[Article ID : 01/I/24/0121]

PATHOGEN ELICITORS RECOGNITION FROM MAMP AND DAMP PERCEPTION TO INDUCED RESISTANCE IN PLANTS

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Abstract

Environment, Pathogen and Host these three components form the disease triangle. Under favourable condition virulent pathogen can cause diseases in a susceptible host. We can manage the diseases of the plants by applying various chemical pesticides like fungicides, bactericides but there are so many disadvantages associated with this like chemical compounds cause environmental pollution through their residual effects and also form resistance to the pathogen due to continuous application. So, in spite of that if we focus on the plant pathogen interaction and molecular basis behind the pathogen detection, signal transduction and defense response of the plant, that will be more sustainable and ecofriendly approach. Plants' different molecules can recognise the microbe associated pattern and after successful recognition several resistance genes of the plant get activated. Recent advances and research activities in this aspect will surely increase the agricultural production and explain the complex molecular functions involved in host pathogen interaction in a simpler way.

Key Words : PRR, MAMP, Induced Resistance, Defense response.

Introduction

Agricultural revolution totally depends on the molecular basis of host pathogen interaction. Host pathogen interaction means how pathogens infect the plants, how they survive inside the plants' physiology and finally how plants can recognise and develop the defense response against the infection of the pathogens. There are so many resistance genes (R-genes) of plants and avirulent genes (Avr-genes) of pathogens which are involved in the plant defense response development and cause compatible or incompatible reactions according to their interactions (Rhoades, 1935). These R-genes are heritable in nature and through the various breeding methods like selection, introduction, mutation, somaclonal variation, hybridization, genetic engineering, resistant plants can be developed. Resistance genes can be introduced from related, unrelated species, germplasm collection, known variety, unrelated organisms like viral coat protein genes for viral resistance etc. By inoculating biocontrol agents like *Pseudomonas* sp. in the plant rhizospheres, we can incorporate the induced systemic resistance (ISR) in the plants. Biocontrol agents increase the production of reactive oxygen species (ROS), phenyl alanine ammonia lyase (PAL), phenol, superoxide dismutase enzyme (SOD) etc thus induce the resistance in plants and prevent the pathogens infection. So, it is better to emphasise more on the host pathogens interaction and proper execution of that scientific strategies than application of chemical pesticides because prevention is better than cure.

Defense response of plants, a multicomponent system

Pathogens attack the plants in a few steps like attachment, germination of spores, formation of infection structure like appressoria, infection peg, haustoria etc., penetration, infection, colonization of the host and finally multiplication, symptoms development. These sequential events form the pathogenesis. There may be two types of interaction between plants and pathogens. In susceptible reaction characteristic symptoms are developed and in resistant reaction multiplication

and symptoms development get restricted. Resistance is a rule and susceptibility is the exception. According to the Gene for Gene hypothesis 'for every resistance gene in the host there is a corresponding avirulence gene in the pathogen'. This hypothesis was postulated by H. H. Flor (1956), with his experiment on linseed rust (*Melampsora lini*) **(Flor, 1942) (Table- 1).**

	A (Dominant, Virulent)	a (Recessive, Avirulent)
R (Resistant)	AR (Incompatible)	aR (Compatible)
r (Susceptible)	Ar (Compatible)	ar (Compatible)

Table-1 Gene for Gene Hypothesis, H.H. Flor (1956)

Compatible and incompatible reaction

Homologous or compatible interaction occurs when elicitor released by the pathogens are not recognised by the plants' receptors. There are two types of elicitors like: Non specific elicitors (Glycoprotein, Lipopeptide, Amphiphilic glycolipid, Glycoprotein, Toxins, Fatty acids, Extracellular microbial enzymes-protease, pectic enzymes) and Specific elicitors (Avr gene products, hrp gene products, suppressor molecules). Sometimes degraded polysaccharides of the plant cell wall which are formed due to enzymatic activity of the pathogens are also act as elicitors. In heterologous or incompatible reaction elicitors of the pathogens interact and are recognised by plant cell receptors and resistant reaction takes place.

