



How do plants defend themselves against pathogens-Biochemical mechanisms and genetic interventions

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Abstract In agro-ecosystem, plant pathogens hamper food quality, crop yield, and global food security. Manipulation of naturally occurring defense mechanisms in host plants is an effective and sustainable approach for plant disease management. Various natural compounds, ranging from cell wall components to metabolic enzymes have been reported to protect plants from infection by pathogens and hence provide specific resistance to hosts against pathogens, termed as induced resistance. It involves various biochemical components, that play an important role in molecular and cellular signaling events occurring either before (elicitation) or after pathogen infection. The induction of reactive oxygen species, activation of defensive machinery of plants comprising of

enzymatic and non-enzymatic antioxidative components, secondary metabolites, pathogenesis-related protein expression (e.g. chitinases and glucanases), phytoalexin production, modification in cell wall composition, melatonin production, carotenoids accumulation, and altered activity of polyamines are major induced changes in host plants during pathogen infection. Hence, the altered concentration of biochemical components in host plants restricts disease development. Such biochemical or metabolic markers can be harnessed for the development of “pathogen-proof” plants. Effective utilization of the key metabolites-based metabolic markers can pave the path for candidate gene identification. This present review discusses the valuable information for understanding the biochemical response mechanism of plants to cope with pathogens and genomics-metabolomics-based sustainable development of pathogen proof cultivars along with knowledge gaps and future perspectives to enhance sustainable agricultural production.

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Introduction

The biotic stress constantly affects the agro-ecosystem which includes viruses, mycoplasmas, bacteria and fungi which directly alter the soil health and fertility and productivity of crops. Pathogens disturb the physiological and metabolic processes and pathways in plants resulting in loss of yield and quality in plants (Kumar and Verma 2018). Among all the processes, components of biochemical pathways play a vital part in safeguarding plants against pathogens. In general, when there is no stress, the plants

exhibit optimal growth and development by using the available oxygen. However, during stress like pathogen attack, usage of oxygen results in the production of reactive oxygen species (ROS) in plant tissues (Singla et al. 2019), which in turn causes photo-oxidative damage to biomolecules and the internal cellular structures (Xie et al. 2016; Mittler 2017). The plants respond to such interaction of microbes by inducing a plethora of biochemical changes associated with stress signaling and thus activating their defense pathways. The induced defense mechanism includes various non-enzymatic components comprising phenolic compounds, flavonoids, lignins and enzymes for phenol metabolism like phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidases (POX) and glutathione reductase (GR) (Debona et al. 2012; Akter et al. 2015), accumulation of tannins and phytoalexins. Besides, changes in cell wall composition act as a passive structural fence against pathogen attack. Das and Roychoudhury (2014a, b) reported that carotenoids and lipophilic organic compounds also serve as a defense mechanism for the detoxification of several types of ROS. Another defensive compound such as phytoalexins is also produced in host plants as secondary metabolites by the hypersensitive response. During the pathogen infection, phytoalexins accumulate at the infection site and prevent the fungal growth and also of other pathogens *in-vivo* and thus, considered as an important plant-defensive compound against many necrotrophic and biotrophic pathogens (Bizuneh 2020). Apart from this, polyamines (PAs) also play a key function in the plant's cellular metabolism and hence act as a protective barrier to pathogens by alteration of their activities (Hussain et al. 2011). Hence, the plants are well equipped with natural biochemical compounds to cope with the plant pathogens. Therefore, the present review is aimed to deliver a synthesis of the literature that discusses the function of various plant biochemical components in self-defense against pathogens. Furthermore, we also aimed to discuss the plant defense systems against pathogens through elaborating on the production of ROS, alteration in metabolites, antimicrobial compounds and their role in defense and modification of cell wall composition. Besides, the use of metabolic/biochemical markers for the development of pathogen-resistant cultivars has also been discussed.

Plant disease resistance genes

During the plant-pathogen interaction, the plant releases several types of elicitors. These elicitors are recognized by plant resistance genes and trigger various biochemical and physiological changes in plants. Plant pathogens, pathogen-associated molecular patterns (PAMPs) are recognized by

pattern recognition receptors (PRRs) and damage-associated molecular patterns (DAMPs) by wall-associated kinases (WAKs) within the cell membrane. Recognition and signaling cascade lead to pathogen triggered immunity (PTI). Other elicitors, generally called effectors are intercepted nucleotide-binding domains and leucine-rich repeats (NLRs) and this kind of recognition by R gene and defense response is called effector-triggered immunity (ETI) (Jones and Dangl 2006). PTI and ETI are interconnected and complement each other defense pathways instead of the earlier “Zig-Zag” way (Ngou et al. 2021). PRRs, WAKs, and NLRs mediated interconnected signaling cascades regulated Mitogen-activated protein kinases (MAPKs), ubiquitin, transcription factors (TFs), calcium, hormones, G-proteins expression in the plant (Gururani et al. 2012; Meng and Zhang 2013; Andersen et al., 2018). This leads to various defense responses that reduce pathogen spread; cell wall modification, closure of stomata, production of ROS, hypersensitive response, or the production of various anti-pathogen proteins and compounds (e.g., protease inhibitors, chitinases, defensins, and phytoalexins) (van Loon et al. 2006).

Plant defense system against plant pathogens

In natural habitats, plant defense systems serve a pivotal role in safeguarding plants against pathogens and in nutrient mobilization (Miller et al. 2010). In addition to self-defense of plants, useful microbes and plant growth-promoting rhizobacteria also activate the defense mechanism via two different pathways, systemic acquired resistance (SAR) and induce systematic resistance (ISR). The ISR may be strengthened through beneficial microorganisms, whereas SAR implies an altered gene expression at molecular levels and is related to PR proteins. Nawrocka and Matolepsza (2013) reported that both ISR and SAR, have different gene expression and induction mechanisms that depend on the regulatory pathway. Under biotic stress, beneficial microbes stimulate SAR which includes an accumulation of PR protein and salicylic acid (SA), however, ISR depends on jasmonate and ethylene regulated pathways (Salas-Marina et al. 2011). The biochemical mechanism of self-defense in plants (induced by themselves) against pathogens has been covered under the following sections.

Production of ROS and its role in protection mechanism

ROS production is significant to carry out a hypersensitive response for host defense. The balance between synthesis and removal of ROS is directly interrupted by biotic or

abiotic stress (Mittler 2017). A common consequence in the cell under any stress is the production of ROS, viz., hydrogen peroxide (H_2O_2), superoxide anion radical ($\text{O}_2^{\bullet-}$), singlet oxygen ($^1\text{O}_2$) and hydroxyl radical ($\bullet\text{OH}$) that could lead to extreme oxidative loss to plant tissues. In higher plants, lower levels of ROS have been found to regulate differentiation, redox homeostasis, stress signaling and systemic responses, however, elevated levels of ROS harm cellular components through protein damage, lipid peroxidation and membrane destruction (Das and Roychoudhury 2014a, b). Thus, high ROS affects normal cellular functioning (Asthir et al. 2010). Under stress conditions, plants send signals to alter their metabolism for the synthesis/activation of defensive genes in affected plant parts (Gill et al. 2019).

During stressful conditions, the ROS production rate dramatically increases in plants' mitochondria, chloroplast, endoplasmic reticulum, apoplast, peroxisomes, plasma membrane and cellular walls (Sharma et al. 2012; Mittler 2017). ROS affects the lipids, proteins as well as DNA in plant cells. During stressful conditions, lipid peroxidation increases significantly through the formation of lipid radicals (Meo et al. 2016). ROS also causes oxidation and modifications of the proteins directly or indirectly. Direct changes include nitrosylation, carboxylation, the formation of disulfide bonds and glutathionylation. Interaction of lipid peroxidation products with protein may also lead to indirect changes. Various amino acids like proline, lysine, threonine, arginine, methionine and cysteine are highly susceptible to ROS attack (Petrov et al. 2015). ROS also causes damage to DNA at multiple sites that include changes in nitrogenous bases, breakage of DNA strands, oxidation of deoxyribose sugar, etc. If the cross-linking between DNA and protein is not repaired in time it is very harmful and deadly to the plant (Poprac et al. 2017). ROS detoxifications are carried out when plant cells, enzymes and redox metabolites function synergistically to protect themselves from adverse effects. Oxidative stress tolerance is an integrated mechanism associated with the changes in antioxidative/defensive enzymes, free radical scavenging activities, non-enzymatic antioxidants, osmolytes and signaling molecules (Caverzan et al. 2016). The induced defense is facilitated via defensive enzymes i.e., CAT, SOD, POX, PAL, PPO, ascorbate peroxidase (APX) and tyrosine ammonia-lyase (TAL) along with secondary metabolites such as phenols and condensed tannins and also through the utilization of H_2O_2 and malondialdehyde (MDA) (Bhaduri and Fulekar 2012). During various environmental stresses or pathogenic attacks, the first line of defense is formed by SOD against ROS-induced loss in barley (Torun et al. 2019).

Major antioxidative enzymes that perform a significant role in plant-defense mechanism are discussed in Table 1 and Fig. 1.

Structural modifications in host plants

The plant cell wall is the primary target site of infection by the pathogen. Over a million years of co-evolution, plants have evolved a multilayered defense mechanism against microbes, of which the cell wall is a vital component. Microbes establish a pathogenic relationship with host plants by avoiding the plant cell wall which requires suitable host recognition tactics followed by the appropriate infection structures and/or chemical exudates formation (Turra et al. 2015). The microbes that fail to evolve suitable tactics to bypass the cell wall of a host plant remain as non-pathogens or non-adapted pathogens. In the case of pathogenic microbes, with the ability to overcome preformed barriers, the host plant uses the cell wall as a defense barrier. Some of the mechanisms include:

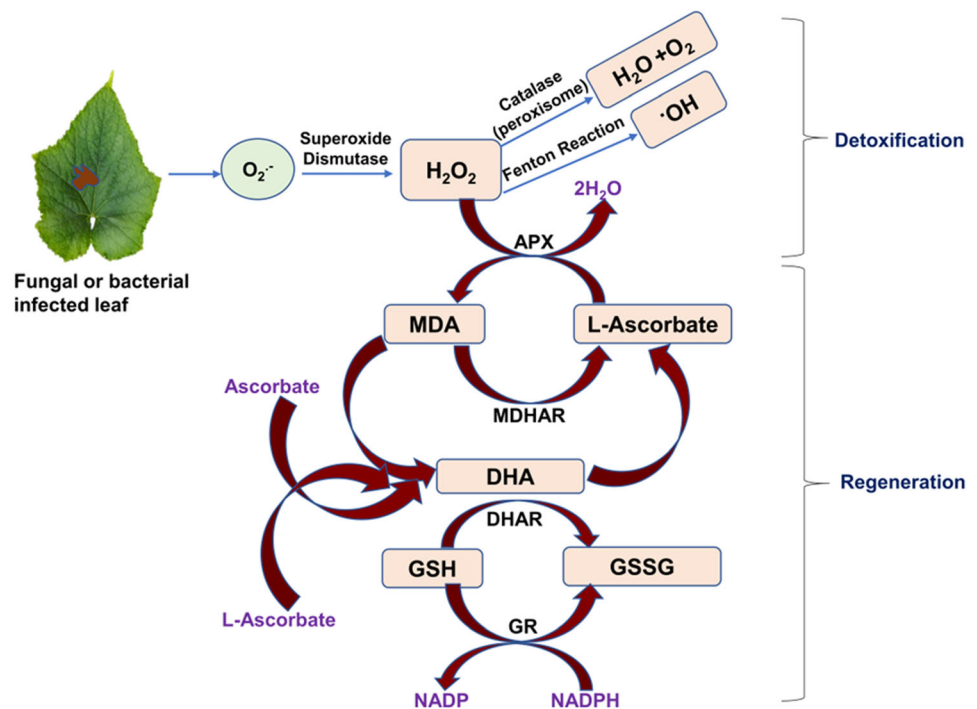
Release of elicitors

Boller and Felix (2009) reported the release of oligosaccharide elicitors during infection from the cell wall of a host plant DAMPs or of a pathogen PAMPs as a part of the process of degradation. Plants recognize these elicitors with a help of immune receptors present on the plasma membrane which eventually trigger signaling cascades to activate various DAMP or PAMP-triggered immunity defense mechanisms, these are also known as Defense Triggered Immunity (DTI) or PTI, respectively (Jones and Dangl 2006). One of the common defense responses among DTI or PTI is the cell wall reinforcement to develop more resistance to physical pressure and/or hydrolytic enzyme produced by the pathogens (Malinovskiy et al. 2014). Moerschbacher and Mendgen (2012) reported that the reinforcement of the cell wall process may take place by various means depending upon the type of interaction with elicitors such as cross-linking and rearrangement of pre-existing cell wall components, the inclusion of cross-linked polymerized materials to the existing cell wall and local cell wall components deposition at the site of infection. For example, the elicitors induce thickening of outer layer parenchyma cells and produce amorphous, fibrillar material to trap the bacteria (Keane 2012). In contrast to bacteria, various defensive substances like callose, hydroxyproline amino acid-rich glycoproteins (such as an extension), phenolic compounds (also lignin as well as suberin) and mineral elements (e.g., calcium and silicon) are produced and deposited into the cell wall to safeguard against fungus attack (Deepak et al. 2010).

