), supported the earlier observations that older *Plantago lanceolata* were more

vulnerable to environmental stresses and pathogen attacks. Exogenous application of Salicylic acid (SA) in sweet cherry fruits resulted in increased induction of resistance *Penicillium expansum* in younger plants [Chan *et al.,* 2008]. Ripening of tomato was found to be associated with susceptibility towards *Botrytis cinerea* [Cantu *et al*., 2009]. Millett *et al*. (2009) too reported that the younger potato plants are more resistant than the senescing plants towards the Foliar Blight disease. The increase in severity of Brown streak disease along with age in *Cassava* varieties has been studied by Rwegasira and Rey (2012).

However most of the plants are more resistant to pathogens as they mature. Griffin and Hunt (1972) observed that older *Alfalfa* plants had fewer galls. Matured groundnut plants were resistant to rust (*Puccinia arachidis*) [Savary, 1986]. The plant-pathogen systems of wheat/ *Puccinia recondite* f.sp. *tritici* [Pretorius *et al*., 1988] and tobacco/ *Peronospora tabacina* [Reuveni *et al*., 1986] exhibited more resistance in old plants. The gradual increase in resistance of pepper plants to *Phytophthora capsici* with increasing age is also a function of ARR [Kim *et al*., 1989]. Decrease in susceptibility with increasing leaf age in the rice/*Xanthomonas campestris* pv. *oryzae* model system has been observed by Koch and Mew (1991). Roumen (1992) and Roumen *et al*. (1992) observed the decrease in the number of sporulating lesions in the leaves of all rice genotypes with the increase in age. Dickson and Petzoldt (1993) reported that resistance in broccoli against downy mildew was dependent on plant age. The presence of ARR in stems of Korean tomato cultivars was reported by Hwang and Hwang (1993). Similarly, plant's physiological age influenced the expression of genetic resistance in celery to beet armyworm [Diawara *et al*., 1994]. Hong *et al*. (2001) demonstrated that the penetration of *Colletotrichum coccodes* in leaf of Pepper plants at mature stage was very limited thereby reducing infection. The association of ARR during transition from vegetative to flowering stage and subsequent induction of resistance against *Peronospora parasitica* in *Arabidopsis* was studied by Zhao *et al.* (2005). Subsequent study by Hansen *et al.* (2005) also supported the possible role of ARR in plant defense. Zeier (2005) demonstrated that younger leaves of *Arabidopsis* had predominantly more pronounced inducible defenses than older but non-senescent leaves to achieve a similar degree of resistance against *Pseudomonas syringae.* The synthesis of the defense signal SA was also greater in younger leaves. The results obtained by Mandal *et al*. (2007) demonstrate that mature tobacco plants were more resistant against *Tomato spotted wilt virus* (TSWV) infection. The observations of Lemessa and Zeller (2007) on pepper

and tobacco plants indicated the existence of higher resistance in older leaves against *Ralstonia solanacearum*. Younger tomato leaves were susceptible to *Yellow leaf curl virus* as compared to the mature aged leaves [Levy and Lapidot, 2008]. The positive correlation between resistance and age has been reported in *Solanum tuberosum* [Mutty and Hossenkhan, 2008], where four out of six test varieties showed increased resistance against *Phytophthora infestans* when the plants reach mature stages of growth. Ando *et al*. (2009) observed an increase in resistance in Cucurbits against *Phytophthora capsici* as the leaves matured from young waxy green to mature green stage. Shibata *et al.* (2010) observed that the younger leaves of *Nicotiana benthamiana* exhibited lesser amount of resistance than mature leaves against *Phytophthora infestans* which could be increased by foliar application of a SA analog.

Age-related resistance can have implications for appropriate disease control strategies and timing for different biocide applications to target susceptible stages of plant development for their protection from invading pathogens.

2.2. Biological induction of SAR in plants:

Induction of systemic resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by extracts from spinach and rhubarb leaves was demonstrated by Doubrava *et al*. (1988). An aqueous formulation of concentrated extracts from leaves of the giant knotweed (*Reynoutria sachalinensis*) when applied weekly at a concentration of 2%, provided control of powdery mildew (*Sphaerotheca fuliginea*) on long English cucumber [Daayf *et al*., 1995]. Foliar application of methanolic leaf extracts of *Datura metel* effectively reduced the incidence of sheath blight and bacterial blight diseases of rice under greenhouse condition [Kagale *et al*., 2004]. Aqueous extract (10%) from leaves of zimmu (*Allium sativum* L. X *Allium cepa* L.) when applied as foliar spray to first and second leaves of cotton plants induced systemic resistance in third and fourth leaves when challenged with *Xanthomonas campestris* pv. *malvacearum* [Satya *et al*., 2007]. The inhibitory effects of powdered leaves of *Hyptis suaveolens, Withania somnifera, Eucalyptus citriodora,* peel powder of *Citrus sinensis, Punica granatum* and *Citrus medica, Pongamia* cake and neem cake on the growth of *Aspergillus flavus* in soybean seeds during storage has been reported [Krishnamurthy *et al*., 2008]. Severity of *Phytophthora infestans* in tomato seedlings was substantially reduced by foliar spray with garlic juice [Portz *et al*., 2008].

Leaf extracts of *Datura metel* protected pearl millet crops by inducing resistance against downy mildew disease (*Sclerospora graminicola*) [Devaiah *et al*., 2009]. Aqueous plant extracts of *Punica granatum, Hibsicus sabdariffa* and *Eucalyptus globulus* significantly reduced the severity of bacterial wilt disease in potato [Hassan *et al*., 2009]. Mixtures of plant growth promoting rhizobacteria (PGPR) strains and Zimmu leaf extract were effective in induction of systemic resistance in tomato plants against *Alternaria solani* [Latha *et al*., 2009]. Application of leaf extracts of *Mirabilis jalapa, Datura metel* and neem oil reduced incidence of *Mungbean Yellow Mosaic Virus* (MYMV) with increased yield in black gram under field conditions [Venkatesan *et al*., 2010]. *Adathoda vasica* leaf extract possessed the ability to induce the activity of defense enzymes in rice which can be associated with induction of resistance against bacterial leaf blight [Govindappa *et al*., 2011]. Subsequently Kagale *et al*. (2011) demonstrated induction of systemic resistance in rice by leaf extracts of *Ipomoea carnea* and *Zizyphus jujube* against *Rhizoctonia solani.* Leaf extract of *Podophyllum hexandrum* was effective in minimizing the disease incidence and producing better yield upon foliar application in potato against late Blight [Majeed *et al*., 2011]. Ethanolic leaf extracts of *Cymbopogan citrus* and *Ocimum sanctum* induced systemic resistance in Rice against Sheath Blight Disease [Pal *et al*., 2011]. Nashwa and Abo-Elyousr (2012) reported that application of leaf extracts of *A. sativum* reduced disease severity as well as increased fruit yield in tomato plants. Aqueous extract of leaves from *Bauhinia variegata* could control *Bipolaris sorokiniana* and protected barley plants through increased levels of salicylic acid [Bach *et al*., 2012]. Antimicrobial activities of methanolic extracts of *Aframomum melegueta* and *Zingiber officinale* could control fungal diseases of tomato fruits, thereby increasing their shelf life [Chiejina *et al*., 2012].