There are some specific avirulent genes of pathogens which resist pathogens to infect the particular crop. Like Tomato infecting bacterial spot caused by *Xanthomonas campestris pv. vesicatoria* also causes disease on pepper. Avirulent gene *avrBsT* inhibit the disease causing potential of the pathogen. When pathogen losses that avirulent gene, it becomes able to cause disease on both pepper and tomato.

Avirulent genes function in three different ways like:

1) The *avr* gene product itself functions as an elicitor and interact with the receptor in the host cell membrane. Ex. *avr9* gene of *Cladosporium fulvum* .
2) The *avr* gene regulates the synthesis of elicitor and that elicitor interacts with the receptor in plant cell membrane. Ex. avrD gene of *Pseudomonas syringae* pv. *tomato*.
3) The *avr* gene products enter into the host cell and act as elicitor or direct the synthesis of elicitor.

A) Pathogen Detection:

Plant has two layers of defense mechanism. First layer is the waxy cuticular layer, cell wall and preformed antimicrobial compound, which act as basal resistance. Second layer is the two tier innate immune response. Two tier innate immune response consists of three types of receptor. Those are Pattern recognition receptors (PRRs), Wall associated kinases (WAKs) and Nucleotide-binding domain- leucine rich repeats (NLRs).

1) Recognition of PAMPs and DAMPs by PRRs and WAKs

Pattern recognition receptors (PRRs) detect the Pathogens associated molecular pattern (PAMP) or Microbes associated molecular pattern (MAMP) (Zipfel, 2014). PRR can detect wide range of fungal, bacterial and viral components like Fungal carbohydrate (Xylanase, Chitin), Bacterial protein (Flagellin, Peptidoglycon, Elongation factor EF-Tu), Viral Nucleic Acid (DS RNA), Oomycete (elicitins, β-glucan), Insect (Aphid derived Elicitors). Fuction of PRRs depend on some regulatory proteins like somatic embryogenesis receptor like kinases (SERKs) and Brassinosteroid insensitive 1 associated receptor kinase 1 (BAK1) (Monaghan *et al*., 2012 and Prince *et al*., 2014). A prominent example of

PRR associated resistance is in wheat TaLRK10, TaRLP1.1 and TaRLK-R1-3 PRRs lead to resistance against rust, caused by *Puccinia* sp (Zhou *et al*., 2007). Transmembrane domain of PRRs helps to remain the PRRs in plsma membrane and cytoplasmic kinase domain helps in signal transduction (Zipfel, 2014).

Wall associated kinases can detect the Damage associated molecular pattern (DAMP). Cell wall components which are degraded by the pathogenic infection, act as elicitors and are recognised by the WAK (Decreux *et al.*, 2005). Fungal enzymes degrade the plant cell wall component pectin and produce oligogalacturonic acid and WAK1, WAK2 recognise that component with their N-terminal extracellular galacturonan binding domain as receptors (Brutus *et al.*, 2010). WAK contains cytoplasmic kinase domain also. PRRs and WAKs contain some specialised lectin domains, which can recognise the carbohydrate components like peptidoglycans, lipopolysaccharides, cellulose etc (Lannoo et al., 2014). Sometimes extracellular DNA, NADP, Atp also act as elicitors after pathogenic infection. In Arabidopsis WAK1 recognize the oligogalacturonides , DORN1/LecRK-I.9 percept the extracellular ATP (Choi *et al*., 2014).

2) Recognition of pathogen effectors by NLRs

Nucleotide-binding domain- leucine rich repeats (NLRs) detect the pathogen effectors. Pathogens increase the rate of infection by secreting the effectors. When NLRs can sense the presence of pathogenic effectors they change their structural appearance from a ADP bound condensed state to an exposed N-terminal domains with open ATP bound state (Takken *et al.,*2012). The NB-ARC contains various conserved motifs like Kinase-2/Walker-B, P-loop/Walker-A, MHDV, GLPL, resistance nucleotide-binding site A (RNBS-A), RNBS-B, RNBS-C, RNBS-D, and MHDV. In monocot and dicot plants CC-NBS-LRR genes (CNL genes) (Coiled coil- Nuecleotide binding site- Leucine rich repeat) and in only dicot plants TIR-NBS-LRR genes (Toll interleukin receptor- Nuecleotide binding site- Leucine rich repeat) present. *Xanthomonas translucens* using type-3 secretion system, secretes 20-40 effectors and they transfer those effectors into the Wheat Cytoplasm (Boch *et al*., 2010).

