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REVIEW ARTICLE

Endogenous Peptide Signals in *Arabidopsis thaliana*, their receptors and their role in innate immunity

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ABSTRACT: In plant innate immunity, the first line of defense against microbial pathogens is triggered by the perception of molecular signatures of the pathogens, by a highly sensitive membrane resident immune receptors. These pathogen-associated molecular patterns (PAMPs) are perceived by pattern-recognition receptors (PRRs) of the host to initiate pattern-triggered immunity (PTI). The endogenous plant signals, which are called damage-associated molecular patterns (DAMPs), are generated under different circumstances such as wounding, biotic and abiotic stresses. The DAMPs can activate the PTI and subsequently trigger the immune system in plants. These peptide signals called plant elicitor peptides (Peps) first discovered in *Arabidopsis thaliana* and later their orthologues were identified in different plant species. Peps are involved in immunity against diverse biotic and abiotic stresses and can fine-tune immune signaling pathways. So far, eight endogenous signals (AtPep1 to AtPep8) are discovered in the model plant *A. thaliana*. Recent studies revealed that the Pep members are not redundant and each of them has a specific function. AtPeps-triggered immunity is emerging as a highly complex, dynamic and a coordinated process involved in immune signaling cascades and consequently induces adequate defense responses. Therefore, it is possible to apply synthetic Peps to induce the immune system against microbial infections in plants. Here, the recent researches and progresses on Pep-triggered signaling are presented from their first discovery until now. Furthermore, the finding of their corresponding receptors AtPEPR1 and AtPEPR2 is explained in detail. Moreover, the subsequent events in the cells as the consequence of AtPeps perception are highlighted.

KEYWORDS: AtPeps, AtPEPR1, AtPEPR2, Endogenous signals, Plant immunology, PTI

INTRODUCTION

Innate immune system is triggered by the perception of pathogenic microbes by pattern recognition receptors of the host plant [1, 2, 3, 4]. Plants and other multicellular organisms such as mammals possess a sophisticated system to monitor cellular integrity and to detect the presence of damaged cells [2, 3, 4, 5]. In plants as well as in mammals, this trigger is based on the recognition of endogenous host-derived elicitors, the so-called "damage-associated molecular patterns" (DAMPs) [2, 4, 5, 6, 7]. The perception of DAMPs results in the induction of similar downstream defense cascades same as the perception of microbe-associated molecular patterns (MAMPs), such as the changes of ion fluxes in the plasma membrane, phosphorylation of mitogen-activated protein kinases via MAP kinase cascades, activation of 1-amin-cyclopropane-1- carboxylate (ACS) synthase, activation of defense-related hormones such as ethylene (ET), salicylic acid (SA) and jasmonic acid (JA) and transcriptional reprogramming of the cell [2, 4, 5, 7].

*At*Pep1 is a small immunomodulatory peptide which was isolated from an extract of wounded *A. thaliana* leaves, which could activate defense-related genes and also the

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synthesis of reactive oxygen species (ROS) [6, 7]. *At*Pep1 is a 23-amino acids (aa) peptide from *A. thaliana* which is derived from a 92-aa precursor protein encoded within a small gene called *AtPROPEP1* [2, 7]. This gene is induced by wounding and also methyl jasmonate (MeJA) treatment [8, 9, 10]. Further investigation showed that the *AtPROPEP1* gene has seven paralogues which are named *AtPROPEP1-AtPROPEP8* [2, 10, 11]. Except for *AtPROPEP6* which is located on chromosome 2, all the others are located on chromosome 5 [11]. The protein products of *AtPROPEPs* are conserved at the C-terminal region (Fig. 1) [9]. Importantly, genome analysis has presented evidence that *AtPROPEPs* have orthologs in other plant species including monocots and dicots [12-15]. The discovery of the endogenous immunomodulatory peptide signal *At*Pep1 in Arabidopsis has opened a new field of plant innate immunity research to identify their orthologues in other plant species.

AtPeps perception induce expression of its own precursor which can boost PTI-related defense responses [2, 4]. Therefore, it is possible to protect plants against bacterial infection by artificially treating the plants with synthetic elicitor immunomodulatory peptides. Considering the importance of this family, until now, AtPeps orthologues have been found in different plant species including seven Peps in *Zea mays* [12], 18 Peps in Rosaceae [13, 14, 15], seven Peps in Fabaceae [16] and six Peps in the Solanaceae[13, 14, 15]. Therefore, considering above-mentioned points, in depth investigation of AtPROPEPs and their corresponding receptors is needed to formulate adequate defense responses. In this paper, the recent finding of these endogenous immunomodulatory peptide signals in *A. thaliana*, their corresponding receptors and their role in innate immunity will be reviewed in detail.

AtPROPEPs are conserved across different plant species

Since endogenous immunomodulatory peptide elicitors similar to the *At*Pep family have been identified in different species across the plant kingdom, it seems that

