Review Paper:

Molecular basis of insect resistance in plants: current updates and future prospects

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Abstract

Insect pests are one of the major threats to plant productivity and survival. Plants respond to insect attack through perception of insect effectors and activation of multiple signaling components including reactive oxygen species, Ca^{2+} and MAP kinases. The defense signaling in turn is regulated by phytohormones, secondary metabolites, volatile compounds, physical barriers, defense proteins and transcription factors. The emergence of genomics and genetic engineering has facilitated the identification of multiple molecular components and their usage in inducing insect resistance.

In the present review, we discuss about various strategies used by plants in response to insect herbivory with special reference to various molecular and genomic approaches towards insect resistance. Expansion of molecular approaches to understand plant-insect interaction will be a priority in future towards development of novel insect resistant plant varieties.

Keywords: Insect herbivory, defense signaling, plant-insect interactions, secondary metabolites, genomic technologies.

Introduction

Plants being sessile are constantly exposed to an array of biotic stresses including pathogens and insects which challenge their growth and vigor. Insect herbivory often results in compromising plant homeostasis and development, eventually causing plant death. Majority of crop species with high economic significance are infested by diverse insect pests worldwide resulting in huge crop losses accounting to billions of dollars[7]. Both plants and insects are believed to have co-evolved, thus have complex interaction dynamics.

Unlike animals, plants do not have a well characterized and systematic immune system to overcome such stresses and in stead rely on multiple molecules and signaling substances to develop a network of defense response. Usually, the outermost epidermal layer of plants acts as a corporal wall for the external stress and threats. Deposition of lignin, resins and silica on the epidermal layer further strengthenes it. Aditionally, development of modified leaves such as trichomes, spines, thrones and prickles restricts and discourages insect herbivory. The diverse and complex repository of plant metabolites also take part in defense response against insect attacks. These phytochemicals are not only being bitter and indigestible and also reduce the plant palatability, but at times act as toxins to insects[20].

Hypersensitive responses (HR), programmed cell death, tissue reinforcement at the site of attack and expression of defense-responsive genes are associated with plant defense in response to pathogen and insect attacks[8]. Insect feedings on plant tissues often result in oxygen burst inside the cell thereby releasing intermediate signal molecules such as reactive oxygen species (ROS), superoxides (O_2^-), nitric oxide (NO) and hydrogen peroxide (H_2O_2) which in turn induce the defense responses through activation of downstream targets.

Similarly, several phytohormones like abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and gibberellins (GA) regulate defense responses and modulate expression of many downstream target genes[4].

Additionally, calcium-dependent protein kinase (CDPK), cyclin-dependent protein kinase (CDK) and mitogen activated protein kinase (MAPK) serve as an important component of the defense signaling cascades[4]. Moreover, plant defense responses against insects can be much diversified and may be employed constitutively or transiently. Further, plant defense responses against insects exhibit temporal dynamics having some defense responses being elicited within minutes of insect herbivory whereas other being activated in days[20].

Over the last few decades, significant progress has been made in understanding insect defense strategies, identification of insect resistance genes and unraveling the molecular mechanism of host-insect interaction across plant species. These defense strategies adopted by plants are yet to be characterized and categorized according to their nature of elicitation.

In this review, we have summarized the numerous plant defense responses against insect herbivory and categorized them as per their nature of defense. Furthermore, we provide a detail account of the genetic and molecular mechanism of insect resistance and the application of multiple genomic technologies towards enhancement of insect resistance in plants.

Plant response to insect herbivory

The counter defense responses against insect attacks can be of constitutive, induced, direct and indirect in nature. Plants have evolved multi-tier defense systems to counter act against insect pest (Fig. 1). This diversified defense system exhibits protective strategies starting from physical barriers to chemical secondary metabolites and inducible/adaptive defenses. At times, when the direct defense via production of toxic secondary metabolites against the attacking insects fails, plant secrets compounds that attract the scavengers of the attacking insects[16]. During this indirect defense, plants harbor and nourish the scavengers of the attacking insects to diminish the herbivory pressure.

Depending upon the ability and context of adapting strategies to self-defend, a plant can be considered to be tolerant or resistant. Briefly, when the physical and chemical entities of a plant can intimidate insects and abate the damage caused by herbivory, it is considered to be resistant. The resistant plants try to deter insect growth and nourishment and usually impose strong selection pressure on the attacking herbivores.

On the other hand, tolerant plants are unable to discourage the insects, but can reduce the detrimental effects of herbivory. The selection pressure exerted by tolerant plants is generally low and the growth and sustainability of the insects are unchallenged. Insect herbivory, oviposition and colonization can induce an array of plant defense responses such as strengthening or modifying physical fencings, production of antagonistic secondary metabolites, secretion of herbivore-induced plant volatiles (HIPVs), HR/ROS generation, defense signaling and expression of defense-related genes. Additionally, the priming of plant defense against insects facilitates rapid triggering of defense responses[5]. Therefore, plants respond to insect attack by initiating any one kind or a cocktail of the strategies to diminish or discourage herbivory.

Physical barriers and structural modifications in insect defense

To counteract the insects of different feeding guilds, plants have developed modified structural traits such as trichomes, waxes, cuticle depositions and spines that stand stout as physical barriers against insect attachments, herbivory and oviposition (Fig. 1). Formation of a film or crystal by the epicuticular waxes discourages insects to land on the plant surface, to feed or lay eggs[5]. Under insect induced stress, the production and composition of waxes in the plants vary from its natural properties. For example, the oviposition of cabbage white butterfly on *A. thaliana* induces the change in wax composition by raising the concentration of tetra-triacontanoic acid (C34) and lowering that of tetracosanoic acid (C24)[6]. This change in the wax composition attracts the egg parasitoid wasps *Trichogramma brassicae*.

Similarly, plants prevent herbivory by enhancing the leaf and root toughness. The hardening of plant epidermal parts discourages the insect feedings. The toughness of the roots is often strengthened by the deposition of lignin polymers to limit insect attacks. Further, deposition of compounds like silica, suberin, callose and cellulose results in cell wall reinforcement which restricts insect feedings.

Plants also promote extensive regrowth of roots under herbivore attack which results in the increase of root number and root density. Modification of plant leaves to thorns and spines and emergence of adapting structures like trichomes also protect the plant from insects. While thorns and spines discourage the larger herbivores to feed on the plants, the trichomes check the insect attachment and their mobility[1]. Both the glandular and non-glandular trichomes contribute towards pest management, the former by decreasing the plant palatability whereas the later limits pest mobility.

In *N. attenuate*, the glandular trichomes produce abundant quantities of O-acyl sugars that indirectly cause the larva of *M. sexta* to release volatile metabolites, which in turn attract its predators[8]. Similarly, the high leaf trichome density repels and reduces oviposition of the mite *Tetranychus uticae* in raspberry[35].

Secondary metabolites in insect defense

A wide range of bioactive compounds and secondary metabolites are synthesized by the plants which act antagonistic to insects (Fig. 1). These plant-made chemicals not only reduce the insect attacks, but also limit the extent of herbivory damage and modulate downstream defenses. The secondary metabolites, mainly contribute towards the direct defenses, however, can also take part in indirect defenses like housing the predators of the specific insects[16].

Several plant-produced bioactive compounds act as toxins to insects and affect their digestive and nervous tissues, resulting in retarded growth or death. Furthermore, they have a key role in regulating the taste, odor and color of a plant or plant parts[37].

Alkaloids are found in almost all plants and play an important role in defense against insects. Feedings of aphids were restricted in *Festuca arundinacea* due to the production of pyrrolizidine alkaloids (PA) by the endophytic fungi *Acremonium coenophialum*[32]. The PAs get reduced to their toxic forms, once they reach the alkaline digestive tracts of insects, enabling PAs as potent anti-feeders which mostly fend off aphids and other bugs[32]. Deglycosylation of 2-β-D-glucopyranosyloxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) generates HDMBOA, which prevents herbivory attacks from the moths *S. frugiperda* and *S. littoralis*[23].