Zig-Zag Model

Pathogens try to defend the plant's recognition system and overall defense mechanism for successful infection, so they change the properties of elicitors, so that plant's immune system cannot recognise it. This is how plant microbe interaction takes place. This model is known as Zig-Zag Model (Fig- 1). This was proposed by Jones and Dangl, 2006 (Jones *et al.,* 2006). The entire mechanism can be divided into four stages like:

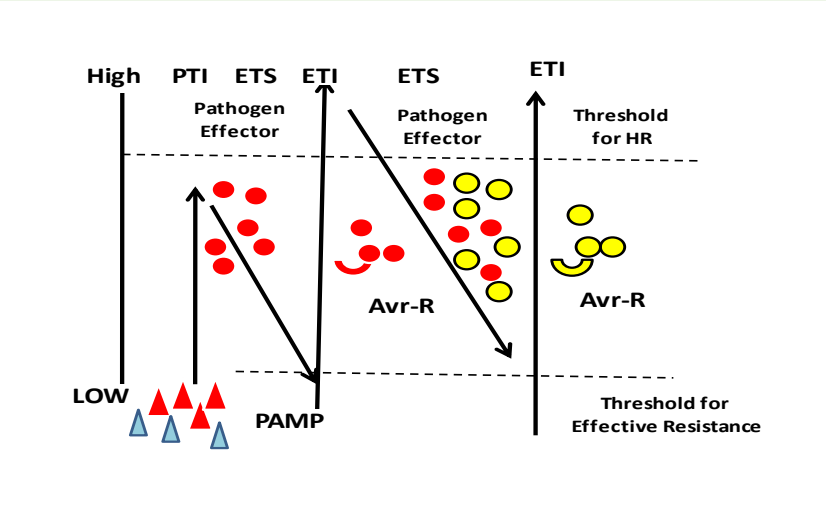


Fig-1 Zig Zag Model, Jones and Dangl, 2006

Phase 1 : Plant's immune response get activated, PRRs detect the MAMP and trigger PAMP-triggered immunity (PTI).
Phase 2 : After successful infection pathogens deliver effectors that prevent the PTI, and plant immune mechanism fails to recognise the newly secreted effectors and resulting in effector-triggered susceptibility (ETS).
Phase 3 : The newly formed effectors can be recognized by NB-LRR protein, activating effector-triggered immunity (ETI), and at threshold level plant induces resistance through hypersensitive cell death (HR).
Phase 4 : Pathogen strains that have lost certain effectors and secret new set of effectors, are selected (Fig-2).

Through Horizontal gene transfer pathogen can transfer the effectors producing genes from one species to another and increase their virulence. Some disadvantages associated with this model are Time scale and environmental effects have not been taken into consideration. This interaction mechanism is only confined to the biotrophic pathogens.

