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REVIEW ARTICLE

Elicitins as molecular weapons against pathogens: consolidated biotechnological strategy for enhancing plant growth

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ABSTRACT

To fight against pathogens, defense systems in plants mainly depend upon preformed as well as induced responses. Pathogen detection activates induced responses and signals are transmitted for coordinated cellular events in order to restrict infection and spread. In spite of significant developments in manipulating genes, transcription factors and proteins for their involvement in immunity, absolute tolerance/resistance to pathogens has not been seen in plants/crops. Defense responses, among diverse plant types, to different pathogens involve modifications at the physio-biochemical and molecular levels. Secreted by oomycetes, elicitins are small, highly conserved and sterol-binding extracellular proteins with PAMP (pathogen associated molecular patterns) functions and are capable of eliciting plant defense reactions. Belonging to multigene families in oomycetes, elicitins are different from other plant proteins and show a different affinity for binding sterols and other lipids. These function for sterols binding to catalyze their inter-membrane and intra- as well as inter-micelle transport. Importantly, elicitins protect plants by inducing HR (hypersensitive response) and systemic acquired resistance. Despite immense metabolic significance and the involvement in defense activities, elicitins have not yet been fully studied and many questions regarding their functional activities remain to be explained. In order to address multiple questions associated with the role of elicitins, we have reviewed the understanding and topical advancements in plant defense mechanisms with a particular interest in elicitin-based defense actions and metabolic activities. This article offers potential attributes of elicitins as the biological control of plant diseases and can be considered as a baseline toward a more profound understanding of elicitins.

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Introduction

Plants innate immunity systems are able to restrict pathogen growth and infection spreads and ensures plant safety [1,2]. Mainly, the plant immunity consists of two layers named as PTI (pathogen-associated molecular patterns (PAMPs)-triggered immunity) and ETI (effector-triggered immunity) [1,3,4]. PTI is regarded as a chief mediator of plant basal defense [5]. PAMPs/MAMPs recognized by plants are usually peptides, numerous secreted proteins or polysaccharides from bacteria, fungi and oomycetes [6]. Secreted by oomyctes, e.g. *Phytophthora, Pythium* sp., elicitins are small, highly conserved and sterol-binding proteins with PAMP functions [7,8]. Elicitins were first discovered in the 1980s [9,10]. Certain elicitins obtain sterols from plants and fulfill sterol demand in oomycetes e.g. *Phytophthora* and *Pythium* which are unable to synthesize sterols [11,12]. In several plant-pathogenic oomycetes, the multifunctional elicitins expedite infection by triggering the necrosis of plant tissue. These usually result in induction of a hypersensitive response (HR) and systemic acquired resistance (SAR) in several plants and is, therefore, reckoned as the most distinguished oomycetes PAMPs [13–19]. These small proteins may perform the role like fungal hydrophobins and probably function as pathogenicity factors in other plant–microbe interactions.

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Elicitins comprise 1–98 amino acid domains without arginine, histidine and tryptophan [20,21]. Six cysteine residues have been observed in conserved positions that make three disulfide bridges [18,22–24]. So far, elicitins have been divided into five diverse classes depending upon their primary structure. Members of class I only possess 98 amino acids elicitin domains [15]. Belonging to the same *Phytophthora* sp., elicitins of class I can be further classified as acidic (α-elicitins class 1A) or basic (β-elicitins Class 1B) [8,15,25]. Both α- and β-elicitins have the same affinity to bind with the binding site on the cell membrane. Elicitins are also grouped on the basis of source species as cryptogein, capsicein, parasiticein and INF1 from *Phytophthora cryptogea, P. capsici, P. parasitica* and *P. infestans* correspondingly.