2.3. Broad spectrum antimicrobial activity of Neem extracts:

Neem extracts are known to exhibit a wide range of antifungal, antibacterial, antiviral and antioxidant properties [Subapriya and Nagini, 2005]. Due to the broad spectrum applicability of neem extracts in different forms against a wide array of pathogens, it has been called "Neem- a green treasure" [Girish and Shankara, 2008]. Preliminary studies carried out by several investigators showed significant inhibitory effects of neem extracts on several bacterial strains [Chopra *et al*., 1952; Rao *et al*., 1986]. The bark extracts of neem could suppress the *in vitro* growth of *Streptococcus sobrinus* [Bhuiyan *et al*., 1997]. Wet rot disease of *Amaranthus* sp.

caused by *Choanephora cucurbitarum* was controlled with bark and root extracts of *A. indica* [Olufolaji, 1999]. The results of work by Hoque *et al.* (2007) suggested that neem extracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and spoilage organisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Alcaligenes faecalis* and *Aeromonas hydrophila*. Neem oil suppressed *in vitro* growth of several species of pathogenic bacteria such as *Staphylococcus aureus, Mycobacterium tuberculosis*, *Salomonella paratyphi* and *Vibrio cholerae*. *In vitro* antibacterial activity of neem oil showed 92% susceptibility against *Pseudomonas aeruginosa, Streptococcus pyogenes, Escherichia coli* and *Klebsiella aerugenes* [Jahan *et al.,* 2007]. Ethanolic extracts of neem leaf showed considerable antibacterial activity against *Xanthomonas* and antifungal activity against *Fusarium* [Perumal *et al*., 2008].

Neem formulations were found effective in protecting the roots of tomato against nematode *Meloidogyne javanica* [Javed *et al*., 2008]. Rajasekaran *et al.* (2008) reported the antibacterial efficacy of neem leaf extracts against several Gram positive and Gram negative bacteria. Moslem and El-Kholie (2009) demonstrated *in vitro* antifungal activities of neem leaf and seed extracts against *Fusarium oxysporum, Alternaria solani, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The alcoholic extracts of neem leaf inhibited different fungal species [Mondall *et al*., 2009]. The constituents like azadirectin and nimbin isolated from the methanolic extracts of seed, leaf and bark of neem were found to exhibit antioxidant properties [Amal *et al*., 2009]. The methanolic extracts of *Azadirachta indica* exhibited anti microbial property against many species of microbes [Grover *et al*., 2011]. Leaf extract of *A.indica* could inhibit the rot fungi (*Aspergillus niger, F. oxysporum, Rhizopus stolonifer* and *Geotrichum candidium*) isolated from the rotten tomatoes [Yeni *et al*., 2010]. The antibacterial activity of methanolic extract of neem leaves against *Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus pumilus* has been reported by Maragathavalli *et al.* (2012). Reddy *et al.* (2012) demonstrated that aqueous extract of vermi-composted neem leaves had *in vitro* inhibitory effect against *Xanthomonas campestris* and recommended for use to control tomato bacterial spot in fields. The antibacterial effect of *A. indica* bark extract against *Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella ozanae* and *Escherichia coli* was reported by Abalaka *et al*. (2012). The methanol extract of *Azadirachta indica* leaves exhibited pronounced activity against *Bacillus subtilis* [Hashmat *et al*., 2012].

2.4. Induction of resistance in plants by Neem extracts:

Neemazal, a product of neem (*A. indica*), induced resistance in pea (*Pisum sativum*) against *Erysiphe pisi* [Singh and Prithviraj, 1997]. Oil extract of seeds and aqueous ethanol leaf extracts of *A. indica* significantly reduced spread of blast disease in rice plants under greenhouse conditions [Amadioha, 2000]. Neem seed kernel extract was effective in controlling the powdery mildew disease and increasing the grain yield of blackgram under pot culture experiment [Rettinassababady *et al*., 2000]. Aqueous extract of leaves of neem provided control of leaf stripe pathogen (*Drechslera graminea*) on barley indirectly by inducing plant defense reactions [Paul and Sharma, 2002]. Greenhouse-grown tomato and pepper plants sprayed with aqueous suspensions of neem oil and then inoculated with *Xanthomonas campestris* pv. *vesicatoria* showed lesser disease symptoms than the water-treated controls [Abbasi *et al.,* 2003]. Neem seed powder significantly reduced the disease severity of *Fusarium* and root-knot in both screenhouse and field grown tomato plants [Agbenin *et al*., 2004]. Aqueous leaf extract of neem could control *Alternaria sesami* on sesame (*Sesamum indicum* L: *Syn. S. orientale* L) [Guleria and kumar, 2006]. Javed *et al*. (2007) reported the persistence of systemic effects of neem formulations against root-knot nematode, *Meloidogyne javanica.* Trilogy, a product of neem, induced resistance in Cucumber against *Podosphaera xanthii* [Aboellil, 2007]. Reduced incidence of *A. flavus* after the application of neem cake in soybean during storage conditions was observed by Krishnamurthy *et al*. (2008). All concentrations of neem extract effectively suppressed the mycelial growth of pathogenic fungi and this effect gradually increased with its increasing concentration against early blight and wilt diseases of tomato [Hassanein *et al*., 2008]. The post-harvest deterioration of plum (*Prunus salicina*) or Yali pear (*Pyrus bertschneideri*) inoculated with the respective pathogens could be prevented remarkably by treating harvested fruits with Neem extract [Wang *et al*., 2010]. Results have shown that neem seed powder significantly reduced the disease severity of *Fusarium* and root-knot nematode in tomato [Hadian *et al*., 2011]. Spraying cabbage plants with neem extracts significantly reduced the numbers of pests as compared to the control plants [Baidoo and Adam, 2012]. It was recommended by Nwogbaga and Utobo (2012) that neem seed extract be used as an alternative to synthetic inorganic chemicals in combination with black beauty eggplant variety for the management of fungal diseases in eggplant. Bhuvaneshwari *et al*. (2012) have shown that neem fruit extract induced systemic resistance in barley against *D. graminea*.

2.5. SAR in plants:

An analysis of hypersensitive and induced resistance responses have shown that plants employ an array of defense strategies for survival, from the sacrifice of some cells to salvage the plant as a whole, to the synthesis of antimicrobial compounds [Develey-Riviere and Galiana, 2007].