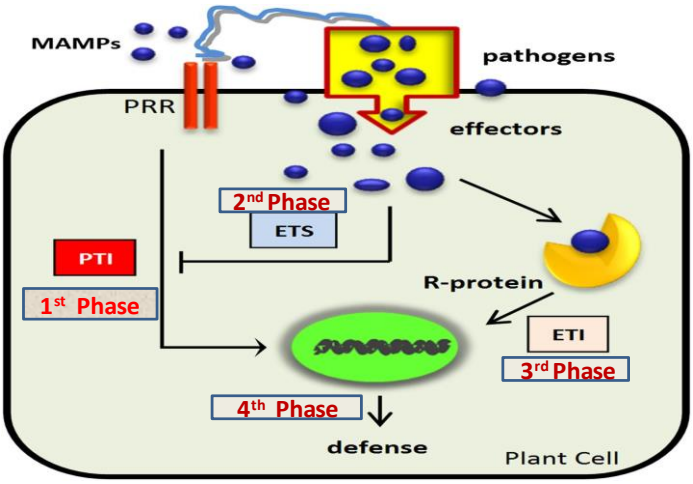


Fig-2 Steps of Zig Zag Model

B) Signal transduction

1) MAPK Signalling

After successful recognition, receptors induce some signalling mechanism. Among them one of the most prominent signalling systems is Mitogen activated protein kinase (MAPK) signalling. In initiation of MAPK signalling, Ras protein which is located in the membrane of plant cell, facilitate the formation of GDP from GTP and phosphorylates the MAPKKK (Raf) proteins which further phosphorylates the MAPKK (MEK) proteins and MAPK (ERK) proteins (Meng *et al.*, 2013). Interaction of bacterial flagellin and elongation factor with PRRs FLS2 and EFR with receptor BAK1 triggers the MAPK signalling (Chinchilla *et al.,* 2007). MAPK signalling also get activated through the perception of degraded pectin components by WAK1 and WAK2 (Kohorn *et al.,* 2012). Presence of MAPK genes

has been found in *Arabidopsis* on which 20 MAPKs, 10 MAPKKs and 60 MAPKKKs are present (Fig-3) (Ichimura *et al.,* 2002).

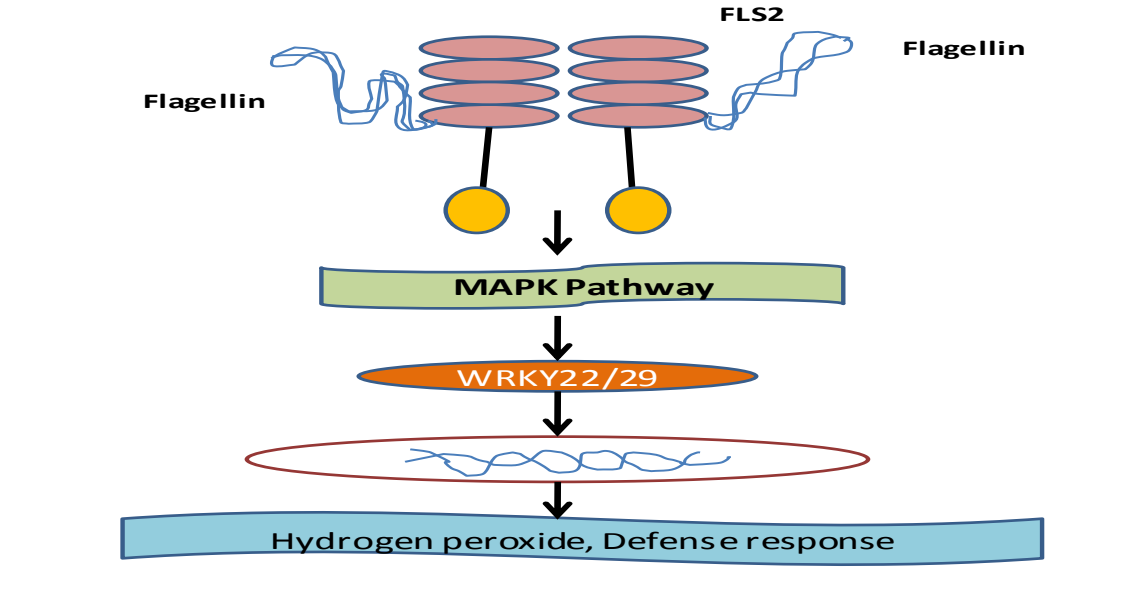


Fig-3 MAPK Signalling cascade

2) G – protein

Heteromeric G-protein and G protein coupled receptor (GPCR) are closely involved in signal transduction. GPCR interacts with the extracellular ligands and α subunit of G-protein complex get exchanged for GTP from GDP and α subunit is dissociated from β-γ subunit, this is how signalling cascade get initiated (Temple *et al.,* 2007). α subunit again get associated with β-γ subunit after GTP hydrolysis.

3) Ubiquitin

Ubiquitin is a small protein molecule found in most of the eukaryotes. Ubiquitination means deactivation of protein by proteasome activity. Proteasome is the complex of proteinases which can degrade the protein molecules. That degraded protein molecules act as signalling molecules and pathogens simultaneously produce effectors of different nature to disrupt the signalling mechanism. Plants use small ubiquitin like modifiers (SUMOs) to regulate the signal transduction (Marino *et al*., 2012).