Although elicitin binding is essential for inducing plant defenses such as the interaction of AVR9 with Cf-9 in *Lycopersicum esculentum* or NIP1-Rrs1 interaction in *Hordeum vulgare*, the active response cannot be recorded in the absence of a third interactive partner e.g. INF1 (Inverted formin 1)- NbLRK1 kinase interaction [26,27]. The elicitins activity appears dependent on definite residues. Lysine residues in A and D helices are the essential components for the activity of elicitins [28]. This statement was confirmed by the necrotic index and pI correlation [29] as well as a strong effect of the Lys13Val mutation in helix A on the induction of tobacco defense response [30]. Cell surface receptor mediated elicitin response activates a signal transduction leading to HR for restricting pathogen growth. On the other hand, initiation of SAR causes effective defense against pathogen attack and spread on sites other than the infection sites. A closer look at the literature, however, unravels many gaps. For example, elicitins have yet to be considered as compounds of benefit to oomycetes only. Sterol binding is an important function performed by elicitins for oomycetes, but this should not be accompanied as an integral part of immunity induction. Likewise, confusion exists between elicitins and effectors due to their protein nature. Very little information is available regarding phytohormones and their interaction with elicitins. Therefore, we have focused these aspects on elicitins in connection with plant life cycle. In this article, elicitin perception and plants responses for broad range immunity events have been focused. This demonstrates the distinguished functions of elicitins in plants and bacteria and show differences between elicitins and plant/bacterial proteins performing diverse roles in the life cycle of both organisms. In addition to structural and functional significance, the unique involvement of elicitins in triggering immunity, interaction with plant hormones and other cellular compounds like sterols, proteins have been emphasized. We expect the use of this knowledge in cell recognition by elicitin and succeeding signaling actions for engineering plants with resistance.

Elicitins, effectors and plant proteins, do not confuse

Elicitins are members of complex multigene families in oomycetes (Figures 1 and 2) [7]. These genes are divided into different subclasses namely elicitin (ELI), and elicitin-like (ELL) genes. ELI and ELL genes differ among species displaying distinct expression patterns and HR [7,21,31]. The host PRRs (pattern recognition receptors) perceives PAMPs/MAMPs [32]. Defense against pathogens can be triggered by PAMPs or pathogens may overpower host immunity by means of specialized

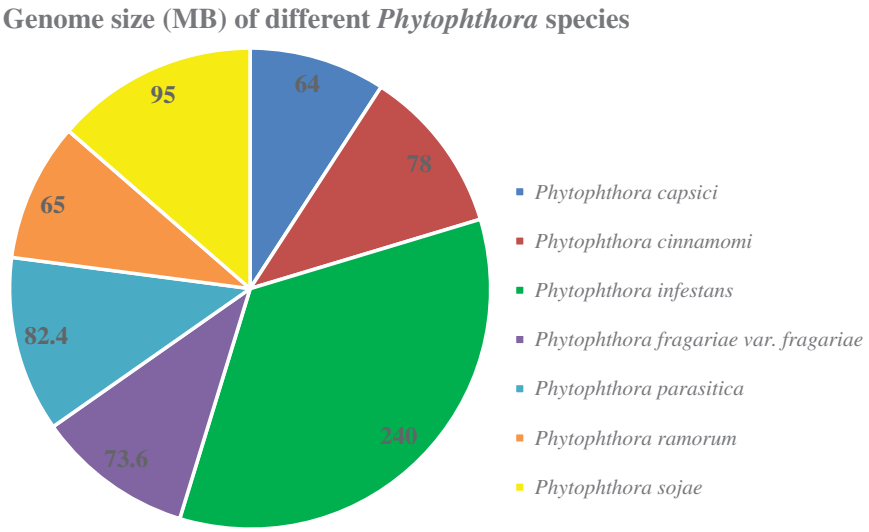

Figure 1. Genome size (Mb) of different *Phytophthora* species.

