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Systemic acquired resistance (SAR): A novel strategy for plant protection with reference to mulberry

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Abstract
Exclusive reliance on pesticides, fungicides and herbicides resulted in pesticide and herbicide, resistance, pest resurgence, residues, environmental pollution. Plants have developed various resistance mechanisms to help them adapt to pathogen and insect attack. Systemic acquired resistance (SAR) is a form of induced resistance that is activated throughout a plant after being exposed to elicitors from virulent, avirulent, or nonpathogenic microbes, or artificial chemical stimuli such as chitosan or salicylic acid (SA) (Gozzo and Faoro, 2013). It is a mechanism of induced defense that confers long-lasting protection against a broad spectrum of microorganisms. SAR requires the signal molecule salicylic acid (SA) and is associated with accumulation of pathogenesis-related proteins, which contribute resistance to the plants. They can be used as fungicide alternative without any threat of developing resistance and being safe and ecofriendly (Najar *et al*, 2010). The elicitor, β-Amino butyric acid induces greater systemic resistance to mulberry in addition to enhancement in biochemical parameters and NPK contents of mulberry leaves (Mazal, 2014). Therefore, in order to control the diseases of mulberry without adverse effect on environment, humans and silkworm's health attention needs to be given to promote SAR chemicals. A model needs to be framed to promote the use of these chemicals in order to make sericulture more profitable. This is an ecofriendly approach of disease and pest management. The chitinase genes of mulberry induced by insect wounding and fungal infection, suggesting that these chitinases help the mulberry plant to cope with the challenges from insects and fungi (Wang *et al*., 2015). Jasmonic acid (JA) is an important plant defense signal mediating resistance to herbivores. Presently disease control is largely depending on the use of fungicides, bactericides and insecticides. The hazardous nature of these chemicals on the environment, human health and silkworm strongly necessitates the search for new, harmless means of disease control. Induced resistance like SAR can diminish the use of toxic chemicals for disease control and thus could be proposed as an alternative, non-biocidal, ecologically-friendly approach for plant protection and hence for sustainable Sericulture. Induced resistance is increased expression of Natural defense mechanisms against different pathogens provoked by external factors of various types. Systemic acquired resistance (SAR) is a "whole-plant" resistance response and can be distinguished from other disease resistant responses by both the spectrum of pathogen protection and the associated changes with gene expression.

Keywords: Ecofriendly, gene expression, Jasmonic acid (JA), pest resurgence, Systemic acquired resistance (SAR), Sericulture and whole-plant resistance

1. Introduction
Induced resistance can be split broadly into systemic acquired resistance (SAR) and induced systemic resistance (ISR). ISR is phenotypically similar to pathogen-SAR in that it confers an enhanced defensive capacity against diseases caused by fungi, bacteria, viruses, and nematodes (Ran *et al.* 2005) [42, 43]**.** Both localized acquired and systemic acquired resistance (ISR, SAR) were extensively studied by Ross (1961) [44, 45, 46, 47] who was the first-to introduce definitions of these phenomena. Systemic acquired resistance is a form of induced resistance that is activated throughout a plant after being exposed to elicitors from virulent, avirulent, or nonpathogenic microbes, or artificial chemical stimuli such as chitosan or salicylic acid (SA) (Gozzo and Faoro, 2013) [17, 18]. But it can take several days for SAR to develop throughout the host plant (Kuc, 1982) [32]. Due to their action in stomatal closure, SA as ABA have been proposed to mediate drought tolerance and induced ROS accumulation has been proposed as an integrator between SA and ABA signaling on the regulation of stomatal closure (Miura *et al*., 2013) [36].

Either SA or ABA causes a reduction of transpiration by inducing stomatal closure, thus allowing the storage of water in leaves for survival under drought conditions. Moreover, SA can stimulate ABA accumulation, and so both hormones may have synergistic effects on stomatal closure and drought tolerance acquisition. The onset of SAR involves the generation of mobile signal(s) at the site of local infection, and this occurs within 4–6h of primary infection. The signal(s) then translocate presumably through the phloem to the systemic tissues, where it activates defence response. Several chemical inducers of SAR have been identified including, Salicylic acid (SA) and its methylated derivative or MeSA, Jasmonic acid or JA**,** Auxin, Pipecolic acid or Pip, Dehdryoabietinal or DA**,** Azelaic acid or AzA and BABA.Besides, several other abiotic (heavy metal, detergents etc.) and biotic compounds like carbohydrates, lipid, proteins have also been studied for their role in activation of plant defense responses. These compounds are known as plant elicitors or activators which elicit or activate host response against pathogens attack. Activation of resistance in plants by new generation crop protection agents is an emerging approach in the management of diseases. These plant response acts specifically throughout the plant to reduce the severity of wide range of diseases caused by fungi, bacteria and viruses. These chemicals are highly effective in preventing bacterial, fungal and viral infection. The introduction of SAR in disease management of mulberry is a new concept. A primary local infection can trigger not only effector-triggered immunity.