4) Calcium ion fluctuation

In response to the PAMP or DAMP, receptors trigger the calcium ions fluctuation and activate the signalling cascade. Calcium dependent protein kinases, Calmodulin, and Calcineurin B-like protien detect Calcium ions fluctuations and activate Calmodulin-binding transcription activators (Poovaiah *et al.,* 2013). Calmodulin produces reactive oxygen species as defense response in plant immune system.

5) Hormone

There are so many hormones which are closely involved in plant immune system and defense reaction. Like:

Salicylic acid plays an important role in systemic acquired resistance against biotrophic and hemi biotrophic pathogenic infection. NDR1 effector and PAD4 (Phytoalexin deficient 4), EDS1 effectors

are recognised by the CNL receptors and TNL receptors respectively and after that recognition signals are transmitted and finally salicylic acid get activated (Aarts *et al.,* 1998).

Jasmonic acid (JA) and Ethylene (ET) play role in necrotrophic pathogen infection. In case of caterpillar predation, JA and ET induce volatile compound production which acts as signalling molecule. Production of ethylene is also get increased in presence of bacterial flagellin (Denoux *et al.,*2008).

Nitric oxide, cytokinins, abscisic acid, gibberellins, brassinosteroids also have role in defense response. ABA induces the stomatal closure in response to pathogen infection to the plant tissues (Lind *et al*., 2015).

6) Transcriptional factors

Transcriptional reprogramming acts in different level in defense mechanism like: a) Expression of basic components associated with resistance like kinases, receptors etc. b) Receptor proteins initiate the transcriptional factor's activity, c) Downstrean of receptor initiation through transcriptional factors activity (Qiu *et al*., 2008). bHLH, MYB, AP2/ERF, WRKY , TGA/bZIP and NAC these six families of transcriptional factors are directly associated with plant defense mechanism.

7) Nucleic acid activity and Pathogen derived resistance

Pathogenic infection causes changes in chromatin structure. Histone methylation/acetylation, DNA methylation, RNA interference induce the plant defense response. This kind of changes in genetic level causes downregulation of resistance inhibitors and upregulation of resistance inducers (Holeski *et al.,* 2012).

Cross protection or pathogen derived resistance can be an important source of plant defense mechanism. By inoculation of weaker, less virulent strain of pathogen to the plant, resistance can be induced against more virulent strain of the pathogen.

C) Defense response

After successful signal transduction, plants produce different type of defense response, like:

1) HR, ROS, Cell wall modification

Hypersensitive response is the most common defense response in plant immune system which further causes Programmed cell death (PCD). By PCD plants restrict the nutrient supply to the pathogen, so that growth of the pathogen is inhibited.

Reactive oxygen species formation makes the environment unfavourable for the growth of the pathogens. Peroxidase, NADPH oxidase, Amine and oxalate oxidase enzymes are directly involved in production of ROS (Karkonen *et al.,* 2015). NADPH oxidase produces superoxide, after that peroxidase generates hydrogen peroxide and it further produces ROS. Rapid transient production of huge amount ROS, is known as oxidative burst. Plants which lack the ability to detoxify ROS, have more strong immune response to the pathogens.

In post infection cell plants strengthen the cell wall through some modification like formation of Abscission layer in *Prunus* sp. against shot hole disease caused by *Xanthomonas campestris pv. pruni* and Cork layer formation against canker of potato caused by *Rhizoctonia solani* , Tyloses formation in xylem vessels inhibit the pathogen multiplication. Plants deposit lignin, callose to strengthen cell wall.

2) Enzymes and enzymatic inhibitors

Pathogenic enzymes are encountered by the plant receptors and detection of the effectors is facilitated by the plant immune system. Pathogens use different enzymes to degrade the plant cell components like cellulases, pectinases, xylanases. Similarly plants evolve enzymes like chitinases, β-1-3-glucanases to degrade pathogen's carbohydrate (Bellincampi *et al.,* 2014). Proteases enzymes are used by both plants and pathogens to reduce the enzymatic activity of Cellulase, pectinase and Chitinase respectively.

