Plant Archives Volume 20 No. 1, 2020 pp. 1169-1174 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PLANT IMMUNE RESPONSE STRATEGIES AGAINST PATHOGENS

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Abstract

All plants have evolved innate immunity to recognize invading pathogens and to elicit successful defense response against invading pathogen, but the magnitude of such defense response may vary among susceptible and resistant varieties. Further successful pathogens can develop disease symptoms, because they might be able to evade recognition mechanism of host or suppress the host plant defense response. The key objective of this review article is to explain the defense strategies of the plants against pathogens to make precise predictions about the abiotic and biotic elicitors of disease resistance. In this way it is anticipated that such abiotic and biotic elicitors could be used as eco-friendly alternative measures for inducing disease resistance in plants of agricultural and horticultural importance within the framework of Integrated Pest Management. Further genetic engineering of plants could be done to express disease resistance proteins that can recognize and respond against pathogen molecules responsible for development of pathogenicity.

Key words : Abiotic elicitors, Biotic elicitors, Integrated Pest Management, Disease resistance.

Introduction

In natural environment plants represent a nutrient rich culture media for a diverse range of pathogens including Virus, Viroids, Bacteria, Mycoplasma and Fungi. Thus plants are most frequently attacked by pathogens which can have devastating effects on host plants. But plants are not the defenseless creatures; they have an amazing diversity of evolved defense mechanisms being utilized by plants to survive on the onslaught of pathogen and herbivore attack. Plants can respond to attempted infection by activating a broad range of defense mechanisms, which may be local (e.g. the hypersensitive response, H.R) or systemic (e.g. systemic acquired resistance, SAR). Cellular necrosis, either in form of H.R or as symptom of infection by a necrotizing pathogen, is associated with the induction of systemic resistance (Grant *et al.,* 2013). In SAR, the elicitors enhance the levels of translocatable signaling chemicals which in turn results into coordinated induction of the genes controlling a diverse array of defense pathways in tissues spatially at distance from the initial sites of challenge. An overview of these constitutive and inducible defense responses are represented in fig. 1. When plants are challenged by pathogens, these biotic stresses can lead to induction of physical, biochemical and molecular changes in plant system, such as the physical strengthening and rigidity of plant cell walls through lignin deposition, suberization, callose deposition and by producing secondary metabolites like phenolic compounds, pathogenesis-related (PR) proteins and phytoalexins which deals to prevent pathogen invasion and disease setup.

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Phenolic compounds are responsible for induced systemic disease resistance and mechanical constitutive defenses against variety of pathogens (Harakava, 2005;Chen *et al.,* 2007) and insect herbivores (War *et al.,* 2012).) Plants enhance defense responses by means of inducing activities of a wide spectrum of defense enzymes such as peroxidases, β-1, 3-glucanases, chitinases, phenylalanine ammonia lyase and polyphenol oxidases which can slow down the rate of disease spread and infection setup (Kumari and Vengadaramana, 2017). Lignin and phenolic secondary metabolites plays most significant roles in providing plant disease resistance. Phenolic secondary metabolites includes a diverse range of defensive compounds including flavonoids, isoflavonoids, anthocyanins, tannins, lignin, phytoalexins, and furanocoumarins. These phenolic compounds imparts