Furthermore, the derivatives of glucosinolates such as the indole glucosinolate in *Arabidopsis* confers enhanced resistance to the aphid *M. persicae*[16]. Plant terpenoids contribute towards plant defense acting as toxins, insect repellents and anti-feeders. Terpenoids present in the form of volatile compounds, resins and vital components of plant essential oils also adversely affect the insect attack.

Many plant peptides and other compounds help in plant defense by impairing the insect digestion ability. Plant products like lectins, chitinases and α-amylase inhibitors either act as anti-digestive proteins by interfering with the digestion of consumed plant parts or as anti-nutritive compounds by limiting the consumption of plant parts by the herbivores. Proteinase inhibitors (PIs) synthesized by the plants help to impair the digestive process in the insects. Cysteine proteases and metalloproteinase are key enzymes found in insects from the class Hemiptera and inhibition of these key enzymes in the insect guts can result in fatal consequences[16].

Likewise, the plant α-amylase inhibitors (α-AI) block the α-amylases of attacking herbivores restricting their starch catabolism. In wheat, the α-AIs were found to restrict the mealworms, beetles and wheat weevils from attacking the plant and grains[20]. The heterologous expression of bean *α-amylase inhibitor1* in *Pisum sativum* conferred resistance against the weevil *Bruchus pisorum*[52]. Additionally, plant produce chitinase to neutralize pest attacks as chitin is a major compound in the insect exoskeletons. The transgenic tomato lines expressing poplar chitinase exhibited resistance to the beetle, *Leptinotarsa decemlineata* by inhibiting their development[41]. Similarly, polyphenol oxidase (PPO) enzymes also contribute towards plant defense, more frequently upon mechanical injury or wounding.

During insect feedings, the disruption of plant cells releases PPOs which in turn produces ROS[48]. Overexpression of tomato PPO conferred enhanced resistance to the moth *S. litura* by decreasing its development and increasing its mortality rate[48]. Many plants possess laticifers and resin ducts which store latex and resins. During insect attacks, these ducts get ruptured and the latex is secreted out at the wound site to discourage or to trap the insects. Besides being sticky to entrap insects, the latex of some plants can also be toxic. For instance, the latex of *A. cannabinum* consists of phenolics, alkaloids, terpenoids and PIs, which act as toxins or anti-nutrients when ingested by insects[16].

Specialized chemicals in insect defense: Plants produce several specialized compounds including the VOCs, food bodies and nectars which attract, nurture and accommodate the scavengers of insects, thus indirectly contributing towards insect's defense (Fig. 1). VOCs are produced majorly in flowers and roots to attract pollinators and insect scavengers. In maize, more than 30 volatile compounds including sesquiterpenes, (E) -α-bergamotene and other aromatic compounds were produced under herbivory by the leaf-worm *S. littoralis*[15]. Interestingly, the VOCs or HIPVs also help in intra- and intercommunication among plants and priming the defense responses against insects. *Artemisia tridenta* and *N. attenuate* plants were reported to share this behavior, where injured *A. tridenta* plants released VOCs induced chemical defense in *N. attenuate*[37]. Similarly, exposure of volatiles in the undamaged leaves released from wounded leaves exhibited elevated defensive responses against the larval feeding of the moth *Lymantria dispar*[46].

Plants produce the nutrient rich food bodies (FBs) to commence a mutualistic association with other organisms which in turn can give protection against insects. The association between *Piper fimbriulatum* and *Pheidole bicornis* ants is mutualistic as the ants protect the plant from several insects, while feeding on its FBs[18]. Numerous plants produce nectars to attract pollinators, predators of insects and pests and parasitoids which contribute largely to the indirect defense[16]. Though nectars are produced at flowers, EFNs are produced and deposited on shoots and leaves of plants. The synthesis and secretion of these EFNs increases when the plant is challenged by insects.

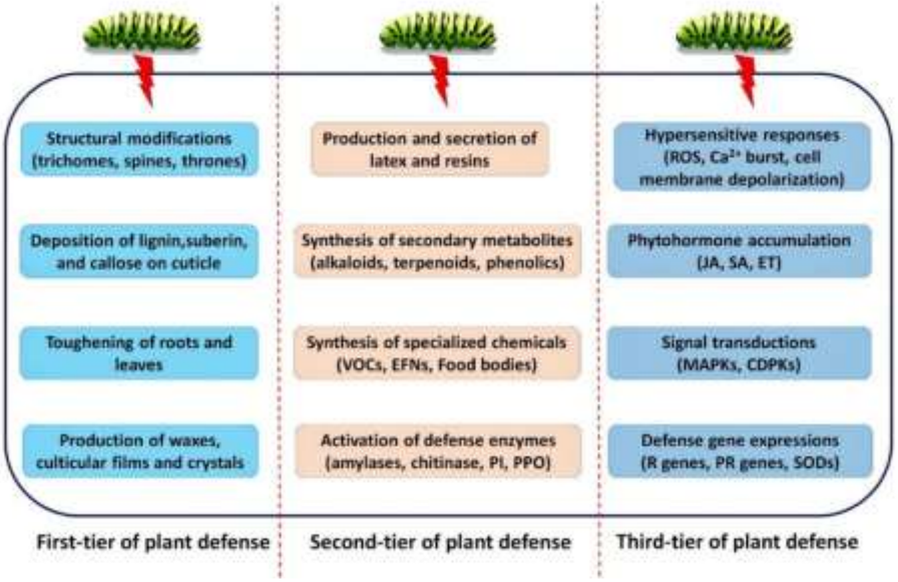

Figure 1: The multi-tiered plant defense system against insects.
(R gene- resistance gene, PR gene- pathogenesis-related gene)

Perception and signaling in plant-insect interactions

Alongside structural and chemical defenses, plants rely on many intrinsic physiological processes like recognition of insect effectors, ion flux gradients across the plasma membrane, Ca^{2+} burst, ROS generation or oxidative burst and signaling cascades for perception and response to herbivory[15] (Fig. 2). The activation of these signaling events subsequently induces the accumulation of phytohormones, expression of defense response genes, phytohormone biosynthesis genes and phytohormone regulated genes[5]. The perception of the stimuli of insect feedings is usually done at the site of the attack, but can spread to adjacent cells and throughout the plant to initiate systemic defense responses.

Often, the attacking insects release oral secretions (OS) or elicitors into the plant cells. To perceive these elicitors, several specific receptors are present on the cell membrane[46]. The earliest signals generated by herbivory include depolarization of the plasma membrane, ionic influxes or effluxes and oxidative or Ca^{2+} bursts. Most of the insect OS contains fatty acid-amino acid conjugates (FACs) which act as potential elicitors for defense responses. Feeding on plant parts and OS by cotton leafworm in lima beans rapidly induced the depolarization of plasma membrane potential, thereby initiating defense responses against the insect[6].

Mousavi et al[53] demonstrated the role of depolarization of membrane potentials and specific membrane proteins like glutamate receptor-like (GLRs) proteins in modulating the JA-induced gene expressions and communication of wound signals. These OS and FACs in turn activate the kinase signaling cascades that play a crucial role in modulating defense against pest attacks. In *N. attenuata*, the exogenous treatment of *Manduca sexta* derived FACs to the injured leaves activated MAPKs, wound-induced protein kinase (WIPK), salicylic acid-induced protein kinase (SIPK) and resulted in accumulation of phytohormones like JA, SA and ET[72]. Similarly, the feeding of brown planthoppers in rice induced the expression of multiple *OsMPKs*[55].

