

Biotic Elicitors in Inducing Disease Resistance in Plants against Pathogen Infection: A Review

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Review Article

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Article History

Received: 24.10.2018

Accepted: 31.10.2018

Published: 30.11.2018

DOI:

10.21276/haya.2018.3.11.1



Abstract: Elicitors are the synthetic or naturally occurring molecules, which capable of trigger the plant defense mechanism against the plant pathogens and environmental hazards. Plants deploy a number of defense mechanism against the phytopathogens and elicitors can able to induce the production of secondary metabolites to facilitate the biochemical defense mechanism, which eventually leads to plant resistance. Biotic elicitors are the type of elicitors that have the biological origin and derived from the pathogen or from the plant itself. To induce the plant resistant, initially biotic elicitors need to go through with microbial-associated molecular patterns (MAMPs, also called PAMPs). As well, the plants have evolved other receptors to detect the elicitors and leads to gene mediate resistance mechanism against the pathogens. This review discusses such biotic elicitors and their way to induce the disease resistance.

Keywords: Elicitors, Defense mechanism, Phytopathogens, Plant secondary metabolites, Plant resistance, Microbial-associated molecular patterns.

INTRODUCTION

Through the past decades to protect the crop plants from the pathogen diseases, it used various synthetic bactericides, fungicides, and antiviral agents. However, many such synthetic compounds are highly negative effects on environments and the consumers. This leads to people to change their attitude towards to produce natural origin products, which are comfortable with less adverse side effects [1].

Elicitors are the synthetic or naturally occurring small molecules or more complex macromolecules derived from plants or microbial sources and capable of inducing plant defense mechanisms which eventually, leads to enhanced resistance in plants against the pathogens and other hazards [2, 3]. Plants recognize microorganisms as harmful, with plant-pathogen interactions and through elicitors. Plant receptors are responsible for detecting the derived elicitors from the phytopathogens. Plant cells show physiological and morphological responses to those elicitors [4].

Many of elicitors are constitutively present as the structural components in the pathogen cell wall (e.g. glucan and chitin fragments as well as bacterial flagellin and lipopolysaccharides-LPS) and other types of elicitors are playing a role in virulence determinants such as harpins, products of *avr* genes. As well, the function of some elicitors' remains elusive yet [3]. The elicitors can stimulate the stress responses in plants and have the ability to produce secondary metabolites to give the protection against the stress to the plant and insignificant in plant growth and developments. Plant secondary metabolites are the diverse low molecular weight compounds, which have the ability to initiate the

biochemical defense mechanism against the pest, parasitic nematodes, herbivores mammals, and other phytopathogens [5, 6].

There are many different types of elicitors are present and were classified as; biotic or abiotic, general or race specific and exogenous or endogenous elicitors. In the industry, elicitors are called as 'Plant activators'. The elicitors which are having biological origin are called as biotic elicitors and they may be derived from the pathogens or from the plant itself [7, 8, 6]. In the production of secondary metabolites, biotic elicitors have an important role [6]. Elicitation is one of the techniques or process that used various microbial, chemical and physical factors (elicitors) as a trigger for enhancing the production/synthesis and accumulation of secondary metabolites [9, 4].

Nature of biotic elicitors

Biotic elicitors are directly released by the microorganisms and it recognized by the plant cell such as enzymes (or Receptors) and cell wall fragments. The biotic elicitors also formed by the action of microorganisms on plant cell wall such as Fragments of pectines [7]. Some biotic elicitors are may be the components of pathogens cell wall such as beta-

glucans, chitin, or chitosan and they released with the effect of host enzymes such as beta-glucanase and/or chitinase. However, those elicitors have broad host ranges [10]. The biotic elicitor compounds have endogenous and constitutive in nature. These are formed or released by the plant cells as a response to various stimuli [7].

Biotic elicitors inducing defense resistant against pathogen

MAMP/ PAMP

MAMP, called the microbial-associated molecular pattern or the pathogen-associated molecular pattern/ PAMPs [11]. The MAMP is recognized by the plant innate immune system through the action of pattern recognition receptors/PRRS [12, 13]. These PRRS are attached to the plasma membrane of the plant cell [14].

In MAMP, plant cell receptors (PRRs) are identify the microbial elicitors such as bacterial flagella (bacterial motility structure), bacterial lipopolysaccharide or LPS (LPS is a glycolipid component of Gram-negative bacterial outer membranes and this is important to induce NO synthase AtNOS1 and also activate several other defense genes and as well as this is a general elicitor) and even fungal chitin and ergosterol (main structural components of higher fungi cell walls and membranes). Identify those MAMPs is broadly can see among as many of plant families. Several studies indicated that different type of beta-glucan cell wall components of Phytopathogenic fungi and oomycetes are also serve as MAMP and induce the plant defenses against the pathogens. For as an classical example, branched heptagluco-side components (general-elicitor) of oomycetes are making plant defence against only in family Fabaceae (e.g. soybean). Another experiment shows that cell wall component (transglutaminase GP42) of oomycete and a soil-borne plant pathogen - *Phytophthora sojae* elicits defence responses in potato and parsley. But anyhow some of microbial molecules which is important for pathogenicity are not satisfy the MAMPs or effector pathway, and they are inducing HR. Fungi *Trichoderma viride*, act as invasive pathogen by producing enzyme xylanase(xylanases also role as PAMPs by inducing defense responses and ability to promote the necrosis). But, existing researches indicated that specific cultivars of tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*) show defense responses which created by with the help of the EIX or elicitor ethylene-inducing xylanase [15-17].