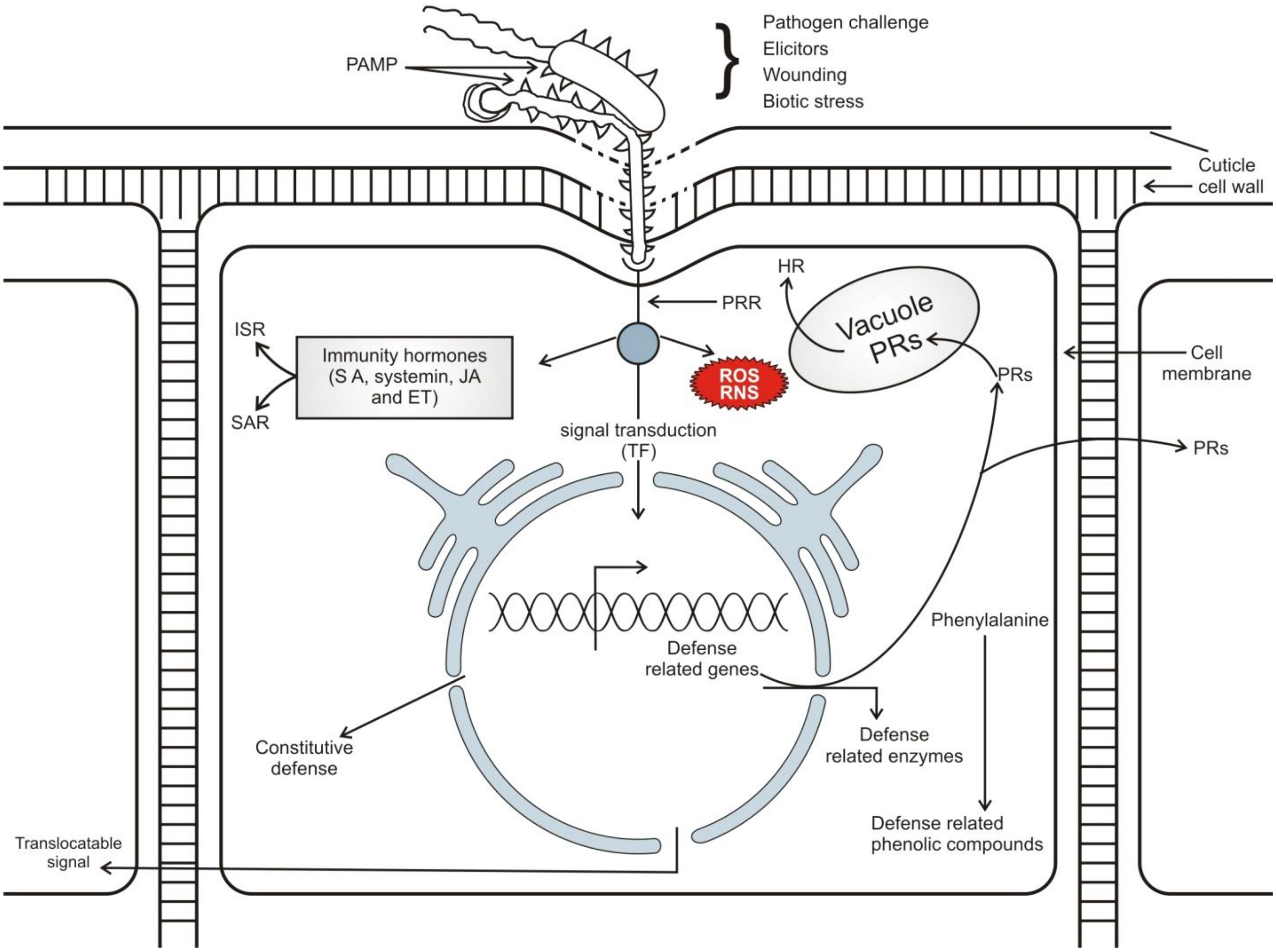

Fig. 1: Plant immune responses execute at physical, biochemical and molecular levels. Pathogenesis Associated Molecular Pattern (PAMP) of encountered pathogen are recognized by transmembrane Pattern Recognition Receptor (PRR) leading to a signal transduction cascade for induction of immune responses execute at physical, biochemical and molecular levels. Physical defense involves cuticle, physiological changes in cell wall and lignin deposition, biochemical defenses includes synthesis of defense-related enzymes, antimicrobial phenolic secondary compounds, synthesis of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Synthesis and activation of immunity hormone may also occur to execute Induced Systemic Resistance (ISR) and/or Systemic Acquired Resistance (SAR). Molecular mechanisms include the regulation of genes coding for PR- proteins and defense related enzymes.