Oxidative burst or production of ROS is another rapid response of plants against the insect attacks. ROS plays a key role in modulating anti-herbivory plant defense via redox potential based signaling[16]. The role of ROS in herbivore defense has been well demonstrated in *N. attenuata*[74]. Wounding of the *N. attenuate* plants resulted in transcript accumulation of NaRBOHD, a member of the respiratory burst oxidase homolog (RBOH) family. Further, the treatment of OS from *M. sexta* resulted in elevated transcription of NaRBOHD. The production of ROS even after the OS treatment was remarkably decreased in the NaRBOHD-silenced plants and they were more susceptible to herbivory.

The plant oxidases like RBOHs have the capacity to integrate with Ca^{2+} and MAPK signaling alongside ROS generation indicating its nexus in herbivory-induced defense responses. Combination of ROS production and Ca^{2+} signaling has already been demonstrated in *Arabidopsis* where the binding of Ca^{2+} synergistically activated the RBOH[12]. ROS-mediated defense response against phloem and sap-sucking insects is often realized through the accumulation of H_2O_2 and enhanced activity of peroxidases (POD), superoxide dismutases (SOD), catalases (CAT) in a variety of plant species[36].

Amongst several ion species, Ca^{2+} ion plays a pivotal role in the alteration of cell membrane potential and signal transductions during herbivory. Under normal circumstances, the concentration of Ca^{2+} ions in the cytoplasm and in the apoplast stays in nanomolar and micromolar range respectively[11]. Upon insect feeding, the Ca^{2+} homeostasis get disturbed and a surge of Ca^{2+} ions flows into the cytosols. This alteration of the ionic concentrations by the Ca^{2+} burst initiates downstream signaling cascades for defense responses[11]. Ca^{2+} signals are often perceived by dedicated calcium sensor proteins such as calmodulin (CaM) and CDPKs, which further communicate with downstream targets to propagate the received signal to the nucleus[14].

The binding of Signal responsive1 (*AtSR1*) transcription factor protein to CaM induces insect resistance in *Arabidopsis thaliana* while the *atsr1* mutants were susceptible to herbivory[40]. The defense responses against aphid feedings in *Arabidopsis* (vs. *Myzus persicae*) and wheat (vs. *Diuraphis noxia*) were found to be regulated by the expression of CaM-binding proteins[64]. Likewise, AtCPK3 and AtCPK13 modulated the CPK-mediated Ca^{2+} signaling regulating the defense responses against generalist herbivore *S. littoralis*[34].

Phytohormones play an explicit role in fine tuning plant defenses. JA acts as a key player in modulating defenses during insect infestations by contributing to both direct and indirect defenses[75] (Fig. 2). Herbivory or wounding of plant parts results in the rise of intracellular concentration and accumulation of JA in the plant cells. Defense responses induced by JA accumulation can range from physical modifications like the formation of trichomes to chemical dynamics like the liberation of volatile organic compounds (VOCs), secretion of extra foliar nectars (EFNs), production of secondary metabolites and expression of JA-responsive genes[70]. Likewise, SA pathway is ubiquitous in vascular plants and plays a significant role in rapid adaptation to insect herbivory (Fig. 2).

SA modulates the defense response against the bollworm *Helicoverpa armigera* in tomato by inducing the production of ROS[57]. SA induced H_2O_2 accumulation also discourages the herbivore to feed on the plant parts as higher concentration of H_2O_2 adversely affects the insect gut and development[47].

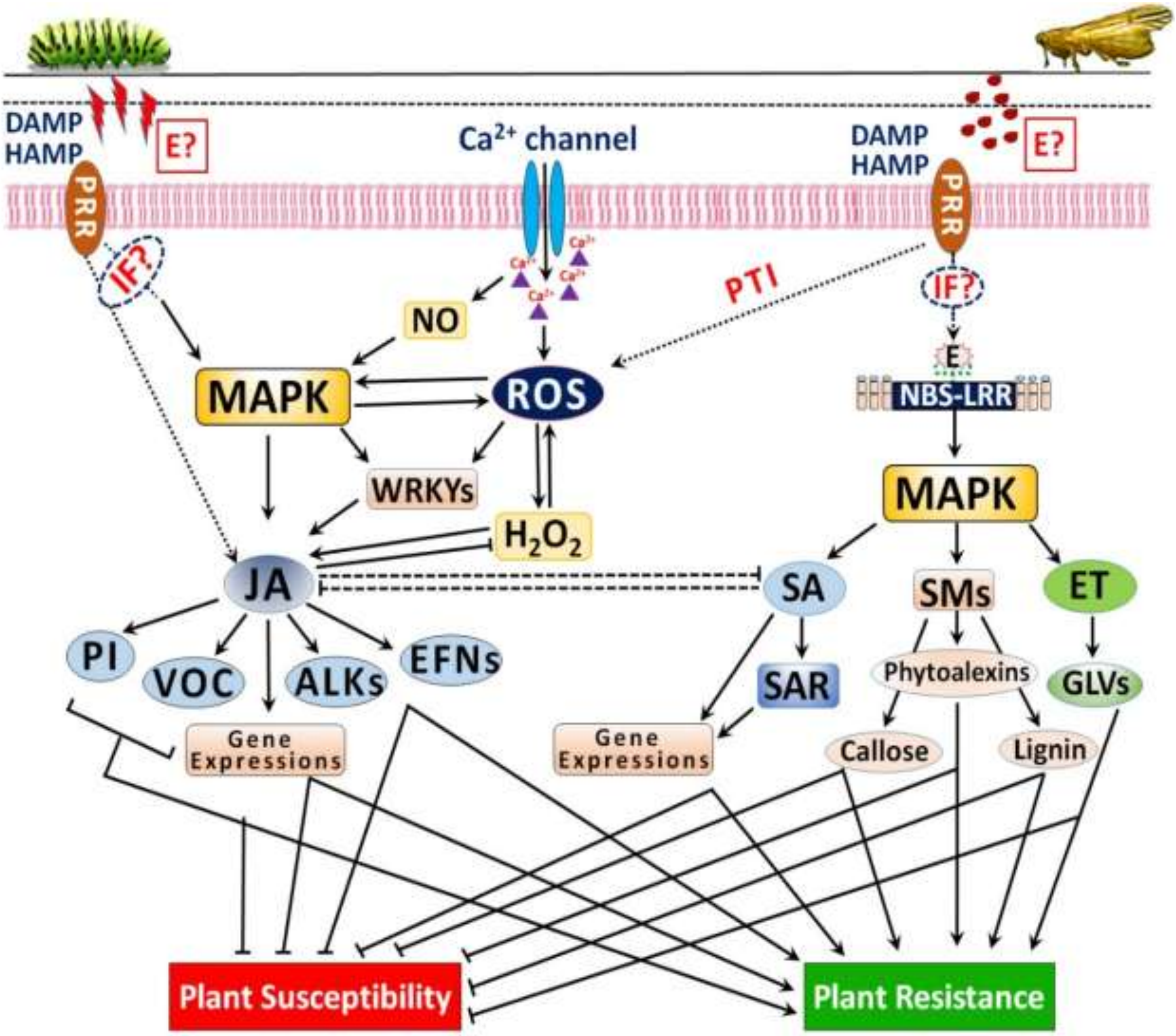

Figure 2: Molecular mechanism of insect resistance in plants. Plant pathogen recognition receptors (PPRs) recognizes the HAMPs or DAMPs and activate the PAMP-triggered immunity (PTI) through the MAPK cascade. Alternatively, the insect resistance proteins identifies the insect effectors activating the effector triggered immunity (ETI). For the sucking insect infestation, MAP kinase cascade activate the SA and ET signaling which results in increased expression of SA response genes, accumulation of phytoalexins and deposition of callose and lignin. In case of chewing insect infestation, MAP kinase activate the JA signaling leading to insect defense through the production of plant volatile organic compounds (VOCs) and extra foliar nectars (EFNs) which attracts natural enemies of insects. MAPK, Mitogen activated protein kinase; NO, Nitric oxide; PPR, HAMP, Herbivore associated molecular patterns; DAMP, damage induced molecular patterns; SA, Salicylic acid; JA, Jasmonic acid; SAR, systemic acquired resistance.