Table 1 Description of defensive enzymatic machinery of plants against pathogens

Name of enzyme	Description	Isoforms/types	Function	Reaction catalyzed	References
Superoxide dismutase (SOD)	Family of metalloenzymes present in all organisms. During various environmental stresses or pathogenic attacks, SOD forms the first line of defense against ROS induced damages	<ol style="list-style-type: none"> 1. Mn-SOD (localized in mitochondria) 2. Fe-SOD (present in chloroplasts) 3. Cu/Zn-SOD (residing in cytosol, peroxisomes, and chloroplasts) 4. Increased activity of SOD provides resistance against <i>Alternaria solani</i> in tomato 	Removal of $O_2^{\bullet -}$ via dismutation it into O_2 and H_2O_2	$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+$ \downarrow $2H_2O_2 + O_2$	<p>Torun et al. (2019)</p> <p>Gulzar et al. (2021)</p>
Catalase (CAT)	Tetrameric heme-containing enzyme and has high affinity for H_2O_2 and has a completely high turnover rate (6×10^6 molecules of H_2O_2 to H_2O and O_2 in one minute)	<ol style="list-style-type: none"> 1. CAT1 which is expressed in pollen and seeds (residing in peroxisomes and cytosol) 2. CAT2 in photosynthetic tissues (present in peroxisomes and cytosol) 3. CAT3 which is present in leaves and vascular tissues (localized within the mitochondria) 4. CAT activity increases in leaves of barley and provides resistance against <i>Bipolaris sorokiniana</i> 5. Induction of CAT activity provide resistance against <i>Sclerotium rolfsii</i> in chickpea 	Dismutation of H_2O_2 into H_2O and O_2	H_2O_2 \downarrow $H_2O + (1/2) O_2$	<p>Das and Roychoudhury (2014a, b)</p> <p>Sandalio et al. (2021)</p> <p>Bhaduri and Fulekar (2012)</p> <p>Kaur et al. (2021)</p> <p>Sahni and prasad (2020)</p>
Ascorbate peroxidase (APX)	Class I superfamily of heme peroxidases	<p>Exists in diverse isoforms viz. cytosolic, stromal, thylakoidal, mitochondrial and peroxisomal</p> <p>Up-regulated activity of peroxidase in rice provides resistance against <i>Xanthomonas oryzae</i></p> <p>Upregulated activity of peroxidase shows resistance against powdery mildew disease in cucumber</p>	It utilizes ascorbate as H-donor to breakdown H_2O_2 and releases water and monodehydroascorbate (MDHA) (Fig. 1)	$H_2O_2 + AA$ \downarrow $2H_2O + DHA$	<p>Chiang et al., (2015)</p> <p>Caverzan et al. (2016)</p> <p>Kalaivani et al. (2021)</p> <p>Jogaiah et al. (2020)</p>
Glutathione reductase (GR)	GR is a flavoprotein and an oxidoreductase located in both eukaryotes and prokaryotes	<p>Mainly, it is present in chloroplasts with little amounts present inside the mitochondria and cytosol</p> <p>Increased activity of GR provide resistance against <i>Alternaria solani</i> in tomato</p>	It catalyses reduction of GSSG in NADPH dependent manner and thus is critical in maintaining GSH pool	$GSSG + NADPH$ \downarrow $2GSH + NADP$	<p>Bela et al., (2018)</p> <p>Dey et al. (2016)</p> <p>Gulzar et al. (2021)</p>

Fig. 1 Antioxidant defense system in plants to detoxify the reactive oxygen radicals generated during the stress condition. The induced defense is facilitated via defensive enzymes such as Peroxidases, Catalase, Superoxide Dismutase (SOD) and Ascorbate peroxidase (APX). SOD catalyzes the dismutation of O_2^- to H_2O_2 , catalase (CAT) dismutates H_2O_2 to oxygen and water, and ascorbate peroxidase (APX) reduces H_2O_2 to water by utilizing ascorbate (ASC) as the specific electron donor. This antioxidant defense system is considered the main enzymatic system for protecting cells against oxidative damage



Deposition of papillae

The local depository material of the cell wall is called papillae, which is formed between plant membranes and inside the plant cell wall. Papillae composition varies between plant species but phenolics, ROS, cell wall proteins and polymers are most commonly present among species. Among cell wall polymers (1, 3)- β -glucan callose is plentiful and invasive (Voigt 2014). For instance, Chowdhury et al. (2014) utilized microarray expression profiling to reveal the involvement of major polysaccharides, such as callose, arabinoxylan and cellulose in barley papillae produced in response to the pathogen- *Blumeria graminis* f. sp. *Hordei* (Bgh). They found that the effective papillae that prevent Bgh's penetration contain more polysaccharides than ineffective papillae. The inner core of papillae is made up of callose and arabinoxylan and the outer layer consists of arabinoxylan and cellulose. The established interconnection between arabinoxylan and cellulose to penetration resistance offers new avenues for further refinement of the Composition of papillae and the development of cultivars/plants with better resistance against diseases. Recently, Li et al. (2018) showed the promising function of multi-vesicular bodies (specialized late formed endosomes) in papillae formation in response to bacteria and fungus (Li et al. 2018). Figure 2 explained the biochemical defense response by plant cells in response to a plant pathogen.

Metabolic alteration and synthesis of antimicrobial compounds

Many plant species produce a wide variety of toxic secondary metabolites for pathogens. The functions of secondary metabolites in plant defense include deterrence, toxicity and as a precursor to the physical defense system. The metabolic alteration in hosts consists of the synthesis of many antimicrobial proteins, enzymes and metabolites. All these host responses provide enough strength and rigidity to decrease the wounds caused by pathogens. Some important plant metabolites, enzymes and proteins are discussed here under the following section.

Production of melatonin and its ecological roles against pathogens

Melatonin is derived from serotonin that acts as an effective biocide against pathogens e.g., bacterial and fungal. The low dose of melatonin confers antimicrobial resistance against gram-positive and negative pathogenic bacteria (Tekbas et al. 2008). Melatonin confers antimicrobial activity by upregulating the pathogen-related, SA and ethylene signaling-associated gene and reducing the plant's susceptibility. Melatonin has also been found to be effective in decreasing the rate of infection after entry of the pathogens in plants (Arnao and Hernandez-Ruiz 2018), for example, the melatonin application also reduced the number of *Pseudomonas syringae* (the virulent bacterial pathogen) in infected leaves of *Arabidopsis thaliana* and

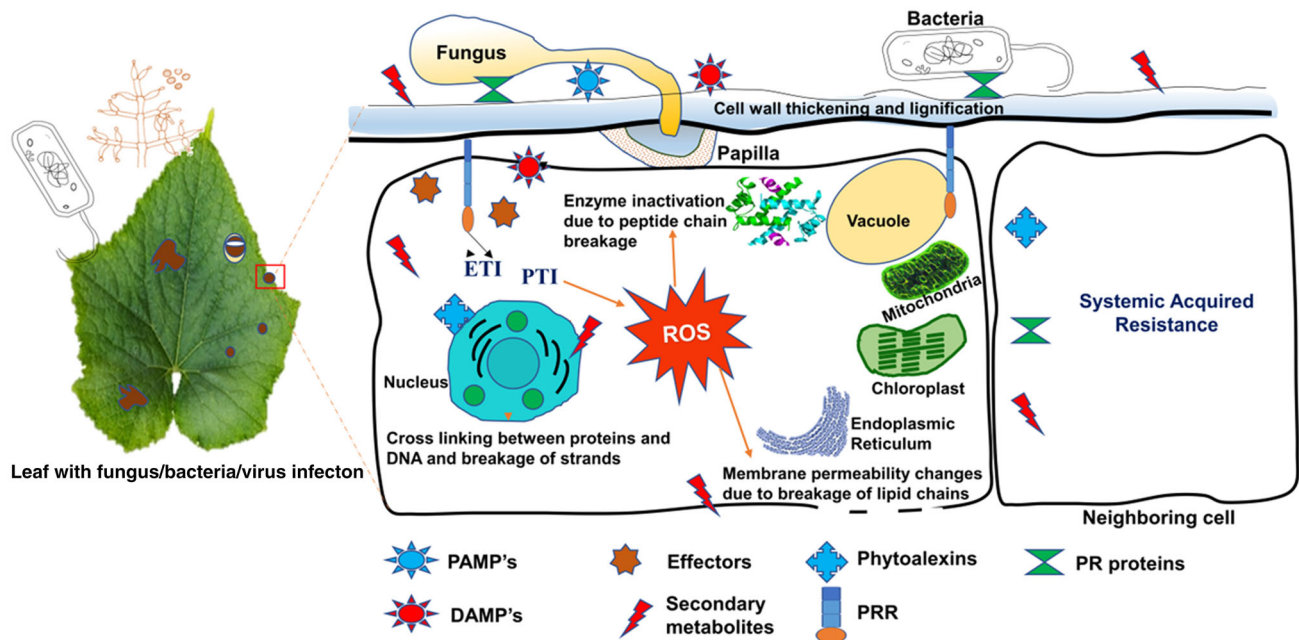


Fig. 2 Schematic representation of biochemical defense response by plant cell in response to plant pathogen (fungus, bacteria, etc.). Fungal germ tube growth is restricted by thickening of cell walls and formation of papilla. Biochemical defense response is triggered by PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) by the production of reactive oxygen species (ROS),

phytoalexins, pathogenesis related (PR) protein and secondary metabolites like phenylalanine ammonia lyase, polyphenol oxidase, flavonoids etc. PAMPs and DAMPs initiate the PRR mediated immune response. Various secondary metabolites also act as signal molecules and intermediates for systemic acquired resistance (SAR) against pathogens in plants

also revealed the involvement of melatonin, especially serotonin (Fujiwara et al. 2010) and N-acetyl serotonin (Lee et al. 2014) in activating defense signal molecules (elicitor) that triggers the expression of numerous defense-related genes against *P. syringae* in *Arabidopsis* and tobacco (Lee et al. 2015). Furthermore, these findings were validated using diverse *Arabidopsis* mutants and hence revealing the involvement of melatonin action in the upstream of defense genes signaling pathway to biosynthesize various phytohormones i.e., salicylic acid, jasmonic acid and ethylene (Zhu et al. 2015) that together bring out disease resistance in a well-coordinated manner. Hence, the initial phase of plant-pathogen interaction results in enhanced ROS production (oxidative burst), which the plant takes care of by enhanced endogenous melatonin production (Lee et al. 2014). There are some interesting examples available, where fungi and bacteria live in plants in a mutualistic endophytic relationship and possess a higher level of melatonin than those plants where such mutualism does not occur (Jiao et al. 2016). Hence, this indicates an interesting area to examine the functions of melatonin levels in mutualistic relationships between plants and fungi to tackle the attack/infection against harmful microbes.

Elicitation of phytoalexins

Phytoalexins are de novo synthesized antimicrobial compounds by plants, as part of the action to defend against invading pathogens. Phytoalexins provide disease—resistance against pathogens but the mode of action varies depending on the types of host–pathogen interaction. The majority of phytoalexins are toxic and repress the growth of pathogenic fungi, nematodes and bacteria. Accumulation of phytoalexin is controlled by the relevant biosynthetic enzymes that are induced by biotic and abiotic stress-generated elicitors. The enhanced expression of biosynthetic enzyme coding genes increases the levels of phytoalexins. The response of the elicitors to induce the phytoalexin synthesis quickly (within minutes) stimulates de novo transcription of the corresponding genes. For example, Glyceollin (pathogen elicited phytoalexins) regulates resistance against *Phytophthora sojae* in soybean (Jahan et al. 2020). Like the localized cellular compartmentalization of phytoalexins during pathogen interaction, induction of the phytoalexin biosynthetic enzyme genes is also localized to the cells neighbouring the infection site. For example, Cocoa resistance to *V. alboatrum* has been linked with the localized phytoalexins production in the vicinity of the vessel (Cooper et al. 1996; Laouane et al. 2011). This helps to produce phytoalexins at

Table 2 List of the different Phytoalexins identified in plants against various pathogens

Plants	Pathogens or elicitors	Biosynthesis pathways, signaling components and other defense responses	Phytoalexins	References
Alfa-alfa	<i>Fusarium oxysporum</i> f. sp. <i>medicaginis</i>	Flavonoid biosynthesis	Medicarpin and 7,4'-dihydroxyflavone	Gill et al. (2018)
Pea	<i>Nectria haematococca</i> and <i>Mycosphaerella pinodes</i>	Pisatin biosynthesis, Pisatin tolerance	Pisatin	Coleman et al. (2011a, b)
Soybean	<i>Fusarium solani</i>	Phenylpropanoid pathway	Glyceollins	Abdul, M and Al-Muwayhi (2020)
	<i>Colletotricum truncatum</i>	Fatty acid synthesis pathway	Octanoic Acid	Nose et al. (2022)
Tobacco	<i>Botrytis cinerea</i> and <i>Phytophthora nicotianae</i>	Superoxide release, HR cell death	Scopoletin and capsidiol	El Oirdi et al. (2009)
	<i>A. tenuissi</i>	Adverse effects on mycelial growth	Biphenyl	Song et al. (2021)
Grape	<i>Agrobacterium rhizogenes</i>	Tyrosine phosphorylation, cell death	Resveratrol	Kiselev et al. (2007)
Maize	<i>Rhizopus microsporus</i> , <i>Colletotrichum graminicola</i> , <i>Fusarium graminearum</i> , <i>Cochliobolus heterostrophus</i> and <i>Aspergillus flavus</i>	Kauralexin synthesis and jasmonic acid-ethylene synergy	Kauralexins and zealexins	Schmelz et al. (2011)
	<i>Fusarium graminearum</i> and <i>Fusarium verticillioides</i>	Flavonoid Biosynthesis	Xilonenin	Forster et al. (2022)
Oat	<i>Puccinia coronata</i>	Avenanthramide biosynthesis	Avenanthramides	Yang et al. (2004)
Rice	<i>Magnaporthe oryzae</i>	Phytocassanes, momilactones and oryzalexin synthesis, and HR-associated phytoalexin biosynthesis	Momilactone A and momilactone B, phytocassane A, phytocassane E and sakuranein	Hasegawa et al. (2010); Ahuja et al. (2012)
		Diterpenoids	<i>Ent-10-oxodepressin</i>	Liang et al. (2021a, b)
		Flavonoids biosynthesis	Tangeretin	Liang et al. (2021a, b)
Sorghum	<i>Colletotrichum sublineolum</i> and <i>Cochliobolus heterostrophus</i>	Flavone biosynthesis from flavanones, H ₂ O ₂ accumulation, papilla formation, callose deposition, HRGP cross-linking, cell death	Luteolin, apigenin and 3-deoxyanthocyanidins	Liu et al. (2010)
	<i>Fusarium</i>	Anthocyanins synthesis pathway	3-deoxyanthocyanidin	Nida et al. (2021)
Sugarcane	<i>Colletotrichum falcatum</i>	Phenyl propanoid biosynthesis pathway	3-deoxy anthocyanidin	Nandakumar et al. (2021)
Barely	<i>B. sorokiniana</i> and <i>Fusarium graminearum</i>	Chalcone synthesis	Methoxylchalcones	Ube et al. (2021)
Lettuce	<i>Rhizoctonia solani</i> and <i>Olpidium virulentus</i>	Shikimate pathway	Benzoic acid and lettucenin A	Windisch et al. (2021)

higher rates and hence quickly controlling the pathogen proliferation in plants. The general function of the phytoalexin involves puncturing the cell wall of pathogens,

disrupting their metabolism and reproductive functions and hence arresting the growth and development of invaded pathogens.