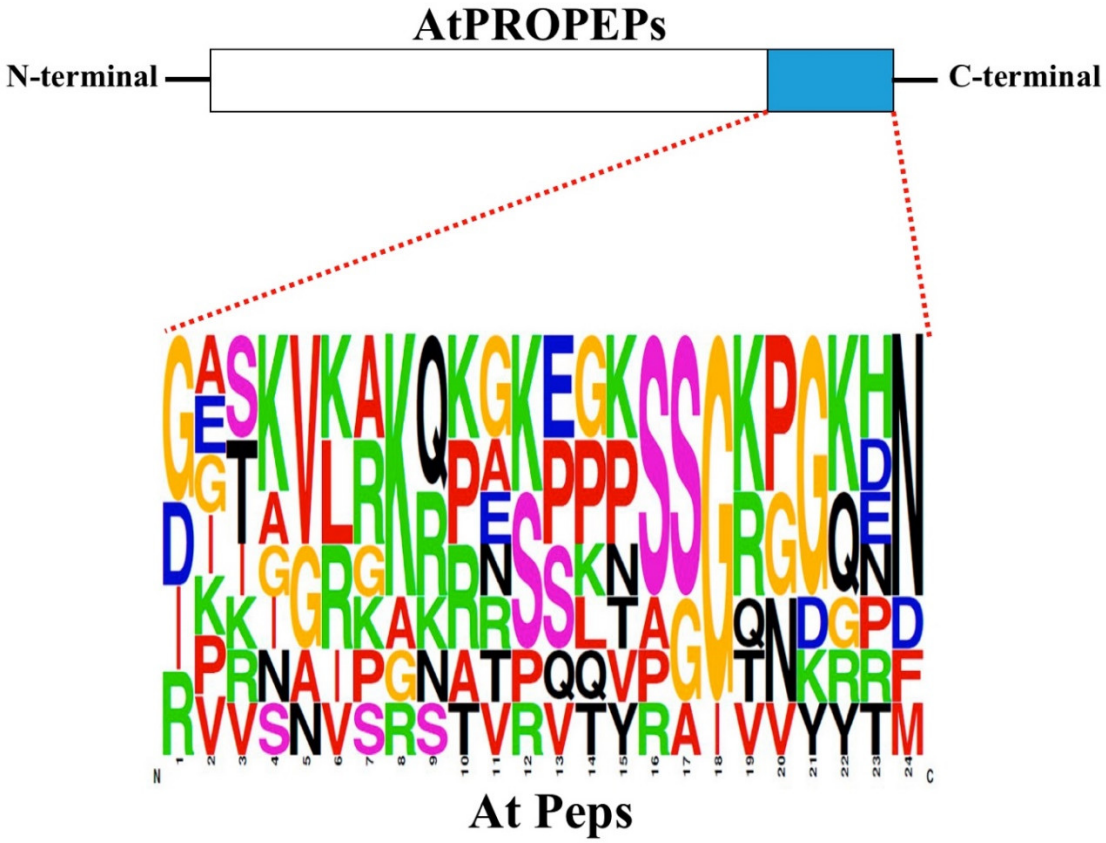

Figure 1. Schematic representation of the AtPROPEPs precursor protein, the position of the signal sequence peptides and comparison of the consensus of highly conserved Pep family. The 24 amino acid sequences from the C-terminal region of AtPROPEPs (AtPROPEP1-AtPROPEP8) were used for comparison. The amino acid sequences are represented by their alphabetical codes. The size of these alphabets indicates that there is higher sequence conservation at that position.

they have been maintained over evolution [2, 7, 8, 15, 16]. They play a role in regulating and balancing the immune system against any attack by pathogens, nematodes and also herbivores [16 - 19]. Interestingly, it has been proposed that DAMPs signaling (such as *At*Peps in *A. thaliana*) intensifies or prolongs the stereotypical defense response triggered by MAMPs [2, 20]. Thus, it seems that DAMPs are important for the fine-tuning of the defense response [2, 20, 21]. So far, several classes of plant-derived molecules, which elicit defense responses, have been identified [2, 5, 16]. Endogenous immuno-modulatory peptide elicitors in plants are classified into three major groups based on the structure of their precursor proteins, which include different processing mechanisms to release the active signal [5, 16].

The differences between the amino acid sequences of these endogenous immunomodulatory peptide signals in different plant families and species indicate the diversity of receptor partners that perceive these elicitors and also show that there is a diversity in processing and also different export mechanisms for activation of these immunomodulatory peptide signals in the cell [5, 16, 20]. Therefore, endogenous immunomodulatory peptide signals can be classified into three major groups as following:

(I) Peptides from Precursor Proteins Without an N-terminal Secretion Signal

The best example of this group is systemin which was identified in tomato. Systemin was the first endogenous peptide signal which was identified in plants [7]. Systemin, a peptide with 18 aa residues, induces various defense responses in tomato leaves and cell cultures [4]. It is formed from the C-terminal domain of a 200-aa precursor protein. Recently SYR1 was identified as the receptor that can perceive the systemin [21]. SYR1 receptor in tomato belongs to the class of leucine-rich repeat kinases (LRR-RLKs) and it is important for defense against herbivory attacks [21]. Recent studies showed that systemin is not the only peptide molecule that elicits defense responses [21]. As mentioned, apart from systemin, a well-studied family of endogenous peptide elicitors, there are the *At*Peps from *A. thaliana*. They are derived from the family of AtPROPEPs, which do not have an N-terminal secretion signal [2, 8]. Furthermore, recent investigations showed that in response to wounding, the cysteine protease METACASPASE4 (MC4) has role in Peps maturation in *A. thaliana* [22]. Moreover, recent studies showed that Peps perception not only enhance resistance against microbial pathogens but also induce resistance against nematodes [17]. Recent investigations showed that among the Pep family, AtPep3, compared to other members of this family is more active in response to different biotic and abiotic stimuli [23]. Furthermore, Nakaminani et al., (2017) showed that synthetic AtPep3 can enhance bleaching of chlorophyll [24]. Additionally, it was found that salt treatment can induce the transcription of AtPROPEP3 [24].

(II) Peptides from Precursor Proteins with an N-terminal Secretion Signal

In tobacco (*Nicotiana tabacum* L.) two 18-aa glycopeptides induce defense responses [8]. These peptides are named NtHypSysI and NtHypSysII [25]. They are hydroxyproline-rich systemins, and both are derived from the same precursor protein Ntprepro-HypSys [12], which carries an N-terminal secretion signal [26]. Also, orthologs of these peptides have been identified in other Solanaceae taxa [27].

(III) Cryptic Peptide Signals Derived from Proteins with Separate Primary Functions

The terms “cryptic peptides” is used to indicate the pool of peptides formed through the proteolytic action of peptidases on precursor proteins [28]. Cryptic peptides may have different biological activities that can be discriminated from the function of their precursor proteins [8, 28]. Formerly, Pearce *et al.*, (2010) [28] identified a 12-aa peptide from soybean which can activate the expression of defense genes upon herbivory attack. Since it is derived from a member of the subtilisin-like protease (subtilase) family, it was named Glycine max Subtilase Peptide (GmSubPep). Perception of the peptide by its corresponding receptor leads to the initiation of defense signaling cascades [28]. It has been also confirmed that the gene encoding GmSubPep was not induced by defense-related phytohormones or wounding and is constitutively expressed in all actively growing tissues [28]. Furthermore, recent investigations showed that GmPep1, GmPep2 and GmPep3 perception in *Glycine max* can enhance resistance against *Meloidogyne incognita* which is regarded as one of the most destructive nematodes [17].