Similarly, ET signaling functions in concert with JA and/or SA to activate or repress specific branches of defense network during insect attack. For instance, ethylene biosynthesis supported the growth of fall armyworm *Spodoptera frugiperda* in maize[25].

Alternatively, the impairment of ethylene signaling facilitated lower aphid growth in tomato and higher resistance to *S littoralis* in *Arabidopsis*[50]. Beside, ET is responsible for the induced emission of many VOCs during plant-insect interactions as seen in the European alder plants, lima beans and maize[16].

Molecular approaches towards insect resistance

Insect resistance in plants usually involves two major defense strategies. On one hand, constitutive defenses protect plants from insect attacks by forming different physical barriers and chemical metabolites, induced defense facilitate perception of insect elicitors or effector molecules resulting in the production of specific chemicals, activation of downstream signaling modules and genetic reprograming of transcriptional mechanisms[13]. Majority of the insect resistance genes in plants encode plasma membrane-localized/ intracellular-localized receptors implying that induced defense is critical to plant immunity against insect attack[13].

In other words, plant defense response against insects exhibits a great similarity to that against the pathogens. In the last decade, several important studies have made it possible understand the insights of the molecular mechanism of resistance to insect herbivory in plants.

Plant immunity to multiple phytopathogens is often illustrated by a zig- zag model[33]. However, such a model is not fully established for the plant-insect interactions and the extent up to which it is applicable for the same, is yet unknown. Insect herbivory often results in the production of conserved molecules known as herbivore-associated molecular patterns (HAMPs) or damage-associate molecular patterns (DAMPs), which are similar to the pathogen associated molecular patterns (PAMPs) (Fig. 2). These molecules can be recognized by the pattern recognition receptor (PRRs) in plants and trigger the PTI (PRR-triggered immunity), which is similar to that of the PAMP-triggered immunity[8].

HAMPs include secretory proteins from insect saliva, oral secretions (OS) and oviposition fluid including fatty acid-amino acid conjugates (FACs), volicitin, carliferins, bruchins, alkaline phosphatase and glucose oxidase that induce defense response through the JA signaling pathway[16].

Likewise, the DAMPs released from the damaged tissue upon insect herbivory include oligogalacturonides, cutin monomers and endogenous peptides including systemin, VOC, HypSys and RALF. Beside HAMPs and DAMPs, insect secreted effectors such as endo-β-1,4-glucanase N1EG1 from brown planthoppers (BPH) or HARP1 from cotton ballworm can suppress or surpass the PTI to induce the effector-triggered susceptibility (ETS)[49] (Fig. 2).

Contrary to this, plant produces specific receptors or resistance (R) proteins that can recognize these insect effector molecule thereby triggering the effector-triggered immunity (ETI). In rice, *Bph14* encodes a NB–LRR protein that acts as a specific receptor for the candidate effectors from BPH during rice-BPH interactions[13]. In other words, while the PTI based response to insect attack in plant includes cell wall callose deposition (structural), activation of ROS signaling (chemical) and triggering of signaling cascades (MAPK), ETI involves a more like gene-for-gene interaction for defense response through the activation of specific genes or transcription factors[13].

Based on the recent studies on transcriptome and proteome dynamics, several genes involved in insect resistance have been cloned and characterized in myraids of plant species. While some of these genes exhibit a clear gene-for-gene relation with the insect effectors, others do not go by this hypothesis. For instance, NB-LRR class *R*-gene *Mi-1.2* from tomato and vat from melon encode protein that directly confers resistance to *Macrosiphum euphorbiae* and *Aphis gossypii* respectively[59, 66] (Table 1).

Alternatively, a rice long chain base gene OsLCB1a facilitated defense against herbivore infestation not by directly interacting with the elicitor but by increasing the concentration of the defense protein across the membranes[3]. Nevertheless, plants do possess several genes that participate in the plant-insect interactions and regulate plant defenses irrespective of their relationship with the insect effectors. In rice, three lectin receptor kinases (OsLecRK1, OsLecRK2 and OsLecRK3) and multiple OsMPKs were reported to be involved in rice resistance against BPH infestations[44] (Table 1).

Similarly, LecRK1 in *N. attenuate* acts as a crucial player in conferring defense against *M. sexta* by inhibiting the accumulation of SA and elevated concentration of specific secondary metabolites including nicotine, diterpene-glucosides and trypsin protease inhibitors[22]. OsLRR-RLK1 a leucine-rich repeat receptor-like kinase in rice was reported to initiate defense responses against the chewing herbivore *Chilo suppressalis*[27].

While the transcription of OsLRR-RLK1 was significantly upregulated by the insect attack, silencing of gene demonstrated reduced resistance to *C. suppressalis*. Further, the MAPK cascade operates downstream to OsLRR-RLK1 and is positively regulated by OsLRR-RLK1 controlling the expressions of MAPK and WRKY transcription factors[27].

Likewise, the identification of specific effectors in insects and their manipulations via different functional genomic tools revealed new insights in plant-insect interactions. In an earlier study, the transcriptome analysis of the salivary glands of the pea aphid *Acyrthosiphon pisum* has led to the identification of C002, a major effector of herbivore infestation[54]. Silencing of C002 resulted in increased aphid lethality as the aphids were unable to reach the plant sieve tube elements. Interestingly, when the C002 ortholog MpC002 from green peach aphid was overexpressed in transgenic *Arabidopsis* plants, it promoted aphid virulence confirming its role in eliciting the plant defenses[58]. Similarly, overexpression of candidate aphid effectors Me10 and Me23 also resulted in the enhanced aphid virulence in *N. benthamiana*[2].

Insect herbivory causes burst of jasmonic acid leading to significant transcriptional reprogramming suggesting the involvement of multiple transcription factors (TFs) in inducing insect resistance[13] (Fig. 2). It is well established that binding of jasmonates-Isoleucine (JA-Ile) to Coronatine Insensitive 1 (COI1) causes the degradation of JAZ proteins and activation of the basic helix loop helix (bHLH) TF MYC2 during jasmonates signaling[56].

Recent studies have thrown in new insights into the role of different TFs in transcriptional reprogramming during JA signaling. MYC2 together with MYC3 and MYC4 has an additive response towards defense against herbivory[17].

Table 1
List of prominent genes targeted for realizing insect resistance in plants.