ETI which is often associated with programmed cell death (PCD) of the infected cells, but also production of the immune signal salicylic acid (SA) in chloroplasts through the activity of isochorismate synthase ICS1.Mobile immune signals are also produced, including Azelaic acid (AzA), glycerol-3-phosphate (G3P), methyl salicylic acid (MeSA), and dehydroabietinal amines (DA). AzA regulates the expression of AZI1, which encodes a predicted secreted protease-inhibitor/seed-storage/lipid-transfer family protein, and AzA, G3P, and DA all require DIR1, a putative lipid transfer protein, for their functions. Accumulation of SA affects the cellular redox and the NPR1 nuclear translocation through S-nitrosoglutathione (GSNO) and thioredoxins (TRXs). The nuclear NPR1 concentration is controlled by SA levels through the SA receptor proteins NPR3 and NPR4. A high concentration of SA in the local infection site promotes NPR1-NPR3 interaction and NPR1 degradation to allow PCD and ETI to occur, whereas in the neighboring cells, the intermediate level of SA disrupts NPR1-NPR4 interaction, resulting in the accumulation of NPR1.NPR1 can interact with transcription factors (TFs) to activate the expression of ER genes to facilitate protein secretion; the expression of antimicrobial PR proteins such as PR1, PR2, and PR5; and resistance to secondary infection. The SAR primed state is associated with acetylation (Ac) of H3K9 and methylation (Me) of H3K4 at SAR-associated gene promoters. DNA methylation and proteins affecting chromatin architecture (e.g., SNI1) and DNA repair (e.g., RAD51 and BRCA2) may function in priming plant defense genes and protecting genome stability not only in the current generation but also in the progeny. An avirulent pathogen not only triggers defense responses locally but also induce the production of signals such as salicylic acid (SA), methyl salicylic acid (MeSA), Azelaic acid (AzA), glycerol-3-phosphate (G3P), and abietane diterpenoid dehydroabietinal (DA). These signals then lead to systemic expression of the antimicrobial PR (pathogenesis-related) genes in the un inoculated distal tissue to protect the rest of the plant from secondary infection. This phenomenon is called systemic acquired resistance (SAR). SAR can also be induced by exogenous application of the defense hormone SA or its synthetic analogs 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole S-methyl ester (BTH). SAR provides broad-spectrum resistance against pathogenic fungi, oomycetes, viruses, and bacteria. SAR-conferred immune "memory" in plants can last for weeks to months, and possibly even the whole growing season. In contrast to ETI, SAR is not associated with PCD, and instead promotes cell survival. The onset of SAR is associated with massive transcriptional reprogramming, which is dependent on the transcription cofactor NPR1 (nonexpresser of PR genes 1) and its associated transcription factors (TFs) such as TGAs. SAR is believed to be conferred by a battery of coordinately induced antimicrobial PR proteins whose secretion requires significant enhancement of endoplasmic reticulum (ER) function. Mobile signal, moves systemically, found in phloem exudates of infected leaves, and is required for SAR. Accumulation of salicylic acid induces the secretion of pathogenesis related (PR) proteins with antimicrobial activities. SAR requires SABP2's MeSA esterase activity in the systemic tissue to convert biologically inactive MeSA to active SA. (SABP2'S = Receptor in systemic tissue). In *Nicotiana tabacum* contains has N resistance gene that governs gene –for – gene type resistance to TMV, MeSA functions as enhance the resistance to subsequent infection by TMV.Oxylipns, synthesized from polyunsaturated fatty acid. Methyl jasmonate (MJ) function as volatile signal and also translocated through the vasculature. Methyl jasmonate (MJ) activates gene encoding protease inhibitor which protect plants against insect attack. Treatment of potato with jasmonate increase resistance to Phytophthora infestans. Pathogen infection induces the release of free carbon 18 fatty acids (C18 FA) from membrane lipids, these results in Azelaic acid (AzA) production. AZI1 (AZELAIC ACID INDUCED1) gene, which is expressed at elevated level in Azelaic acid-treated plant, was required for defence priming by Azelaic acid.PR protein is plant protein that are induced in pathological and related situation. These proteins are accumulated 7-10 days after infection and indicate the attainment of SAR. It is accumulated in the intercellular spaces (first line of defence) and vacuole (second line of defence by lytic enzyme). Defensins are small cationic peptides of 45-54 amino acid residues with anti-microbial activities. They inhibit the growth of wide range of phytopathogenic microorganisms. They are the new member of thionine family. Defensins are expressed in most but not all plants and are key members of a plants immune system and provide a first line of defense against pathogen attack. They work at level of innate nonspecific immunity against the varied pathogens. Nontoxic to most animal and plant cells and are thermostable. Defensins have shown satisfactory efficacy against pathogens.