*At*PEPR1 and *At*PEPR2 Receptors Are Responsible for Arabidopsis Endogenous Peptide Signal (Peps) Perception and Contribute to Innate Immunity

A few months after *At*Peps discovery in *Arabidopsis thaliana*, using a photo-affinity labeling technique with synthetic homologs of *At*Pep1, the corresponding receptor that exclusively perceives AtPeps is identified [29]. It was called the AtPEPR1 receptor which is specific to the AtPeps family and only perceives AtPep members [29]. AtPEPR1 is a leucine-rich repeat receptor a member of the plasma membrane-localized receptor-like kinase (LRR-RLKs) [29]. Later, in another investigation AtPEPR2 was identified and characterized as a second plasma membrane receptor for *At*Peps [30, 31]. Like AtPEPR1, AtPEPR2 is a plasma membrane LRR-RK; it has 76% amino acid similarity with AtPEPR1 [31]. This indicates close phylogenetic similarity of AtPEPRs with several receptors involved in endogenous peptide signaling (Fig. 2). Recent investigations showed that signaling mechanisms downstream of the Pep-PEPRs system are highly conserved and AtPEPRs are only highly sensitive to Peps members [16].

Both AtPEPR1 (At1G73080) and AtPEPR2 (At1G17750) belong to RLKs super-family [32]. The *At*PEPR1 has 1123 amino acids while *At*PEPR2 contains 1088 amino acids [33]. The AtPEPR1 contains 28 LRR, whereas AtPEPR2 has 26 LRR [33]. Both have three domains including: an extracellular domain (29-769 amino acids positions for *At*PEPR1 and amino acids 27-739 for *At*PEPR2); a helical transmembrane domain (amino acids 770-790 for *At*PEPR1 and amino acids 740-760 for *At*PEPR2); and a cytoplasmic protein kinase domain (amino acids 791-1123 for AtPEPR1 and amino acids 761-1088 for AtPEPR2). [33].

Peptide structure-function investigation and ligand-receptor interaction showed that structural features are required for the proper perception of AtPeps with AtPEPR1 and ATPEPR2 [32, 33]. In AtPEPR1, 833-841 amino acids are involved in nucleotide binding while in AtPEPR2 the nucleotide-binding site is within amino acids 800-808 [29, 30, 34]. In both receptors there are two modified residues (amino acids or nucleotides that are derivatives of the standard amino acids or nucleotides are called modified residues; PDB term definition) at the cytoplasmic domain [29, 30, 32, 34]. Remarkably, protein-protein interaction studies using the yeast two-hybrid assay, showed that AtPEPR1 and AtPEPR2, interact with BAK1 co-receptor [35].

Binding assays using *At*Pep peptides and AtPEPR1 and AtPEPR2 indicate that AtPEPR1 can perceive *At*Pep1 to *At*Pep8 while AtPEPR2 can only perceive *At*Pep1 and *At*Pep2 [31]. Transcription of both AtPEPR1 and AtPEPR2 are up-regulated upon treatment with *At*Peps, MAMP, wounding, and treatment with methyl jasmonate (MeJA) [2]. However, it was shown experimentally that AtPEPR1 is can sense all eight *At*Peps, whereas AtPEPR2 can only recognize *At*Pep1 and *At*Pep2 [2, 30]. These data provide evidence that *At*PEPR1 and *AtPEPR*2 have differential responses to the Pep members and therefore may have different roles in defense signaling [2, 30]. However, the exact mechanisms underlying Pep peptides perception by AtPEPR1 and AtPEPR2 receptors and how they influence defense responses are largely unknown [1, 2, 7].

Importance of co-receptor BAK1 with AtPEPRs receptor for proper perception and signal transduction

For the proper perception of *At*Peps by AtPEPR1/AtPEPR2, BAK1 is needed as a co-receptor (or, alternatively, other members of the SERKs protein family) which consequently activate the same downstream signaling cascade such as the MAPK cascade, oxidative burst, or induce the expression of defense-related marker genes [9, 36, 37, 38]. Based on *in vitro* and *in vivo* studies, it has also been recently reported that AtPEPR1 specifically interacts with the receptor-like cytoplasmic kinases BIK1 and PBS1-like 1 (PBL1) to trigger Pep1-induced signaling [1, 37].

Structural Basis of *At*Peps Perception by the AtPEPR1 Receptor

Recently, the crystal structure of the ectodomain of AtPEPR1 in complex with *At*Pep1 has been determined [33]. The crystallography results show that *At*Pep1 adopts a fully extended conformation, and it binds to the inner surface of the super-helical AtPEPR1 [33]. Furthermore, biochemical assays indicate that *At*Pep1 is capable of inducing AtPEPR1-BAK1 hetero-dimerization [1, 33]. Studies have shown that the deletion of the last residue of *At*Pep1 significantly affects *At*Pep1 interaction and plays a crucial role in hetero-dimerization [33].

In that research, FLS2 (protein data bank code: 4MN8) was used as the initial search model and the electron