Genes	Plant(s)	Insect pest(s)	Bio-technique used	Function
Defense genes				
Bph1-38	Rice	*Nilaparvata lugens*	Marker-assisted selection, Positional cloning, Molecular breeding	Resistance to *N. lugens*
R-genes (NBS-LRR) (*Mi1.2, Vat, Ra, Bph9/14/18/26*)	Tomato, Melon, Lettuce, Rice	*Bemisia tabaci, Aphis gossypii, Pemphigus bursarius, N. lugens*	Overexpression, gene knock-out, RNAi, Molecular breeding	Improved insect resistance[13]
Proteinase inhibitors (PIs)	Tobacco, Arabidopsis, *Solanum Nigrum*	*Trichobaris mucorea, Trichoplusia ni, M. sexta*	Gene knockout, RNAi	Improved insect resistance[40]
Signaling genes				
OsLecRK1-3	Rice	*N. lugens, Sogatella furcifera*	Genetic transformation, Marker-assisted selection, RNAi	Enhanced resistance to *N. lugens* and *S. furcifera*, broad-spectrum resistance[44]
OsLRR-RLK1	Rice	*Chilo suppressalis*	Overexpression, RNAi	Enhanced resistance to *C. suppressalis*[27]
CDPKs (*NaCDPK4-5*)	Tobacco	*Manduca sexta*	Gene knockout	Resistance by activating JA pathway[75]
NADPH oxidase (*RBOHD/F*)	Arabidopsis, Tobacco	*M. persicae, S. littoralis*	RNAi	Resistance response regulation
MAPKs (*OsMPK3-4, OsMAPK20_5, OsMKK3, SIPK, WIPK, LeMPK1-3, CaMPKs*)	Rice, Tobacco, Tomato, Chickpea	*N. lugens, C. suppressalis, Cnaphalocrocis medinalis, M. sexta, Helicoverpa armigera*	Transcript evaluation, Overexpression, RNAi, Gene knockout	Defense modulations and downstream channeling[44,55,72]
SA pathway genes (*ICS1, NPR1, PAD4*)	Arabidopsis	*Pieris brassicae, S. littoralis*	Gene knockout, Transcript evaluation	Improved resistance
JA pathway genes (*AOC, AOS, LOX, COI1, JAZ*)	Arabidopsis, Rice, Tobacco	*M. sexta, T. ni, S. littoralis, N. lugens, B. tabaci, T. ni*	Gene knockout, RNAi	Resistance response regulation[7,8,45]
ET pathway genes (*ACS2, EIN2/3, EIL1*)	Rice, Arabidopsis	*N. lugens, C. suppressalis, S. littoralis, S. exigua*	RNAi, Gene knockout	Improved resistance
OsGID1	Rice	*N. lugens*	Overexpression, RNAi	Improved resistance
Transcription factors				
*WRKY*s	Rice, Arabidopsis, Tomato	*C. suppressalis, P. brassicae, S. littoralis, M. euphorbiae*	RNAi, Gene knockout	Resistance response regulation[27]
MYBs	Arabidopsis, Tobacco	*M. sexta, S. exigua,*		

Earlier, *Arabidopsis* MYB102 TF has been found to be necessary for defense against *Pieris rapae* although its mechanism of action and relation to JA signaling is uncertain[10]. Similarly, the overexpression of AtMYB75 significantly reduced the growth of *Spodoptera frugiperda* in *Arabidopsis*[31]. In a subsequent experiment, the heterologous expression of MYB12 conferred enhanced resistance to *Spodoptera litura* and *Helicoverpa armigera* in tobacco[51] (Table 1).

In a recent study, Schweizer et al[62] performed a systemic transcriptome profiling to demonstrate the significant resistant effect of nine TFs including WRKYs, NACs and ERFs in resistance to *S. littoralis* herbivory. However, compared to myc234 triple mutant, the knockout lines of these TFs were only partially sensitive to *S. littoralis* suggesting that MYC2, MYC3 and MYC4 are the master regulators of resistance to herbivory in *Arabidopsis*. Contrarywise, the WRKY TFs were found to be prominently responsible for generalist insect resistance in rice. OsWRKY89 conferred enhanced WBPH resistance through increased leaf wax deposition, culm lignification and SA accumulation[67] (Table 1).

OsWRKY70 reported enhanced resistance to stripped stem borer through positive regulation of JA synthesis and sensitivity to BPH through negative regulation of gibberalic acid (GA)[43]. Similarly, OsWRKY45 facilitated BPH resistance through increased production of H_2O_2 and ET[29] and OsWRKY53 demonstrated SSB resistance in rice through negative regulation of OsMPK3/6 signaling[28]. Most recently, OsbHLH61 and OsbLHL96 reported significant induction of defense responsive genes leading to BPH resistance in rice[69]. All these studies establish the involvement of multiple novel TFs in plant defense against insect attack.

RNA interference or the antisense mediated silencing of homologous genes using double stranded RNA (dsRNA) intermediates is a prominent reverse genetic tool that has been exploited to understand the functions of genes and biological management of agriculturally important insect pests[76]. dsRNA targeting essential genes in insects have been introduced into plants which when ingested by the insects feeding on the plants result in reduced growth or death of the insect[76]. Since the first proof of concept study toward the usage of RNAi towards growth inhibition and death of the western corn rootworm (WCR) *Diabrotica virgifera virgifera,* the technology has been effectively employed towards development of resistance against multiple Coleopteran and Lepidopteran insects.

Li et al[42] have reported that BPH or Asian corn borers fed with rice or maize dipped in a solution containing *dsCes* (carboxylesterase gene) or *dsKTI* (Kunitz-type trypsin inhibitors gene) demonstrated significant reduction in their survival rate. Similarly, the expression of *dsNlMLP* (mucin like protein gene) in rice plant protected it from BPH infestation due to impairment of salivary sheath and reduced rate of survival of insects fed on these plants[63].

In another interesting development, an RNAi based insecticide named SmartStax Pro has been developed by Monsanto and Dow Agrosciences[26]. This plant incorporated protectant used a pyramided strategy employing multiple Bt proteins and dsRNA targeting WCR *Snf7* gene resulting in significant control of *Diabrotica virgifera virgifera* infestation in maize[26].

Although the delivery of RNAi through transgenic plants is a reality, it is also expected that the RNA based products are developed in a non-transformative approach to avoid the regulatory issue associated with GM products. One such study reported the exogenous spraying of siRNA molecules against the diamondback moth *Plutella xylostella. Brassica* spp. leaves sprayed with siRNAs targeting the *acetylcholine esterase* genes *AchE2* of *Plutella xylostella* led to more than 60% of the feeding larva[24]. Similarly, the foliar application of naked dsRNA targeting the *actin* gene resulted in significant control of Colorado potato beetle, *Leptinotarsa decemlineata*[60]. These advances indicate that RNAi based gene silencing is a feasible and efficient approach to turn off essential genes in the insect pest leading to crop protection.

MicroRNAs (miRNAs), the endogenous small RNAs that negatively regulate gene expression are implicated in multiple biological processes including plant growth, development and responses to environmental stresses[39]. As like siRNAs, the miRNAs have also been associated in insect related response in plants. For example, the fecundity of aphids was tremendously repressed in *Arabidopsis thaliana* lines mutated with DCL1 and ARGONAUTE1 (AGO1), the two key enzymes involved in miRNA processing[38]. Recent evidences also indicate that miRNAs act as the regulatory modulators of insect resistance in agriculturally important plants.

Thirty two resistance specific miRNAs were identified through high-throughput sequencing of *Solanum lycopersicon* post infection with whitefly, *Bemisia tabaci*[68]. A distinct profile of resistance specific miRNAs has been identified in response to *Aphis gossypii* aphid attack in *Cucumis melo*[61]. Likewise, over 150 miRNAs were differentially expressed in response to herbivory of the tea plant, *Camellia sinensis* by caterpillars of the moth *Ectropis oblique*[30]. In rice, 104 resistance specific and 80 basal defense responsive miRNAs were detected post infection with brown plant hopper (BPH) under compatible and incompatible interaction[72].

Among these miRNAs, OsmiR156 and OsmiR396 have been recognized as primary regulators of BPH resistance in rice. OsmiR156 negatively regulates BPH resistance through the regulation of JA biosynthetic pathway[21], OsmiR396 increases rice sensitivity to BPH by regulating the expression of the *OsF3H* (flavanone 3-hydroxylase), the rate limiting enzyme in the flavonoid biosynthetic pathway[9]. Most recently, small RNA profiling of rice line incorporated with the BPH resistance gene, BPH6 detected 29 opposite expressed and 9 specifically expressed miRNAs during early or late feeding stages suggesting their involvement in BPH6-mediated resistance to BPH in rice[65]. All these findings suggest that plant miRNAs are important in the resistance response against insect and act as useful resource in understanding the role of post-transcriptional silencing components in plant-insect interactions.