Oxidative burst

Immediately downstream of pathogen recognition, early events in plant cell may activate receptor-associated, plasma membrane-bound, heterotrimeric GTP-binding (or simply G) proteins, as noted for a wide variety of animal transmembrane receptors, a family of proteins involved in second messenger cascade. Activation of G proteins may be coupled to ROS generation by the influx of Ca^{2+} from the apoplast due to the opening of calcium channels. The increase of intracellular Ca

$^{2+}$ concentration activates a Ca $^{2+}$ dependent protein kinase that, in turn, stimulates ROS generation. In particular, phosphorylation of a plasma membrane-bound enzyme, a NADPH-dependent oxidase, sharing homology with its mammalian counterpart, stimulates the production of superoxide anion. This radical species is then dismutated to hydrogen peroxide (H_2O_2), by superoxide dismutase (SOD). The role of H_2O_2 is pivotal in plant defence mechanisms, because it is a non-radical, non-charged and membrane permeable species. Therefore, it (i) contributes to create a hostile environment to the pathogen because of its direct toxicity (ii) participates to the oxidative cell wall strengthening and (iv) acts as a signal molecule (second messenger) for the activation of defence genes. However, the cellular H_2O_2 concentration has to be maintained under a cytotoxic threshold by cell antioxidant defences, because this species can react with transition metals (Cu or Fe), according to Fenton or Haber Weiss reactions, to form hydroxyl radical (OH.), the most reactive and dangerous ROS. Enzymes that regulate the H_2O_2 homeostasis include mainly catalases (CATs), ascorbate peroxidase (APX) and peroxidases (POXs), whereas the main non-enzymatic ROS scavengers are ascorbic acid, glutathione, tocopherols, carotenoids and polyphenols (Apel and Hirt, 2004; Yoshioka *et al*., 2009) [59]. Similarly, to animals, nitric oxide (NO) is an important signal molecule in plants too. In mammals, NO is produced by the enzyme NO synthase (NOS) that converts L-citrulline to L-arginine. In plants, there are not homologue genes of animal NOS, though the activity of NOS like enzymes has been reported in these organisms (Chandok *et al*., 2003, 2004; Guo *et al*., 2003, 2005) [5, 6]. Alternatively, plants generate NO from nitrite by nitrate reductase (NR) or via non-enzymatic reduction of apoplastic nitrite (Yamamoto *et al*., 2003; Bethke *et al*., 2004) [58]. The physiological role of NO is still not entirely known, though its involvement in stomatal closure, seed germination, fruit ripening, senescence and root organogenesis has been reported. Interestingly, during the pathogen attack, NO may mediate induction of HR and SAR by interacting with H_2O_2 and salicylic acid (SA) (Delledonne *et al*., 2001; [9] Buonaurio *et al*., 2003; [4] Polverari *et al*., 2003; [41] Wendehenne *et al*., 2004; [56] Zaninotto *et al*., 2006) [60].

The hypersensitive reaction

Resistant plants often respond with a HR at site of fungal penetration, by localised programmed cell death followed by a wide range of both local and systemic defence reactions, such as lignification, phytoalexin and PR-protein synthesis. This resistance mechanism, which is often associated with both gene-for-gene resistance (effector-triggered immunity) and non-host resistance (microbial-associated molecular-pattern immunity), might involve just a single cell (invisible HR) or extensive and visible tissue areas, to deprive an invading pathogen of an adequate nutrient supply. Additionally, the release of antimicrobial compounds from dying cells and defence responses triggered in cells immediately surrounding infection site contribute to poison and restrict (biotrophic or hemibiotrophic) fungi. Another event at the onset of HR is the generation of molecular signals (SA, ethylene and jasmonic acid) which may alert distal parts of the plant and induce SAR (Williams and Dickman, 2008) [57]. PCD in plants shows striking similarities to the hallmarks observed in apoptosis, a typical form of PCD in animals, including chromatin condensation, DNA cleavage (ladders) and activation of caspase (cysteine-aspartic proteases)-like proteases (metacaspases). By contrast, plant cells display unique features lacking in their animal counterparts, such as the presence of a rigid cell wall, chloroplasts and vacuolar proteases (Williams and Dickman, 2008)[57]. Depending on pathogen lifestyle, PCD/HR may be either beneficial or detrimental to the host. As previously introduced, in biotrophic pathogen-plant interactions, HR prevents infection, because biotrophs require living cells for growth and colonization. Conversely, in response to necrotrophic pathogens, which feed on dying or dead tissues, PCD is advantageous to the pathogen and not to the plant (Glazebrook, 2005). This divergence can be explained considering PCD as an essential pathogenicity factor of certain necrotrophic pathogens, which evolved fine strategies to subvert and induce inappropriate PCD in host cells.