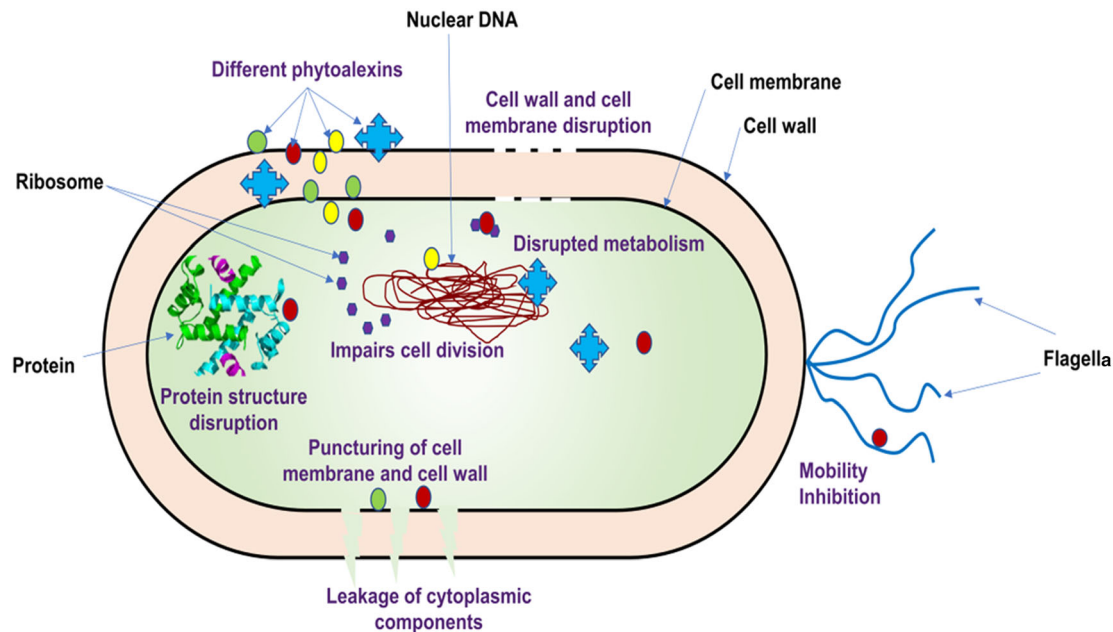


Fig. 3 Diagrammatic representation of mechanisms of phytoalexins against bacteria. Phytoalexins act in many ways, with each phytoalexin having a specific mode of action. It can either directly affect

the cell via membrane disruption and cell metabolites or indirectly by movement or cell multiplication

To date, over 300 chemicals having phytoalexin-like characteristics have been identified belonging to over 30 plant families. The phytoalexins are family-specific. For example, sulfur-containing phytoalexins (like brassin) are most common among all Brassicaceae, while the Poaceae family possesses different phytoalexins like oryzalexins, zealexins, kauralexins, sakuranetin and phenyl amides (Arruda et al. 2016). Similarly, phenylpropanoid-related compounds, steroid glycoalkaloids sesquiterpenoids and coumarins are found in Solanaceae; and isoflavones, coumestans, phaseollin, stilbenes/resveratrol are restricted to the family Leguminosae. The list of phytoalexins identified against different diseases in various crops is presented in Table 2 and the mechanism of phytoalexins against bacteria is represented in Fig. 3. Chemically, phytoalexins are different, however, the majority of phytoalexins are the product of the Shikimic acid pathway through which the majority of the secondary metabolites are derived such as anthocyanins, flavonoids and lignin. The key enzymes such as PPO, PAL, 4-coumarate-CoA ligase (4CL), cinnamate 4-hydroxylase (C4H) and chalcone synthase (CHS) involved in this pathway serve a significant part in defense- mechanisms.

Phenylalanine ammonia lyase (PAL) role in plant resistance

PAL, consisting of tetramer with each subunit of 77–83 kDa, is an extensively studied enzyme (Jendresen

et al. 2015) as its the chief enzyme in the metabolism of phenylpropanoid and aids in synthesizing several secondary metabolites which include phenols (coumarins, flavonoids, lignins), phenolic derivatives and lignin (Bhattacharjee et al. 2017). The activity of PAL increases with the infection in several pathosystems (You et al. 2020). PAL activity in the leaves of resistant cultivars of barley genotypes increased against infection spot blotch pathogen *B. sorokiniana* (Kaur et al. 2021). Resistant reaction mainly occurs due to the defense of the cell walls by lignin intensification and the cell wall-bound phenolic compounds accumulation (Jun et al. 2018). In transgenic tobacco, reduction in lesion size and the number has also been witnessed in plants with PAL over-expression after getting infected with *P. syringae*. Similarly, bread wheat exhibited PAL gene *AevPAL1* confers resistance to cereal cyst nematode by affecting the synthesis of salicylic acid and downstream secondary metabolites (Zhang et al. 2021). These studies indicate that PAL over-expression in plants provides disease resistance.

Role of polyphenol oxidase (PPO) in plant resistance

PPO has three domains which include: i) an N-terminal plastid transit peptide ii) a highly conserved type-3 copper center and iii) a C-terminal part. The PPO catalyzes the oxidation of monophenols and o-diphenols to o-quinones and is broadly scattered amongst bacteria, fungi, plants and animals (Boeckx et al. 2015). Direct effect of PPO on

photosynthesis in walnut (*Juglans regia*) and also as an oxygen buffer or water-water cycle to facilitate reactive oxygen scavenging (Araji et al. 2014). For example, in different pearl millet cultivars, the level of PPO was positively correlated with the incidence of downy mildew resistance (Raj et al. 2006). In another investigation, potato plants resistant to bacterial wilt exhibited relatively enhanced expression of PPO after infection with *Ralstonia solanacearum* than the susceptible plants (El-Argawy and Adss 2016). In tomato plants, the over-expressed PPO exhibited increased resistance to *P. syringae*. Khodadadi et al. (2020) reported transgenic tobacco lines reduced the severity of disease symptoms with a reduced population of bacteria.

Flavonoids

Knowingly, higher plants are rich in flavonoids that occur within the leaves and floral organs. Based on their chemical structure, they are categorized under four groups, namely, flavonols, flavones, isoflavones and anthocyanins. Flavonoids perform important roles in providing protection from pathogens as well as in pigmentation to flowers and other plant parts (Fini et al. 2011). In plants, flavonoids are regarded as one of the secondary ROS scavenging systems experiencing destruction to the photosynthetic pigments because of extra excitation power. Agati et al. (2012) reported that they are important in scavenging $^1\text{O}_2$ and mitigating the destruction that happened to the outer envelope of the chloroplast membrane. For example, antibacterial flavonoids selectively target bacterial cells, inhibit virulence factors and reduce biofilm formation by interfering with quorum sensing (Gorniak et al. 2019). A study was carried out on the role of flavonoids on cotton wilt resistance. Metabolomics and transcriptomic analysis displayed flavonoids enrichment in leaves because of the upregulation of flavonoid biosynthesis genes. Also, in the red cotton cultivar, the fungal-pathogen invasion activity of *Verticillium dahliae* was suppressed because of the enhanced levels of flavonoids (Long et al. 2019). Identified Bayogenin 3-O-cellobioside (Norvinyeku et al. 2020), probenazole-inducible protein 1 (PBZ1), and phenylpropanoid accumulated (Ma et al. 2020) by metabolomics study of rice, are correlated with blast resistance. Metabolomics showed the accumulation of flavonoid compounds and plants coping with *C. gloeosporioides* by biosynthesis of flavonoid compounds providing potential targets for resistance breeding (Jiang et al. 2021).

Lignin deposition

Plants tend to deposit the lignin and callose in the cell wall to encounter the attack from pathogens. Enzymes such as

CAD, PAL and POX are involved in the biosynthesis of lignin. Enhanced activity of these enzymes during pathogen infection indicates their substantial role in plant defense (Scott-Craig et al. 1995). Hence, one of the common responses of plant defense is enhanced lignification (Vance et al. 1980). For instance, wheat varieties that were infected with spot blotch-pathogen exhibited a better lignification response (Eisa et al. 2013). The lipid peroxidation leads to MDA production in response to stress conditions, therefore, MDA content is a lipid peroxidation indicator (Malencic et al. 2004) and used as a marker for cellular damage caused due to stress. Phenolic compounds can also contribute to strengthening host cell components with the aid of lignin and suberin biosynthesis providing act as a physical barrier to pathogens. For example, in olive plantations, “Olive Quick Decline Syndrome” tolerant cultivar has been shown to decrease in disease progression through lignification of cell wall against *X. fastidiosa* (Sabella et al. 2018). Similarly, in potatoes, leaf xylem tissue lignifications (induced by the biocontrol fungus *Trichoderma viride*) have been found to decrease the severeness of late blight disease (Purwantisari et al. 2020). The various biochemical metabolites and cell wall components that have major contributions to disease resistance in different crops are presented in Table 3.

Role of carotenoids as non-enzymatic antioxidants

Carotenoids are yellow, red and orange color pigments. C_{40} isoprenoids possess a long-conjugated polyene chain that is accountable for their coloration and biological activities. The unique characteristic of carotenoids, i.e., a polyene backbone together with a chain of conjugated $\text{C} = \text{C}$ bonds is responsible for both their pigmentation properties and ability to interact with free radicals and singlet oxygen. This property makes carotenoids powerful antioxidants in the plant system (Paznocht et al. 2017). Carotenoids show their anti-oxidative potential by safeguarding the photosynthetic system in the following manners (i) it reacts with the products of lipid peroxidation to end the chain reactions, (ii) scavenges $^1\text{O}_2$ and heat dissipation, (iii) prevents $^1\text{O}_2$ formation by reacting with $^3\text{Chl}_*$ and excited chlorophyll (Chl_*) (Young and Lowe 2018). According to the studies of Mohamad and Bahman (2018), potato plants challenged with *Rhizoctonia solani* and treated with SA showed an increase in carotenoid content which further provided strong antioxidants to the potato plant system resulting in lower disease incidence.

Polyamines and their response towards biotic stresses

Polyamines alter in plant cells when they interact with fungal, bacterial and viral pathogens (Asthir et al. 2004)

Table 3 List of studies of various biochemical metabolites and cell wall components for providing disease resistance in different crops

S. No	Crop	Pathogen	Response	References
1	Barley	<i>Puccinia striiformis</i> f. sp. <i>Hordei</i> (causing stripe rust in barley)	Leaves of the resistant cultivar (RD 2901) showed an increase in activities of NADPH oxidase, catalase, peroxidase, and enzymes of ascorbate–glutathione pathway at the seedling stage	Singla et al. (2019)
2	Chickpea	<i>Helicoverpa armigera</i> (Insect pest)	Resistant genotypes showed the integrative effect of up-regulated defensive components in leaves, pod walls and seeds such as enhanced activities of catalase, peroxidase, glutathione reductase. Polyphenol oxidase and phenylalanine ammonia lyase, and accumulation of H ₂ O ₂ and total phenols	Kaur et al. (2017)
3	Castor	<i>Fusarium oxysporum</i> f. sp. <i>ricini</i>	Thickening of the cell wall, Increased activities of defense enzymes viz, superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and β-1,3-glucanase (PR protein) in resistant cultivars as compared to the susceptible cultivar	Bharathi et al. (2019)
4	Barley	<i>Puccinia striiformis</i> f. sp. <i>Hordei</i> (causing stripe rust in barley)	RD2901 (resistant behavior) depicted increased levels of PR proteins, phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and accumulated β-glucan and lignin in the plant cell wall during plant-pathogen interaction	Singla et al. (2020)
5	Rice	<i>Magnaporthe oryzae</i>	Activity of phenylalanine ammonia lyase (PAL) was induced in rice plants in response to the fungal pathogen Bayogenin 3-O-cellobioside accumulated	Giberti et al. (2012) Norvienyeku et al. (2020)
6	Wheat	<i>Alternaria trititina</i>	Probenazole-inducible protein 1 (PBZ1), and phenylpropanoid accumulated and provide blast resistance	Ma et al. (2020)
7	Tomato	<i>Ralstonia solanacearum</i>	Total phenol contents were significantly higher in resistant varieties compared to the susceptible ones	Mishra et al. (2011)
		<i>Alternaria alternata</i>	Activities of phenylalanine ammonia lyase (PAL) and polyphenol oxidase were significantly higher in resistant cultivars along with the increase in total phenolic content as compared to the susceptible cultivars	Vanitha et al., (2009)
8	Arabidopsis	<i>Powdery mildew</i>	Chitinase and β-1,3-glucanase induction in tomato cause fruit defense mechanism against <i>A. alternata</i> infection	Cota et al. (2007)
		<i>Pseudomonas syringae</i>	Elevated early callose deposition results in complete penetration resistance	Ellinger et al. (2013)
			Glycosylation and accumulation of N-hydroxy pipelicolic acid provide defense against <i>Pseudomonas syringae</i>	Holmes et al. (2021)
9	<i>Eruca sativa</i>	<i>Alternaria brassicicola</i>	Induction of β-1,3- glucanase and chitinase activities (PR proteins) in the resistant cultivars	Gupta et al. (2013)
10	Stylo	<i>Colletotrichum gloeosporioides</i>	Metabolomics showed the increased accumulation of flavonoid compounds and cope with <i>C. gloeosporioides</i>	Jiang et al. (2021)

e.g., spermine (Spm), performs a vital function as a mediator in providing defense against the various pathogens thus providing resistance to plants (Takahashi et al. 2004). The accumulation of spermidine in barley leaves was observed after infection with *Puccinia hordei* and Bgh(powdery mildew pathogen). However, it is difficult to investigate the contribution of polyamines in both host plants as well as in pathogenic fungi against stress. But studies exist that indicate the possibilities of controlling fungal diseases in plants by specifically inhibiting the biosynthesis of polyamines (Hussain et al. 2011).

Polyamines role in host–pathogen interaction revealed that accumulation of hydrogen peroxide because of spermidine induced degradation of polyamines and nitric oxide served a vital function in the interaction between host plant and pathogens (Yamasaki and Cohen 2006; Di Martino et al. 2013). Hence, manipulation of the key genes or upstream of the polyamine-biosynthesis pathway can impart tolerance against pathogens in plants.