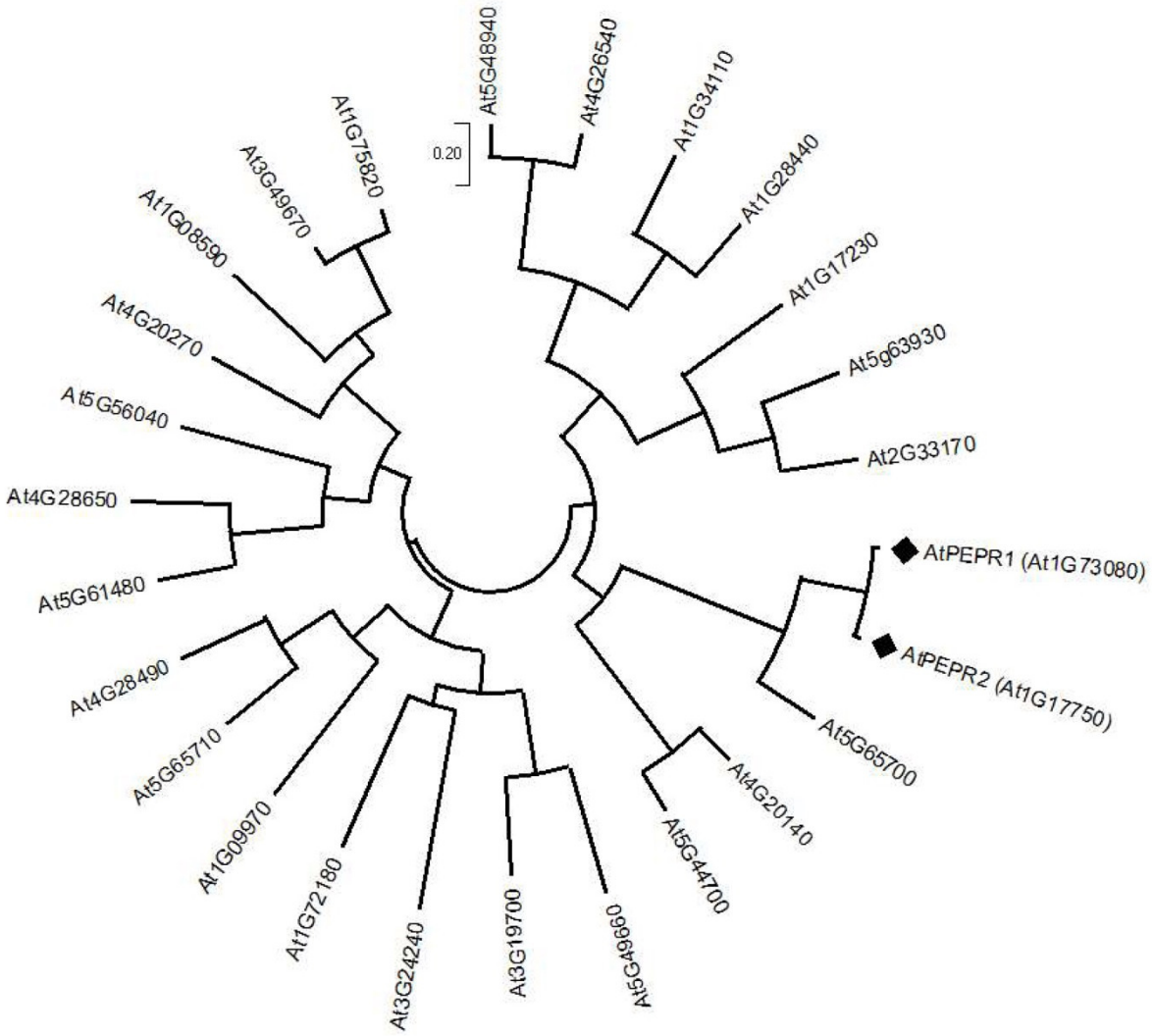

Figure 2. Phylogenetic analysis from amino acid sequences of the leucine-reach repeat receptors of the *Arabidopsis thaliana* which have the highest amino acid sequence similarity with AtPEPR1 and AtPEPR2 receptors. AtPEPR1 and AtPEPR2 receptors are labeled. Protein sequences from AtPEPRs were used as queries using BLASTP search to identify the most similar proteins in *A. thaliana*. Protein sequences with more than 70% sequence identity were downloaded from the NCBI database and multiple alignment analyses was performed by employing the ClustalW software. Phylogenetic analyses and graphical representation were carried out using MEGA software version X.

density was used to build the model of *At*Pep1 (amino acids 7-23). In that research, it was observed that in parallel with the central axis of the AtPEPR1 super-helix, *At*Pep1 had a fully extended conformation and interacted with the inner side of the helical structure running across 15 LRR of AtPEPR1 (from LRR4 to LRR18).

Among the LRRs of AtPEPR1, many amino acids are highly conserved, but interestingly *At*Pep1 selectively makes contact with the variable residues on the inner surface of AtPEPR1 [33]. This indicates that these variable residues are the structural determinants for ligand specificity [1, 33]. It is also noteworthy that at the primary sequence level, the *At*Pep1-interacting amino acids are from the third, fifth, seventh, and eighth positions of each LRR motif [33]. A similar observation was also made for the binding of flg22 to FLS2-LRR [39, 40].

Subsequent events as a consequence of Pep perception

As the consequence of Pep perception, several events may occur. The ligand binding with AtPEPRs leads to hetero-merization with their co-receptor BAK1 [39], and afterwards, downstream signaling cascades lead to the release of AtPEPR-bound BIK1 [41]. In the next step, Ca^{2+}-influx is changed, and as a result, the cytosolic Ca^{2+} levels increase [42]. This affects the activation of the

RbohD protein, which has a crucial role in the oxidative burst, i.e. the formation of ROS [20, 30, 43]. In addition, as AtPEPRs contain a cytosolic guanylyl cyclase (GC) domain, the ligand perception may lead to the production of cyclic GMP (cGMP) [42, 44]. In addition, the ROS that are generated may themselves have a role in different defense signaling pathways and also in membrane depolarization [30, 45, 46]. Concomitantly, phosphorylation of mitogen-activated protein kinases (MAPKs), especially MPK3 and MPK6 takes place [43, 47]. This may lead to the activation of defense-related transcription factors and ultimately to the induction of many defense-related genes and an increase in the levels of the defense hormones ET, jasmonic acid and also salicylic acid (SA) [19, 20]. After ligand perception and signal transduction, endocytosis and degradation of the receptor may occur, in part via PUB-mediated processes [48]. Ultimately the Pep perception also leads to callose deposition, seedling growth inhibition [47, 49] and production of secondary metabolites [50]. Fig. 3, provides an overview of downstream events as a consequence of Pep perception.

Pep peptides are secreted to amplify defense responses triggered by MAMPs

It has been hypothesized that Pep peptides are secreted to amplify defense responses triggered by MAMPs, based on the following observations: first, Peps and MAMPs reprogram the transcriptional level of almost the same genes [2, 5, 9, 50]; second, defense responses triggered by the perception of MAMPs and Peps are similar [4, 5, 9, 30, 31, 52]; third, AtPEPR receptors are cell surface receptor kinases able to detect extracellular Pep peptides [29, 30, 31] and finally, overexpression of *AtPROPEPs* genes leads to constitutive defense gene expression in the absence of infection or wounding and enhances disease resistance [9, 33, 53].