Cell-wall strengthening

As its basal structure, the primary cell wall is composed of a framework of cellulose micro fibrils that are embedded in a matrix of hemicelluloses, pectins and structural proteins. In the epidermis, it constitutes one of the first lines of defence against fungal pathogens, and it typically represents a preformed physical barrier, although the ex-novo induction of structural defences can strengthen the cell wall. The cell-wall appositions include an array of structures that are involved in the accretion of new cell-wall material. In some pathosystems, the attempted penetration of leaves by phytopathogenic fungi is accompanied by deposition of a plug of material, known as a papilla, directly beneath the penetration site. The epidermal cell wall surrounding the papilla can be modified to form a characteristic disc-shaped zone or halo. The materials involved in the thickening of host cell wall range from minerals, such as silicon, calcium and sulphur (the fungicidal activity of which is well known), to more or less complex organic polymers, mainly including callose and lignin, polymers of β-1,3-glucose and monolignols, respectively. Moreover, papillae can be impregnated with oxidized phenols, which are directly toxic to pathogens (Huckelhoven, 2007; Hematy *et al*., 2009) [24, 25]. Extensions (hydroxyproline-rich glycoproteins) are the main structural proteins of plant primary cell wall. They have similarities to animal collagen, they form a defined scaffold that sets the spacing of cellulose micro fibrils, and they are characterized by post-translationally hydroxylated proline, which makes up about 40% of amino-acid residues. Oxidative crosslinking of hydroxyproline-rich glycoproteins involves the re-arrangement of these pre-existing cell-wall components by peroxidases and hydrogen peroxide (H_2O_2), further improving their resistance to both enzymatic hydrolysis and the physical pressure that can be exerted by pathogens (Cannon *et al*., 2008). Lignin is a polymer that comprises different phenylpropenol units (monolignols) that are connected by covalent linkages. Peroxidases and H_2O_2 are essential for its random polymerization, which takes place in muro, with growing lignin polymer infiltrating the primary cell wall. Lignin is extremely difficult to attack enzymatically, and very few organisms can degrade lignified tissues, as can white rot fungi. Therefore, induced lignification represents an optimal inducible structural barrier for plants, and lignified tissues are also a poor and hostile substrate for pathogen growth and development (Bhuiyan *et al*., 2009) [3].

Phytoalexins

Phytoalexins are low-molecular-weight antimicrobial plant secondary metabolites, and they are synthesized de novo from