Role of phenolics in defense mechanism

Phenols guard the plant against pathogen attack or ultraviolet radiation (Shahidi and Yeo 2016). Phenols can interfere with the oxidation process by reacting with free radicals, chelating-metal ions and by scavenging oxygen (Masisi et al. 2016). As antioxidants, phenolic compounds prevent oxidative damage to cellular organelles and organic molecules such as proteins, membrane lipids, DNA and RNA. Additionally, they function as reducing agents, hydrogen donors and singlet oxygen quenchers (Wang et al. 2018). When pathogens attack, phenolic compounds are produced which are considered as a part of the active defense response in plants (Holub et al. 2019). Cherif et al. (1992) reported that the early and rapid phenolic accumulation at the site of injection results in isolation and limits the progression of pathogens.

Accumulating phenolics possess low molecular weight i.e. benzoic acid and the phenylpropanoids in reaction to infection and leads to slower growth of *B. sorokiniana* in barley and activation of various phytoalexins (Bashyal et al. 2011). Barley possesses higher antioxidant activity than other cereals which are contributed by higher contents of phenolic acids like trans-cinnamic, salicylic, ferulic, chlorogenic, p-hydroxybenzoic, protocatechuic, coumaric and vanillic acids. The different resistant wheat varieties possessed significantly higher total phenol contents as compared to those susceptible to *Alternaria triticina* (Mishra et al. 2011). The activity of PAL expression and the accumulation of phenolic compounds at the infection site has been linked to the resistance mechanism (Nicholson and Hammerschmidt, 1992). In wheat, the level of total phenolic content has been correlated with host resistance to numerous diseases e.g., Karnal bunt (Gogoi et al., 2001) and *Alternaria* blight (Mishra et al. 2011). Metabolomics studies revealed the involvement of phenolic compounds in plant-pathogen interactions (Castro-Moretti et al. 2020; Lopez-Fernandez et al. 2020). Phytohormone SA is the most studied defense-responsive phenolic compound (Lefeverre et al. 2020). A comparative metabolomic study showed that the accumulation of N-OH-Pip (N-hydroxy-pipecolic acid) after bacterial infection, imparts SAR (Chen et al. 2020).

Activation of pathogenesis-related (PR) proteins

In plants, PR proteins are produced to retaliate against various diseases such as fungal, bacterial, viral and viroid diseases, as well as some chemicals. PR proteins were first recognized in tobacco plants infected by the *tobacco mosaic virus* (TMV) (Dani et al. 2005). Most PR proteins exist within the intercellular areas, while, primary PR proteins occur inside the vacuole (Arabi et al. 2019). Some

PR proteins are basic and sensitive to degradation by proteolytic enzymes (in the case of tomato and potato). Carbohydrates aid in the synthesis of several defense biomolecules like phenolics and phytoalexins. The metabolism of sucrose, a major translocator of carbon in plants, gets seriously affected during disease (Kosova et al. 2014). Hence, the quality and quantity maintenance of sugars (as well as PR proteins generation and accumulation) against invading pathogens is pivotal.

PR proteins accumulate locally inside the infected and surrounding uninfected tissues hence, restricting the spread of infection to infected parts only. Liu et al. (2010) classified PR-proteins into 17 families like β -1, 3-glucanases, chitinases, peroxidases, thaumatin-like proteins, ribosome-inactivating proteins, thionins, non-specific lipid switch proteins, oxalate oxidase and oxalate oxidase-like proteins. Chitinases, a PR-1 family protein cleaves the bond between C1 and C4 of chitin's consecutive N-acetylglucosamine (NAG) monomers. Plant chitinases are usually endo-chitinases having the ability to degrade chitin (Suarez et al. 2001). Extracellular chitinases quickly block the spreading of the hyphae that invade internal areas. It also helps to release fungal elicitors that induce the synthesis of several other chitinases inside the host (Stangarlin and Pascholati 2000). Unlike other plant proteins, PR proteins are stabilized through disulfide linkages and hence are resistant to proteolysis and increased temperatures (Gorjanovic 2009). Here we have elaborated the functions of the chitinase and glucanase PR proteins family in disease-resistance.

Role of chitinase and glucanase enzymes during pathogen infection

In plants, β -1, 3-glucanases belong to the PR-2 family of PR proteins (Ji et al. 2000). β -1, 3-glucanases can cleave the β -1, 3-glycosidic bond in β -1, 3-glucan, a major cell wall component of Oomycetes. In contrast to chitinases, β -1, 3-glucanase (called callose in plants) is more important in plant life as indicated from their role in various other physiological functions, apart from plant defense (Anguelova et al. 2001). β -1, 3-glucanase plays direct as well as indirect actions to safeguard the plants from fungal pathogens via causing hydrolysis and lysis of fungal cell walls and oligosaccharide elicitors formation for the generation of various PR proteins or phytoalexins, respectively (Ebrahim et al. 2011). Chitinases and β -1, 3-glucanase are the most significant hydrolytic enzymes amongst PR proteins produced in several plant taxa after encountering various pathogen infections (Sels et al. 2008), for example, their increased concentration offers protection to the plants against fungal pathogens via degradation of the cell wall as it contains the vital substances i.e., chitin and β -1, 3-glucan (Santen et al. 2005). After fungal infection, β -1,

3-glucanases expressed in coordination with chitinases as cited from different crops like the bean, pea, tomato, maize, tobacco, soybean, wheat, barley and potato (Sels et al. 2008). Wheat infection with stripe rust fungus (*P. striiformis* f. sp. *tritici*) has increased chitinase activity by upregulating TaBZR2, which confer broad-spectrum resistance (Bai et al. 2021).

Transcription factors and regulatory elements

Transcription factors (TFs) regulate gene expression through binding to specific sequences in the promoters of their target genes. TFs play roles at several levels of resistance by transcriptional reprogramming: (a) activate receptor proteins directly by TF (b) basal resistance components expression (e.g., response suppression proteins, receptors, kinases), (c) activation of downstream of receptor initiation (i.e., MAPK cascade leading to TF activation via phosphorylation) (Franco-Zorrilla et al. 2014; Lu et al. 2011). Upon infection to pathogens, plants respond via altered transcriptional reprogramming of the TF families like AP2/ERF, bHLH, bZIP, MYB, NAC and WRKY (Samota et al. 2017; Tsuda and Somssich 2015). For eg. in rice, Ideal Plant Architecture 1 (IPA1) TF imparts the resistance against rice blast infection by regulating (activating) the expression of WRKY45, a pathogen defense gene (Wang et al. 2018). Similarly, OsWRKY53 imparts resistance against rice blast in rice (Chujo et al. 2007). This indicates WRKY53 plays a prominent role in regulating the release of ROS, throughout the hypersensitive response. TF TaRIM1 (*R. cerealis*-induced MYB confers resistance to sharp eyespot disease in wheat via modulating defense genes (Shan et al. 2016). Similarly, ORK10/LRK10 are defense regulator receptor kinases that impart resistance against fungal diseases in cereal crops (Marcel et al. 2010).

The regulatory element, GCC-box elements represent the hallmark of the promoters of aphid- and pathogen-responsive genes (Dong et al. 2010). Conclusively, defense genes such as *WRKY53* impart broad-spectrum resistance through the transfer of wide-stream responsive signals to the other defense-related genes in proximity (upstream and downstream). Hence, the signaling crosstalk of the TFs for disease resistance seems a promising domain of research in crops.

How can plant metabolites be used in crop improvement?

Metabolomics measures the metabolite abundance and environmentally induced changes in metabolites concentration, as a predictive biomarker for disease diagnosis.

Metabolic markers are a sub-category of biomarkers, which reflect the compounds involved in plant metabolism (Fernandez et al. 2016; Zaynab et al. 2019). During biotic stresses, plants accumulate numerous metabolites often tissue and species-specific, that can function as biomarkers for biotic stress resistance (Razzaq et al. 2019). Such metabolites that can be used to provide tolerance against biotic or abiotic stresses are regarded as defensive diagnostic or metabolite markers. In plants, single metabolic markers have been suggested to assess the stress intensity, e.g., proline, which accumulates drought stress-prone plant species (Hayat et al. 2012). Later, metabolic variables were found to be useful markers to detect stress damage or resistance and hence the diagnostic markers have been developed in the form of enzymes (Gibon et al. 2004), metabolites (Korn et al. 2010; Duan et al. 2021), transcripts (Tamaoki et al. 2013) and amino acids (Zhao et al. 2021).

Metabolic markers for disease resistance in crops

Fridman et al. (2000) first proposed metabolic markers in 2000, as a tool to map the metabolite quantitative trait loci (mQTLs) and find the related candidate genes. In 2007 for the very first time, metabolic profiling was carried out to evaluate the biomass performance in *Arabidopsis thaliana* (with a coefficient of correlation of 0.58) that opened new avenues in plant breeding where metabolic markers can be explored to find the allelic combinations for better plant performance (Meyer et al. 2007). Hence, metabolomics became an emerging technique for studying plant immunity, especially in interpreting the functions of small-sized molecules associated with plant—microbe interactions (Feussner and Polle 2015). Phytoalexins and pathogenesis-related proteins are potential metabolite markers against wilt pathogen, *Fusarium oxysporum* in chickpea roots (Kumar et al. 2016). Similarly, Desalegn et al. (2016) identified the function of pisatin and pisatin biosynthesis-associated proteins in conferring resistance against *Didymellapinodes* in *Pisum sativum*. Lately, Seybold et al. (2020) performed metabolomics in wheat to elucidate the resistance mechanism against *Z. tritici* causing disease-resistant metabolites, i.e., trehalose, asparagine, phenylalanine, myoinositol, and L-alanine have been reported to serve as unique metabolite markers against *Fusarium graminearum* causing head blight in wheat (Cuperlovic-Culf et al. 2018). Zhou et al. (2019) reported smiglaside and smilaside as potential biomarkers against the *Fusarium graminearum* (head blight) in maize. Khizar et al. (2020) identified metabolites like phenylpropanoids (stilbenes and furanocoumarin), flavonoids (phlorizin and kaempferol), alkaloids (indolizine and acetylcorynoline) and terpenoids (azelaic acid and oleanolic acid) for leaf spot resistance (*Aspergillus tubingensis*) in cotton. Lately, Zhao et al.

Table 4 Different metabolic markers associated with plants to impart resistance against stress

Plants	Resistant against	Statistical approach	Metabolite accumulated/Reduced	References
Wheat	<i>Fusarium graminearum</i>	Fold change/ Correlation Network	Phenolic acid, Phenylpropanoids, Trehalose, Asparagine, Phenylalanine, Myoinositol, 3-hydroxybutyrate, and L-alanine, Spermine, Putrescine, GABA, Inositols, Galactose, and Lactic acid	Cuperlovic-Culf et al. (2016, 2018) Gunnaiyah et al. (2012)
	Wheat streak mosaic virus	PCA, KEGG, METLIN, MetFrag and MetaboAnalyst	Reduction in some amino acids such as L-tyrosine, tryptophan, isoleucine and phenylalanine	Farahbakhsh et al. (2019)
	<i>Triticum turgidum</i>	PCA, XCMS and CAMERA	Benzoxazinoids	Shavit et al. (2018)
	<i>Fusarium graminearum</i>	PLS-DA	Proline and Alanine	Zhou et al. (2021)
Rice	<i>Rhizoctonia solani</i>	-	Jasmonic acid, mucic acid, and glyceric acid	Suharti et al. (2016)
	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	KEGG, MassHunter, GeneSpring-MS 1.2 and METLIN	Phenylalanine and glutamine, linoleic acid lipids, carbohydrates, alkaloids, xanthophylls, and acetophenone	Sana et al. (2010)
	<i>Orseolia oryzae</i>	ANOVA	Heneicosanoic acid, Threonic acid, Palmitoleic acid, Palmitic acid, Nonadecanoic acid and Linoleic acid	Agarrwal et al. (2014)
	<i>Magnaporthe oryzae</i>	PCA, partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA)	Shikimate, galactinol, trehalose, D-mannose, linolenic acid, dopamine, tyramine, and L-glutamine	Duan et al. (2021)
Maize	<i>Fusarium graminearum</i>	ANOVA and SAS software	Smiglaside and Smilaside	Zhou et al. 2019
Cotton	<i>Aspergillus tubingensis</i>	PCA, OPLS-DA, PLS-DA	Phenylpropanoids (stilbenes and furanocoumarin), flavonoids (phlorizin and kaempferol), alkaloids (indolizine and acetylcorynoline) and terpenoids (azelaic acid and oleanolic acid)	Khizar et al. (2020)

(2021) identified proline and alanine as important metabolic markers for head blight resistance in wheat. In a recent study by Duan et al. (2021), metabolites namely shikimate, galactinol, trehalose, D-mannose, linolenic acid, dopamine, tyramine, and L-glutamine were identified as metabolic markers imparting plant defense response against rice blast. The list of different plant metabolic markers identified to provide resistance against the diseases has been given in Table 4.