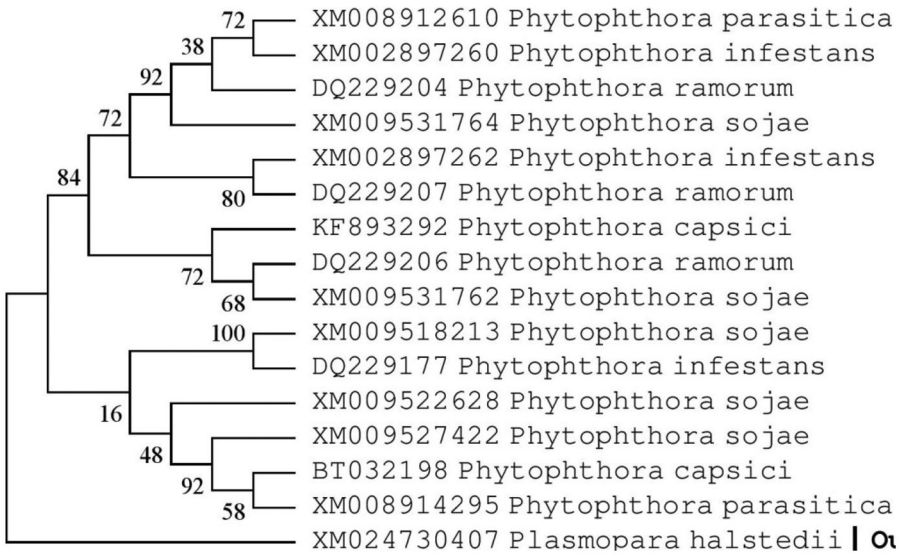

Figure 2. Phylogenetic relationship among elicitins secreted by different oomycetes. Closely related oomycete species possess small difference among their sequences while less related oomycetes display more difference in their elicitin sequences. This lineage not only maps the evolutionary history of oomycetes in terms of secreting elicitins but also present lineage of oomycetes. All elicitins in *Phytophthora* species are related not only to each other but may also be linked with other oomycete species.

effectors molecules evolved during the course of evolution (Tables 1 and 2) [32,61]. Interestingly, among several microbial molecules peculiarity between MAMPs and effectors is not always very clear, and such molecules do not sternly fit in any group [62]. Although elicitins resemble MAMPs in many ways, yet we appraise that elicitins are entirely different from plant proteins. We support this notion on the basis of two points. Firstly, elicitins are structurally more conserved with characteristic cysteine spacing patterns. Secondly, they are significantly different in sequence as compared to plant proteins by possessing C-terminal domains of different size that may have high proline, serine, or threonine, content. This proposes a tendency to be associated with the cell wall [63]. More generally, sequential difference is very much related with their identification by plants. Detailed analysis of protein domain database has confirmed sequences unique to elicitin only that are not found in any other organism [64]. Consequently, in case of interaction, hosts view them as non-self molecules. It is pertinent to mention that some nonspecific lipid transfer proteins (nsLTP) resemble elicitins. However, they do not share any phylogenetic linkage with elicitins [63,65]. Because some oomycetes need external sterol sources for life cycle, elicitins function as sterol transporters [15]. In a defense perspective, we notice that HR in plants restricts the pathogens growth and HR-inducing activity is definitely not the main function of ELIs in *Phytophthora* sp. But no such function can be attributed to all plant proteins or effectors. Biochemical analyses revealed that the intrinsic biological role of ELIs is linked to lipid binding [66]. The intrinsic jobs of ELLs are largely unidentified [21]. The discussed data advocate sterol binding/transport as a very important and distinct function performed of elicitins contrary to the effectors or plant proteins. Besides, the presence and functioning of plant proteins is not limited to an interaction with microbes or other organisms. An entirely convincing argument comes from PRRs based recognition. Effectors as well as plant proteins are independent of their recognition by PRRs. As MAMPs, elicitins are appropriately recognized by membrane bound PRRs for triggering immune response [20]. Moreover, INF1 perception is determined by SERK3/BAK1 modulating PRR mediated immunity [5,67].