3) Defensins and Thaumatin like proteins

Defensins are small molecule of plant protein. It directly inhibit the invading pathogens. *Triticum aestivum* defensin 1 (Tad1) in wheat crown shows antipathogenic property (Koike *et al*., 2002). Defensin triggers the production of ROS.

Thaumatin like protein named from *Thaumatococcus daniellii* . Thaumatin like protein in barley binds to 1,3-β-D-glucans and induce resistance against powdery mildew. Some thaumatin like proteins are zeamatin (maize), osmotin (tobacco), avematin (oat), hordomatin (barley) and trimatin (wheat) (Osmond *et al.,* 2001).

4) Phytoalexin and PR Protein

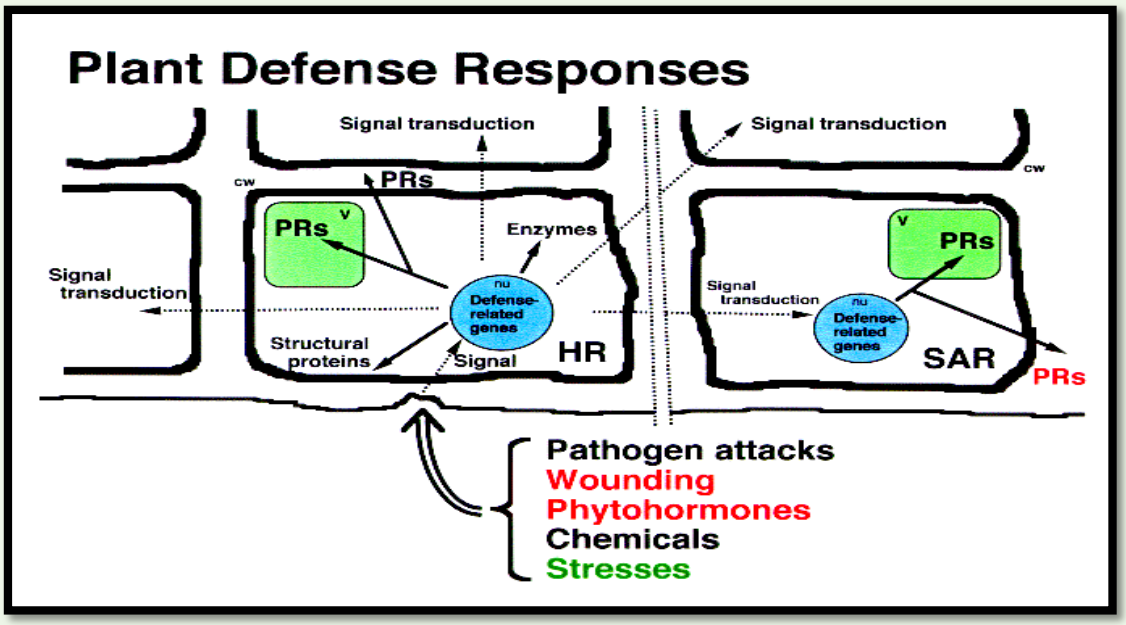
Phyto = "plant" and alexin = "to ward off; Phytoalexins are low molecular weight antimicrobial and often antioxidative substances synthesized *de novo* by plants that accumulate rapidly at areas of pathogen infection.

Pathogenesis related protein (PR protein), toxic to invading fungal pathogens, is produced intracellularly after pathogenic infection. PR proteins like PR1 (Antioomycetes, Antifungal), PR2 (β-1-3-glucanase), PR3 (Chitinase), PR4 (Antifungal), PR6 (Proteinase inhibitors), Thaumatin, Defensin, Cystein rich proteins etc.

5) Biological weapons

VAM (Arbuscular mycorrhiza) increases the DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) production in corn which induce the resistance against insect pests and pathogens.