Defense metabolites targeted breeding approaches

Metabolomics based genome-wide association studies (mGWAS) and metabolic QTLs (mQTLs) are powerful and potential tools in detecting genetic variations associated

with different metabolites in plants. Metabolic profiling helps in refining the genotype–phenotype association through the investigation of different important metabolites that provide biotic stress resistance. The mQTL/mGWAS helps in the identification of SNP markers-metabolites association resulting in pointing out the candidate genes governing biotic stress tolerance (Fernandez et al. 2016; Wen et al. 2016). However, the profiling of metabolites using electrospray ionization tandem mass spectrometry in mapping or GWAS (the sample size is quite large) is very costly and hence, is expensive to apply for a large sample size (Gieger et al. 2008; Gibon et al. 2012). Therefore, to study the genotype-metabolite associations, the bulk-sequencing-based approach like QTL-Seq can be a relatively more effective approach. In the bulk-seq approach, the bulk

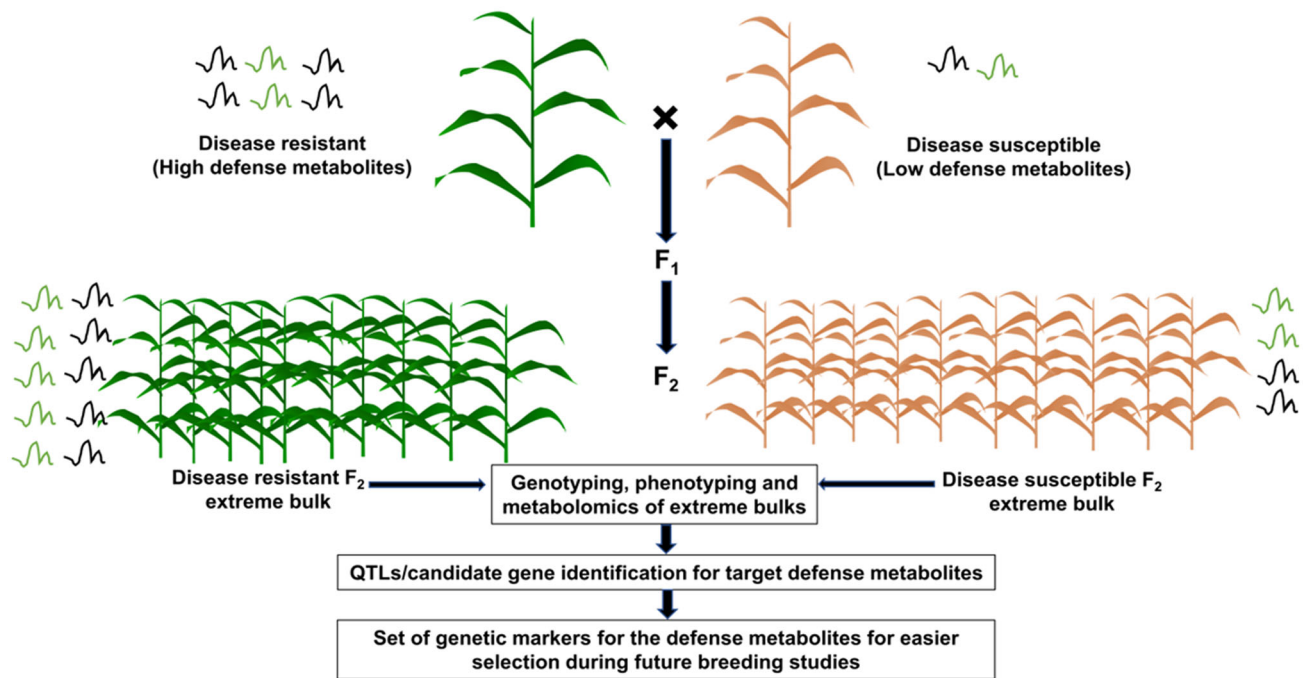


Fig. 4 Genomics assisted breeding approach for utilization of defense metabolites. The F₂ population is generated by crossing the two contrasting genotypes and the extreme bulks identified on screening under artificial inoculation conditions (disease resistant and susceptible) are subjected to whole genome sequencing and metabolomics. One of the parents genome sequence is used as

a reference to identify putative candidate genes and associated SNPs. The identified SNPs can serve as effective markers for the selection of resistant genotypes under future breeding programs such as marker assisted selection. Similarly, mGWAS concept can be applied to a representative diverse set of germplasm and establish marker-trait associations

of around 15–10 extreme phenotypes-resistant and susceptible (based on plant-pathogen reaction) can be selected and used for the metabolomics (Zou et al. 2016). Another approach is to use the subpanels of the germplasm but by ensuring the presence of diversity in sub-panels as the true indicator of the whole germplasm. Such approaches allow identifying a small set of reliable metabolite markers (hence reducing the cost) which can be employed by plant breeders for the effective selection of tolerant germplasm. Later, identification of key genetic markers related to such metabolomics markers can further help to reduce the cost of metabolomics-based breeding programs as sequencing-based markers are cheaper as compared to metabolite markers. Further, forward and reverse genetics can help validate the identified candidate genes for their role in the targeted metabolites synthesis.

The greater use of metabolites in crop improvement has been witnessed due to two important reasons. First, is the development of advanced next-generation sequencing platforms that provided ultra-high-density maps for the identification of mQTLs/candidate genes (Scossa et al. 2015). The second reason is the development and availability of easy-to-use, open-source and efficient statistical platforms for fast-forward analysis of metabolic and phenotypic data. For example, MetabR ([http://metabr.r-forge.r-](http://metabr.r-forge.r-project.org/)

[project.org/](http://metabr.r-forge.r-project.org/)), MetaboAnalystR (<https://github.com/xialab/MetaboAnalystR>), and MetaboDi (<http://github.com/reasmock/MetaboDi/a>) are preferred R software packages for analysis of metabolomics due to open-source nature. The metabolomics can be integrated with the genomic sequencing technologies-based approaches (like transcriptomics and pan-genomics) to exploit the metabolic diversity in plants (Zhou and Liu 2022). The process of genomics assisted breeding approach for utilization of defense metabolites is represented in Fig. 4

Concluding remarks and future perspectives

The optimum and sustainable strategy for the management of plant diseases should be the targeted manipulation of naturally occurring defense mechanisms of host plants. In the recent past, remarkable efforts have been made for the identification of metabolites providing disease resistance in different crops. Different metabolites like enzymes, amino acids, lipids and organic acids have been identified as metabolic markers for disease resistance in different crops. Hence, the target of disease breeding programs should focus on the identification of metabolite markers involved in providing the resistance. The metabolic markers being

more realistic performance indicator of the plants can be more effective than the molecular markers. However, the use of metabolite markers is a costly affair, especially when dealing with larger germplasm. Hence, such established metabolite markers can further be used to identify the closely linked genetic markers for diseases. The identified markers can be further validated and used for the identification of mQTLs and mGWAS. Such metabolites can also be used for genome-wide prediction to enhance the genetic gains in disease resistance breeding. Furthermore, the use of metabolites can be extended to epigenome-wide association studies and pan-genomics. The availability of the phenotypic data on the genotype's performance from the high throughput phenomics approach would also add an additional layer to correlate and establish relationships with the different metabolites. Hence, the collaborations between plant breeders, pathologists, statisticians and bioinformaticians would prove crucial to explore the omics (genomics, phenomics, metabolomics) to their fullest potential. Recently, the emerging approach of metabolome network studies (interactions between the different metabolites) opens the avenue for exploring the interaction of different metabolites in imparting disease. The utilization of resistance-related constitutive and induced metabolites in imparting plant disease resistance would be an interesting research domain to explore in near future. Therefore, it's the need of the hour to explore the natural metabolites in host plants to manage plant diseases effectively and sustainably.

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Declarations

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References

- Abdul M, Al-Muwayhi R (2020) Biotic stress as a defense mechanism in soybean (*Glycine max* L.) toward microbial pathogen: biochemical and physiological pathways study. *Life Sci J* 17(5):24–36. <https://doi.org/10.7537/marslsj170520.02>
- Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: location and functional significance. *Plant Sci* 196:67–76
- Agarwal R, Bentur JS, Nair S (2014) Gas chromatography mass spectrometry based metabolic profiling reveals biomarkers involved in rice-gall midge interactions. *J Integr Plant Biol* 56(9):837–848. <https://doi.org/10.1111/jipb.12244>
- Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. *Trends Plant Sci* 17:73–90
- Akter S, Huang J, Waszczak C, Jacques S, Gevaert K, Breusegem FV (2015) Cysteines under ROS attack in plants: a proteomics view. *J Exp Bot* 66:2935–2944
- Andersen EJ, Ali S, Byamukama E, Yen Y, Nepal MP (2018) Disease Resistance Mechanisms in Plants. *Genes* 9(7):339
- Anguelova MV, Westhuizen VD, Pretorius ZA (2001) Beta-1,3-glucanase and chitinase activities and the resistance response of wheat to leaf rust. *J Phytopathol* 149:381–384
- Arabi E, Jawahar M, Al-daoude A, Al-shehadah E, Shoaib A, Imad M (2019) Differential expression of defense related genes in susceptible versus resistant barley genotypes challenged with *Pyrenophora teres*. *Mycopath* 15:61–66
- Araji S, Grammer TA, Gertzen R (2014) Novel roles for the polyphenol oxidase enzyme in secondary metabolism and the regulation of cell death in walnut (*Juglans regia*). *Plant Physiol* 164:1191–1203
- Arnao MB, Hernandez-Ruiz J (2018) Melatonin and its relationship to plant hormones. *Ann Bot* 121(2):195–207
- Arruda RL et al (2016) An approach on phytoalexins: function, characterization and biosynthesis in plants of the family Poaceae. *Cienc Rural* 46(7):1206–1216
- Asthir B, Spoor W, Duffus CM (2004) Involvement of polyamines, diamine oxidase and polyamine oxidase in resistance of barley to *Blumeriagraminis* f. sp *Hordei*. *Euphytica* 136:307–312
- Asthir B, Koundal A, Bains NS, Mann SK (2010) Stimulation of antioxidative enzymes and polyamines during stripe rust disease of wheat. *Biol Plant* 54:329–333
- Bai X, Zhan G, Tian S, Peng H, Cui X, Islam MA, Goher F, Ma Y, Kang Z, Xu Z-S, Guo J (2021) Transcription factor BZR2 activates chitinase Cht20.2 transcription to confer resistance to wheat stripe rust. *Plant Physiol* 187(4):2749–2762. <https://doi.org/10.1093/plphys/kiab383>
- Bashyal BM, Chand R, Kushwaha C, Joshi AK, Kumar S (2011) *Bipolaris sorokiniana* of barley: infection behaviour in different members of Poaceae. *Indian Phytopath* 46:28–31
- Bela K, Riyazuddin R, Horvath E, Hurton A, Galle A, Takacs Z, Zsigmond L, Szabados L, Tari I, Csiszar J (2018) Comprehensive analysis of anti-oxidant mechanism in *Arabidopsis* glutathione peroxidase -like mutants under salt and osmotic stress reveals organ specific significance of the At GPXL's activities. *Environ Exp Bot* 150:127–140
- Bhaduri AM, Fulekar MH (2012) Antioxidant enzyme responses of plants to heavy metal stress. *Rev Environ Sci Biotechnol* 11:55–69

- Bharathi E, Lakshmi PS, Yadav P, Hameeda B (2019) Defense responses to fusarium oxysporum f. sp. ricini infection in castor (*Ricinus communis* L.) cultivars. *Indian Phytopath* 72:647–656
- Bhattacharjee P, Sarkar J, Chakraborty AP, Chakraborty U (2017) Biochemical responses in *Sorghum bicolor* and *Triticum aestivum* to spot blotch disease and induction of resistance by plant growth promoting rhizobacteria. *J Mycol Plant Pathol* 47:323–332
- Bizuneh GK (2020) The chemical diversity and biological activities of phytoalexins. *Tradit Med Adv*. <https://doi.org/10.1007/s13596-020-00442-w>
- Boeckx T, Winters AL, Webb J, Smith AHK (2015) Polyphenol oxidase in leaves: is there any significance to the chloroplastic localization. *J Exp Bot* 66:3571–3579
- Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu Rev Plant Biol* 60:379–406
- Castro-Moretti FR, Gentzel IN, Mackey D, Alonso AP (2020) Metabolomics as an emerging tool for the study of plant-pathogen interactions. *Metabolites* 10(2):52
- Caverzan A, Casassola A, Brammer SP (2016) Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. *Genet Mol Biol* 39:1–6
- Chen J, Ullah C, Reichelt M, Beran F, Yang Z-L, Gershenzon J et al (2020) The phytopathogenic fungus *Sclerotinia sclerotiorum* detoxifies plant glucosinolate hydrolysis products via an isothiocyanate hydrolase. *Nat Commun* 11(1):3090
- Cherif M, Benhamou N, Menzies JG, Belanger RR (1992) Silicon-induced cellular defense reactions in cucumber plants attacked with *Pythium ultimum*. *Physiol Mol Plant Pathol* 41:411–425
- Chiang CM, Chien HL, Chen LFO, Hsiung TC, Chiang MC, Chen SP, Lin KH (2015) Over expression of the genes coding ascorbate peroxidase from *Brassica campestris* enhances heat tolerance in transgenic *Arabidopsis thaliana*. *Biol Plant* 59:305–315
- Chowdhury J, Henderson M, Schweizer P, Burton RA, Fincher GB, Little A (2014) Differential accumulation of callose, arabinoxylan and cellulose in non penetrated versus penetrated papillae on leaves of barley infected with *Blumeria graminis* f.sp. *hordei*. *New Phytol* 204:650–660
- Chujo T, Takai R, Akimoto-Tomiya C, Ando S, Minami E, Nagamura Y, Kaku H, Shibuya N, Yasuda M, Nakashita H, Umemura K, Okada A, Okada K, Nojiri H, Yamane H (2007) Involvement of the elicitor-induced gene OsWRKY53 in the expression of defenserelated genes in rice. *Biochim Biophys Acta* 1769:497–505
- Coleman JJ, White GJ, Rodriguez-Carres M, Van Etten HD (2011a) An ABC transporter and a cytochrome P450 of *Nectria haematococca* MPVI are virulence factors on pea and are the major tolerance mechanisms to the phytoalexin pisatin. *Mol Plant Microbe Interact* 24(3):368–376. <https://doi.org/10.1094/MPMI-09-10-0198>
- Coleman JJ, White GJ, Rodriguez-Carres M, Etten HDV (2011b) An ABC Transporter and a cytochrome P450 of *nectria*haematococca MPVI are virulence factors on pea and are the major tolerance mechanisms to the Phytoalexin Pisatin. *Mol Plant Microbe Interact* 24(3):368–376
- Cooper RW, Resende MLV, Flood J, Rowan MG, Beale MH, Potter U (1996) Detection and cellular localization of elemental sulphur in disease-resistant genotypes of *Theobroma cacao*. *Nat* 379(6561):159–162
- Cota IE, Troncoso-Rojas R, Sotelo-Mundo R, Sánchez-Estrada A, Tiznado-Hernández ME (2007) Chitinase and β -1,3-glucanase enzymatic activities in response to infection by *Alternaria alternata* evaluated in two stages of development in different tomato fruit varieties. *Scientia Horticulturae* 112(1):42–50. <https://doi.org/10.1016/j.scienta.2006.12.005>
- Cuperlovic-Culf M, Wang L, Forseille L, Boyle K, Merkley N, Burton I, Fobert P (2016) Metabolic biomarker panels of response to fusarium head blight infection in different wheat varieties. *PLoS One*. 11:e0153642
- Cuperlovic-Culf M, Vaughan M, Vermillion K, Surendra A, Teresi J, McCormick S (2018) Effects of atmospheric CO₂ level on the metabolic response of resistant and susceptible wheat to fusarium graminearum infection. *Mol Plant Microbe Interact*. <https://doi.org/10.1094/MPMI-06-18-0161-R>
- Dani V, Simon WJ, Duranti M, Croy RD (2005) Changes in the tobacco leaf apoplast proteome in response to salt stress. *Proteomics* 5:737–745
- Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci*. <https://doi.org/10.3389/fenvs.2014.00053>
- Das K, Roychoudhury A (2014b) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci* 2(53):1–13
- Debona D, Rodrigues FA, Rios JA, Nascimento KJT (2012) Biochemical changes in the leaves of wheat plants infected by *Piricularia oryzae*. *Phytopathology* 102:1121–1129
- Deepak S, Shailasree S, Kini RK, Muck A, Mithofer A, Shetty SH (2010) Hydroxyproline-rich glycoproteins and plant defense. *J Phytopathol* 158(9):585–593
- Desalegn G, Turetschek R, Kaul HP, Wienkoop S (2016) Microbial symbionts affect *Pisum sativum* proteome and metabolome under didymellapinodes infection. *J Proteomics* 30(143):173–187
- Dey S, Sidor A, Rourke BO (2016) Compartment-specific control of reactive oxygen species scavenging by antioxidant pathway enzymes. *J Biol Chem* 291:11185–97
- Di Martino ML, Campilongo R, Casalino M, Micheli G, Colonna B, Prosseda G (2013) Polyamines: emerging players in bacteria-host interactions. *Int J Med Microbiol* 303(8):484–491
- Dong N, Liu X, Lu Y, Du L, Xu H, Liu H, Xin Z, Zhang Z (2010) Overexpression of TaPIEP1, a pathogen-induced ERF gene of wheat, confers host-enhanced resistance to fungal pathogen *Bipolaris sorokiniana*. *Funct Integr Gen* 10:215–226
- Duan G, Li C, Liu Y, Ma X, Luo Q, Yang J (2021) Magnaportheorzyae systemic defense trigger 1 (*MoSDT1*)-mediated metabolites regulate defense response in Rice. *BMC Plant Biol* 21(1):1–12
- Ebrahim S, Usha K, Singh B (2011) Pathogenesis related (PR) proteins in plant defense mechanism. *Sci Microb Pathol* 2:1043–1054
- Eisa M, Chand R, Joshi AK (2013) Biochemical and histochemical traits: a promising way to screen resistance against spot blotch (*Bipolaris sorokiniana*) of wheat. *Eur J Plant Pathol* 137:805–820
- El Oirdi Trapani MA, Bouarab K (2009) The nature of tobacco resistance against *Botrytis cinerea* depends on the infection structures of the pathogen. *Environ Microbiol* 12:239–253
- El-Argawy E, Adss I (2016) Quantitative Gene Expression of Peroxidase, Polyphenoloxidase and Catalase as Molecular Markers for Resistance against *Ralstonia Solanacearum*. *Am J Mol Biol* 6 : 88-100
- Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrow T, Manisseri C, Somerville S, Voigt C (2013) Elevated early callose deposition results in complete penetration resistance to powdery mildew in arabidopsis. *Plant Physiol* 161(3):1433–1444
- Farahbakhsh F, Hamzehzarghani H, Massah A, Tortosa M, Yasayee M, Rodriguez VM (2019) Comparative metabolomics of temperature sensitive resistance to wheat streak mosaic virus (WSMV) in resistant and susceptible wheat cultivars. *J Plant Physiol* 237:30–42
- Fernandez O, Urrutia M, Bernillon S, Giauffret C, Tardieu F, Le Gouis J, Langlade N, Charcosset A, Moing A, Gibon Y (2016)