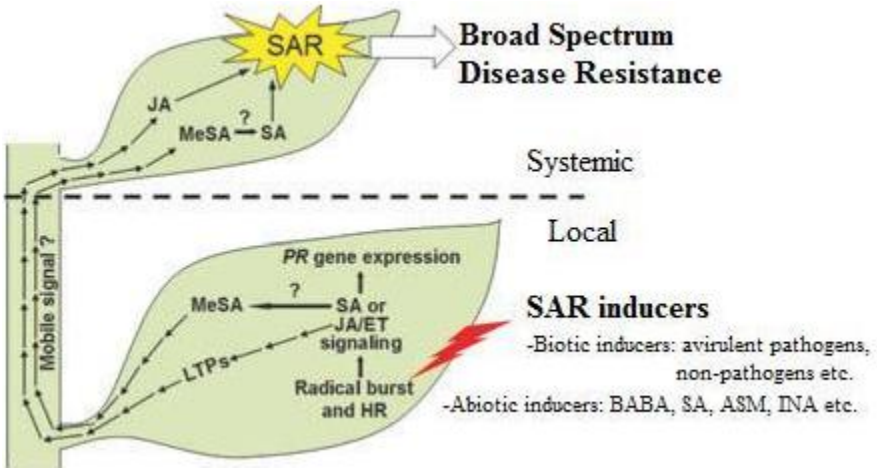

Figure 2.1. The sequence of defense signalling events from the recognition of biotic and abiotic inducers to the establishment of systemic acquired resistance (SAR) in pepper plants.

[Source: Choi and hwang, 2011]

Systematic study of this phenomenon in tobacco (*Nicotiana tabacum*) revealed that SAR persists for at least 20 days [Ross, 1961]. SAR in plants can be induced by pathogens [Funnel *et al*., 2004], natural or synthetic chemicals [Kessman *et al*., 1994], wounding [Schweizer *et al*., 1998] and plant extracts [Hassan *et al*., 2009]. This induction is followed by increased *de novo* synthesis and distribution of Salicylic acid, both locally and systemically [Meuwly *et al*., 1995], thereby acting as a signal responsible for systemic induction of resistance [Gaffney *et al.*, 1993]. Jasmonic acid (JA), its derivative Methyl jasmonate (MeJA) and ethylene have also been implicated in signaling for induction of SAR [Pieterse and Loon, 1999; Park *et al*., 2007].

2.5.1. Priming:

The first systematic investigation of priming in plant cell suspension cultures was carried out by Kauss *et al.* (1992). The first evidence that priming plays a role in rhizobacteria-mediated ISR came from experiments with carnation (*Dianthus caryophyllus*), where treatment with *Pseudomonas fluorescens* WCS417r mediated an accelerated rise in phytoalexin levels upon inoculation with *Fusarium oxysporum* f.sp. *dianthi* [Van Peer *et al.*, 1991]. Priming 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]**.** In *Arabidopsis*, it was demonstrated that treatment with the rhizobacteria does not directly activate defense-related genes, rather confers priming for enhanced expression of JA- and ethylene-inducible genes upon infection by *P. syringae* [Van Wees *et al.*, 1999; Verhagen *et al.*, 2004]. It was reported that the application of certain chemicals indirectly accelerated the expression of Phenylalanine ammonia lyase (PAL) and other PR proteins [Mur *et al.*, 1996; Kohler *et al.*, 2002]. β-amino butyric acid (BABA) primes SA-inducible *PR-1* expression in *Arabidopsis* [Zimmerli *et al.*, 2000; Zimmerli *et al.*, 2001; Ton *et al*., 2009]. Slaughter *et al.* (2012) demonstrated that treatment of *Arabidopsis* with BABA or inoculation with avirulent bacteria induces priming against *P. syringae* pv. *tomato* DC3000 which was correlated with the increased levels of SA-dependent gene transcripts of *PR1*, *PR2* and *PR5* upon infection, indicating general changes in the regulatory mechanisms of defense gene expression.

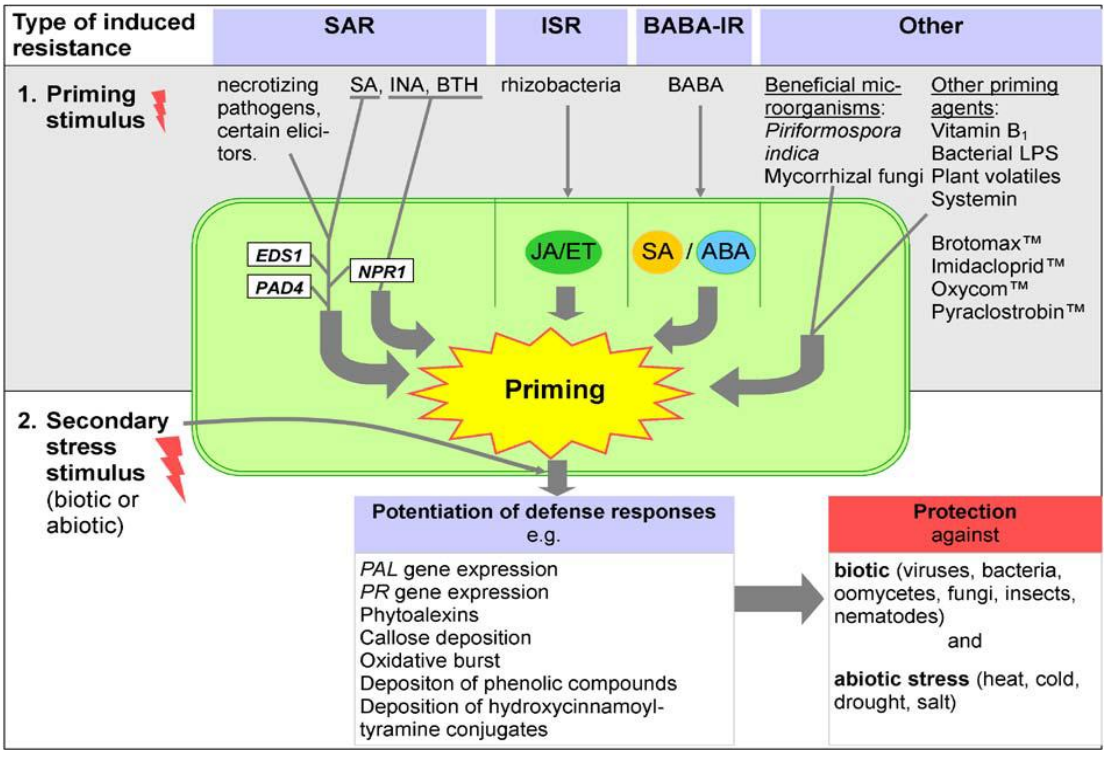

Figure 2.2. Events associated with induced resistance and priming phenomena in plants.