resistance to pathogen challenge by protecting the plant from invasion of pathogen and also gives strength and rigidity to plant system. Phenolics are the large group of secondary metabolites produced by the plants to acquire defense from pathogen attack. They are synthesized primarily by shikimic acid and the malonic acid pathways in plants. Shikimic acid pathway is involved in biosynthesis of majority of plant phenolic compounds. Shikimic acid dependent pathway causes conversion of simplest carbohydrate intermediate derived from glycolysis and oxidative pentose phosphate pathway to synthesize aromatic amino acids. The most significant and major classes of phenolic compounds in plants are synthesized from phenylalanine by the elimination of one ammonia molecule thus form cinnamic acid. Conversion of phenylalanine to cinnamic acid is catalyzed by phenylalanine ammonia lyase (PAL), which is the regulatory and well studied enzyme in secondary metabolism of plant. Biochemical activity of PAL is enhanced by many environmental factors, like as lower nutrient levels, light intensity (through its effect on phytochrome), and fungal infection (Kumar, D.; 2015). PAL activity can be also elicited by the growth hormone ethylene and signaling molecules like jasmonic acid and the salicylic acid (Kim *et al.*, 2007). The malonate pathway has less significance in higher plants, but it is common source of phenolic secondary products in certain bacteria and fungi. Flavonoids and isoflavanoids are the predominent classes of phenolics. Anthocyanins are colorful water-soluble flavonoids pigments produced in plants to protect the foliage from harmful effects of ultraviolet radiation. Anthocyanins are also responsible for the showy colors of many plants and are present in high concentrations in flowers, fruits, and the coloured leaves of deciduous plants in fall. Phytoalexins are isoflavonoids with antifungal and antibacterial properties that are synthesized in response to pathogen attack. These toxic compounds disrupt pathogen metabolism and are usually have pathogen specific toxicity. Some examples of these defensive compounds are: medicarpin synthesized by alfalfa (*Medicago sativa*), camalexin synthesized in *Arabidopsis thaliana* and rishitin synthesized by potatoes and tomatoes.

Plant defense response against pathogens

In response to challenge by pathogens, plants have evolved variety of defensive strategies falling in categories of constitutive and inducible defenses to discourage or neutralize pathogens. Constitutive defenses are the first line of plant defenses and they includes preformed anatomical barriers such as lignified cell walls, waxy and cuticularised epidermis and bark tissues. These constitutive defenses protect the plant system from invasion of pathogen and also give rigidity and strength to the plant. Further plants have physiological strategy of closing the stomata to prevent the entry of pathogen within the plant system. If this first line of defense response is breached by the pathogen, the plant resorts to a second line defenses called inducible defenses. They can include hypersensitive response, synthesis of antimicrobial secondary metabolites, and antimicrobial enzymes that have ability to fight against the pathogens.

Plants most often wait until the interaction of pathogens before the production of antimicrobial secondary metabolites and/or defense related enzymes due to the high maintenance energy costs and associated nutrient requirements for their production as they are specialized traits. Pathogen interaction with host plant can cause the induction of protective mechanisms in the damaged tissues and can elicit the synthesis of diffusible signaling that travels a long distance and offers signal transduction to activates genes encoding PR proteins and enzymes of other protective mechanisms at sites far from the sites of injury, wound or challenge. Some defensive reactions may occur within time period of minutes, while some others may take hours to execute systemic resistance. Abiotic and biotic elicitors could be used as an agent of preconditioning prior to infection as a stimulus to evoke inducible defenses. Thus disease intensity can be reduced or disease can be suppressed due to trigger of induced resistance mechanism in plants by priming stimulus. Induced Systemic Resistance (ISR) mechanism and the Systemic Acquired Resistance (SAR) mechanism are the two strategies of induced defenses in plant. Combination of both ISR and SARmechanisms could enhance the defense response against those pathogens which have resistance against either of ISR or SAR mechanisms (Choudhary, 2007). Inducible defenses includes the production of toxic compounds, pathogen-degrading enzymes such as chitinases and glucanases. These defense related compounds are usually stored in form of inactive precursors and are later converted to their bioactive forms by the enzymes stored in different cellular compartment in response to either pathogen attack or the tissue damage.