- Fortune telling: metabolic markers of plant performance. *Metabolomics* 12(10):1–14
- Fuussner I, Polle A (2015) What the transcriptome does not tell—Proteomics and metabolomics are closer to the plants' pathophenotype. *Curr Opin Plant Biol* 26:26–31
- Fini A, Brunetti C, Ferdinando D, Ferrini F, Tattini M (2011) Stress induced flavonoid biosynthesis and the antioxidant machinery of plants. *Plant Signal Behav* 6:709–11
- Forster C, Handrick V, Ding Y, Nakamura Y et al (2022) Biosynthesis and antifungal activity of fungus-induced O-methylated flavonoids in maize. *Plant Physiol* 188(1):167–190
- Franco-Zorrilla JM, López-Vidriero I, Carrasco JL, Godoy M, Vera P, Solano R (2014) DNA-binding specificities of plant transcription factors and their potential to define target genes. In: Proceedings of the national academy of sciences of the United States of America, vol. 111(6): pp. 2367–2372. Doi: <https://doi.org/10.1073/PNAS.1316278111/-DCSUPPLEMENTAL>
- Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. *Proc Natl Acad Sci* 97(9):4718–4723
- Fujiwara T, Maisonneuve S, Isshiki M, Mizutani M, Chen L, Wong HL, Kawasaki T, Shimamoto K (2010) Sekiguchi lesion gene encodes a cytochrome P450 monooxygenase that catalyzes conversion of tryptamine to serotonin in rice. *J Biol Chem* 285(15):11308–11313
- Giberti S, Berteaux CM, Narayana R, Maffei ME, Forlani G (2012) Two phenylalanine ammonia lyase isoforms are involved in the elicitor-induced response of rice to the fungal pathogen *Magnaportheoryzae*. *J Plant Physiol* 169(3):249–254
- Gibon Y, Blaessing OE, Hannemann J, Carillo P, Hohne M, Hendriks JH, Palacios N, Cross J, Selbig J, Stitt M (2004) A robot-based platform to measure multiple enzyme activities in arabidopsis using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. *Plant Cell* 16(12):3304–3325
- Gibon Y, Rolin D, Deborde C, Bernillon S, Moing A (2012) New opportunities in metabolomics and biochemical phenotyping for plant systems biology. In: Roessner DU (Ed.) *Metabolomics*. Rijeka: INTECH pp. 213–240.
- Gieger C, Geistlinger L, Altmair E, Hrabe de Angelis M, Kronenberg F, Meitinger T, Mewes HW, Wichmann HE, Weinberger KM, Adamski J, Illig T, Suhre K (2008) Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genet* 4(11):e1000282
- Gill US, Uppalapati SR, Gallego-Giraldo L, Ishiga Y, Dixon RA, Mysore KS (2018) Metabolic flux towards the (iso)flavonoid pathway in lignin modified alfalfa lines induces resistance against *Fusarium oxysporum* f Sp *Medicaginis*. *Plant Cell & Environ* 41(9):1997–2007. <https://doi.org/10.1111/pce.13093>
- Gill MB, Zeng F, Shabala L, Zhang G, Yu M, Demidchik V, Shabala S, Zhou M (2019) Identification of QTL related to ROS formation under hypoxia and their association with waterlogging and salt tolerance in barley. *Int J Mol Sci* 20:1–15
- Gogoi R, Singh DV, Srivastava KD (2001) Phenols as a biochemical basis of resistance in wheat against Karnal bunt. *Plant Pathol* 50:470–476
- Gorjanovic S (2009) A review: Biological and technological functions of barley seed pathogenesis—related proteins (PRs). *J Inst Brew* 115:334–360
- Gorniak I, Bartoszewski R, Krolczewski J (2019) Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochem Rev* 18:241–272
- Gulzar N, Ali S, Shah MA, Kamili AN (2021) Silicon supplementation improves early blight resistance in *Lycopersicon esculentum* Mill by modulating the expression of defense-related genes and antioxidant enzymes. *Biotech* 11(5):232
- Gunnaiah R, Kushalappa AC, Duggavathi R, Fox S, Somers DJ (2012) Integrated metabolite-proteomic approach to decipher the mechanisms by which wheat QTL (Fhb1) contributes to resistance against *Fusarium graminearum*. *PLoS ONE*. 7:e40695
- Gupta P, Ravi I, Sharma V (2013) Induction of β -1,3-glucanase and chitinase activity in the defense response of *Eruca sativa* plants against the fungal pathogen *Alternaria brassicicola*. *J Plant Interact* 8:155–161
- Gururani MA, Venkatesh J, Upadhyaya CP, Nookaraju A, Pandey SK, Park SW (2012) Plant disease resistance genes: current status and future directions. *Physiol Mol Plant Pathol* 78:51–65. <https://doi.org/10.1016/J.PMPP.2012.01.002>
- Hasegawa M, Seo IMS, Lmai T, Koga J, Okada K, Yamane H, Ohashi Y (2010) Phytoalexin accumulation in the interaction between rice and the blast fungus. *Mol Plant Microbe Interact* 23(8):1000–1011. <https://doi.org/10.1094/MPMI-23-8-1000>
- Hayat R, Ahmed I, Sheirdil RA (2012) An overview of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. In: Ashraf M, Ozturk M, Ahmad MSA, Aksoy A (eds) *Crop production for agricultural improvement*. Springer, Berlin, pp 557–579
- Holmes EC, Chen YC, Mudgett MB, Sattely ES (2021) Arabidopsis UGT76B1 glycosylates N-hydroxy-pipecolic acid and inactivates systemic acquired resistance in tomato. *Plant Cell* 33(3):750–765
- Holub P, Nezval J, Stroch M, Spunda V, Urban O, Jansen MA, Klem K (2019) Induction of phenolic compounds by UV and PAR is modulated by leaf ontogeny and barley genotype. *Plant Physiol Biochem* 134:81–93
- Hussain SS, Ali M, Ahmad M, Siddique KH (2011) Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnol Adv* 29(3):300–311
- Jahan MA, Harris B, Lowery M, Infante AM, Percifield RJ, Kovicich N (2020) Glyceollin transcription factor GmMYB29A2 regulates soybean resistance to *Phytophthora sojae*. *Plant Physiol* 183(2):530–546. <https://doi.org/10.1104/pp.19.01293>
- Jendresen CB, Stahlhut SG, Li M, Gaspar P, Solvej S, Forster J, Maury J, Borodina I, Nielsen AT (2015) Highly active and specific tyrosine ammonia-lyases from diverse origins enable enhanced production of aromatic compounds in bacteria and *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 81:4458–4476
- Ji C, Norton RA, Wicklow DT, Dowd PF (2000) Isoform patterns of chitinase and β -1,3-glucanase in maturing corn kernels (*Zea mays* L.) associated with *Aspergillus flavus* milk stage induction. *J Agric Food Chem* 48:507–511
- Jiang L, Wu P, Yang L, Liu C, Guo P, Wang H, Wang S, Xu F, Zhuang Q, Tong X et al (2021) Transcriptomics and metabolomics reveal the induction of flavonoid biosynthesis pathway in the interaction of *Stylosanthes-Colletotrichum gloeosporioides*. *Genomics* 113:2702–2716
- Jiao J, Ma Y, Chen S, Liu C, Song Y, Qin Y, Yuan C, Liu Y (2016) Melatonin-producing endophytic bacteria from grapevine roots promote the abiotic stress-induced production of endogenous melatonin in their hosts. *Front Plant Sci* 7:1387
- Jogaiah S, Satapute P, De Britto S, Konappa N, Udayashankar AC (2020) Exogenous priming of chitosan induces upregulation of phytohormones and resistance against cucumber powdery mildew disease is correlated with localized biosynthesis of defense enzymes. *Int J Biol Macromol* 162:1825–1838
- Jones JDG, Dangl JL (2006) The plant immune system. *Nat* 444:323–329
- Jun S-Y, Sattler SA, Cortez GS, Vermerris W, Sattler SE, Kang C (2018) Biochemical and structural analysis of substrate