[Source: Goellner and Conrath, 2008]

A role for *NPR1* gene in priming of SA-mediated defense response was demonstrated by Kohler *et al.* (2002) and Ahn *et al*. (2007). Exogenous application of Riboflavin activates jasmonate mediated priming of phenylpropanoid pathway during the induction of resistance in rice against *R. solani* [Taheri and Tarighi, 2010]. The observations made by Sharifi-Sirchi *et al*. (2011) demonstrated that green tea extract induces resistance in lime plants against *Xanthomonas citri* subsp. *citri* by priming the expression of PR proteins. A precise control of synthesized Nitric Oxide (NO) in cooperation with reversible s-nitrosothiols (SNO), storage and epigenetic modifications might play an important role in integrating and coordinating potato defense responses in the priming phenomenon [Floryszak-Wieczorek *et al*., 2012]. The *PR1* and *PR2* target genes are upregulated locally and systemically in tobacco plants following treatment with grape marc extract [Goupil *et al*., 2012]. Recent studies also suggest that SAR can be inherited from one generation to another. Compared to progeny from control, progeny from *Pst*DC3000-inoculated *Arabidopsis* were primed to activate salicylic acid (SA)-inducible defense genes and were more resistant to the (hemi)biotrophic pathogens *Hyaloperonospora arabidopsidis* and *Pst*DC3000. This transgenerational SAR stayed over one stress-free generation, indicating an epigenetic basis of the phenomenon [Luna *et al*., 2012].

2.6. Expression of Pathogenesis related (PR) proteins during SAR:

Fraser (1982) pointed out that PRs became apparent in non-inoculated leaves distinctly later than the manifestation of acquired resistance. However, in tissues already primed to express PRs, challenge inoculation might lead to their earlier and faster accumulation. Moreover, a hybrid between *N. glutinosa* and *N. debneyi* constitutively expressed PRs and was highly resistant against *Tobacco Necrosis Virus* (TNV) [Ahl and Gianinazzi, 1982]. Induction of PRs has since been found to be invariably linked to necrotizing infections giving rise to systemic resistance development, and has been accepted as a marker of the induced state [Van Loon, 1997]. Alexander *et al*. (1993) demonstrated that constitutive high-level expression of *PR-la* in transgenic tobacco results in tolerance to infection against oomycete pathogens. Pre-treatment of tomato fruit with methyl salicylate (MeSA) or methyl jasmonate (MeJA) induces the synthesis of some stress proteins (such as PR proteins), which leads to increased chilling tolerance and resistance to pathogens, and thus decreasing the incidence of decay [Ding *et al*., 2002]. The susceptible wheat heads pre-treated with an autoclaved mycelial wall preparation showed

induced resistance against Fusarium head blight (FHB) along with increased activities of POX and PPO [Mohammadi and Kazmi, 2002]. The seeds treated with *Pseudomonas fluorescens* lead to accumulation of higher phenolic compounds and higher activities of POX and PPO which may play a role in defense mechanism of maize plants against *R. solani* f. sp. *sasakii* [Sivakumar and Sharma, 2003]. A new variety of *Nicotiana edwardsonii*, named *N. edwardsonii* cv. *Columbia*, expressed PR proteins in a temporal manner and also exhibited enhanced resistance to *Tobacco necrosis virus*, *Tobacco mosaic virus*, and *Tomato bushy stunt virus* [Cole *et al*., 2004]. POX activity appeared to be an important factor especially through its action on lignification, which seemed to be involved in the resistance of apple fruit to *P. expansum* [Valentines *et al*., 2005]. Accumulation of PR proteins and other defense related compounds was observed during induction of SAR in rice by different plant extracts [Kagale *et al*., 2004; Kagale *et al*., 2011]. The activities of POX and PPO were positively correlated to the enhanced disease resistance against bacterial wilt in *Eucalyptus urophylla* [Longxian *et al*., 2004]. Wang *et al*. (2005) reported the presence of a novel lysozyme in mung bean (*Phaseolus mungo*) seeds with both antifungal and antibacterial activities. Role of LOX for plant defense under different stress conditions have been enumerated by Nemchenko *et al*. (2006). LOX activity showed a positive relationship with resistance in Brazilian rice cultivars [Sandhu *et al*., 2007]. The application of plant extracts could induce systemic resistance in many plants through accumulations of PR-proteins [Aboellil, 2007]. Spraying of cacao plants with a heterogeneous chitosan suspension (MCp) from *Crinipellis perniciosa* mycelium showed a significant increase of oxidative POX and PPO activities [Cavalcanti *et al*., 2008]. The pepper 9-Lipoxygenase Gene *CaLOX1* functions in defense and cell death responses to microbial pathogens [Hwang and Hwang, 2010]. The induction of PR proteins due to the interactions between rice plants and *Xanthomonas oryzae* pv. *oryzae* suggested that these proteins were involved in the defense mechanism [Wu *et al.,* 2011]. POX and PPO have been reportedly involved in defense response of tomato [Abdel-Fattah and Al-Amri, 2012]. Taheri and Tarighi (2010) elaborated the role of PR proteins in tomato rescue from *Rhizoctonia solani*.

2.6.1. Peroxidase in plant defense:

In tomato, more than 12 peroxidase isoenzymes have been described and 7 of the coding genes have been mapped [El Mansouri *et al*., 1999], *CEVI1* [Mayda *et al*., 2000], *APX20* [Gadea *et al*.,

1999], *TPX1* [Quiroga *et al*., 2000], *TPX2*, *TAP1* and *TAP2* [Yoshida *et al*., 2003], being some of them. The *CEVI-1* gene has been shown to be induced in susceptible varieties of tomato plants under stress conditions in a salicylic acid–independent manner [Mayda *et al*., 2000] and also noted to be activated only upon compatible plant-pathogen interactions [Pilloff *et al*., 2002].