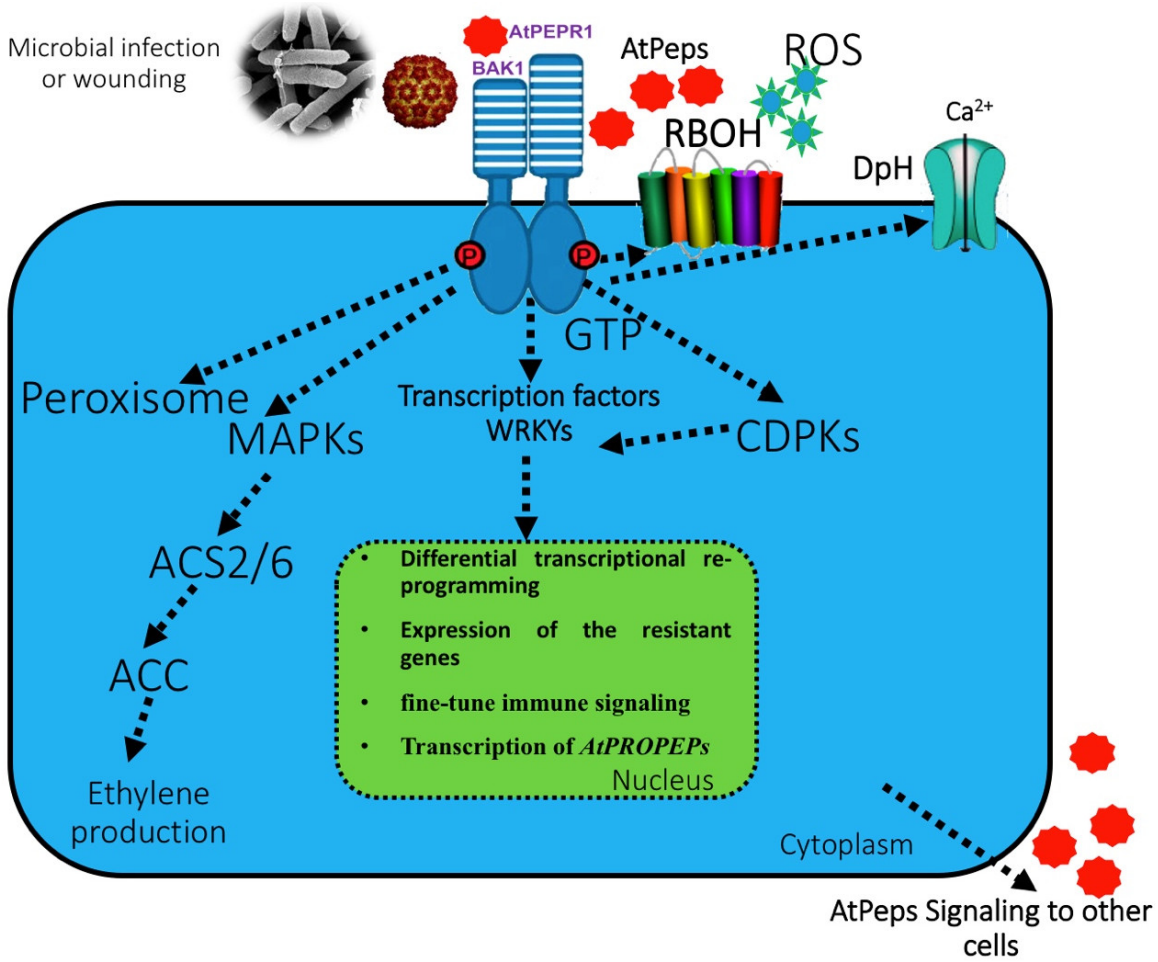

Figure 3. A brief overview of the downstream events as a result of AtPeps perception by their corresponding receptors AtPEPR1 and AtPEPR2. As a consequence of AtPeps perception, the AtPeps signals to the neighbor cells.

Classification of AtPep family into four major groups

Considering the importance of the AtPROPEPs, their classification was a major question for researchers for decades. There is a need for the classification of AtPPROPEPs and their corresponding receptors into several groups. Recent studies showed that *AtPROPEP3* expression is highly induced in response to NaCl treatments [54]. It seems that each member of the Pep family has a specific function in response to abiotic stresses. Furthermore, in response to bacterial infection, the expression of *AtPROPEP3* is highly induced [55]. Recently Safaeizadeh and Boller (2019) showed that it is possible to subdivide the Pep family into four groups, based on their observation made using promoter-GUS reporter lines in which the promoters of the various *AtPROPEP* genes were fused with the GUS gene.

Safaeizadeh and Boller (2019) could classify *AtPROPEP1* in one group; *AtPROPEP2* and *AtPROPEP3* in a second group; *AtPROPEP4*, *AtPROPEP7* and *AtPROPEP8* in a third group and *AtPROPEP5* in a fourth group. These findings, confirm non-redundant roles among the members of the AtPROPEP family and their corresponding receptors. Safaeizadeh and Boller (2019) could also show that among the AtPROPEP family, the AtPROPEP3 is the most active Pep which is highly expressed under different stimuli. Furthermore, yellow fluorescent protein (YFP) study which was fused to the AtPROPEP proteins, to determine protein localization showed that AtPROPEP3 was found to be present in the cytosol, while AtPROPEP1 and AtPROPEP6 were observed in tonoplast [47]. As the AtPROPEPs showed different expression patterns and seemed to be present and active in different regions of the cell, it can be speculated that they do have specific roles and functions.

CONCLUSION

The discovery of the AtPeps along with their corresponding receptors has opened a new field in innate immunity and a novel approach to understand the effect of plant endogenous peptides with regard to their induction upon biotic and abiotic stresses. Safaeizadeh and Boller (2019), clearly showed that the activation of the Pep-family is not redundant and each has a specific function under several forms of biotic and abiotic stresses including MAMP/DAMP treatments, hormone treatments, and salt treatments. In addition, the interplay between MAMP and DAMP signaling has been a question for researchers over several years, and many theories have been proposed so far, such as the amplifier theory, as proposed by Boller and Felix, (2009).