C-terminal domains in ELIs and ELLs

In most of the ELIs and ELLs, a signal peptide has been predicted at the N-terminus. In 14 out of 17 clades, ELIs and ELLs possess C-terminal domains of 17–291 amino acids [7]. But 3 clades i.e. ELL-7, ELL-9, and ELL-10 were recognized with small C-terminal domains comprising of maximum seven amino acids [63]. Most of the ELI-1 proteins exclusively consist of a signal peptide along with conserved elicitin domain of 98-amino acid [21]. Together with the present information, we argue that C-terminal domains in ELIs as well as ELLs exhibit clade specific characteristics in the composition of amino acids and in the structure of repeats. Interestingly, the amino acid configuration has also been reported in many of the C-terminal domains. These appear to be

rich in residues such as threonine, serine, and proline. Often these residues have been observed as part of the repeat. The predicted O-GalNAc-glycosylation sites propose glycosylated C-terminal domains [68]. In the C-terminal domains, presence of Thr and Ser residues advocates wide-ranging O-glycosylation and association with the cell wall [63]. Planned comparisons of existing information on structural attributes of elicitins revealed hydrophobic regions at C-terminal end among different ELL classes i.e. ELL-1, ELL-2, and ELL-13 [69]. According to Eisenhaber et al. [70], these hydrophobic regions are a part of the GPI anchor site. The GPI based anchoring to the plasma membrane is a common ELL strategy for tethering to the cell exterior e.g. ELL-3. Zoospore stage cells lack cell wall [63]. Therefore, due to specific genes expression at this stage, ELL-3 proteins can be fastened to the plasma membrane of the motile zoospores with the help of the GPI anchor. The zoospore surface may be coated with oligosaccharides by using putative O-linked glycosylation.

Is sterol binding necessarily required for elicitin actions and the plant defense?

Optimum growth of oomycete and sporulation require sterols [71]. Elicitins show different affinity for binding sterols and other lipids. These bind to sterols for catalyzing their inter-membrane and intra- as well as inter-micelle transport. With particular reference to stoichiometry, only one sterol can bind an individual elicitin molecule [71,72]. Some oomycetes largely depend upon external sterol sources during their life cycle e.g. *Phytophthora* needs sterols for reproduction [11,71]. It is well understood that elicitins scavenge sterols from plant membranes and liposomes. These are also reckoned as sterol carriers [12]. The point of interest is assessment of sterol carrying activity and its relation with HR response for the role of these proteins in plant immune responses. Normally, the growth and sporulation of *P. ramorum* display two general inclinations with reference to sterols. The first is the different response curves with respect to sterol present. Secondly, high sterols levels may cause decreased growth and sporulation in *P. ramorum* [71,73]. Inevitably, the mechanistic reasons for the diverse response curves or sterol discrimination are largely unidentified. But evidence has supported the dependence of this attribute upon variances in the uptake and metabolism of sterol. Moreover, it has been revealed with the help of crystal structure of a sterol–elicitin complex that sterol binding is critically dependent upon "the x-loop" a highly conserved region [22]. We propose that elicitin binding with sterol disrupt plasma membrane and, as a consequence, HR is induced. However, elicitin (cryptogein) mutants failed in sterols binding and can also elicit cell death response in tobacco [28]. The ability of cryptogein and its mutants to bind sterol as well as associated conformational modification in the ω-loop might not be chief factors in either the production of ROS or induction of resistance in plants [28,72]. On these bases, it is affirmed that defense elicitation and sterol binding are not dependent on each other.

Molecular interaction between elicitins and phytohormones

Elicitin usually induces HR and activate JA (Jasmonic acid) and ET (Ethylene) signaling. In tobacco, SA (salicylic acid) signaling and SAR (systemic acquired resistance) along with HR cell death is on record against several pathogens in elicitin treated plants [20,74]. Elicitins like cryptogein and megaspermin may activate SA as well as JA and ET signaling. But, in parallel, compromised SAR was noticed in cryptogein and γ-megaspermin treated nahG *Nicotiana* plants expressing a SA degrading salicylate hydroxylase that inhibits SA accumulation and causes the up-regulation of PR genes [74,75]. Interestingly, INF1 may induce resistance, without HR, against *Ralstonia* by activating signaling pathways mediated by JA and ET in *L. esculentum*. The cysteine at position 3 is necessary for inducing HR in tobacco. The reported replacement of Cys by Ser at position 3 revise the HR induction process and the defense can be compromised [76]. In *Arabidopsis thaliana*, the JA signaling pathway was activated in non-host resistance against *P. infestans* without any HR [77]. Therefore, in *P. infestans* infected tomato, INF1 can be regarded as PAMP triggering basal defense mediated by JA and ET independent of HR cell death [20]. But such basal defense responses are not sufficient to suppress the pathogen growth. An unsolved question is the molecular mechanism of elicitin recognition and its downstream signaling components. We need to determine these by more systematic and theoretical analyses. To reinforce the information [78], it is inferred that elicitin capability to bind sterol is directly related with the induction of HR and SAR in tobacco. Hence, the ability of INF1 to induce *R. solanacearum* resistance and JA and ET signaling activation in plants such as tomato helps us to infer its probable dependence upon the sterol binding ability. Thus, we argue that the identification of receptor or receptor complex for recognizing elicitins along with related elements in plants would elucidate regulatory mechanisms for differential signal