All the defense response has been summarily presented in **Fig-4.**



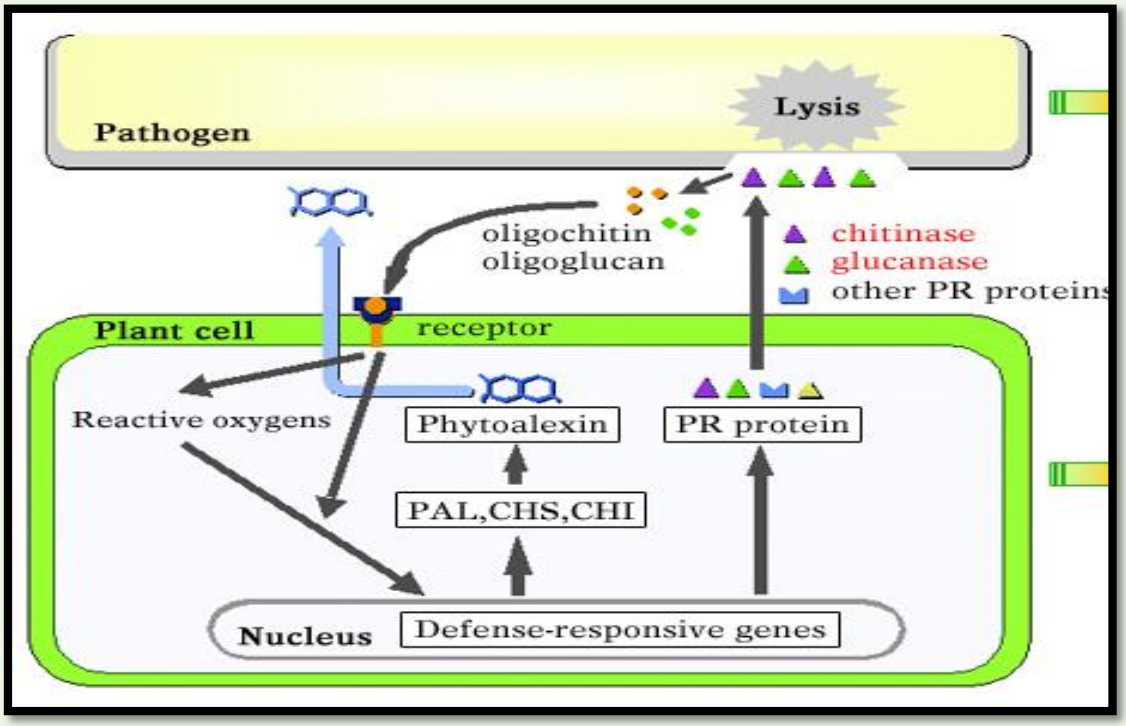


Fig-4 Defense Response in Plant after successful signal transduction

Conclusion

Detail study of plant-pathogen interaction and their molecular basis is highly important in twenty first century to maintain the production of crop with a sustainable disease management strategy. In this review the aspect of plants receptors PRRs, WAKs and pathogens effectors, PAMP, DAMP have been emphasised. The interactions are effected by several factors like Plant ontogenic resistance, plant phenology and physiology, plant genotype nature of PAMP, DAMP, environmental factors like UV radiation, climate etc. This topic is a very relevant issue in sustainable agricultural practices.

References

Aarts N, Metz M, Holub E, Staskawicz B.J, Daniels M.J and Parker J.E (1998). Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in Arabidopsis. Proc. Natl. Acad. Sci. USA 95:10306–10311.

Bellincampi D, Cervone F and Lionetti V (2014). Plant cell wall dynamics and wall-related susceptibility in plant–pathogen interactions. Front. Plant Sci. 5:228.

Boch J and Bonas U (2010). Xanthomonas AvrBs3 family-type III effectors: Discovery and function. Ann. Rev. Phytopathol. 48: 419–436.

Brutus A, Sicilia F, Macone A, Cervone F and De Lorenzo G (2010). A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. Proc. Natl. Acad. Sci. USA 107:9452–9457.

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones J.D, Felix G and Boller T (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448:497–500.

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee S.Y and Stacey G (2014). Identification of a plant receptor for extracellular ATP. Science 343:290–294.

Decreux A and Messiaen J (2005). Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. Plant Cell Physiol. 46:268–278.

Denoux C, Galletti R, Mammarella N, Gopalan S, Werck D, De Lorenzo G, Ferrari S, Ausubel F.M and Dewdney J (2008). Activation of defense response pathways by OGs and Flg22 elicitors in Arabidopsis seedlings. Mol. Plant 1:423–445.