Although till date, there are some fragments of information about the activation of the Pep-PEPR system, there is still a lack of information about the regulation of *At*PROPEPs and their corresponding receptors (*At*PEPR1 and *At*PEPR2) in response to biotic and abiotic stresses. Recent studies showed that AtPROPEPs display different expression patterns and also exhibit different localizations, although all of them seem to function in a similar way by inducing defense responses [2]. Although *AtPROPEPs* were classified into different groups based on the promoter-reporter lines, more studies are needed to classify them in a better way and comprehensively understand the mechanisms underlying DAMP perception. Furthermore, it is recently reported that some of the genes encoding AtPROPEPs are not very active upon MAMP/DAMP elicitors [2]. Thus, each member of this family has specific functions which can be activated under different circumstances and different conditions. Therefore, each AtPROPEPs should be studied in more detail.

Furthermore, recent studies showed that external treatment at very low concentrations (with nanomolar dose) of chemically synthetic Peps can induce multiple defense responses against microbial infections [13, 57]. Moreover, investigations showed that Peps pre-treatment enhances resistance to herbivore and also nematode attacks [17, 21, 53, 58, 59]. Therefore, the application of Pep peptides opens a new way as the potential strategy to increase resistance against microbial, herbivore and nematode attacks.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

REFERENCES

[1] Couto, D., and Zipfel, C. 2016. Regulation of pattern recognition receptor signaling in plants. Nat. Rev. Immunol, 16: 537–552.

[2] Safaeizadeh, M., and Boller, T. 2019. Differential and tissue-specific activation pattern of the AtPROPEP and AtPEPR genes in response to biotic and abiotic stress in Arabidopsis thaliana. Plant Signal Behav, 14, e1590094.

[3] Li, Q., Wang, C., and Mou, Z. 2020. Perception of Damaged Self in Plants. Plant Physiol, 182(4): 1545-1565. .

[4] Safaeizadeh, M., Boller, T., and Becker, C. 2021.Transcriptomic profiling uncovers novel players in innate immunity in *Arabidopsis thaliana* Cold Spring Harbor. Biorxiv.. 01.02.425067.

[5] Dressano, K., Weckwerth, P.R., Poretsky, E., Takahashi, Y., Villarreal, C., Shen, Z., Schroeder, J.I., Briggs, S.P., and Huffaker, A. 2020. Dynamic regulation of Pep-induced immunity through post-translational control of defence transcript splicing. Nat Plants, 6(8): 1008-1019.

[6] Colaianni, N.R., Parys, K., Lee, H.S., Conway, J.M., Kim, N.H., Edelbacher, N., Mucyn, T.S., Madalinski, M., Law, T.F., Jones, C.D., Belkhadir, Y., and Dangl, J.L. 2021. A complex immune response to flagellin epitope variation in commensal communities. Cell Host Microbe, 29: 1-15.

[7] Boller, T., and Felix, G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol, 60: 379–406.

[8] Yamaguchi, Y., and Huffaker, A. 2011. Endogenous peptide elicitors in higher plants. Curr. Opin. Plant Biol, 14: 351–357.

[9] Huffaker, A., Pearce, G., and Ryan, C.A. 2006. An endogenous peptide signal in Arabidopsis activates components of the innate immune response. Proc. Natl Acad. Sci. USA, 103: 10098–10103.

[10] Safaeizadeh, M., Bartels, S., and Boller, T. 2016. Regulation of the *AtPROPEP* and *AtPEPR* Genes under Biotic and Abiotic Stress, Studied with Promoter-GUS-reporter Constructs in *Arabidopsis thaliana.* Epigenetic and Chromatin Regulation Congress P.51. Strasburgh, France.

[11] Bartels, S., and Boller, T. 2015. Quo vadis, Pep? Plant elicitor peptides at the crossroads of immunity, stress, and development. J Exp Bot, 66(17): 5183-93.

[12] Poretsky, E., Dressano, K., Weckwerth, P., Ruiz, M., Char, S.N., Shi, D., Abagyan, R., Yang, B., and Huffaker, A. 2020. Differential activities of maize plant elicitor peptides as mediators of immune signaling and herbivore resistance. Plant J, 104(6): 1582-1602.

[13] Ruiz, C., Nadal, A., Montesinos, E., and Pla, M. 2018. Novel Rosaceae plant elicitor peptides as sustainable tools to control Xanthomonas arboricola pv. pruni in Prunus spp. Mol Plant Pathol, 19(2): 418-431.

[14] Foix, L., Nadal, A., Zagorščak, M., Ramšak, Ž., Esteve-Codina, A., Gruden, K., and Pla, M. 2021. Prunus persica plant endogenous peptides PpPep1 and PpPep2 cause PTI-like transcriptome reprogramming in peach and enhance resistance to Xanthomonas arboricola pv. pruni. BMC Genomics, 18;22(1):360.

[15] Ruiz, C., Nadal, A., Foix, L., Montesinos, L., Montesi,nos, E., and Pla, M. 2018. Diversity of plant defense elicitor peptides within the Rosaceae. BMC Genet, 23;19(1):11.

[16] Lori, M., van Verk, M.C., Hander, T., Schatowitz, H., Klauser, D., Flury, P., Gehring, C.A., Boller, T., and Bartels, S. 2015. Evolutionary divergence of the plant elicitor peptides (Peps) and their receptors: interfamily incompatibility of perception but compatibility of downstream signalling. J Exp Bot, 66(17): 5315-25.

[17] Lee, M.W., Huffaker, A., Crippen, D., Robbins, R.T., and Goggin, F.L. 2017. Plant elicitor peptides promote plant defences against nematodes in soybean. Mol Plant Pathol. 19(4): 858-869.

[18] Furumizu, C., Krabberød, A.K., Hammerstad, M., Alling, R.M., Wildhagen, M., Sawa, S., and Aalen, R.B. 2021. The sequenced genomes of nonflowering land plants reveal the innovative evolutionary history of peptide signaling. Plant Cell, 24;33(9): 2915-2934.

[19] Ross, A., Yamada, K., Hiruma, K., Yamashita-Yamada, M., Lu, X., Takano, Y., Tsuda, K., and Saijo, Y. 2014. The Arabidopsis PEPR pathway couples local and systemic plant immunity. EMBO Journal, 33: 62–75.

[20] Flury, P., Klauser, D., Schulze, B., Boller, T., and Bartels, S. 2013. The anticipation of danger: microbe-associated molecular pattern perception enhances AtPep-triggered oxidative burst. Plant Physiol, 161: 2023–35.