MAMP recognition at the cell surface by receptors

Extracellular ligand-binding domain (often comprised leucine-rich repeats or LRR and as receptor-like kinases or RLK) are involved with the pattern recognition at the cell surface. These ligand-binding domains act as activators in pattern recognition in receptors [13].

MAMP triggered defense responses

Flagellar motility of pathogenic bacteria is important for them to enter into plant tissues [13]. Study of Melotto *et al.*, [18] shows that, pre-invasive resistance in plant *Arabidopsis* sp against the flagellar pathogenic bacteria. Unlike the fungal pathogen, bacteria need wound or natural opening of the plant tissue to enter. In the study of Melotto *et al.*, [18], further discovered that entrance of pathogen bacteria through the stoma in to plant tissue *Arabidopsis* sp is restricted by the innate immunity of the plant. In this study of them, it proved as initial responses against the plant and animal pathogen, stomata of *Arabidopsis* sp is actively closed. The biotic stresses are important to the activity of guard cell to close the stomata. As well, they suspected *Pseudomonas syringae* pv. Tomato strain DC3000 (PstcDC3000) have the ability to induce a natural virulence mechanism/s which counter PAMP induced stomatal closure. This innate immunity helps to prevent bacteria from entering the plant leaf. The study further shows that lipopolysaccharide (LPS)-triggered nitric oxide (NO) production and flagellum perception by its receptor FLS2 contribute to *Arabidopsis* sp resistance.

MAMP signaling and regulation

Study of Rasmussen *et al.*, [19], explains that Mitogen-activated protein kinase (MAPK) signaling plays main roles in such intracellular immunity pathways. MAP kinase kinase kinase (MAP3K; also called MEKK) activation is allowing to stimuli the MAP kinase signaling. MAPK stimuli included the flagellin receptor FLS2, the bacterial elongation factor EF-Tu receptor EFR, and the chitin receptor CERK1. Rasmussen and co-workers [19], are further identified that complete MAP kinase cascade mediating flg22 signaling in *Arabidopsis* sp. They further found that three types of (MPK3, MPK4, and MPK6) MAP kinase cascade is activated by flg22 and elf18 (elongation factor-Tu peptide) and these three types of MAPK cascades are differently regulated already at the PRR level.

Furthermore, MPK3/6 pathway indicates positively regulate MAMP responses but the MPK4 exerts a negative regulatory function [13].

Some of the biotic elicitors and their action against the plant pathogen by inducing disease resistance

EIX (ethylene-inducing xylanase) - This is a fungal elicitor and *LeEix* genes from tomato acts as a receptor for EIX. There are two types of *LeEix* genes and called as *LeEix1* and *LeEix2*. EIX elicitor is suppressing the *LeEix* gene expression and elicitor responses in Vivo and to the both of *LeEix* (1 and 2) proteins restored the binding of elicitor EIX. *LeEix* genes show homology to genes encoding plant proteins with leucine-rich repeat (LRR) region. The (LRR) region shows the role with resistant protein by showing resistance against the pathogen infection. For as an

example, polymorphic TIR-NBS-LRR resistance proteins are encoding with the flux rust resistant gene *L* and finally control the fungi rust disease [20, 16, 21].

Branched (1, 3-1, 6)- β -glucans – oomycetes is the source for this general biotic elicitor (oligosaccharide) and effects on the plant by inducing Phytoalexin producing in soybean(glyceollin- a phytoalexin type) and rice [22]. As much works are done with the cotyledons in soybean, it was found that glucan elicitors are also capable with elicited the synthesis of several types of phytoalexins in a wide range of other plant species too [1]. Many funguses also produce elicitors with beta glucan structure, which can be isolated from their mycelial cell wall. Anyhow, one of the best-characterized beta-glucan elicitors is released from the cell walls of *Phytophthora megasperma*, species of oomycetes [22].

Chitin (*N*-acetyl-D-glucosamine polymer) and chitosan- Chitin and chitosan are fungal cell wall component and plant defense system activate against it. In addition, those elicitors may release by the fungal pathogen during their invasion of host plants [23]. Both elicitors are quite active but they are not functionally effective like β -glucan elicitors. But chitosan is the most active elicitor of pisatin(a phytoalexin type) which obtained from the *Fusarium solani* cell wall [22].

The first plant PRR was found in rice plant (*Oryza sativa*). In the case of rice, CEBiP PRR protein recognizes the chitin from the fungal cell wall. Several studies show that WAK genes are participating to induce resistance against the fungal and bacterial pathogen in many species or plant, even in rice [24].