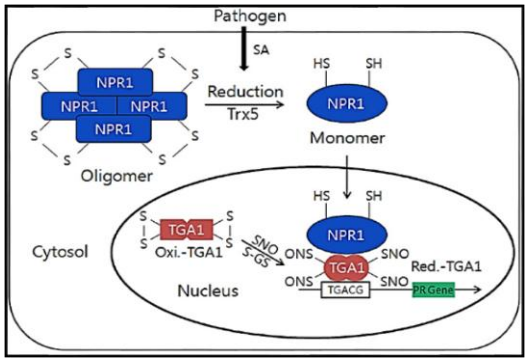
essential substrates, including phenylalanine, malonyl-CoA, acetylCoA, mevalonic acid and other amino acid (Iriti and Faoro, 2009) [26]. They have been identified in the majority of plant families, and members of specific plant families usually produce similar types of phytoalexins. They are synthesized and accumulate locally around infection sites, and although they have never been found in systemically protected tissues before a challenge inoculation, they can rapidly accumulate in induced tissues after a challenge (Van Loon, 2000). Interestingly, they accumulate both in resistant and susceptible hosts at the same concentrations, though with a different kinetic, thus showing that their efficacy strictly depends on the timing of their synthesis at infection site. The antimicrobial compounds in healthy plant tissues are known as phytoanticipins (Van Etten *et al.*, 1994). Phenylpropanoids, arising from phenylalanine deamination by the enzyme phenylalanine ammonia-lyase (PAL), include several classes of well-studied phytoalexins, for instance isoflavonoids from the Leguminoseae family (Dixon and Paiva, 1995). The pathosystem Phaseolus vulgaris-Colletotrichum lindemuthianum provided a good model for studying the role of phytoalexins in plant resistance against pathogen attack. C. lindemuthianum, the causal agent of bean anthracnose, is a hemibiotrophic fungus whose colonization is restricted, in resistant hosts, because of isoflavonoid production, including phaseollin, phaseollidin and kievitone (Mansfield, 2000). Similar examples include medicarpin and pisatin, two isoflavonoid phytoalexins from alfalfa (Medicago sativa) and pea (*Pisum sativum*), respectively (DiCenzo and VanEtten, 2006). However, broad bean (Vicia faba) provides a notable exception. Like most other legumes, it produces isoflavonoid phytoalexins, but the main induced antimicrobial compounds are furanoacetylenic wyerone derivatives (Mansfield, 2000). In the *Vitaceae* family, phytoalexins which have been well characterized constitute a rather restricted group of molecules belonging to the stilbene family, synthesized as a general response to fungal attack. These compounds possess the skeleton based on the trans-resveratrol (3, 5, 4′-trihydroxystilbene) structure, including piceids, pterostilbenes and viniferins, that are, respectively glucosides, dimethylated derivatives and oligomers of resveratrol (Jeandet *et al*., 2002). In grapevine (*Vitis vinifera*), activities of chalcone synthase (CHS) and stilbene synthase (STS), enzymes respectively involved in flavonoid and stilbene biosynthesis, are differentially regulated, according to plant developmental stage. During the initial phase of berry ripening (veraison), resveratrol accumulation in cells of berry exocarp declines, while anthocyanin synthesis increases, due to competition between two branches of the same pathway. As a consequence, after veraison, anthocyanin accumulation confers colour to berry skin, whereas lowering levels of the powerful phytoalexin resveratrol make the grape bunches more susceptible to *Botrytis cinerea* (gray mould) infections (Jeandet *et al*., 1995). The resistance of Vitis spp. to B. cinerea infection has been shown to correlate with trans-resveratrol content (Mlikota Gabler *et al*., 2003). Interestingly, open-field treatment with the plant activator BTH can reverse, to a certain extent, the inverse relationship between resveratrol and anthocyanin content at veraison, reducing CHS and STS competition for the same substrate and avoiding metabolic switch from one pathway to the other. Thus, higher levels of resveratrol protect grapes from gray mould after veraison, without interfering with the colouring phase, which is an important qualitative trait (Iriti *et al*., 2004). Following BABA treatment of grapevines, sporulation of Plasmopara viticola was strongly reduced and the accumulation of phytoalexins of the stilbene family increased with time after infection. Camalexin is a N and S- containing indole phytoalexin synthesized from tryptophan via indole-3-acetaldoxime, a branch point metabolite that also leads to the biosynthesis of glucosinolates, the plant hormone indole acetic acid (IAA) and melatonin (Glawischnig *et al*., 2004).

Pathogenesis-related proteins

Some decades ago, it was shown that infection of tobacco plants with tobacco mosaic virus leads to the accumulation of a set of PR proteins (Gianinazzi *et al*., 1970). Acidic extracellular forms of these PR proteins accumulate at the onset of plant resistance, indicating that they have a role as molecular markers for the expression of SAR. PR proteins have a low molecular weight (5-75 KDa), and they are thermostable, highly resistant to proteases, extractable, and stable at low pH (<3). They have a dual cellular localization, as vacuolar (for basic forms) and apoplastic, the latter being the most important site for their accumulation. First detected in tobacco, PR proteins are now considered to be common-place in the plant kingdom and have been detected across different genera in all organs of monocotyledonous and dicotyledonous species: leaves, where they are particularly abundant in both mesophyll and epidermis, stems, roots, flowers and seeds. Additionally, exogenous application of SA, or of its functional analogues 2, 6-dichloroisonicotinic acid and BTH, can activate PR gene expression and resistance in plants without pathogen inoculation (Edreva, 2005; van Loon *et al*., 2006) [42]. PR proteins are categorized into structurally homologous families. Some of these families have direct antimicrobial activities, whereas, for others, no intrinsic antimicrobial effects have been found yet, suggesting that the latter might have different functions. An important common feature of most antimicrobial PR proteins is their antifungal activity, although some of them also have antibacterial, insecticidal and antiviral properties. Originally, five main groups of PR proteins (PR-1 to PR-5) were characterized in tobacco. Since then, the number of PR protein groups has increased up to PR-17 across many plant species (Sels *et al*., 2008) [48].