transduction pathways. Further studies are required to completely understand the key principles of elicitin-phytohormone interaction.

Oxidative burst is induced by elicitins but not always accompanied with HR cell death

HR can extremely differ in appearance and timing at macro-/microscopic levels during various plant–pathogen interactions [79]. Such differences are partly attributed to different infection strategies adopted by diverse pathogen types eliciting HR e.g. oomycetes. Certainly, we can observe differences in fundamental HR cell death mechanism(s) [80]. In addition to elicitin recognition by PRRs, transmembrane proteins like BAK1/SERK3 and SOBIR1 are also involved in host defense responses [81]. It has been suggested that elicitins normally cause HR cell death in some plants but not in all. For example, tobacco, potato and pepper plants responded significantly to elicitins application but radish and turnip cultivars did not respond to elicitin application in terms of tissue necrosis (Yu, 1995). This has been discussed by different researchers that a reactive oxygen species (ROS) burst is usually observed during recognition (Table 1) [82,83]. In fact, such a burst includes events like the influx of Ca^{2+}, activation of MAPK (mitogen activated protein kinase) cascade and NADPH oxidase (RBOHA and RBOHB). However, these molecular events do not result in cell death all the time. *NtRBOHD* loss of function analysis exhibits the forfeiture of ROS production following elicitor treatment [5,84]. Notably, reduced ROS production is directly correlated with compromised plant resistance to pathogens such as those reported in *P. infestans*- potato interaction [82]. Research has provided evidence of second/late ROS burst involved in elicitin induced HR cell death [5,80]. This aspect of research can be more easily explained with the help of MAPKs phosphorylated TFs i.e. *WRKY7*, *WRKY8*, *WRKY9*, *WRKY11* that also causes elicitin-specific late ROS burst. After analysis, it appears that the promoter of RBOHB possess WRKY binding motif and the activation of the *WRKY8* and *WRKY11* TFs enhanced the RBOHB expression [61,85]. Upon elicitin perception, such activation leads to sustained ROS burst ending at cell death. Contrarily, it is not necessary that elicitin perception ends in HR cell death. It is conceivable that INF1 activated plant defense against *R. solanacerum* without triggering HR in tomato [20]. Despite activated JA- and ET-mediated defense responses, pathogen growth was not suppressed [20]. According to other studies, cell wall protein fraction (CWP) containing elicitin-like proteins of *Pythium oligandrum* could also activate plant defense without HR [86,87]. Even though elicitins belonging to diverse oomycete species exhibit different HR-inducing activities, elicitins in *Phytophthora* sp. typically elicit HR in the similar kind of plants e.g. members of family Solanaceae showing recognition of elicitins by responsive plants as a conserved characteristic of this genus. Consequently, elicitins appear as an intermediate among general and specific elicitors. Largely, we have come to a conclusion that some plants may respond to elicitins by activating defense but independent of HR. But interactive partners involved in this response are yet to be identified.