The recent emergence of genome editing technologies (GETs) has opened up new avenues for insect resistance in crop plants. GETs are represented by a collection of advance molecular biology techniques that enable targeted modification of genomic loci in a precise and efficient manner[77]. Among other platforms, CRISPR/Cas9 is the most simplistic and revolutionary tool with wider applicability in crop improvement[77]. Although, many crops have been edited by this technique for multiple traits improvement including resistance to bacterial, viral and fungal phytopathogens, its usage for insect resistance is being exploited only recently. A significant study has been recently performed towards inducing BPH and SSB resistance in rice through CRISPR/Cas9 mediated suppression of serotonin[45].

In rice, the cytochrome P450 monooxygenase gene *CYP71A1* induces the activity of tryptamine 5-hydroxylase enzyme and catalyses the conversion of tryptamine to serotonin. A CRISPR/Cas9 mutation of *CYP71A1* gene in rice resulted in higher SA levels, no serotonin production and improved resistance to SSB and BPH[45]. Genome editing tools primary aims at editing susceptibility genes that support the infection and support compatibility with insect or pathogen. Taking this into account, genome editing has the potential to convert susceptible alleles into resistant types avoiding the need of traditional backcross breeding for resistance introgression. Currently, GETs are increasingly being considered to design gene drives among insect pest to prevent them from herbivore infestation. However, more experiments are required to fully exploit this technology towards insect resistance in plants.

Conclusions and future perspectives

The interactions between plant-insect are highly complex and multi-faceted. The co-evolution of plants and insects and their competing arm races for survival is very fascinating. The multi-tiered defense strategies as discussed in the review are deployed by plants to check insect attacks. Significant advancement has been made in the recent times to understand the molecular mechanism of insect resistance in plants and its subsequent utilization in the resistance breeding programmes. Transgenic plants with elevated callose depositions have been found to exhibit improved resistance against the phloem sucking insects like plant hoppers. Overexpression of specific metabolite genes has also resulted in improved insect resistance.

In recent times, significant technological advancement has been made towards understanding of plant and insect genomes, proteomes and transcriptomes. Functional genomics and genetic engineering have facilitated the cloning and characterization of resistance genes, identification of putative insect effectors and exploration of signaling pathways in plant-insect interactions. The genetic system of plant insect interaction is still incomplete in many plants. For instance, while in some plants insect resistance *R* genes have been cloned, their putative effector is unknown. In other plant insect systems, the effector molecules have been identified while the *R*-genes have not been characterized.

More in-depth and exhaustive studies are required to be performed towards identification of host genes and insect secreted effectors to develop a distinctive regulatory network associated with effector triggered signaling mediated resistance against insect pests. New emerging technologies such as RNAi and CRISPR/Cas9 genome editing are promising tools for insect pest control.

However, several limitations makes them unfeasible for use beyond laboratories. For example, higher genetic variability in the natural pest population could result in more variable results for RNAi under field trail conditions. Further, high concentration of dsRNA may not be possibly administered into insects in spite of its requirements for gene silencing at it may lead to imbalanced dietary options. RNAi demands further studies with respect to dsRNA stability and effective field trials to be considered as efficient insect pest control strategies. Likewise, GETs demand precise knowledge about susceptibility factors which would be effectively mutated for realizing insect management. Nevertheless, these technologies will be vital for unravelling the roles of plant *R*-genes and insect effectors in the modulation of plant immunity to insect pests.

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References

1. Aljbory Z. and Chen M.S., Indirect plant defense against insect herbivores: a review, *Insect Sci*, **25(1)**, 2–23 **(2018)**

2. Atamian H.S., Chaudhary R., Cin V.D., Bao E., Girke T. and Kaloshian I., In planta expression or delivery of potato aphid *Macrosiphum euphorbiae* effectors Me10 and Me23 enhances aphid fecundity, *Mol Plant Microbe Interact*, **26(1)**, 67–74 **(2013)**

3. Begum M.A., Shi X.X., Tan Y., Zhou W.W., Hannun Y., Obeid L., Mao C. and Zhu Z.R., Molecular characterization of rice *OsLCB2a1* gene and functional analysis of its role in insect resistance, *Front Plant Sci.*, **7**, 1789 **(2016)**

4. Berens M.L., Berry H.M., Mine A., Argueso C.T. and Tsuda K., Evolution of hormone signaling networks in plant defense, *Annu. Rev. Phytopathol.*, **55,** 401–425 **(2017)**

5. Blenn B., Bandoly M., Küffner A., Otte T., Geiselhardt S., Fatouros N.E. and Hilker M., Insect egg deposition induces indirect defense and epicuticular wax changes in *Arabidopsis thaliana*, *J Chem Ecol.*, **38(7)**, 882–892 **(2012)**

6. Bricchi I., Bertea C.M., Occhipinti A., Paponov I.A. and Maffei M.E., Dynamics of membrane potential variation and gene expression induced by *Spodoptera littoralis*, *Myzus persicae* and *Pseudomonas syringae* in *Arabidopsis*, *PLoS One,* **7(10)**, e46673 **(2012)**

7. Cheng C., Gao X., Feng B., Sheen J., Shan L. and He P., Plant immune response to pathogens differs with changing temperatures, *Nat Commun.*, **4**, 2530 **(2013a)**

8. Cheng X., Zhu L. and He G., Towards understanding of molecular interactions between rice and the brown planthopper, *Mol. Plant*, **6(3)**, 621–634 **(2013b)**

9. Dai Z., Tan J., Zhou C., Yang X., Yang F., Zhang S., Sun S., Miao X. and Shi Z., The OsmiR396-OsGRF8-OsF3H-flavonoid pathway mediates resistance to the brown planthopper in rice (*Oryza sativa*), *Plant Biotechnol J.*, **17(8)**, 1657–1669 **(2019)**

10. De Vos M., Denekamp M., Dicke M., Vuylsteke M., Van Loon L., Smeekens S.C. and Pieterse C.M., The *Arabidopsis thaliana* transcription factor AtMYB102 functions in defense against the insect herbivore *Pieris rapae*, *Plant Signal Behav*., **1(6)**, 305–311 **(2006)**

11. Dodd A.N., Kudla J. and Sanders D., The language of calcium signaling, *Annu Rev Plant Biol.*, **61**, 593–620 **(2010)**

12. Drerup M.M., Schlücking K., Hashimoto K., Manishankar P., Steinhorst L., Kuchitsu K. and Kudla J., The Calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the *Arabidopsis* NADPH oxidase RBOHF, *Mol Plant,* **6(2)**, 559–569 **(2013)**

13. Du B., Chen R., Guo J. and He G., Current understanding of the genomic, genetic and molecular control of insect resistance in rice, *Mol. Breeding*, **40**, 1-24 **(2020)**

14. Du L., Yang T., Puthanveettil S.V. and Poovaiah B.W., Decoding of calcium signal through calmodulin: Calmodulin-binding proteins in plants, In Luan S., (eds., Coding and decoding of calcium signals in plants, Signaling and communication in plants, Springer, Berlin, 177–233 **(2011)**

15. Erb M., Meldau S. and Howe G.A., Role of phytohormones in insect-specific plant reactions, *Trends Plant Sci.*, **17(5)**, 250–259 **(2012)**

16. Erb M. and Reymond P., Molecular interactions between plants and insect herbivores, *Annu Rev Plant Biol.*, **70**, 527–557 **(2019)**