- specificity of a *Phenylalanine Ammonia-Lyase*. *Plant Physiol* 176(2):1452–1468. <https://doi.org/10.1104/pp.17.01608>
- Kalaivani K, Maruthi-Kalaiselvi M, Senthil-Nathan S (2021) Seed treatment and foliar application of methyl salicylate (MeSA) as a defense mechanism in rice plants against the pathogenic bacterium, *Xanthomonas oryzae* pv. *Oryzae*. *Pestic Biochem Physiol* 171:104718
- Kaur A, Grewal SK, Singh R, Kaur J (2017) Defense system in chickpea genotypes differing in tolerance to *Helicoverpa armigera* infestation. *Indian J Plant Physiol* 22:324–331
- Kaur S, Bhardwaj RD, Kaur J, Kaur S (2021) Induction of defense-related enzymes and pathogenesis-related proteins imparts resistance to barley genotypes against spot blotch disease. *J Plant Growth Regul.* <https://doi.org/10.1007/s00344-021-10333-2>
- Keane P (2012) How pathogens attack plants. *Microbiol Aust* 33(1):26–28
- Khizar M, Shi J, Saleem S, Liaquat F, Ashraf M, Latif S, Haroon U, Hassan SW, Rehman SU, Chaudhary HJ, Quraishi UM (2020) Resistance associated metabolite profiling of *Aspergillus* leaf spot in cotton through non-targeted metabolomics. *PLoS one* 15(2):e0228675
- Khodadadi F, Tohidfar M, Vahdati K, Dandekar AM, Leslie CA (2020) Functional analysis of walnut polyphenol oxidase gene (*JrPPO1*) in transgenic tobacco plants and PPO induction in response to walnut bacterial blight. *Plant Pathol* 69(4):756–764. <https://doi.org/10.1111/ppa.13159>
- Kiselev KV, Dubrovina AS, Veselova MV, Bulgakov VP, Fedoreyev SA, Zhuravlev YN (2007) The *rolB* gene-induced overproduction of resveratrol in *Vitis amurensis* transformed cells. *J Biotechnol* 128(3):681–692
- Korn M, Gartner T, Erban A, Kopka J, Selbig J, Hinch DK (2010) Predicting Arabidopsis freezing tolerance and heterosis in freezing tolerance from metabolite composition. *Mol Plant* 3:224–235
- Kosova K, Vitamavas P, Prasil IT (2014) Proteomics of stress responses in wheat and barley—search for potential protein markers of stress tolerance. *Front Plant Sci* 5:1–14
- Kumar A, Verma JP (2018) Does plant Microbe interaction confer stress tolerance in plants: a review. *Microbiol Res* 207:41–52
- Kumar Y, Zhang L, Panigrahi P, Dholakia BB, Dewangan V, Chavan SG, Kunjir SM, Wu X, Li N, Rajmohanan PR, Kadoo NY, Giri AP, Tang H, Gupta VS (2016) *Fusarium oxysporum* mediates systems metabolic reprogramming of chickpea roots as revealed by a combination of proteomics and metabolomics. *Plant Biotechnol J* 14(7):1589–1603
- Laouane H, Lazrek HB, Sedra MH (2011) Synthesis and toxicity evaluation of cinnamyl acetate: A new phytotoxin produced by a strain of *Verticillium dahliae* pathogenic on olive tree. *Int J Agric Biol* 13:444–446
- Lee HY, Byeon Y, Back K (2014) Melatonin as a signal molecule triggering defense responses against pathogen attack in Arabidopsis and tobacco. *J Pineal Res* 57:262–268
- Lee HY, Byeon Y, Tan DX, Reiter RJ, Back K (2015) Arabidopsis serotonin N-acetyltransferase knockout mutant plants exhibit decreased melatonin and salicylic acid levels resulting in susceptibility to an avirulent pathogen. *J Pineal Res* 58(3):291–299
- Lefevre H, Bauters L, Gheysen G (2020) Salicylic acid biosynthesis in plants. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2020.00338>
- Li X, Bao H, Wang Z, Wang M, Fan B, Zhu C, Chen Z (2018) Biogenesis and function of multivesicular bodies in plant immunity. *Front Plant Sci* 9:979
- Liang J, Shen Q, Wang L, Liu J, Fu J, Zhao L, Xu M, Peters RJ, Wang Q (2021a) Rice contains a biosynthetic gene cluster associated with production of the casbane-type diterpenoid phytoalexin ent-10-oxodepressin. *New Phytol* 231(1):85–93
- Liang M, Ye H, Shen Q, Jiang X, Cui G, Gu W, Zhang LH, Naqvi NI, Deng YZ (2021b) Tangeretin inhibits fungal ferroptosis to suppress rice blast. *J Integr Plant Biol* 63(12):2136–2149
- Liu H, Du Y, Chu H, Shih CH, Wong YW, Wang M, Chu IK, Tao Y, Lo C (2010) Molecular dissection of the pathogen-inducible 3-deoxyanthocyanidin biosynthesis pathway in sorghum. *Plant Cell Physiol* 51(7):1173–1185
- Long L, Liu J, Gao Y, Xu FC, Zhao JR, Li B, Gao W (2019) Flavonoid accumulation in spontaneous cotton mutant results in red coloration and enhanced disease resistance. *Plant Physiol Biochem* 143:40–49
- Van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants vol. 44: pp. 135–162. <https://doi.org/10.1146/Annurev.Phyto.44.070505.143425>
- Lopez-Fernandez O, Dominguez R, Pateiro M, Munekata PES, Rocchetti G, Lorenzo JM (2020) Determination of polyphenols using liquid chromatography tandem mass spectrometry technique (LC-MS/MS): a review. *Antioxid (Basel, Switz)* 9(6):479
- Lu J, Ju H, Zhou G, Zhu C, Erb M, Wang X, Wang P, Lou Y (2011) An EAR-motif-containing ERF transcription factor affects herbivore-induced signaling, defense and resistance in rice. *Plant J* 68(4):583–596. <https://doi.org/10.1111/J.1365-313X.2011.04709.X>
- Ma Z, Wang L, Zhao M et al (2020) ITRAQ proteomics reveals the regulatory response to *Magnaportheorizae* in durable resistant vs. susceptible rice genotypes. *PLoS ONE* 15:1–20
- Malencic DJ, Vasic D, Popovic M, Devic D (2004) Antioxidant systems in sunflower as affected by oxalic acid. *Biol Plant* 48:243–247
- Malinovskiy FG, Fangel JU, Willats WG (2014) The role of the cell wall in plant immunity. *Front Plant Sci* 5:178
- Marcel S, Sawers R, Oakeley E, Angliker H, Paszkowski U (2010) Tissue-adapted invasion strategies of the rice blast fungus *Magnaportheorizae*. *Plant Cell* 22:3177–3187
- Masisi K, Beta T, Moghadasian MH (2016) Antioxidant properties of diverse cereal grains: a review *in vitro* and *in vivo* studies. *Food Chem* 196:90–97
- Meng X, Zhang S (2013) MAPK Cascades in plant disease resistance signaling vol 51: pp. 245–266. <https://doi.org/10.1146/Annurev-Phyto-082712-102314>
- Meo SD, Reed TT, Venditti P, Victor VM (2016) Harmful and beneficial role of ROS. *Oxid Med Cell Longev* 2016:1–3
- Meyer RC, Steinfath M, Lisek J, Becher M, Witucka-Wall H, Torjek O, Fiehn O, Eckardt A, Willmitzer L, Selbig J, Altmann T (2007) The metabolic signature related to high plant growth rate in Arabidopsis thaliana. *Proc Natl Acad Sci U S A* 104(11):4759–4764
- Miller GAD et al (2010) Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ* 33:453–467
- Mishra VK, Biswas SK, Rajik M (2011) Biochemical mechanism of resistance to *Alternaria* blight by different varieties of wheat. *Int J Plant Pathol* 2:72–80
- Mittler R (2017) ROS are good. *Trends Plant Sci* 22:11–19
- Moerschbacher B, Mendgen K (2012) Structural aspect of plant defense. In: Slusarenko AJ, Fraser RSS, van Loon LC (eds) *Mechanisms of Resistance to Plant Diseases*. Springer Science & Business Media, Berlin
- Mohammad RH, Bahman K (2018) Changes in some antioxidant enzymes activities and carotenoid content in potato plants infected by *Rhizoctonia solani* treated with salicylic acid. *Arch Phytopathol Pflanzenschutz* 51:649–661

- Nandakumar M, Malathi P, Sundar AR, Viswanathan R (2021) Host-pathogen interaction in sugarcane and red rot pathogen: exploring expression of phytoalexin biosynthesis pathway genes. *Indian Phytopathol* 74:529–535
- Nawrocka J, Malolepsza U (2013) Diversity in plant systemic resistance induced by *Trichoderma*. *Biol Control* 67:149–156
- Ngou BPM, Ahn HK, Ding P, Jones JDG (2021) Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature* 592(7852):110–115
- Nicholson RL, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. *Annu Rev Phytopathol* 30:369–389
- Nida H, Lee S, Li Y et al (2021) Transcriptome analysis of early stages of sorghum grain mold disease reveals defense regulators and metabolic pathways associated with resistance. *BMC Genomics* 22:295
- Norvienyeku J, Lin L, Waheed A et al (2020) Bayogenin 3-O-cellobioside confers non cultivar-specific defense against the rice blast fungus *Pyriculariaoryzae*. *Plant Biotechnol J*. <https://doi.org/10.1111/pbi.13488>
- Nose NP, Dalcin MS, Bruna Dias L, Toloy RS et al (2022) Noni essential oil associated with adjuvants in the production of phytoalexins and in the control of soybean anthracnosis. *J Med Plant Res* 16(1):1–10
- Paznocht L, Kotikova Z, Sulc M, Lachman J, Orsak M, Eliasova M, Martinek P (2017) Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains. *Food Chem* 240:670–678
- Petrov V, Hille J, Mueller-roeber B, Gechev TS (2015) ROS-mediated abiotic stress-induced programmed cell death in plants. *Front Plant Sci* 6:1–16
- Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M (2017) Targeting free radicals in oxidative stress-related human diseases. *Trends Pharma Sci* 38:592–607
- Purwantisari S, Priyatmojo A, Sancayaningsih RP, Kasiamdari RS, Budihardjo K (2020) Lignification on potatoes by application of *trichoderma viride*. In: IOP conference series earth environment science vol. 518 pp. 012075
- Raj S, Niranjan BR, Sarosh., and Shetty H.S. (2006) Induction and accumulation of polyphenol oxidase activities as implicated in development of resistance against pearl millet downy mildew disease. *Funct Plant Biol* 33(6):563
- Razzaq A, Shamsi S, Ali A, Ali Q, Sajjad M, Malik A, Ashraf M (2019) Microbial proteases applications. *Front Bioeng Biotechnol* 7:110
- Sabella E, Luvisi A, Aprile A, Negro C, Vergine M, Nicoli F, Miceli A, De Bellis L (2018) *Xylella fastidiosa* induces differential expression of lignification related-genes and lignin accumulation in tolerant olive trees cv. Leccino. *J Plant Physiol* 220:60–68
- Sahni S, Prasad BD (2020) Management of collar rot disease using vermicompost and a PGPR strain *Pseudomonas* sp. and their effect on defense-related enzymes in chickpea. *Indian Phytopathol* 73:301–311
- Salas-Marina MA et al (2011) Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur J Plant Pathol*. 131:15–26
- Samota MK, Sasi M, Awana M, Yadav OP, Amitha MSV, Tyagi A, Kumar S, Singh A (2017) Elicitor-induced biochemical and molecular manifestations to improve drought tolerance in rice (*Oryza sativa* L.) through seed-priming. *Front Plant Sci* 8:934
- Sana TR, Fischer S, Wohlgenuth G, Katrekar A, Jung KH, Ronald PC, Fiehn O (2010) Metabolomic and transcriptomic analysis of the rice response to the bacterial blight pathogen *Xanthomonas oryzae* pv *oryzae*. *Metabolomics* 6(3):451–465
- Sandalio LM, Peláez-Vico MA, Molina-Moya E, Romero-Puertas MC (2021) Peroxisomes as redox-signaling nodes in intracellular communication and stress responses. *Plant Physiol* 186(1):22–35. <https://doi.org/10.1093/plphys/kiab060>
- Santen K, Marttila S, Liljeroth E, Bryngelsson T (2005) Immunocytochemical localization of the pathogenesis-related PR-1 protein in barley leaves after infection by *Bipolaris sorokiniana*. *Physiol Mol Plant Pathol* 66:45–54
- Schmelz EA, Kaplan F, Huffaker A, Dafoe NJ, Vaughan MM, Ni X, Rocca JR, Alborn HT, Teal PE (2011) Identity, regulation, and activity of inducible diterpenoid phytoalexins in maize. *Proc Natl Acad Sci* 108(13):5455–60
- Scossa F, Brotman Y, de Abreu E, Lima F, Willmitzer L, Nikoloski Z, Tohge T, Fernie AR (2016) Genomics-based strategies for the use of natural variation in the improvement of crop metabolism. *Plant Sci* 242:47–64
- Scott-Craig JS, Kerby KB, Stein BD, Somerville SC (1995) Expression of an extracellular peroxidase that is induced in barley (*Hordeum vulgare*) by the powdery mildew pathogen (*Erysiphe graminis* f. sp. *hordei*). *Physiol Mol Plant Pathol* 47:407–418
- Sels J, Mathys I, De Coninck BMA, Cammue BPA, De BFC (2008) Plant pathogenesis-related (PR) proteins: a focus on PR peptides. *Plant Physiol Biochem* 46:941–950
- Seybold H, Demetrowitsch TJ, Hassani MA, Szymczak S, Reim E, Hauelsen J, Lubbers L, Ruhlemann M, Franke A, Schwarz K, Stukenbrock EH (2020) A fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. *Nat Commun* 11(1):1910
- Shahidi F, Yeo J (2016) Insoluble-bound phenolics in food. *Molecules* 21:1–22
- Shan T, Rong W, Xu H, Du L, Liu X, Zhang Z (2016) The wheat R2R3-MYB transcription factor TaRIM1 participates in resistance response against the pathogen *Rhizoctonia cerealis* infection through regulating defense genes. *Sci Rep* 6(1):28777
- Sharma P, Jha AB, Dubey RS, Pessaraki M (2012) Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. *J Bot* 3:1–26
- Shavit R, Batyrshina ZS, Dotan N, Tzin V (2018) Cereal aphids differently affect benzoxazinoid levels in durum wheat. *PLoS ONE* 13(12):e0208103
- Singla P, Bhardwaj RD, Kaur S, Kaur J (2019) Antioxidant potential of barley genotypes inoculated with five pathotypes of *Puccinia striiformis* f. sp. *Hordei*. *Physiol Mol Biol Plants* 25:145–157
- Singla P, Bhardwaj RD, Kaur S, Kaur J (2020) Stripe rust induced defense mechanisms in the leaves of contrasting barley genotypes (*Hordeum vulgare* L.) at the seedling stage. *Protoplasma* 257(1):169–181
- Song C, Wang X, Yang J, Kuang Y, Wang Y, Yang S, Qin J, Guo L (2021) Antifungal biphenyl derivatives from *Sorbus pohuashanensis* leaves infected by *Alternaria tenuis* and their effect against crop pathogens. *Chem Biodivers*. 18(5):e2100079
- Stangarlin JR, Pascholati SF (2000) Activity of ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco), chlorophyllase, β -1, 3-glucanase and chitinase and chlorophyll content in bean cultivars (*Phaseolus vulgaris*) infected with *Uromyces appendiculatus*. *Summa Phytopathol* 26:34–42
- Suarez V, Staehelin C, Arango R, Holtorf H, Hofsteenge J, Meins JF (2001) Substrate specificity and antifungal activity of recombinant tobacco class I chitinases. *Plant Mol Biol* 45:609–618
- Suharti WS, Nose A, Zheng SH (2016) Metabolite profiling of sheath blight disease resistance in rice: in the case of positive ion mode analysis by CE/TOF-MS. *Plant Prod Sci* 19:1–12
- Takahashi Y, Berberich T, Miyazaki A, Seo S, Ohashi Y, Kusano T (2004) Spermine signaling in tobacco: activation of mitogen-activated protein kinases by spermine is mediated through mitochondrial dysfunction. *Plant J* 36:820–9