Plant response to elicitins enhance disease resistance

Avr gene expression activates the HR. The linked plants defense reactions mimic the avirulent pathogens induced effects [88]. Besides, *R* genes encode specific receptors for direct/indirect interaction with elicitors. This interaction initiates signal transduction pathways resulting in HR and immune responses [88,89]. We have already discussed that plant species differ in their responses to elicitins. In continuation of this, another distinction is the higher resistance displayed by elicitin-responsive plant species to elicitin-producing pathogens in comparison with non-elicitin producing pathogens [76]. In *P. parasitica*, the lack of elicitin production links to virulence on tobacco plants that displays a strong response to elicitins. In two pathosystems i.e. *P. parasitica* – *N. tabacum* and *P. infestans* – *N. benthamiana*, the production of elicitin in low quantity relates with augmented virulence [10]. HR is induced in limited plant species by INF1. Recognition of INF1 is a key element of the *N. benthamiana* defense response to *P. infestans*. In virulence studies involving various *P. infestans* isolates, five *Nicotiana* sp. exhibited resistance responses. [76,90]. These observations prove the character of specific molecules in the *Phytophthora* host range and propose elicitins as avirulence factors dealing with resistance at the species level. γ-megaspermin treated tobacco plants accumulate PR proteins and show SAR. But, SAR was compromised in cryptogein and γ-megaspermin-treated salicylate hydroxylase expressing tobacco plants [74]. Additionally, SA accumulation and PR genes up-regulation was prevented [74]. Therefore, we can infer that elicitins link with other signaling pathways and can control plant defense response. Du et al. [24] cloned and transferred ELR from wild potato to cultivated potato and confirmed the role of elicitins in restricting infection. Enhanced resistance to *P. infestans* strains recommends that elicitins

Table 1. The table presents different oomycete elicitors and their receptors.

Oomycete species	Elicitor	Type: Protein	Type: Carbohydrate	Type: Lipid	Type: Unsaturated Fatty acids	Type: Peptide	Plant	Plant receptor	References
Aphanomyces euteiches	Glucan-chitosaccharides		■				*M. truncatula*	UNKNOWN	[33]
Hyaloperonospora arabidopsidis	nlp11 and nlp24	■					*thaliana*	RLP	[34,35]
Phytophthora	Beta-glucans		■				*Solanaceous plants*	Glucan-dependent CEBiP CERK1	[33,36,37]
Phytophthora cactorum	PcF	■					*S. lycopersicum, Fragari avesca × ananassa*		[38]
	Glycoside hydrolase (XEG1)	■					*G. max, S. lycopersicum, C. annum, N. benthamiana*	SERK3/BAK1	[39]
Phytophthora cryptogea, Phytophthora capsici	Elicitin	■					*N. tabacum*	RLP	[24]
Phytophthora infestans	INF1	■					*S. tuberosum*	BAK1/SERK3-dependent ELR	[5,24,40] savchenko
	Arachidonic acid	■			■		*S. tuberosum, P. vulgaris, Avocado*	NOT KNOWN	[37,40]
	Eicosapentaenoic acid (EPA)	■		■			*S. tuberosum*		[41]
	Arachidonic acid (AA)	■		■			*A. thaliana, S. tuberosum*	UNKNOWN	[41,42]
Phytophthora megasperma	Transglutaminase GP42, Pep-13 and Pep-25	■					*P. crispum*	NOT KNOWN	[43]
	Heptaglucoside		■				*G. max*		[44]
Phytophthora parasitica	OPEL	■					*N. benthamiana, N. tabacum*	NOT KNOWN monomeric, 100-kDa integral plasma membrane protein	[40,45]
	nlp20	■					*A. thaliana, A. alpina, T. arvense, D. rigida, L. sativa*	RLP	[34,46]
Phytophthora parasitica var. nicotianae	CBD2synth	■					*thaliana, N. tabacum*		[47]
	CBEL (Cellulose-binding elicitor lectin)	■					*N. tabacum*	NOT KNOWN, but cellulose dependent	[47]
	(CBEL)/GP34	■					*N. tabacum*	NOT KNOWN	[48]
Phytophthora sojae	Pep-13					■	*S. tuberosum*	NOT KNOWN	[49]
Pythium aphanidermatum	NLP	■					*A. thaliana, N. tabacum*	RLP	[34,50]

Plants depend upon the innate immunity to perceive and tackle pathogens. Elicitors are conserved molecules which stimulate a defense response in a host plant as PAMP. Elicitors are detected by surface receptors (PRRs).