Conformational switching from the oligomers to a monomer of NPR1 by redox changes in plants

Under normal conditions, NPR1 forms an inactive oligomer structure and is oxidized by intermolecular disulfide bonds in the cytosol. However, during pathogen attack, the NPR1 changes its structure from inactive to active form of monomer through the reduction of the intermolecular bridges by h5-type thioredoxin (AtTrx-h5). Then, NPR1 is translocated to the nucleus and interacts with TGA1 transcription factor that can induce PR gene expression. TGA1 in normal condition is inactive and oxidized form with intramolecular disulfide bonds in the nucleus. However, in stress condition TGA1 is reduced and interact with NPR1 and can enhance its DNA binding activity through S-nitrosylation (SNO) and S-glutathionylation (S-GS) by GSNO and glutathione (GSH/GSSG)



Systemic wounding Response (SWR)

Plants produce jasmonic acid and Methyl Jasmonate in response to particularly herbivory and wounding which build up in the damaged parts of the plant. MeJA is also a plant hormone involved in tendril (root) coiling and seed maturation. It acts as a signaling molecule for the production of phytoalexins. MeJA has been used to stimulate traumatic resin duct production in lodgepole pine trees. This can be used as a defense against many insect attackers as a type of vaccine. MeJA is an important cellular regulator involved in diverse developmental processes, such as shoot growth, yield and seed quality (Farouk & Osman, 2009) [13]. In addition, Jasmonates activate plant defence mechanisms in response to insect-driven wounding, various pathogens, and environmental stress (Cheong & Choi, 2003) [3]. MeJA is a suitable candidate for insect control in agriculture. No negative effects on crop yield (Farouk & Osman 2009) [13].

Chitinase genes in mulberry

The mulberry genome encodes 20 chitinase genes which are grouped into two main families and organized into five classes. The genomic structures and phylogenetic relationships of mulberry chitinase genes are analyzed, which provided a genetic basis for understanding the functions of these genes. Expression of mulberry chitinase genes in five different tissues including root, bark, bud, flower, and leaf. Mulberry chitinase genes are used to detect transcriptional differences in their response to insect wounding and fungal infection. These chitinase genes were induced by insect wounding and fungal infection, suggesting that these chitinases help the plant to cope with the challenges from insects and fungi (Wang *et al.*, 2015) [53, 54, 55]. Chitinases are among a group of proteins involved in plant defense response against infection and wounding. The availability of *Morus* genomic data provides a unique opportunity for identifying putative mulberry chitinase genes (He et *al*., 2013). Based on the sequences and conserved domains 20 chitinase genes are reported in the mulberry genome. There is accumulation of Mnchi16 transcripts in the insect-wounded leaves. Mnchi8 and Mnchi19 are upregulated in response to infection. Mnchi19 strongly inhibit the hyphal extension of the fungus. Mnchi8 and Mnchi16 are strongly induced by different factors, suggesting that different mulberry chitinases respond to different biotic stresses. This information will be crucial in advancing our understanding of mulberry chitinase genes for Sericultural improvement. Studies of expression patterns of mulberry chitinase genes induced by insect wounding, fungal infection, and elicitors will provide new information on the interactions between wounding and other signals and help to determine the role of these genes in plant defense responses. Seasonal evaluation of total soluble protein fractions extracted from cortical parenchyma cells of mulberry (*Morus bombycis* Koidz.) tree identified a predominant 18 KDa protein that is directly correlated to periods of cold acclimation. The 18 KDa protein, designated as WAP18 (winter accumulating 18 KDa proteins) increased from September to December and then gradually decreased until June. The maximum levels of WAP18 are detected in mid-winter, which corresponds to the maximum freeze tolerance in cortical parenchyma cells of mulberry tree. Two-dimensional gel electrophoresis confirmed that WAP18 consists of at least three proteins that range between an isoelectric point of 5.0 and 6.0. The purified WAP18 exhibited in vitro cryoprotective activity for the freeze labile l-lactate dehydrogenase (LDH) enzyme. Thus, WAP18 may function in the freezing tolerance mechanism of cortical parenchyma cells of mulberry tree during winter ((UkajI *et al*., 2004).Gupta, *et al*. (2006) reported that in mulberry *(Morus alba* L.), various individual strains of plant growth-promoting rhizobacteria (PGPR) and synthetic analogs of naturally occurring plant activators have potential to elicit induced systemic resistance (ISR) against either brown leaf spot (*Cercospora moricola*) or leaf rust (*Cerotelium fici*) diseases. Three PGPR strains, *Azotobacter chroococcum* strain Azc-3, *Bacillus megaterium* strain Bm-1 and *Pseudomonas fluorescens* strain Psf-4, and plant activators, acetyl-salicylic acid (ASA), sodium salicylate (NaS) and 4-amino-n-butyric acid (ABA) are tested and up to 2000 ppm concentration exhibited their compatibility with the PGPR strains tested. Upon assaying of the elicitors with plant-pathosystem, disease suppression was significantly high with integrated application of PGPR strains and Thus, the integration of PGPR strains and chemical plant activators holds great promise in providing more effective ecofriendly alternatives to the toxic chemical fungicides presently used for the control of brown leaf spot and leaf rust diseases in mulberry and also the solution to protect mulberry plants from multiple/mixed infections of both these diseases