17. Fernández-Calvo P., Chini A., Fernández-Barbero G., Chico J.M., Gimenez-Ibanez S., Geerinck J., Eeckhout D., Schweizer F., Godoy M., Franco-Zorrilla J.M., Pauwels L., Witters E., Puga M.I., Paz-Ares J., Goossens A., Reymond P., De Jaeger G. and Solano R., The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses, *Plant Cell*, **23(2)**, 701-15 **(2011)**

18. Fischer R.C., Richter A., Wanek W. and Mayer V., Plants feed ants: food bodies of myrmecophytic Piper and their significance for the interaction with *Pheidole bicornis* ants, *Oecologia*, **133(2)**, 186–192 **(2002)**

19. Frost C.J., Appel H.M., Carlson J.E., De Moraes C.M., Mescher M.C. and Schultz J.C., Within-plant signaling via volatiles overcomes vascular constraints on systemic signaling and primes responses against herbivores, *Ecol Lett.*, **10(6)**, 490–498 **(2007)**

20. Fürstenberg-Hägg J., Zagrobelny M. and Bak S., Plant defense against insect herbivores, *Int J Mol Sci.*, **14(5)**, 10242–10297 **(2013)**

21. Ge Y., Han J., Zhou G., Xu Y., Ding Y., Shi M., Guo C. and Wu G., Silencing of miR156 confers enhanced resistance to brown planthopper in rice, *Planta*, **248(4)**, 813–826 **(2018)**

22. Gilardoni P.A., Hettenhausen C., Baldwin I.T. and Bonaventure G., *Nicotiana attenuata* LECTIN RECEPTOR KINASE1 suppresses the insect-mediated inhibition of induced defense responses during *Manduca sexta* herbivory, *Plant Cell*, **23(9)**, 3512–3532 **(2011)**

23. Glauser G., Marti G., Villard N., Doyen G.A., Wolfender J.L., Turlings T.C. and Erb M., Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores, *Plant J.*, **68(5)**, 901–911 **(2011)**

24. Gong L., Chen Y., Hu Z. and Hu M., Testing insecticidal activity of novel chemically synthesized siRNA against *Plutella xylostella* under laboratory and field conditions, *PLoS One*, **8(5)**, 1–8 **(2013)**

25. Harfouche A.L., Shivaji R., Stocker R., Williams P.Y. and Luthe D.S., Ethylene signaling mediates a maize defense response to insect herbivory, *Mol Plant Microbe Interact*, **19(2)**, 189–199 **(2006)**

26. Head G.P., Carroll M.W., Evans S.P., Rule D.M., Willse A.R., Clark T.L., Storer N.P., Flannagan R.D., Samuel L.W. and Meinke L.J., Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management, *Pest Manag Sci.*, **73(9),** 1883–1899 **(2017)**

27. Hu L., Ye M., Kuai P., Ye M., Erb M. and Lou Y., OsLRR-RLK1, an early responsive leucine-rich repeat receptor-like kinase, initiates rice defense responses against a chewing herbivore, *New Phytol.*, **219(3)**, 1097–1111 **(2018)**

28. Hu L., Ye M., Li R. and Lou Y., OsWRKY53, a versatile switch in regulating herbivore-induced defense responses in rice, *Plant Signal Behav*., **11(4)**, e1169357 **(2016)**

29. Huangfu J., Li J., Li R., Ye M., Kuai P., Zhang T. and Lou Y., The transcription factor OsWRKY45 negatively modulates the resistance of rice to the brown planthopper *Nilaparvata lugens*, *Int J Mol Sci.*, **17(6)**, 697 **(2016)**

30. Jeyaraj A., Liu S., Zhang X., Zhang R., Shangguan M. and Wei C., Genome-wide identification of microRNAs responsive to *Ectropis oblique* feeding in tea plant (*Camellia sinensis* L.), *Sci Rep.*, **7(1)**, 13634 **(2017)**

31. Johnson E.T. and Dowd P.F., Differentially enhanced insect resistance, at a cost, in *Arabidopsis thaliana* constitutively expressing a transcription factor of defensive metabolites, *J Agric Food Chem.*, **52(16),** 5135–5138 **(2004)**

32. Johnson M.T.J., Evolutionary ecology of plant defences against herbivores, *Funct Ecol.*, **25(2)**, 305–311 **(2011)**

33. Jones J.D. and Dangl J.L., The plant immune system, *Nature*, **444(7117),** 323–329 **(2006)**

34. Kanchiswamy C.N., Takahashi H., Quadro S., Maffei M.E., Bossi S., Bertea C., Zebelo S.A., Muroi A., Ishihama N., Yoshioka H., Boland W., Takabayashi J., Endo Y., Sawasaki T. and Arimura G., Regulation of *Arabidopsis* defense responses against *Spodoptera littoralis* by CPK-mediated calcium signaling, *BMC Plant Biol*., **10**, 97 **(2010)**

35. Karley A.J., Mitchell C., Brookes C., McNicol J., O'Neill T., Roberts H., Graham J. and Johnson S., Exploiting physical defence traits for crop protection: leaf trichomes of *Rubus idaeus* have deterrent effects on spider mites but not aphids, *Ann Appl Biol.*, **168**, 159–172 **(2016)**

36. Kerchev P.I., Fenton B., Foyer C.H. and Hancock R.D., Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signaling pathways, *Plant Cell Environ.*, **35(2)**, 441–453 **(2012)**

37. Kessler A., Halitschke R., Diezel C. and Baldwin I.T., Priming of plant defense responses in nature by airborne signaling between *Artemisia tridentata* and *Nicotiana attenuate*, *Oecologia*, **148(2),** 280–292 **(2006)**

38. Kettles G.J., Drurey C., Schoonbeek H.J., Maule A.J. and Hogenhout S.A., Resistance of *Arabidopsis thaliana* to the green peach aphid, *Myzus persicae*, involves camalexin and is regulated by microRNAs, *New Phytol.*, **198(4)**, 1178–1190 **(2013)**

39. Khraiwesh B., Zhu J.K. and Zhu J., Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants, *Biochim Biophys Acta*, **1819(2)**, 137–148 **(2012)**

40. Laluk K., Prasad K.V., Savchenko T., Celesnik H., Dehesh K., Levy M., Mitchell-Olds T. and Reddy A.S., The calmodulin-binding transcription factor SIGNAL RESPONSIVE1 is a novel regulator of glucosinolate metabolism and herbivory tolerance in *Arabidopsis*, *Plant Cell Physiol*., **53(12)**, 2008–2015 **(2012)**

41. Lawrence S.D. and Novak N.G., Expression of poplar chitinase in tomato leads to inhibition of development in Colorado potato beetle, *Biotechnol Lett*., **28(8)**, 593–599 **(2006)**

42. Li H., Guan R., Guo H. and Miao X., New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests, *Plant Cell Environ*., **38(11)**, 2277–2285 **(2015b)**

43. Li R., Zhang J., Li J., Zhou G., Wang Q., Bian W., Erb M. and Lou Y., Prioritizing plant defence over growth through WRKY regulation facilitates infestation by non-target herbivores, *Elife*, **4**, e04805 **(2015a)**

44. Liu Y., Wu H., Chen H., Liu Y., He J., Kang H., Sun Z., Pan G., Wang Q., Hu J., Zhou F., Zhou K., Zheng X., Ren Y., Chen L., Wang Y., Zhao Z., Lin Q., Wu F., Zhang X., Guo X., Cheng X., Jiang L., Wu C., Wang H. and Wan J., A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice, *Nat Biotechnol*., **33(3)**, 301–5 **(2015)**

45. Lu H.P., Luo T., Fu H.W., Wang L., Tan Y.Y., Huang J.Z., Wang Q., Ye G.Y., Gatehouse A.M.R., Lou Y.G. and Shu Q.Y., Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis, *Nat Plants*, **4(6)**, 338–344 **(2018)**