The anionic peroxidases of tomato appeared to be related to pathogenesis. The expression of POX is induced to high levels in tissues responding to challenge by fungal pathogens, wounding, or exposure to either ABA or fungal elicitor preparations [Sherf *et al.*, 1993]. Hammerschmidt *et al.* (1982) reported the association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium***.** Spraying the lower surface of the first true leaf (leaf 1) of cucumber plants with 50 mM K_2HPO_4 induced systemic resistance to anthracnose caused by *Colletotrichum lagenarium* as suggested by increased POX activity [Irving and Kuc, 1990]. Reuveni *et al.* (1992) denominated Peroxidase activity as a biochemical marker for resistance in Muskmelon against *P. cubensis*. The induction of systemic resistance in tomato against *Phytophthora infestans* by pre-inoculation with tobacco necrosis virus was accompanied by an increase of POX activity in inoculated leaves as well as in upper non-inoculated leaf tissues [Anfoka and Buchenauer, 1997]. The activity of POX was positively correlated to the establishment of SAR against bacterial wilt in *Eucalyptus urophylla* [LongXian *et al*., 2004]. Overproduction of Ascorbate peroxidase and increased peroxidase activity enhanced the active oxygen scavenging system, resulting into oxidative stress tolerance and oomycete pathogen resistance in pepper [Sarowar *et al*., 2005]. Bindschedler *et al.* (2006) demonstrated that POXs played significant role in generating H_2O_2 during the *Arabidopsis* defense response and in conferring resistance against a wide range of pathogens. The significant increase in peroxidase activity in tomato plants exposed to the antagonist and then inoculated with the challenging pathogen, suggested its role in the induction of ISR [Halfeld-Vieira *et al*., 2006]. Disease reduction was accompanied with a gradual increase in peroxidase activity in Faba bean [Hassan *et al.*, 2007]. New POX isoforms were induced as a defense response in *H. trionum* after inoculation with *V. dahlia* [Golubenko *et al*., 2007]. Aboshosha *et al.* (2008) demonstrated the validity of peroxidase activity and peroxidase isozymes pattern as genetic markers for resistance and susceptibility in sunflower to *M. phaseolina*. The induced defense responses and protective effects in Onion against *Stemphylium vesicarium* by application of SA is attributed to the increased expression of POX [Abo-Elyousr *et al*., 2009]. Class III peroxidases may play a role in

ROS generation in resistant wheat and non host rice plants during response to Hessian fly attacks [Liu *et al*., 2010]. The defense related role of POX in the induction of resistance by BABA in Artichoke against White Mould has also been well documented [Marcucci *et al*., 2010]. Bybordi *et al.* (2010) concluded that salinity stress induces POX in canola seedlings. In addition to potential use in marker assisted breeding, enhanced expression of anionic peroxidase through breeding or genetic engineering may lead to enhanced insect or disease resistance [Dowd *et al*., 2010]. The activity of POX was significantly increased in powdery mildew infected leaves of flax lines as compared with either resistant or susceptible parents [Ashry *et al*., 2011]. Gradual increase in POX activity in cotton after fungal infection pointed towards its possible role in acquisition of resistance [Pshenichnov *et al.*, 2011]. The enhanced activitity of the POX may contribute to bioprotection of black gram plants against *B. tabaci* infestation [Taggar *et al*., 2012]. The induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *lycopersici* has been correlated to the increased activity of POX [Ojha and Chatterjee, 2012]. Daudi *et al*. (2012) from his investigations concluded that apoplastic oxidative burst of POX increased immunity in *Arabidopsis*.

2.6.1.1. POX isoforms in plant defense:

The levels of peroxidase expression and its isozyme patterns have been studied in a number of plants found to be altered by stress, chemicals, and pathogenic infection [Gasper *et al*., 1982]. Bashan *et al*. (1987) reported the presence of four different POX isoforms in *P. syringae* pv. *tomato* infected tomato plants as compared to only one in control plants. Wounding of *N. tobaccum* triggered the expression of several cationic POX isozymes in the leaf and both cationic and anionic POX isozymes in pith tissue. Tobacco plants infected with *Tobacco mosaic virus* induced two anionic isozymes in the infected leaves, also systemically induced in leaves which were not inoculated with virus [Lagrimini and Rothstein, 1987]. Ye *et al*. (1990) correlated the induction of new POX isoforms in tobacco to the establishment of induced resistance against blue mold and *Tobacco mosaic virus*. Alcazar *et al*. (1995) demonstrated that in the resistant variety of *Capsicum annum,* increase in intercellular POX activity along with an additional acidic isoperoxidase, could protect the host plant against the fungal attack. It has been suggested that increase in activity of a specific anionic isoform of POX in some resistant inbred lines of maize, induced by virus inoculation, could be related to a defense mechanism against this virus

[De Souza *et al*., 2003]. *Rhizoctonia* infection in Norway spruce resulted in early local and systemic increase in peroxidase activity. The prominent isoforms were basic peroxidases. An increased intensity of similar peroxidase isoforms was found in drought-affected plants also [Nagy *et al*., 2004]. Dehydration and senescence caused disturbance in the redox homeostasis of Ramonda leaves, while inducing different POX isoforms [Veljovic-Jovanovic, 2006]. The *Pseudomonas fluorescens* infected cucumber plants when challenged with *Pseudoperonospora cubensis* showed induction of different isoforms of POX as compared with control plants, an indication of defense response [Anand *et al*., 2007]. Enhancement of resistance in cowpea against *R. solani* by exogenous application of SA is mediated by increase in POX activity as well increased expression of its isoforms [Chandra *et al*., 2007]. Analysis of POX enzymes indicated a variation between resistant and susceptible tomato cultivars [Kavitha and Umesha, 2008]. Endophytic bacterial application to cotton could induce specific isoforms of POX [Rajendran and Samiyappan, 2008]. Cold stress induction of a new POX isoform responsible for cell wall lignification was reported in buckwheat by Lucic *et al.* (2009). In soybean, nematode infected resistant cultivars accumulated higher concentration of POX than the control [Wu and Duan, 2011]. *Tomato ring spot virus* (ToRSV) and *Grapevine fan leaf virus* (GFLV) infected grapevine cultivars showed increased activity of POX as compared to uninfected plants, which is believed to be responsible for induced resistance in the host plant against these viruses [Ahmed *et al*., 2012]. The intensity of POX isoforms was higher in resistant seedlings of pearl millet than the susceptible ones [Lavanya *et al*., 2012]. Zhao *et al.* (2012) suggested that enhanced POX activity and induction of new isozymes may be related to mitigating pathogen-induced oxidative damage which resulted in the decrease of calli decay and resistance against fungal attack in pear.

2.6.1.2. Age-related expression of Peroxidase:

Increase in POX activity with the increase in age of rice plants has been documented by Kar and Mishra (1976). Wielgoss and Kortekamp (2006) reported the dependency of POX gene *PR9* expression on leaf age in grape vines. Expression of new POX isoforms accompanied the transition of plant vegetative to floral stage and its subsequent development in *Capsicum annum* [Bernal *et al*., 1993]. Tobacco anionic peroxidase gene expression was observed to be regulated spatially and developmentally in all parts of the plant, generally increased with age and maturity of the plant, tissue or organ of tobacco [Klotz *et al*., 1998]. In tomato, Peroxidase activity

increased with plant age and was induced only in older plants by Jasmonic acid (JA) application [Cipollini and Redman, 1999]. Similar observations were made by Takahama *et al*. (1999) in the apoplast of Tobacco leaves where expression of POX isoforms increased with ageing. A correlation between age and POX activity was observed by Ahn *et al*. (2005) in *Abutilon theophrasti*. Subsequent study in tobacco showed that the plants naturally became resistant to *Peronospora tabacina* as it aged due to increased expression of several PRs including POX [Ebrahim *et al*., 2011].