[21] Wang, L., Einig, E., Almeida-Trapp, M., Albert, M., Fliegmann, J., Mithöfer, A., Kalbacher, H., and Felix, G. 2018. The systemin receptor SYR1 enhances resistance of tomato against herbivorous insects. Nat Plants, 4(3): 152-156.

[22] Hander, T., Fernández-Fernández, Á.D., Kumpf, R.P., Willems, P., Schatowitz, H., Rombaut, D., Staes, A., Nolf, J., Pottie, R,, Yao, P., Gonçalves, A., Pavie, B., Boller, T., Gevaert, K., Van Breusegem, and F., Bartels, S. 2019. Stael S. Damage on plants activates Ca^{2+}-

dependent metacaspases for release of immunomodulatory peptides. Science, 22;363(6433).

[23] Logemann, E., Birkenbihl, R.P., Rawat, V., Schneeberger, K., Schmelzer, E., and Somssich, I.E. 2013. Functional dissection of the PROPEP2 and PROPEP3 promoters reveals the importance of WRKY factors in mediating microbe-associated molecular pattern-induced expression. New Phytol, 198: 1165–1177.

[24] Nakaminami, K., Okamoto, M., Higuchi-Takeuchi, M., Yoshizumi, T., Yamaguchi, Y., Fukao, Y., Shimizu, M., Ohashi, C., Tanaka, M., Matsui, M., Shinozaki, K., Seki, M., and Hanada, K. 2018. AtPep3 is a hormone-like peptide that plays a role in the salinity stress tolerance of plants. Proc Natl Acad Sci U S A, 29;115(22): 5810-5815.

[25] Pearce, G., Moura, D.S., Stratmann, J., and Ryan, C.A. 2001. Production of multiple plant hormones from a single polyprotein precursor. Nature, 14;411(6839): 817-20.

[26] Pearce, G., Bhattacharya, R., Chen, Y.C., Barona, G., Yamaguchi, Y., and Ryan, C.A. 2009. Isolation and characterization of hydroxyproline-rich glycopeptides signals in black nightshade leaves. Plant Physiol, 150: 1422–1433.

[27] Nietzschmann, L., Gorzolka, K., Smolka, U., Matern, A., Eschen-Lippold, L., Scheel, D., and Rosahl, S. 2019. Early Pep-13-induced immune responses are SERK3A/B-dependent in potato. Sci Rep. 5;9(1):18380. doi: 10.1038/s41598-019-54944-y.

[28] Pearce, G., Yamaguchi, Y., Barona, G., and Ryan, C.A. 2010. A subtilisin-like protein from soybean contains an embedded, cryptic signal that activates defense-related genes. Proc Natl Acad Sci U S A. 107(33):14921-5.

[29] Yamaguchi, Y., Pearce, G., and Ryan, C.A. 2006. The cell surface leucine- rich repeat receptor for AtPep1, an endogenous peptide elicitor in *Arabidopsis*, is functional in transgenic tobacco cells. Proc. Natl Acad. Sci. USA, 103: 10104–10109.

[30] Krol, E., Mentzel, T., Chinchilla, D., Boller, T., Felix, G., Kemmerling, B., Postel, S., Arents, M., Jeworutzki, E., Al-Rasheid, K.A., Becker, D., and Hedrich, R. 2010. Perception of the *Arabidopsis* danger signal peptide 1 involves the pattern recognition receptor *At*PEPR1 and its close homologue *At*PEPR2. J. Biol. Chem, 285: 13471–13479.

[31] Yamaguchi, Y., Huffaker, A., Bryan, A.C., Tax, F.E., and Ryan, C.A. 2010. PEPR2 is a second receptor for the Pep1 and Pep2 peptides and contributes to defense responses in Arabidopsis. Plant Cell, 22: 508–522.

[32] Smakowska-Luzan, E., Mott, G.A., Parys, K., Stegmann, M., Howton, T.C., Layeghifard, M., Neuhold, J., Lehner, A., Kong, J., Grünwald, K., Weinberger, N., Satbhai, S.B., Mayer, D., Busch, W., Madalinski, M., Stolt-Bergner, P., Provart, N.J., Mukhtar, M.S., Zipfel, C., Desveaux, D., Guttman, D.S., and Belkhadir, Y. 2018. An extracellular network of Arabidopsis leucine-rich repeat receptor kinases. Nature, 18;553(7688): 342-346.

[33] Tang, J., Han, Z., Sun, Y., Zhang, H., Gong, X., and Chai, J. 2015. Structural basis for recognition of an endogenous peptide by the plant receptor kinase PEPR1. Cell Research, 25: 110–120.

[34] Pearce, G., Yamaguchi, Y., Munske, G., and Ryan, C.A. 2008. Structure-activity studies of AtPep1, a plant peptide signal involved in the innate immune response. Peptides, 29: 2083–2089.

[35] Postel, S., Küfner, I. Beuter, C., Mazzotta, S., Schwedt, A., Borlotti, A., Halter, T., Kemmerling, B., and Nurnberger, T. 2010. The multifunctional leucine-rich repeat receptor kinase BAK1 is implicated in Arabidopsis development and immunity. Eur. J. Cell Biol, 89: 169–174.

[36] Schulze, B., Mentzel, T., Jehle, A.K., Mueller, K., Beeler, S., Boller, T., Felix, G., and Chinchilla, D. 2010. Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. J. Biol. Chem, 285: 9444–9451.

[37] Tintor, N., Ross, A., Kanehara, K., Yamada, K., Fan, L., Kemmerling, B., Nürnberger, T., Tsuda, K., and Saijo, Y. 2013. Layered pattern receptor signaling via ethylene and endogenous elicitor peptides during Arabidopsis immunity to bacterial infection. Proc. Natl Acad. Sci. USA, 110: 6211–6216.

[38] Huffaker, A., and Ryan, C.A. 2007. Endogenous peptide defense signals in Arabidopsis differentially amplify signaling for the innate immune response. Proc. Natl Acad. Sci. USA, 104:10732–10736.

[39] Sun, Y., Li, L., Macho, A.P., Han, Z., Hu, Z., Zipfel, C., Zhou, J., and Chai, J. 2013. Structural Basis for flg22-Induced Activation of the Arabidopsis FLS2-BAK1 Immune Complex. Science, 6158: 624–628.