Table 2. Effectors suppress host immunity and are considered as a part of a pathogenic bacterial strategy for the nonspecific targeting of host kinases.

Oomycete species	Effector	Host	Known host target(s)	Virulence effect(s)	Reference
Phytophthora infestans	AVR3a	*Solanum tuberosum, Nicotiana benthamiana*	Stabilization of CMPG1 Interaction with *Nicotiana benthamiana* dynamin-related protein 2 (DRP2)	Overexpression suppresses INF1 perception, decreases flg22 & INF1 triggered accumulation of ROS	[51] [52]
	PexRD2	*Solanum tuberosum*	Interaction with the kinase domain of potato MAPKKKε	Suppress cell death due to MAPKKK signaling pathway. Its overexpression enhances susceptibility of plants to *P. infestans*	[53]
	AVRblb2	*Nicotiana benthamiana, Lycopersicon esculentum*	Interacts with papain-like cysteine protease C14 from *N. benthamiana* and tomato	Prevents secretion of the plant defense protease C14 in N. benthamiana and tomato; when overexpressed, enhances susceptibility of N. *benthamiana plants to P. infestans*	[54]
	Pi03192	*Solanum tuberosum*	Interaction with NAC targeted by *Phytophthora* 1 (NTP1) and NTP2	Prevent NTP1 and NTP2 relocalization from the ER to the nucleus, which appears to be key for immunity; silencing of NTP1 or NTP2 cause high *susceptibility to P. infestans*	[55]
Hyaloperonospora arabidopsidis	HaRxL44	*Arabidopsis*	Degradation of MED19a, a mediator in the interaction between transcriptional regulators and RNA polymerase II	Decrease salicylic acid-triggered defense reactions in *Arabidopsis*,	[56]
Phytophthora sojae	PsCRN63	*Nicotiana benthamiana, Glycine max*	Direct interaction with catalases	Overexpression causes cell death and H_2O_2 accumulation	[57]
	PsCRN115	*Nicotiana benthamiana, Glycine max*	Direct interaction with catalases	Co-expression with PsCRN63 suppress cell death and H_2O_2 accumulation	[57]
	PSR1	*Arabidopsis*	Interaction with PINP1 helicase domain	Overexpression increases susceptibility *to potato virus X and P. infestans* Overexpression increases susceptibility *of Arabidopsis to P. capsici*	[58] [59]
	PSR2	*Glycine max*	Unknown target; inhibition of the biogenesis of small RNAs	Silencing reduce virulence of *P. sojae*	[58]
	PsIsc1		Hydrolyzes isochorismate (salicylic acid precursor)	Salicylate metabolism pathway is disrupted leading to suppression of salicylate-mediated innate immunity	[60]

Effectors perform molecular or enzymatic activities that display their capability to alter host targets as well as their intracellular recognition by ETI receptors. Fungi and oomycetes effectors are secreted through the endomembrane system and are afterwards carried into host cells by unknown mechanisms. The comparative analyses of eukaryotic effectors relative to bacterial effectors underline the need for more varied effector roles of eukaryotic pathogens.

perception during infection improves resistance [24]. Moreover, some authors have driven the perception of elicitins for plant protection against succeeding pathogen attack with the help of SAR. For instance, elicit pretreated radish, tobacco, or tomato plants exhibited enhanced resistance against *X. campestris* pv. Armoraciae, *P. parasitica*, and *R. solanacearum*, respectively [10,20]. Overall, our discussion demonstrates the strong role of elicitins in triggering plant immune responses against oomycetes. Broadly translated, elicitins can function alone but their interaction with other signaling elements as well as biochemical triggers is an established fact. This casts light on using elicitins, its interactive proteins, isoforms and other compounds as a new baseline for molecular breeding of crop resistance against pathogens.