Induced Systemic Resistance (ISR)

ISR responses are induced by Plant Growth Promoting Rhizobacteria (PGPR) that are supposed to produce a translocatable signal that induces protection in plant tissues far from the roots at sites where the antagonist pathogen is encountered. Working of ISR is independent of salicylic acid signal and is mediated either by either jasmonic acid or by ethylene ($C_2H_{4)}$. These signaling molecules are also produced upon exposure of some forms of nonpathogenic rhizobacteria of rhizosphere region (He *et al.*, 2004). Action of ISR is executed by the transcriptional activation of a certain set of specific genes which are distinct from the PR protein coding genes. Induced resistance through activation of plant's natural defense pathways has been shown to be effective in plants against wide variety of pathogens. (Lavania *et al.*,, 2006). Induced systemic resistance (ISR) is emerging as a significant defensive strategy in which some non pathogenic plant growth promoting bacteria and some fungi of the rhizosphere region sensitize the whole plant body system for boosting defense response against a broader range of plant pathogens. A large number of root associated mutualists, such as *Trichoderma harzianum*, *Trichoderma viridae, Pseudomonas*, *Bacillus*, and mycorrhiza species are reported to boost the whole plant immunity against pathogens for enhanced plant resistance.

Systemically Acquired Resistance (SAR)

SAR is evoked in plants as a defense response against the initiation of diseases by various biotic stresses caused by necrotizing pathogens, non-pathogens or soil borne rhizosphere forming fungi and bacteria. SAR is a also considered as a mechanism of induced defense responses in plants (Gajanayaka *et al.*, 2014). In SAR involves diffusible signals generated at the sites of challenge and translocated in the whole plant system thus bringing an induced defensive state in all the plant tissues even farthest from the sites of challenge by the elicitor or pathogen. SAR offers long-lasting disease protection against a wide spectrum of pathogens. Salicylic acid is the key signaling molecule in SAR which is associated with the accumulation of more PR proteins responsible to impart systemic resistance. Induction of SAR has associated with a wide variety of defense responses, such as biosynthesis of PR proteins, phytoalexins, rapid changes in physical and biochemical composition of cell walls, and acceleration of activities of a variety of defense related enzymes. SAR can be induced systemically upon challenge inoculation with a necrotizing pathogen of interest or by applying some chemical compounds as elicitors of defense (Prasannath *et al.,* 2014).

Systemic acquired resistance (SAR) is the most significant approach through which various attempts for integrated disease management are being tried worldwide. Various abiotic and biotic elicitors have been worked out for developing SAR. Chemical compounds like benzothiadiazole, saliciylic acid, and oxalic acid and biotic agents like *Trichoderma harzianum* and *T.viride* can elicitise the plants to defend themselves from pathogens by inducing SAR (Kumar, D., 2015). Recent researches have given surprising indication of the presence of memory thus long lasting effects of SAR and inheritance of resistance across generations (Slaughter *et al.,* 2012; Luna *et al.,* 2012). The induction of systemic resistance could be the most important solution to impart disease resistance (Rothmann LA and McLaren NW.; 2018 and Abdel-Monaim *et al.,* 2012). Inducing Systemic Acquired Resistance against variety of plant pathogens is one such environmentally-friendly approach of disease management under framework of Integrated Pest Management program (Prasannath and De Costa, 2015).