46. Maffei M.E., Arimura G.I. and Mithöfer A., Natural elicitors, effectors and modulators of plant responses, *Nat Prod Rep.*, **29(11)**, 1288 **(2012)**

47. Maffei M.E., Mithöfer A. and Boland W., Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release, *Phytochemistry*, **68(22-24)**, 2946–2959 **(2007)**

48. Mahanil S., Attajarusit J., Stout M.J. and Thipyapong P., Overexpression of tomato polyphenol oxidase increases resistance to common cutworm, *Plant Sci*., **174(4)**, 456–466 **(2008)**

49. Malik N.A., Kumar I.S. and Nadarajah K., Elicitor and receptor molecules: Orchestrators of plant defense and immunity, *Int J Mol Sci*., **21(3)**, 963 **(2020)**

50. Mantelin S., Bhattarai K.K. and Kaloshian I., Ethylene contributes to potato aphid susceptibility in a compatible tomato host, *New Phytol.*, **183(2)**, 444-456 **(2009)**

51. Mishra P., Pandey A., Tiwari M., Chandrashekar K., Sidhu O.P., Asif M.H., Chakrabarty D., Singh P.K., Trivedi P.K., Nath P. and Tuli R., Modulation of transcriptome and metabolome of tobacco by *Arabidopsis* transcription factor, AtMYB12, leads to insect resistance, *Plant Physiol.*, **152(4)**, 2258–2268 **(2010)**

52. Morton R.L., Schroeder H.E., Bateman K.S., Chrispeels M.J., Armstrong E. and Higgins T.J., Bean alpha-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions, Proc Natl Acad Sci USA, 3820-5 **(2000)**

53. Mousavi S.A., Chauvin A., Pascaud F., Kellenberger S. and Farmer E.E., GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signaling, *Nature*, **500(7463)**, 422–426 **(2013)**

54. Mutti N.S., Louis J., Pappan L.K., Pappan K., Begum K., Chen M.S., Park Y., Dittmer N., Marshall J., Reese J.C. and Reeck G.R., A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant, Proc Natl Acad Sci USA, 9965-9 **(2008)**

55. Nanda S., Wan P.J., Yuan S.Y., Lai F.X., Wang W.X. and Fu Q., Differential responses of *OsMPK*s in IR56 rice to two BPH populations of different virulence levels, *Int J Mol Sci.*, **19(12)**, 4030 **(2018)**

56. Pauwels L. and Goossens A., The JAZ proteins: a crucial interface in the jasmonate signaling cascade, *Plant Cell*, **23(9)**, 3089–3100 **(2011)**

57. Peng J., Deng X., Huang J., Jia S., Miao X. and Huang Y., Role of salicylic acid in tomato defense against cotton bollworm, *Helicoverpa armigera* Hubner, *Z Naturforsch C J Biosci*., **59(12)**, 856-62 **(2004)**

58. Pitino M. and Hogenhout S.A., Aphid protein effectors promote aphid colonization in a plant species-specific manner, *Mol Plant Microbe Interact*, **26(1)**, 130–139 **(2013)**

59. Rossi M., Goggin F.L., Milligan S.B., Kaloshian I., Ullman D.E. and Williamson V.M., The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid, Proc Natl Acad Sci USA, 9750–9754 **(1998)**

60. San Miguel K. and Scott J.G., The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide, *Pest Manag Sci.*, **72(4)**, 801-9 **(2016)**

61. Sattar S., Addo-Quaye C., Song Y., Anstead J.A., Sunkar R. and Thompson G.A., Expression of small RNA in *Aphis gossypii* and its potential role in the resistance interaction with melon, *PLoS One*, **7(11)**, e48579 **(2012)**

62. Schweizer F., Bodenhausen N., Lassueur S., Masclaux F.G. and Reymond P., Differential contribution of transcription factors to *Arabidopsis thaliana* defense against *Spodoptera littoralis*, *Front Plant Sci*., **4**, 13 **(2013)**

63. Shangguan X., Zhang J., Liu B., Zhao Y., Wang H., Wang Z., Guo J., Rao W., Jing S., Guan W., Ma Y., Wu Y., Hu L., Chen R., Du B., Zhu L., Yu D. and He G., A mucin-like protein of planthopper is required for feeding and induces immunity response in plants, *Plant Physiol*., **176(1)**, 552–565 **(2018)**

64. Smith C.M. and Boyko E.V., The molecular bases of plant resistance and defense responses to aphid feeding: current status, *Entomol Exp Appl*., **122**, 1–16 **(2007)**

65. Tan J., Wu Y., Guo J., Li H., Zhu L., Chen R., He G. and Du B., A combined microRNA and transcriptome analyses illuminates the resistance response of rice against brown planthopper, *BMC Genomics*, **21(1)**, 144 **(2020)**

66. Villada E.S., González E.G., López-Sesé A.I., Castiel A.F. and Gómez-Guillamón M.L., Hypersensitive response to *Aphis gossypii* Glover in melon genotypes carrying the *Vat* gene, *J Exp Bot.*, **60(11)**, 3269–3277 **(2009)**

67. Wang H., Hao J., Chen X., Hao Z., Wang X., Lou Y., Peng Y. and Guo Z., Overexpression of rice *WRKY89* enhances ultraviolet B tolerance and disease resistance in rice plants, *Plant Mol Biol*., **65(6)**, 799–815 **(2007)**

68. Wang K., Su X., Cui X., Du Y., Zhang S. and Gao J., Identification and characterization of microRNA during *Bemsia tabaci* infestations in *Solanum lycopersicum* and *Solanum habrochaites*, *Hort Plant J.*, **4(2)**, 62-72 **(2018)**

69. Wang M., Yang D., Ma F., Zhu M., Shi Z. and Miao X., OsHLH61-OsbHLH96 influences rice defense to brown planthopper through regulating the pathogen-related genes, *Rice*, **12(1)**, 9 **(2019)**

70. Wasternack C. and Hause B., Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development, *Ann Bot.*, **111(6),** 1021–1058 **(2013)**

71. Weinhold A. and Baldwin I.T., Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation, Proc Natl Acad Sci., 7855–7859 **(2011)**

72. Wu J., Hettenhausen C., Meldau S. and Baldwin I.T., Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuate*, *Plant Cell*, **19(3)**, 1096–1122 **(2007)**

73. Wu Y., Lv W., Hu L., Rao W., Zeng Y., Zhu L., He Y. and He G., Identification and analysis of brown planthopper-responsive microRNAs in resistant and susceptible rice plants, *Sci Rep*., **7(1)**, 8712 **(2017)**

74. Wu Y., Zhang X., Kang X., Li N., Wang R., Hu T., Xiang M., Wang X., Yuan W., Chen A., Meng D. and Chen S., Oxidative stress inhibits adhesion and transendothelial migration and induces apoptosis and senescence of induced pluripotent stem cells, *Clin Exp Pharmacol Physiol*., **40(9)**, 626–634 **(2013)**

75. Yang J., Duan G., Li C., Liu L., Han G., Zhang Y. and Wang C., The crosstalks between jasmonic acid and other plant hormone signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses, *Front Plant Sci*., **10**, 1349 **(2019)**

76. Zhang J., Khan S.A., Heckel D.G. and Bock R., Next-Generation Insect-Resistant Plants: RNAi-Mediated Crop Protection, *Trends Biotechnol.*, **35(9)**, 871–882 **(2017)**

77. Zhang Y., Massel K., Godwin I.D. and Gao C., Applications and potential of genome editing in crop improvement, *Genome Biol.*, **19(1)**, 210 **(2018)**.

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