Effectors vs elicitin

Elicitins are totally different from effectors. Sometimes, confusions like the protein nature of both molecule types, production from pathogens (oomycetes) etc. raise questions but there exists significant differences that are adequate to distinguish elicitins from effectors. The main difference between effectors and elicitins lies in interaction of effector proteins as virulence factors

with plant R-proteins for activating plant innate immunity. Normally, these effectors suppress plant defense (Table 2) [91]. INF1 triggered HR cell death is suppressed by AVR3aKI, an effector from *P. infestans* [51]. Prior research has unraveled more than 30 effectors belonging to different oomycetes suppressing defense responses triggered by INF1. In spite of many identified effectors, earlier work focused on AVR3aKI. Mechanistic understandings of elicitin-triggered responses and their suppression is limited to AVR3aKI. This effector operates by modulating the host ubiquitin proteasome system *via* stabilization of the plant E3 ubiquitin ligase CMPG1 [51,91]. This suggests the evolution of an effector toolbox among the oomycete group of plant pathogens to modulate host responses triggered by their elicitins. Another point to be noted is the presence of elicitins in closely related groups/subgroups of oomycetes [92] that is entirely contrary to presence of effector molecules among pathogens. So far, no elicitin homologues have been observed in oomycetes groups with a distant lineage. This also proposes shooting off the oomycetes from their ancient progenitors as a legitimate and essential step in origin and expansion of elicitins among different oomycetes. These can be reckoned as the signature character of oomycetes.

Outstanding questions in crop protection perspectives

Elicitins production and their respective involvement in plant defense have attracted attention across the globe. In spite of multifaceted research efforts, some interesting questions in this context still need to be answered. The answers to these outstanding questions are expected to initiate new and direct existing scientific trends related to plant immunity against pathogens. Although elicitin application has been reported [18] but an interesting question is whether elicitins can be produced synthetically and applied exogenously for exact evaluation of their roles in plant defense. This is of central importance for studying pathogenesis and plant defense reactions. This would also yield information about the best mode of elicitin application. As far as roles of synthetic compounds are concerned in plant protection against pathogens, many compounds are being applied individually as well as in combination. Therefore, we can expect some novel results with the application of different elicitins in combination or with other compounds as broad range defense tactics. HR is usually considered a common component of plant defense [93] and elicitin actions. The growth arrest during plant response to pathogens is actually energy expenditure control. In parallel, different physiological and molecular processes are also modulated. A critical open question is the physiological and ecological cost of elicitins production/application. The base line for addressing such questions should be determined. Such experimentation will recognize and increment agricultural multifunctionality within ecological contexts. Besides, it will be helpful in assessing the potential environmental and ecological impact of pathogens as well as elicitins for apposite regulatory frameworks. Additionally, detection of elicitin by plant surface receptors along with the molecular basis of plant response has not been yet determined. This is arguably a significant question to be addressed. Eventually, the researchers should take interest here in improved elicitin perception as well as an understanding of plant defense responses for engineering crops with broad spectrum immunity to oomycetes.

Concluding remarks

We have shown the capability of elicitins to induce resistance in plants and highlighted the significance of processes involved in elicitin recognition as well as actions in cells. The biological activity of elicitins for inducing systemic resistance is the result of a combination of different factors. For example, elicitins interact with endogenous partners in plants i.e. nsLTP1, Lys13 and Lys39 residues etc. Besides, diffusion of the more acidic elicitins is restricted by the overall surface charge. Achieving developmental and sustainability targets along with new priorities under changing conditions need fundamental changes in agri-technology. Research has reached at exhilarating stage with the identification of the PRRs and NB-LRRs. But many issues have been glossed over in previous studies. Detailed studies involving elicitin application and actions can radically improve crop health and food security by enhancing the performance of agricultural systems. Knowledge regarding a revalorization and interdisciplinary approach can determine the greater extent of elicitin functions in plant defense. Another promising line of research is the assessment of multiple divergent 3-UTR sequences for a given elicitin gene by studying duplication of elicitin genes. The discussed information signals the necessity for further investigation to appraise more about elicitins.

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