Table 1: Chitinase genes in the *M. notabilis* genome (Wang *et al.*, 2015)

Gene Name	Accession No.	Gene Name	Accession No.
Mnchi1	Morus022978	Mnchi11	Morus020224
Mnchi2	Morus007185	Mnchi12	Morus000037
Mnchi3	Morus007186	Mnchi13	Morus007737
Mnchi4	Morus017594	Mnchi14	Morus012010
Mnchi5	Morus022481	Mnchi15	Morus018118
Mnchi6	Morus022482	Mnchi16	Morus018119
Mnchi7	Morus020088	Mnchi17	Morus018124
Mnchi8	Morus011486	Mnchi18	Morus013887
Mnchi9	Morus011484	Mnchi19	Morus014360
Mnchi10	Morus003149	Mnchi20	Morus014362
Mnchi11	Morus020224	Mnchi13	Morus007737
Mnchi12	Morus000037	Mnchi14	Morus012010

Laticifers in mulberry accumulate defense proteins

Laticifer is a unique cell. It is elongated tubular and branched network that runs throughout a plant body. Large amounts of its cytoplasm are exudated when the plant body is cut (Hagel *et al*., 2008) [22]. Laticifer cytoplasm called latex contains toxic compounds or proteins, which are involved in defense against herbivores and microbes (Hagel *et al*. 2008: Konno, 2011) [22]. Insecticidal chitinase-like proteins (LA-a and b) in unlignified tissues of mulberry and antifungal class I chitinase (LA-c) in lignified tissues. Mulberry laticifers accumulate large amounts of biotic-stress-related defense proteins e.g., pathogenesis-related protein-1, β-1-3-glucanase, class V chitinase, osmotin and lectins. They are adapted to different threats. Unlignified soft organs, such as leaves, where laticifers run through veins, may form part of the diet of Lepidoptera caterpillars that cannot eat lignified and hardened branches or trunks, whereas the lignified parts may be the target of pathogenic fungi and bacteria. The different accumulation pattern of lectins and PR-1, are related to different threats faced by these tissues, although there have been no reports on the toxic effects of PR-1 on insects.

Table 2: Different PRP in lignified and unlignified tissues of mulberry.

Unlignified tissues	Lignified tissues
Class V chitinase	β-1,3-glucanase
β-1,3-glucanase	Class I chitinase
Class I chitinase	Lectin
Class I chitinase	galactose-binding
Osmotin	Lectin, galactose-binding
Pathogenesis-related protein 1	Lectin, mannose-binding