However, decrease in the expression of POX with the increase in age of the leaf/plant has also been observed. Over five days of ageing in Radish, in which germination decreased from 100 to 52%, POX activity in integument was higher than that in other parts of the seed, increasing in the initial days of ageing and then decreasing sharply in extremely aged seeds. The POX isoenzyme (MW 29.5 = kDa) activity increased in early days of ageing and decreased thereafter [Scialabba *et al*., 2002]. Two POX genes, *tApx* and *Apx4* exhibited a strong age related decrease of expression in leaves of one rosette as well as in leaves derived from plants of different ages of *A. thaliana* [Panchuk *et al*., 2005]. Chauhan *et al*. (2011) also reported a gradual decrease in peroxidase activities as ageing progressed in all the six varieties of wheat seeds under storage conditions.

2.6.2. Polyphenol Oxidase in plant defense:

In tomato, seven nuclear genes encoding polyphenol oxidase (PPO) have been reported. *Eco* RI and *Hind* III restriction fragment length polymorphisms (RFLP) of these seven genes (PPOs A, A', B, C, D, E and F) fall into three structural classes (I, II, and III). RFLP mapping and PFGE (Pulse field gel electrophoresis) analysis demonstrated that the genes are located on chromosome 8, and are possibly clustered within a 165 kb region [Newman *et al*., 1993]. Thipyapong *et al*. (1997) observed that the expression of PPO gene family is differentially regulated in various vegetative and reproductive organs during growth and differentiation.

PPO genes have been reported to be co-activated systemically in response to wounding via the octadecanoid signal transduction pathway [Constabel *et al*., 1995; Thipyapong and Steffens, 1997; Constabel and Ryan, 1998]. Chen *et al*. (2000) suggested that the plant defense enzymes like PPO could be stimulated in cucumber roots which have been colonized by non-pathogenic rhizobacteria or in a compatible interaction between cucumber and *P. aphanidermatum*. The

over-expression of PPO in tomato leads to a significant increase in resistance against *P. syringae* pv. *tomato* in compatible interactions [Li and Steffens, 2002]. The susceptible falat wheat heads pre-treated with an autoclaved mycelial wall preparation showed induced resistance against *Fusarium graminearum* which could be attributed to the increased activities of POX and PPO [Mohammadi and Kazemi, 2002]. Antisense downregulation of PPO gene resulted in enhanced disease susceptibility in tomato [Thipyapong *et al.*, 2004]. Nakkeeran *et al*. (2006) reported that application of *Bacillus subtilis* strain BSCBE4 and *Pseudomonas chlororaphis* strain PA23 mediated induction of PPO which was effective in controlling damping-off of hot pepper. Temporal analysis of transcript accumulation showed that in the resistant seedlings of pearl millet PPO coding genes were expressed earlier and more abundantly than in susceptible seedlings. PPO was observed to be actively involved in plant defense and can be used as a marker of resistance to downy mildew infection in pearl millet [Raj *et al*., 2006]. Cavalcanti *et al*. (2007) documented that activation of tomato defense responses against *Xanthomonas vesicatoria* by application of aqueous suspension of *Crinipellis perniciosa* mycelium were mediated by enhanced activity of PPO. Elevated activity of PPO were observed after treating plants with Bion followed by pathogen inoculation while inducing systemic resistance and controlling Stemphylium leaf blight of onion [Abo-Elyousr *et al.*, 2008]. Mahanil *et al*. (2008) suggested a critical role of PPO-mediated phenolic oxidation in resistance to common cutworm in tomato. Several fold higher PPO activity was recorded after exposing the Olive trees to cold stresses of different intensities [Ortega-García *et al*., 2009]. The foliar spray of SA induced resistance against bacterial spot in tomato accompanied by the increased PPO activity [Ibrahim, 2012]. The expression of PPO genes was altered and its activity increased after application of commercial preparayion from the brown seaweed *Ascophyllum nodosum* during the control of fungal diseases in cucumber [Jayaraman *et al*., 2011]. *Trichoderma harzianum* treated groundnut plants when challenged with *Macrophomina phaseolina* showed enhanced resistance accompanied with higher expression of PPO [Sreedevi et al., 2011].

2.6.2.1 PPO isoforms in plant defense:

Eight different PPO isozymes were expressed in pathogen-challenged tomato plants [Bashan *et al*., 1987]. The induction of a PPO isoform in tomatoes after infection with *P. syringae* pv. *tomato* and *Alternaría solani* suggested its role in disease resistance [Thipyapong and Steffens,

1997]. Active participation of PPO isozymes in wheat resistance to *A. triticana* was demonstrated by Tyagi *et al*. (2000). Two PPO isozymes were reported in the resistant cultivar of sorghum as compared to one in the susceptible cultivar [Kalappanavar and Hiremath, 2000]. Gelvonauskiene *et al*. (2005) reported that the PPO enzyme systems showed considerable variation among the apple cultivars. Specific bands or their combination were determined for scab-susceptible and scab-immune apple cultivars. Madhavi *et al.* (2005) observed a positive correlation between the expression of different PPO isoforms and resistance in sunflower against *Alternaria helianthi*. Increased expression of specific isoforms of PPO was observed due to induced systemic resistance (ISR) in cucumber against *P. cubensis* and *Erysiphe cichoracearum* [Anand *et al*., 2007]. PPO activity and its isozyme expression were observed to be negatively correlated with disease incidence and yield reduction in *Phytophthora colocasiae* infected *Colocasia esculenta* (L) plants [Sahoo *et al*., 2009]. A significant correlation between the degree of host resistance and PPO level was observed by Kavitha and Umesha (2008). Chatterjee and Ghosh (2008) observed hyperactive expression of PPO in diseased mesta plants. The stress sensitive nature of PPO isozymes has been reported by Nemeth *et al*. (2009). Remarkable difference in the expression pattern of PPO isozymes in resistant and susceptible cultivars of soybean was observed in response to cyst nematode infection [Wu and Duan, 2011]. Isoform analysis of PPO indicated a difference between resistant and susceptible tomato cultivars in number of isoforms and also in the intensity of each isoform in the presence of pathogen infection. The results obtained by Richter *et al*. (2012) clearly indicated a strong contribution of a single, specific PPO isoform to disease resistance in Dandelion.