A receptor-like protein: Chitin elicitor binding protein (OsCEBiP) is containing an extracellular chitin-binding lysin motif (LysM) but is lacking with intracellular signaling domain. The OsCEBiP forms a complex with chitin elicitor receptor kinase1 (a receptor-like kinase contains an active intracellular kinase domain,) which is important to initiate the chitin signaling. AtCERK1 (RLK1/LYK1) is essential in chitin signaling in *Arabidopsis* sp. More recently, it was found that receptor-like kinase (RLK) CERK1 is the key player to identify the fungal chitin. Fungal chitin is activated the generation of reactive oxygen species (ROS), activation of mitogen-activated protein kinases, and expression of defense-related genes like immune responses by induces dimerization of AtCERK1. Anyhow, the biological activity of chitin is depending on their size and for example, high PAMP activity is showing heptamers to octamers [25, 26].

Works done by Egusa *et al.*, [25], along with nanofibrillated polymeric chitin shows that defense-related gene expression against the fungus as well as bacteria and can be a useful tool for a wide variety of plant hosts for defense against plant pathogens. In other

cases, the study done by the Baque and co-workers [27], found that combinations of chitosan and pectin have the ability to induce elicitor-induced oxidative defense mechanism in *Morinda citrifolia*.

Yeast elicitor- works done with Hairy root cultures of *Salvia miltiorrhiza*, identify that yeast elicitors have capable of produce the phenolic acids and tanshinones [28]. And also Zhao and Sakai [29], explained that yeast elicitors have the ability to induce the biosynthesis of a phytoalexin (beta-Thujaplicin – have strong antimicrobial activity) and hence the defense responses in the demonstrated plant, *Cupressus lusitanica*. Anyhow, they further explained yeast elicitors (as well as fungal elicitor) have capable of increasing the level of endogenous jasmonate, which is, followed the defense gene expression and the accumulation of secondary metabolites. The elicitors induced the jasmonate signaling pathway and produced the beta- thujaplicin at the molecular level. Zhao and Sakai [29], further explained that lipoxygenase (key enzyme for jasmonate biosynthesis) plays a critical role in the mediation of elicitor production of beta-Thujaplicin. The study also showed that receptor-coupled G-proteins and Ca^{2+} also involved in this signaling pathway and it regulated the production of lipoxygenase. As well, G- protein activator has the ability to activate NDPK. It believed that NADPK could interact with the G- protein alpha subunit. Anyhow they further explained other than the fungal elicitor, exogenous H_2O_2 (hydrogen peroxide) also has the ability to induce jasmonate biosynthesis. As well the influx of Ca^{2+} also required for beta-Thujaplicin accumulation.

Yeast extract- work done by Cai *et al.*, [30], found that, the yeast extracted elicitor is capable of inducing plant secondary metabolites production in the basis of enhancing the plant defense mechanism. They further shows that yeast extraction is capable of the production of *p*-coumaric acid, a dominant phenolic acid by 5.1-fold within 3 days in the controlled culture. Further studies done by the Ahmed and Baig [31], using *Aspergillus niger* and *Penicillium notatum* extraction shows that higher accumulation of psoralen. And also they showed between both species, *A. niger* elicitor has capable of highest accumulation of psoralen.

Erwinia carotovora - De León *et al.*, [32], demonstrated that *E.c. carotovora*(bacteria) elicitor and *Botrytis cinerea* (necrotrophic fungi)inoculation causes the disease symptom and as well induces the defense responses in *Physcomitrella* sp(small moss). In addition, those pathogens are inducing the defense responses genes such as *PR-1*(pathogenesis-related genes- *PR*), *PAL*, *CHS*, and *LOX*. Compounds produced by *LOX*, *PAL*, and *CHS* are involved with the synthesis of JA(jasmonate), phenylpropanoids and SA(salicylic acid) and flavonoids respectively in vascular planta and those are playing defense responses of *Physcomitrella*

sp as shown in vascular plants. Anyhow study of Govrin *et al.*, [33], on *Arabidopsis thaliana*, show that Elicitor from *B. cinerea* induces the hypersensitive response with host cells to collapse in advance of the hyphae with the induced generation of reactive oxygen species, resulting in reduced photosynthesis, electrolyte leakage, and necrotic lesions. They found that elicitor of *B. cinerea* promotes the Gray Mold Disease in *A. thaliana* and other plants.

CONCLUSION AND RECOMMENDATION

Biotic elicitors have the ability to induce the plant defense mechanism against the broad spectrum of pathogen microbes such as bacteria, fungus, and oomycetes etc. Plants recognize the biotic elicitors or pathogen-derived molecules through the *MAMP/PAMP* mechanism and promote the resistance against harmful pathogens. Biotic elicitors can be used to control the phytopathogenic disease. Biotic elicitors are nontoxic and environmentally friendly. Hence, application of biotic elicitors for plant protection against pathogen microbes could be a better replacement for the many of chemical pesticides, which often harmful to the humans and their environments. As well, it needs to be further researches on this subject to improve the identification of new elicitors and further evolve the identified elicitors for the environmental friendly crop protection.

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