SAR effects on nematodes

Soil drenches of SA and INA and root dip application of SA and BTH inhibited nematode reproduction, at specific dosage ranges, without affecting plant growth. SA and INA are able to reduce root galling as well. Foliar sprays of both SA and BTH are ineffective against nematode attacks. Plants tolerated SA more than the other chemicals tested. BTH at elevated concentrations increased the mortality of nematode juveniles and reduced egg hatching in vitro.SAR activators at concentrations suitable for different plant growth stages and applied by the proper method can possibly be included in IPM programmes for nematode management (Molinari, 2016) [38]. Chinnasri *et al*. (2006) [8] reported that the potency of the inducers of systemic acquired resistance (SAR), acibenzolar-s-methyl, DL-α-amino-n-butyric acid (AABA), DL-β-amino-n-butyric acid (BABA), γ-amino-n-butyric acid (GABA), p-aminobenzoic acid (PABA), riboflavin, and salicylic acid (SA), in reducing reproduction of *Meloidogyne javanica* and *Rotylenchulus reniformis* in pineapple and they reported that foliar sprays to 1-mon-old pineapple plants (20 ml/plant) grown in 22-cm-diam. pots in the greenhouse. Two days after application, 10,000 eggs of *M. javanica* or *R. reniformis* were inoculated onto the plants. Six months after inoculation, nematode reproduction was measured. Acibenzolar decreased *R. reniformis* egg production by 58% compared to the nontreated control. Acibenzolar, BABA, and riboflavin reduced *M. javanica* egg production by 60% to 64% compared to the nontreated control. Foliar application of acibenzolar at 100 and 200 mg/liter decreased by 30% and 60%, respectively, the number of *M. javanica* eggs as compared to the nontreated control. Foliar application of acibenzolar may activate intrinsic resistance of pineapple to *M. javanica* and *R. reniformis* and may have a role in the sustainable management of nematodes in pineapple.

Table 3: List of PR proteins

PR proteins	Plants in which PRP detected	Enzymes	Target pathogen sites
PR 1	Rice, barley, maize, tomato, tobacco		Active against glucan activity Plant cell wall thickening Resistance to the spread of the pathogen on the apoplast
PR 2	Rice, barley, maize, tomato, tobacco, potato, pepper, bean, Brassica, sugar beet	β-1-3-glucanase	Cell wall glucan of fungi
PR 3	Rice, maize, tomato, pepper, sugar beet, rape seed	Chitinase	Cell wall chitin of fungi
PR 4	Tomato, tobacco, rubber tree	Chitinase	Cell wall chitin of fungi
PR 5	Rice, wheat, barley, oats, tomato, tobacco, potato	Thaumatine- like	Against oomycetes
PR 6	barley, tomato, tobacco	Proteinase inhibitor	Against nematodes and insects
PR 7	Tomato	Endoproteinase	Microbial cell wall dissolution
PR 8	Cucumber	Chitinase with lysozyme activity	Cell wall chitin of fungi and mucopepetide of bacteria
PR 9	Tomato, rice, tobacco, wheat	Peroxidase	Indirect anti-microbial activity as cross linking of plant cell wall
PR 10	Potato, asperagus, pea, bean, rice	Ribonucleases	Viral RNA
PR 11	Tobacco	Chitinase	Cell wall chitin of fungi
PR 12	Arabidopsis, pea	Defensin	Antifungal and antibacterial
PR 13	Barley	Thionin	Antifungal and antibacterial
PR 14	Barley	Lipid transfer proteins	Antifungal and antibacterial
PR 15	Barley	oxalate oxidase	H2O2 production
PR 16	Barley and wheat	Oxalate-oxidase like with superdismutase activity	H2O2 production
PR 17	Wheat, barley, tobacco	Peptidase	

Table 4: Effect of SAR chemicals on the percent disease intensity and disease control of the powdery mildew (*Phyllactinia corylea*) disease of mulberry leaves during 2010 and 2011 Kharief seasons (Najar *et al*, 2010).

Chemical compounds	PDI		PDC		Pooled	
	2010	2011	2010	2011	PDI	PDC
Ascorbic acid	6.84	4.18	68.23	72.26	5.51	70.14
Benzoic acid	993	6.08	54.80	59.65	7.90	57.22
Calcium chloride	7.32	5.14	66.00	65.89	6.23	65.94
Salicylic acid	6.17	3.65	71.34	75.77	4.91	73.55
EDTA	7.04	6.84	67.30	54.61	6.94	60.95
Check-I (Carbendazim 50 WP)	2.70	1.24	87.45	91.77	1.97	89.61
Check-II (Untreated)	21.53	15.07	-	-	18.30	

Summary and conclusion

The continued use of traditional chemical fungicides has led to environmental and economic problems. As a consequence, integrated pest management (IPM) is favoured for plant protection. Systemic induced resistance is considered to be one of the important strategies to achieve IPM through manipulation of natural defence mechanisms in plants. Synthetic chemicals for systemic induced resistance have provided practical possibilities for induction of resistance in control of plant disease. Promotion of SAR inducers reduces adverse effect on environment, humans and silkworms as well. All SAR chemicals give good protection against the diseases and are almost equally effective as fungicides. There is a need to promote the use of these chemicals in order to make agricultural crops sustainable. Promotion of these chemicals exploits the own resistance of mulberry against pests and diseases. This is an ecofriendly approach of disease and pest management. Stable, low risk of pathogen populations of developing resistance to SAR**.**

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