2.6.2.2. Age related expression of PPO:

The expression of PPO is differentially regulated at different developmental stages of plant/tissue growth. The multiplicity of genes, their differential expression in different parts of the plant and at different stages of development is one of the most important features of recent work on plant PPO [Mayer, 2006; Aniszewski *et al.*, 2008]. Bucheli *et al*. (1996) stated that the expression of sugarcane PPO mRNA declines with tissue age. It was observed to be highest in the apical meristem and the immature stalk but declined in the mature parts down the stalk. The results obtained by Constabel *et al*. (2000) indicated a much greater responsiveness of younger leaves of hybrid poplar to tissue damage in terms of PPO expression than the older ones which depicted

lesser PPO inducibility. The findings of Mazaferra and Robinson (2000) also supported the existence of negative correlation between developmental stage and PPO expression in coffee. Unique patterns of expression of two PPO genes in a tissue and developmental-specific manner during vegetative and reproductive development in the Fuji apple have been demonstrated earlier using RNA gel blot analysis using gene-specific probes [Kim *et al*., 2001]. Li and Steffens (2002) observed the down regulation of endogenous PPO activity with increasing leaf age in tomato. Expression of PPO was reported to be ontogenically regulated in *Physalis angulata.* The younger plants showed strongest induction of PPO after exogenous application of JA [Doan *et al*., 2004]. Thipyapong *et al*. (2004) subsequently reported that suppression of PPO activity in both the control and drought stressed plants with increasing leaf age is associated with higher stress tolerance in tomato genotypes. However, a positive correlation between leaf age and expression of PPO has been demonstrated by Wang and Constabel (2004) in both the transgenic and control *Populus* plants. Dogan *et al*. (2005), from their studies on oregano, also concluded that the expression of PPO is developmentally regulated. In trembling aspen and hybrid poplar, leaf age-dependent local and systemic induction of PPO expression has been detected [Thipyapong *et al*., 2007]. Sahbaz *et al*. (2009) have stated that the expression of specific PPO isoforms played a vital role in plant protection during microbe sensitive developmental stages of cicer milkvetch (*Astragalus cicer* L). The youngest leaves showed the highest PPO activity in *Solanum tuberosum* which gradually decreased as leaf matured [Poiatti *et al*., 2009]. Ethylene responsiveness of tomato PPO B appeared to be tissue and developmental stage-specific [Newman *et al*., 2011]. In most organs, PPO B expression was found to be highest in young tissues and decreased with age. PPO expression was observed to be diminished in older stems.

2.6.3. Lipoxygenase in plant defense:

Lipoxygenase in tomato is encoded by a multigene family comprising of *TomLoxA, TomLoxB, TomLoxC, TomLoxD, TomLoxE and TomLoxF* genes whose expression are tissue-specific. *TomLoxA/B/E* are expressed during tissue ripening and *TomLoxC* is expressed in fruits and leaves. Its products are converted into volatile aldehydes and alcohols which are responsible for the characteristic aroma of tomato plants. *TomLoxD* expression is stimulated by jasmonate, wounding and systemin. This enzyme leads to the synthesis of defense compounds called octadecanoids [Mariutto *et al*., 2011].

Lipoxygenases are widely known for their vital contribution to plant defenses against pathogenic microbes. LOX enzyme activity has been shown to be induced rapidly during a disease-resistance response and more slowly in a susceptible interaction. In plants, LOXs contribute to disease resistance mechanisms through different pathways. According to findings from previous studies, the LOX type II 13-LOX group mediates disease resistance responses in plants primarily through JA signaling cascades. On the other hand, 9-LOX may mediate antimicrobial activity via oxylipin synthesis. LOXs can directly participate in pathogen-induced hypersensitivity reactions in plants [Montillet *et al*., 2002].

The *Arabidopsis LOX1* gene was dramatically induced after infiltration with pathogen *Pseudomonas syringae* [Melan *et al*., 1993]. Differential kinetics of accumulation of LOX mRNA in compatible and incompatible interactions was reported for *P. syringae* infection in tomato [Koch *et al*., 1992]. Expression levels of two LOX genes *PdLOX1* and *PdLOX2* increased following exposure to fungal pathogen and JA and also following injury in *Populus deltoids* [Cheng *et al*., 2006]. Furthermore, by employing antisense approaches, it was advocated that expression of *NtLOX1* gene, a pathogen and elicitor-induced 9-LOX gene was essential for resistance in tobacco [Rance *et al*., 1998; Mene-Saffrane *et al*., 2003]. In tomato leaves, it has been proposed that LOX-induced synthesis of JA activates transcription of genes encoding for protease inhibitors in response to insect attack. Also, LOX activity has been observed to increase in response to mechanical wounding, treatment of plants with cell cultures and elicitors [Fortunato *et al*., 2004]. LOXs are known to be responsible for synthesis of phytoalexins which may exert direct antimicrobial activity and induce or alter wound/pathogen defense gene expression [El-Khallal, 2007]. It has been observed that inoculation of non-pathogenic bacteria was effective in inducing systemic resistance in tomato via LOX pathway stimulation [Akram *et al*., 2008]. Multi fold higher expression of *TomLoxD* mRNA has been observed post wounding in both wounded as well as in systemic leaves of black nightshade by Pearce *et al*. (2009). Subsequently, Delaplace *et al*. (2009) indicated that in tomato several enzymes of the lipoxygenase pathway were involved in response to salt stress. Riboflavin is believed to prime bean plants for prompt activation of the lipoxygenase pathway when challenged with *B. cinerea* and JA triggers the same phenomenon in both beans and tomato [Azami-Sardooei *et al*., 2010]. Enzymatic assays showed that induction of LOX activity occurred locally and systemically in response to insect attacks [Jardim *et al*., 2010]. 9-LOX is known for its inevitable role in positive

regulation of plant defense against microbial pathogens and its systemic induction [Hwang and Hwang, 2010; Lopez *et al.*, 2011; Vellosillo *et al*., 2013]. Implication of *TomloxD* isoform of LOX has been observed to play a vital role as a component of the octadecanoid defense-signaling pathway [Hu *et al*., 2011]. After challenging with *B. cinerea*, the increase in transcription of two LOX genes and higher linolenic acid-consuming LOX activity were associated with a more rapid accumulation of free 13-hydroperoxy-octadecatrienoic and 13-hydroxy-octadecatrienoic acids, two antifungal oxylipins, in bacteria infected tomato plants [Mariutto *et al*., 2011]. The results obtained by Yang *et al*. (2011) showed that stress-induced "JA burst" was accompanied by the activation of LOX and Allene Oxide synthase (AOS) alongwith accumulation of their mRNAs. Their activation was necessary in stress mediated defense response and could enhance the tolerance of pea seedlings to wounding. Recent studies with tomato-*Pst* models have revealed the enhanced susceptibility of *lox1* mutant to the virulent strain *Pst* DC3000 and the partial impairment of *lox1* and *dox1* mutants to activate systemic acquired resistance [Vicente *et al*., 2012]. Joo and Oh (2012) have denominated LOXs as "Potential starting biocatalysts for the synthesis of signaling compounds" during defense responses in plants.