Pathogenesis-related (PR) proteins

Pathogenesis-Related proteins are a class of proteins induced in a plants in response to challenge by fungal, bacterial, viral, viroid and/or by some chemicals or biotic elicitors. PR proteins are structurally and functionally diverse class of plant defense proteins that play most significant roles in executing plant disease resistance mechanism (Mahendranathan *et al.,* 2016). These proteins are greatly specific displays a high degree of pathogen specificity and are their expressions are coordinated at the transcriptional level of regulation. Upon challenge by pathogen or under biotic stress these proteins are produced accumulated in plants in much greater concentrations (Kumar, D. 2015). PR proteins exists within the plant cells intra-cellularly and also repoeted in the intercellular spaces between the cells of different plant tissues (Agrios, 2005). More recently in *Arabidopsis thaliana,* an extracellular PR protein called subtilase, SBT3.3, was characterised that acts as a regulator of Salicylic acid dependent immune modulation (Ramírez *et al.,* 2013). Expression of SBT3.3 is reported to be upregulated upon priming by inducers of defense thus suggesting a role of enzyme in plant immunity.

Defense related enzymes

All defense-related enzymes are PR proteins and are responsible for the development of disease resistance responses in plants. Plant defense-related enzymes includes Phenylalanine Ammonia-Lyase (PAL), Polyphenoloxidases (PPO), class III peroxidases (Prxs), chitinases, and β-1, 3-glucanase. PAL is involved in the biosynthesis of phenolic secondary metabolite of antimicrobial nature compounds and is most essential for disease resistance responses in plant (Waewthongrak *et al.,* 2015). Polyphenol oxidase (PPO), mainly found in cytosol is the main enzyme of phenolic compound oxidation leading to formation of defense related phenolic compound synthesis. Its activity has positive correlation with development of plant disease resistance. PPO is also involved in the biosynthesis of lignin polymers and works to increase the antimicrobial ability of host plants. Chitinase is one of the defense related enzyme that hydrolyzes the cell wall in most phytopathogenic fungi that have chitin in cell wall. Plants enhances defense response by means of inducing expression profile and activity of many defense related enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidases,, chitinases, and β-1, 3-glucanases which can slow down the rate of disease spread and development of disease in plant system (Kumari and Vengadaramana, 2017). Induction of defense related enzyme expression by the use of various abiotic and biotic activators of defense can be environmental-friendly approach of disease management under Integrated-Pest-Management (IPM). Application of various abiotic compounds such as Silicon, salicylic acid, acetyl salicylic acid (aspirin), β-Amino Butyric Acid (BABA), polyacrylic acid, oxalic acid, benzothidiazole, Silicon and biotic inducers of defense like *Trichoderma viride, T. harzianum, Psuedomonas fluorescens, Fusarium oxysporum* are reported to increase the level of defense related enzyme in primed plants against a wide variety of pathogens. Thus priming of plants by such defense inducers will provide a suitable, cheap effective control measure of plant diseases within the framework of integrated disease management system.

Conclusion

Application of chemical pesticides to control plant pathogens has been the most common method of controlling plant diseases worldwide. (Prasannath *et al.*, 2014). Long term use of the chemical pesticides brings negative and long lasting effects on human health and potentially hazardous effects on the environment. This review paper attempts to present future outlook and strategy for inducing Systemic Acquired Resistance by abiotic and biotic elicitors of defense. Elicitors of defense works indirectly against the pathogen through the induction of plant's own immunity. Elicitors are not directly toxic to pathogens, which is the basis of organic pesticides. Thus, Elicitors of defense have the potential to be more environmentally sustainable with no impact on human health. Further most of the farmers in India do not use appropriate safety equipment during the application of harmful chemical pesticides. Furthermore due to regular use of pesticide doses, the pathogens are evolving resistance. Taking into consideration of health, antigenic drift by pathogen and environmental effects of chemical pesticides, it is evident that the need for a new strategy of controlling plant pathogen by means of boosting plant's own immunity is the most urgent need of time.

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