Flor H.H (1942). Inheritance of pathogenicity in melampsora lini. Phytopathology 32: 653–669.

Holeski L.M, Jander G and Agrawal A.A (2012). Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol. Evol. 27: 618–626.

Ichimura K, Shinozaki K, Tena G, Sheen J, Henry Y, Champion A, Kreis M, Zhang S, Hirt H and Wilson C (2002). Mitogen-activated protein kinase cascades in plants: A new nomenclature. Trends Plant Sci. 7:301–308.

Jones J.D and Dang J.L (2006). The plant immune system. Nature 444:323–329.

Kärkönen A, and Kuchitsu, K (2015). Reactive oxygen species in cell wall metabolism and development in plants. Phytochemistry 112:22–32.

Kohorn B.D, Kohorn S.L, Todorova T, Baptiste G, Stansky K and McCullough M (2012). A dominant allele of Arabidopsis pectin-binding wall-associated kinase induces a stress response suppressed by MPK6 but not MPK3 mutations. Mol. Plant 5:841–851.

Koike M, Okamoto T, Tsuda S and Ima R(2002). A novel plant defensin-like gene of winter wheat is specifically induced during cold acclimation. Biochem. Biophys. Res. Commun. 298:46–53.

Lannoo N and Van Damme E.J (2014). Lectin domains at the frontiers of plant defense. Front. Plant Sci. 5:397.

Lind C, Dreyer I, López-Sanjurjo E.J, von Meyer K, Ishizaki K, Kohchi T, Lang D, Zhao Y, Kreuzer I and Al-Rasheid K.A(2015). Stomatal guard cells co-opted an ancient ABA-dependent desiccation survival system to regulate stomatal closure. Curr. Biol. 25: 928–935.

Marino D., Peeters N andv Rivas S (2012). Ubiquitination during plant immune signaling. Plant Physiol. 160:15–27.

Meng X and Zhang S (2013). MAPK cascades in plant disease resistance signaling. Ann. Rev. Phytopathol. 51: 245–266.

Monaghan J and Zipfel C (2012). Plant pattern recognition receptor complexes at the plasma membrane. Curr. Opin. Plant Biol.15: 349–357.

Osmond R I, Hrmova M, Fontaine F, Imberty A and Fincher G.B(2001). Binding interactions between barley thaumatin-like proteins and (1,3)-β-d-glucans. FEBS J. 268:4190–4199.

Poovaiah B, Du L, Wang H and Yang T (2013). Recent advances in calcium/calmodulin-mediated signaling with an emphasis on plant-microbe interactions. Plant Physiol. 163: 531–542.

Prince D.C, Drurey C, Zipfel C and Hogenhout S.A (2014). The leucine-rich repeat receptor-like kinase brassinosteroid insensitive1-associated kinase1 and the cytochrome p450 phytoalexin deficient3 contribute to innate immunity to aphids in arabidopsis. Plant Physiol. 164:2207–2219.

Qiu J.L, Fiil B.K, Petersen K, Nielsen H.B, Botanga C.J, Thorgrimsen S, Palma K, Suarez-Rodriguez M.C, Sandbech-Clausen, S and Lichota J (2008). Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J. 27:2214–2221.

Rhoades V.H (1935). The location of a gene for disease resistance in maize. Proc. Natl. Acad. Sci. USA 21:243–246.

Takken F.L and Goverse A (2012). How to build a pathogen detector: Structural basis of NB-LRR function. Curr. Opin. Plant Biol. 15:375–384.

Temple B.R and Jones A.M (2007). The plant heterotrimeric G-protein complex. Annu. Rev. Plant Biol. 58:249–266.

Zhou H, Li S, Deng Z, Wang X, Chen T, Zhang J, Chen S, Ling H, Zhang A and Wang D (2007). Molecular analysis of three new receptor-like kinase genes from hexaploid wheat and evidence for their participation in the wheat hypersensitive response to stripe rust fungus infection. Plant J. 52:420–434.

Zipfel C (2014). Plant pattern-recognition receptors. Trends Immunol. 35:345–351.