2.6.3.1. Age related expression of LOX:

LOX activity was highest in the early stages of development and it strongly decreased as the age progressed in *Triticum aestivum* seedlings [Hertel *et al*., 1987]. Saravitz and Siedow (1995) reported inhibitory effect of age on the expression of LOX in *Glycine max*. Schmitt and VanMechelen (1997) demonstrated that two lipoxygenases appear during barley grain development, differing in their temporal and spatial expression. Occurrence of ripening specific Lipoxygenase which was expressed exclusively during the fruit development in tomato has been reported [Ferrie *et al*., 1994; Kausch and Handa, 1997]. Presence of different LOXs during germination and in mature seedlings and their different roles in a number of developmental stages of soybean has also been observed [Porta and Rocha Sosa, 2002]. The developmental transition from vegetative growth to flowering in *Arabidopsis* has been observed to be associated with increase in 13-LOX activity, acting as a secondary messenger for oxylipin pathway [Banuelos *et al*., 2008]. The findings of Daosheng *et al*. (2009) suggested that LOX expression is positively correlated with age in *Radix Astragali* plants. The distribution of LOX in plant organs

according to the type of environmental conditions and the age of the plant has been documented by Gigot *et al*. (2010). It has also been reported that high activity of LOX is related to early development stages of plant growth [Vardar and Unal, 2011].

2.6.4. Lysozyme in plant defense:

Antibacterial activity of lysozyme against Gram-positive and Gram-negative bacteria has been well studied and is widely used in agricultural and industrial applications [Proctor and Cunningham, 1988; Utkhede and Koch, 2004; Van Loon *et al*., 2006]. There are several literatures regarding its lytic activity against fungal pathogens also, such as *P. nicotianae, F. oxysporum, P. aphanidermatum, B. cinerea* and *C. albicans* [During *et al*., 1999; Samaranayake *et al*., 2001; Shen *et al*., 2003; Wang *et al*., 2005].

Meyer *et al*. (1946) reported remarkable lysozyme activity in papain and ficin obtained from papaya and fig respectively and hypothesized that its mucolytic activity is possibly directed for defense against insects and other plant parasites. In pea pods, distinct lysozyme isozymes were induced when inoculated with compatible or incompatible strains of *Fusarium solani* or wounded or treated with chitosan or ethylene [Mauch *et al*., 1988]. Metraux *et al.* (1989) observed strong induction of lysozyme/chitinase coding mRNA in cucumber by salicylic acid. Martin (1991) reported that expression of lysozymes rapidly increased following exposure of *Hevea brasiliensis* to elevated levels of the ethylene, fungi, bacteria or virus or a number of other biotic or abiotic stresses leading to their classification as "defense" or "pathogenesis-related" proteins. Likewise, Ebrahim *et al*. (2011) put forward that lysozymes in plant leaves can be used as biochemical markers for identifying plant varieties resistant to fungal infection or other biotic and abiotic factors. The introduction of a human lysozyme gene in tobacco protected it from both bacterial and fungal diseases [Nakajima *et al*., 1997]. The activity of lysozyme was about two fold higher in the BTH-treated plants compared to the controls in tomato [Inbar *et al*., 1998]. Esaka & Teramoto (1998) observed induction of a Class III chitinase/lysozyme in winged bean (*Psophocarpus tetragonolobus*) after treatment with salicylic acid. In *Lupinus*, constitutive expression of Class III chitinase in vegetative organs and developing seeds, as well as after treatment with SA and different abiotic and biotic stresses, was observed by Regalado *et al.* (2000). Subsequently, Ahrenholtz *et al*. (2000) evidenced increased killing of *Bacillus subtilis* on the hair roots of transgenic T4 Lysozyme-producing potatoes.

The lysozyme present in the mung bean seeds demonstrated potent antifungal activity towards a variety of fungal species suggesting its important role in constitutive host defense mechanism against microbial pathogens [Wang *et al*., 2005]. Sawahel and Hagran (2006) reported the generation of white mold disease-resistant sunflower plants which expressed human lysozyme gene. Dong *et al*. (2008) demonstrated that the bacteriophage T4 lysozyme gene confers resistance to both gray leaf spot and brown patch diseases in transgenic tall fescue plants. Lysozyme has been observed in several tuberous plants such as *R. sativus, S. tuberosum, D. carota* and in the latex of *Calotropis procera* which was stable over a large range of temperature and retained its lytic activity at different temperatures [Sakthivel *et al*., 2010].

Sawasdipuska *et al*. (2011) reported the presence of a putative lysozyme in *Pithecellobium dulce* seeds which exerted an antifungal action towards *Macrophomina phaseolina*. A plant lysozyme from *Momordica charantia L*. has been reported to possess antifungal activity against *Rhizoctonia solani* and *Mucorracem osus* apart from an antibacterial action against *Escherichia coli* and *Staphylococcus aureus* [Wang *et al*., 2011]. A novel lysozyme isolated from the Canadian cranberry beans (*Phaseolus vulgaris*) had a potent inhibitory action towards fungal species including *A. alternate* (*Fr*.) *Keissl, Fusarium oxysporum, Pythium aphanidermatum* and *Botrytis cinerea* and an antibacterial action against *Staphylococcus aureus* [Wang *et al*., 2012]. Stacking of several antimicrobial genes including one of lysozyme in potato conferred considerable levels of resistance to the host plant against *Phytophthora infestans*, *Rhizoctonia solani* and *Fusarium solani* [Rivero *et al*., 2012].

2.6.4.1. Age related expression of Lysozyme:

The differential expression of lysozymes/Class III chitinases during different developmental stages in plants has been scantily investigated. However, there are indications that the induction of various isoforms of lysozymes is differentially regulated at the level of gene expression.

While characterizing lysozyme in *in vitro* raised tissues of *Rubus hispidus,* Pilet *et al*. (1983) observed that lysozyme level significantly increased with the increase in growth of the tissue. The appearance of two lysozyme activity possessing chitinases, at a late state of grain development suggested a role for these enzymes in defense against fungi in the quiescent and germinating grain of *Hordeum vulgare* [Jacobsen *et al*., 1990]. Similar results were observed by Leah *et al*. (1991) in Barley seeds. Martin (1991) reported the expression of lysozyme isoforms

in a development-dependent manner in pea pods. Salzer *et al*. (2000) reported the expression of specific Class III chitinases/lysozymes in mycorrhizal roots of *Medicago truncatula*, necessary for nodule formation during the development process. Apart from these, different classes of chitinases/lysozymes have been recorded to be developmentally regulated in several tissues including flowers, roots and leaves of several plants [Collingel *et al*., 1993].