- Tamaoki D, Seo S, Yamada S, Kano A, Miyamoto A, Shishido H, Miyoshi S, Taniguchi S, Akimitsu K, Gomi K (2013) Jasmonic acid and salicylic acid activate a common defense system in rice. *Plant Signal Behav.* 8:e24260
- Tekbas OF, Ogur R, Korkmaz A, Kilic A, Reiter RJ (2008) Melatonin as an antibiotic: new insights into the actions of this ubiquitous molecule. *J Pineal Res* 44(2):222–226
- Torun H (2019) Time-course analysis of salicylic acid effects on ROS regulation and antioxidant defense in roots of hulled and hullless barley under combined stress of drought, heat and salinity. *Physiol Plant* 165:169–182
- Tsuda K, Somssich IE (2015) Transcriptional networks in plant immunity. *New Phytol* 206(3):932–947
- Turra D, ElGhalid M, Rossi F, DiPietro A (2015) Fungal pathogen uses sex pheromone receptor for chemotropic sensing of host plant signals. *Nat* 527:521–524
- Ube N, Katsuyama Y, Kariya K, Tebayashi SI, Sue M, Tohnooka T, Ueno K, Taketa S, Ishihara A (2021) Identification of methoxylchalcones produced in response to CuCl₂ treatment and pathogen infection in barley. *Phytochemistry* 184:112650
- Vance CP, Kirk TK, Sherwood RT (1980) Lignification as a mechanism of disease resistance. *Annu Rev Phytopathol* 18:259–288
- Vanitha S, Niranjana S, Mortensen C, Umesh S (2009) Bacterial wilt of tomato in Karnataka and its management by *Pseudomonas fluorescens*. *Bio Control* 54:685–695
- Voigt CA (2014) Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Front Plant Sci* 5:168
- Wang T, Li Q, Bi K (2018) Bioactive flavonoids in medicinal plants: structure, activity and biological fate. *Asian J Pharma Sci* 13:12–23
- Wen W, Liu H, Zhou Y, Jin M, Yang N, Li D, Luo J, Xiao Y, Pan Q, Tohge T, Fernie AR, Yan J (2016) Combining quantitative genetics approaches with regulatory network analysis to dissect the complex metabolism of the maize kernel. *Plant Physiol* 170:136–146
- Windisch S, Walter A, Moradtalab N, Walker F, Hoglinger B, El-Hasan A, Ludwig U, Neumann G, Grosch R (2021) Role of benzoic acid and lettukenin A in the defense response of lettuce against soil-borne pathogens. *Plants (Basel)* 10(11):2336
- Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R, Tang D (2016) Ferroptosis: process and function. *Cell Death Diff* 23:369–379
- Yamasaki H, Cohen MF (2006) NO signal at the crossroads: polyamine induced nitric oxide synthesis in plants. *Trend Plant Sci* 11:522–524
- Yang Q, Trinh HX, Imai S, Ishihara A, Zhang L, Nakayashiki H, Tosa Y, Mayama S (2004) Analysis of the involvement of hydroxyanthranilate hydroxyl cinnamoyl transferase and caffeoyl-CoA 3-O-methyltransferase in phytoalexin biosynthesis in oat. *Mol Plant Microbe Interact* 17(1):81–89
- You X, Fang H, Wang R, Wang G-L, Ning Y (2020) Phenylalanine ammonia lyases mediate broad-spectrum resistance to pathogens and insect pests in plants. *Sci Bull* 65(17):1425–1427. <https://doi.org/10.1016/j.scib.2020.05.014>
- Young AJ, Lowe GL (2018) Carotenoids-antioxidant properties. *Antioxid* 7:1–4
- Zaynab M, Fatima M, Sharif Y, Zafar MH, Ali H, Khan KA (2019) Role of primary metabolites in plant defense against pathogens. *Microb Pathog* 137:103728
- Zhang H, Huang Q, Yi L, Song X, Li L, Deng G, Liang J, Chen F, Yu M, Long H (2021) PAL-mediated SA biosynthesis pathway contributes to nematode resistance in wheat. *Plant J* 107(3):698–712. <https://doi.org/10.1111/tpj.15316>
- Zhao P, Gu S, Han C, Lu Y, Ma C, Tian J, Bi J, Deng Z, Wang Q, Xu Q (2021) Targeted and untargeted metabolomics profiling of wheat reveals amino acids increase resistance to fusarium head blight. *Front Plant Sci* 12:762605–762605
- Zhou X, Liu Z (2022) Unlocking plant metabolic diversity: a (pan)-genomic view. *Plant Commun.* <https://doi.org/10.1016/j.xplc.2022.100300>
- Zhou S, Zhang YK, Kremling KA, Ding Y, Bennett JS, Bae JS, Kim DK, Ackerman HH, Kolomiets MV, Schmelz EA, Schroeder FC, Buckler ES, Jander G (2019) Ethylene signaling regulates natural variation in the abundance of antifungal acetylated diferuloylsucroses and *Fusarium graminearum* resistance in maize seedling roots. *New Phytol* 221(4):2096–2111
- Zhu Z, Lee B (2015) Friends or foes: new insights in jasmonate and ethylene co-actions. *Plant Cell Physiol* 56:414–420
- Zou C, Wang P, Xu Y (2016) Bulk sample analysis in genetics, genomics and crop improvement. *Plant Biotechnol J* 14(10):1941–1955

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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, Ca²⁺ 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 dollars⁷. 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 instead 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 strengthens it. Additionally, development of modified leaves such as

trichomes, spines, thorns 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 insects²⁰.

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 attacks⁸. 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 (O₂⁻), nitric oxide (NO) and hydrogen peroxide (H₂O₂) 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 genes⁴.

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 cascades⁴. 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 days²⁰.

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 secretes compounds that attract the scavengers of the attacking insects¹⁶. 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 responses⁵. 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 eggs⁵. 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 mobility¹. 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 predators⁸. Similarly, the high leaf trichome density repels and reduces oviposition of the mite *Tetranychus uticae* in raspberry³⁵.

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 insects¹⁶.

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 parts³⁷.

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 bugs³². 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 consequences¹⁶.

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 grains²⁰. 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 development⁴¹. 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 ROS⁴⁸. Overexpression of tomato PPO conferred enhanced resistance to the moth *S. litura* by decreasing its development and increasing its mortality rate⁴⁸. 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 insects¹⁶.

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 tridentata* and *N. attenuate* plants were reported to share this behavior, where injured *A. tridentata* 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 fimbriatum* and *Pheidole bicornis* ants is mutualistic as the ants protect the plant from several insects, while feeding on its FBs¹⁸. Numerous plants produce nectars to attract pollinators, predators of insects and pests and parasitoids which contribute largely to the indirect defense¹⁶. 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.

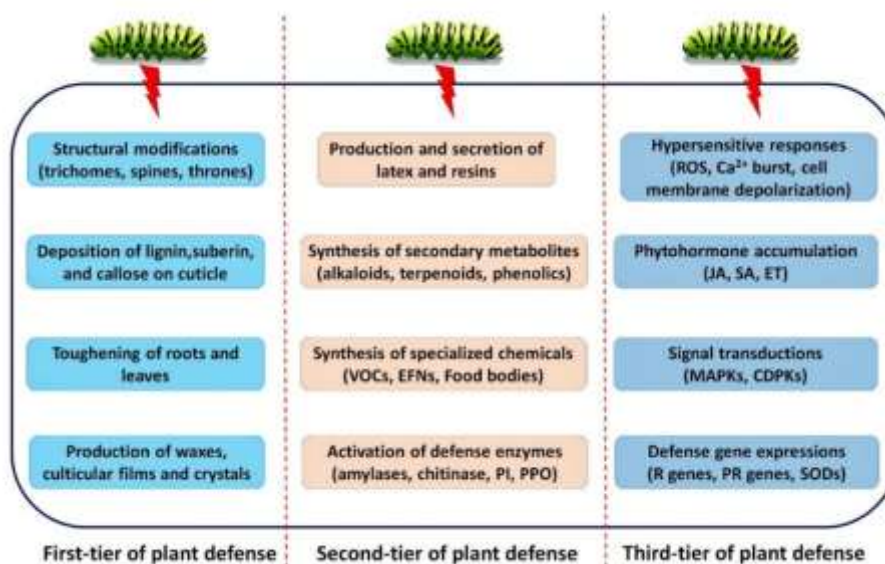


Figure 1: The multi-tiered plant defense system against insects.
(R gene- resistance gene, PR gene- pathogenesis-related gene)

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 herbivory¹⁵ (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 genes⁵. 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 membrane⁴⁶. 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 insect⁶.

Mousavi et al⁵³ 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 ET⁷². 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 signaling¹⁶. 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 RBOH¹². 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 species³⁶.

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 respectively¹¹. 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 responses¹¹. 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 nucleus¹⁴.

The binding of Signal responsive 1 (*AtSRI*) transcription factor protein to CaM induces insect resistance in *Arabidopsis thaliana* while the *atsr1* mutants were susceptible to herbivory⁴⁰. 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 proteins⁶⁴. 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 defenses⁷⁵ (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 genes⁷⁰. 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 ROS⁵⁷. 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 development⁴⁷.

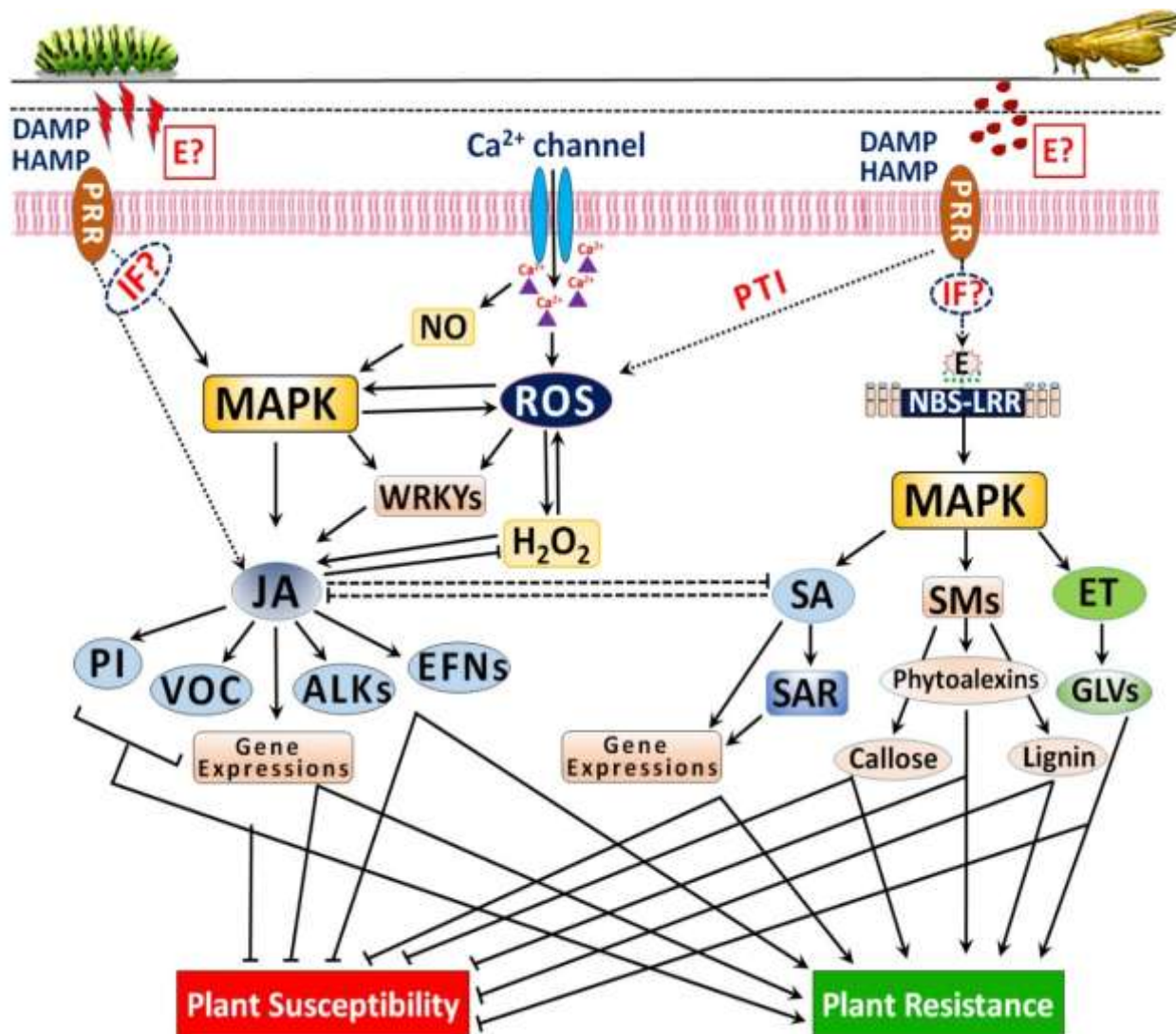


Figure 2: Molecular mechanism of insect resistance in plants. Plant pathogen recognition receptors (PPRs) recognizes the HAMPs or DAMPs and activate the PAMP-triggered immunity (PTI) through the MAPK cascade. Alternatively, the insect resistance proteins identifies the insect effectors activating the 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 maize²⁵.

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 maize¹⁶.

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 reprogramming of transcriptional mechanisms¹³. 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 attack¹³.

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 to 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 model³³. 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-associated 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 immunity⁸.

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 pathway¹⁶.

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 NIEG1 from brown planthoppers (BPH) or HARP1 from cotton bollworm 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 molecules 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 interactions¹³. 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 factors¹³.

Based on the recent studies on transcriptome and proteome dynamics, several genes involved in insect resistance have been cloned and characterized in myriads 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 membranes³. 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 infestations⁴⁴ (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 inhibitors²². *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 factors²⁷.

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 *Acyrtosiphon pisum* has led to the identification of C002, a major effector of herbivore infestation⁵⁴. 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 defenses⁵⁸. 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 resistance¹³ (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 signaling⁵⁶.

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 herbivory¹⁷.

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

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 uncertain¹⁰. 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 tobacco⁵¹ (Table 1).

In a recent study, Schweizer et al⁶² 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 accumulation⁶⁷ (Table 1).

OsWRKY70 reported enhanced resistance to striped 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 H₂O₂ and ET²⁹ and OsWRKY53 demonstrated SSB resistance in rice through negative regulation of OsMPK3/6 signaling²⁸. Most recently, OsbHLH61 and OsbLHL96 reported significant induction of defense responsive genes leading to BPH resistance in rice⁶⁹. 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 pests⁷⁶. 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 insect⁷⁶. 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 al⁴² 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 *dsNIMLP* (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 plants⁶³.

In another interesting development, an RNAi based insecticide named SmartStax Pro has been developed by Monsanto and Dow Agrosiences²⁶. 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 maize²⁶.

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 larva²⁴. 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 stresses³⁹. 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 processing³⁸. 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 interaction⁷².

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 pathway²¹, OsmiR396 increases rice sensitivity to BPH by regulating the expression of the *OsF3H* (flavanone 3-hydroxylase), the rate limiting enzyme in the flavonoid biosynthetic pathway⁹. 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 rice⁶⁵. 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 advanced molecular biology techniques that enable targeted modification of genomic loci in a precise and efficient manner⁷⁷. Among other platforms, CRISPR/Cas9 is the most simplistic and revolutionary tool with wider applicability in crop improvement⁷⁷. 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 serotonin⁴⁵.

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 BPH⁴⁵. Genome editing tools primarily aim 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 arms 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 make 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 trial conditions. Further, high concentration of dsRNA may not be possibly administered into insects in spite of its requirements for gene silencing as 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. Aljibory 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., Berteaux 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., Puthanveetil 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., Berteaux 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., Durey 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. Khraiweh 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, *Acyrtosiphon 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 *OsMPKs* 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. Shanguan 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 *Bemisia 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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