[40] Wang, Z., and Gou, X. 2021. The First Line of Defense: Receptor-like Protein Kinase-Mediated Stomatal Immunity. Int J Mol Sci. 29;23(1):343. doi: 10.3390/ijms23010343.

[41] Choi, J.H., Oh, E.S., and Oh, M.H. 2021. Phosphorylation of BIK1 is critical for interaction with downstream signaling components. Genes Genomics, 43(11): 1269-1276.

[42] Qi, Z., Verma, R., Gehring, C., Yamaguchi, Y., Zhao, Y., Ryan, C.A., and Berkowitz, G.A. 2010. Ca^{2+} signaling by plant Arabidopsis thaliana Pep peptides depends on

AtPepR1, a receptor with guanylyl cyclase activity, and cGMP-activated Ca^{2+} channels. Proc. Natl Acad. Sci. USA, 107: 21193–2198.

[43] Ranf, S., Eschen-Lippold, L., Pecher, P., Lee, J., and Scheel, D. 2011. Interplay between calcium signalling and early signalling elements during defence responses to microbe- or damage associated molecular patterns. Plant Journal, 68: 100–113.

[44] Ma, Y., Walker, R.K., Zhao, Y., and Berkowitz, G.A. 2012. Linking ligand perception by PEPR pattern recognition receptors to cytosolic Ca^{2+} elevation and downstream immune signaling in plants. Proc. Natl Acad. Sci. USA, 109: 19852–19857.

[45] Moreau, M., Lindermayr, C., Durner, J., and Klessig, D.F. 2010. NO synthesis and signaling in plants--where do we stand? Physiologia Plantarum, 138: 372–383.

[46] Baxter, A., Mittler, R., and Suzuki, N. 2014. ROS as key players in plant stress signalling. J. exp. Bot, 65: 1229–1240.

[47] Bartels, S., Lori, M., Mbengue, M., van Verk, M., Klauser, D., Hander, T., Rainer, B., Robatzek, S., and Boller, T. 2013. The family of AtPeps and their precursors in Arabidopsis: Differential expression and localization but similar induction of pattern-triggered immune responses. J. Exp. Bot, 64: 5309–5321.

[48] Stegmann, M., Anderson, R.G., Ichimura, K., Pecenkova, T., Reuter, P., Zarsky, V., McDowell, J.M., Shirasu, K., and Trujillo, M. 2012. The ubiquitin ligase PUB22 targets a subunit of the exocyst complex required for PAMP-triggered responses in *Arabidopsis*. Plant Cell, 24: 4703–4716.

[49] Beck, M., Wyrsch, I., Strutt, J., Wimalasekera, R., Webb, A., Boller, T., and Robatzek, S. 2014. Expression patterns of flagellin sensing 2 map to bacterial entry sites in plant shoots and roots. J. Exp. Bot, 65: 6487–6498.

[50] Huffaker, A., Pearce, G., Veyrat, N., Erb, M., Turlings, T.C., Sartor, R., Shen, Z., Briggs, S.P., Vaughan, M.M., Alborn, H.T., Teal, P.E., and Schmelz, E.A. 2013. Plant elicitor peptides are conserved signals regulating direct and indirect antiherbivore defense. Proc. Natl Acad. Sci. USA, 110: 5707–5712.

[51] Dressano, K., Weckwerth, P.R., Poretsky, E., Takahashi, Y., Villarreal, C., Shen, Z., Schroeder, J.I., Briggs, S.P., and Huffaker, A. 2020. Dynamic regulation of Pep-induced immunity through post-translational control of defence transcript splicing. Nat Plants. 6(8):1008-1019.

[52] Bartels, S., and Boller, T. 2015. Quo vadis, Pep? Plant elicitor peptides at the crossroads of immunity, stress and development. J. Exp. Bot, 66: 5183–5193.

[53] Huffaker, A., Dafoe, N.J., and Schmelz, E.A. 2011. ZmPep1, an ortholog of Arabidopsis elicitor peptide 1, regulates maize innate immunity and enhances disease resistance. Plant Physiol, 155(3): 1325-1338.

[54] Safaeizadeh, M. 2021. Study the effect of salt treatment on the expression of the AtPropep family in Arabidopsis thaliana. Ninth International Congress on Biology, Semnan, Iran. 21: 0797.

[55] Safaeizadeh, M., and Minae, D. 2021. Study the effect of oil extracted *Pseudomonas aeruginosa* to stimulate immune system in *Arabidopsis thaliana*. Ninth International Congress on Biology, Semnan, Iran. 21: 0796.

[56] Poncini, L., Wyrsch, I., Dénervaud, Tendon, V., Vorley, T., Boller, T., Geldner, N., Métraux, J.P., and Lehmann, S. 2017. In roots of Arabidopsis thaliana, the damage-associated molecular pattern AtPep1 is a stronger elicitor of immune signalling than flg22 or the chitin heptamer. PLoS One, 3;12(10): e0185808.

[57] Pastor-Fernández, J., Gamir, J., Pastor, V., Sanchez-Bel, P., Sanmartín, N., Cerezo, M., and Flors, V. 2020. Arabidopsis Plants Sense Non-self Peptides to Promote Resistance Against *Plectosphaerella cucumerina*. Front Plant Sci, 8;11:529.

[58] Huffaker, A. 2015. Plant elicitor peptides in induced defense against insects. Curr Opin Insect Sci, 9: 44-50.

[59] Shinya, T., Yasuda, S., Hyodo, K., Tani, R., Hojo, Y., Fujiwara, Y., Hiruma, K., Ishizaki, T., Fujita, Y., Saijo, Y., and Galis, I. 2018. Integration of danger peptide signals with herbivore-associated molecular pattern signaling amplifies anti-herbivore defense responses in rice. Plant J, 94(4): 626-637.

سیگنال‌های پپتیدی درون زا در *Arabidopsis thaliana*، گیرنده‌های آنها و نقش آنها در ایمنی خود القایی

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چکیده

در ایمنی خود القایی اولین لایه‌ی دفاعی در برابر پاتوژن‌های میکروبی با درک سیگنال‌های مولکولی پاتوژن‌ها توسط گیرنده‌های ایمنی بسیار حساس که در غشاء پلاسمایی سلول‌ها قرار دارند، ایجاد می‌شود. الگوهای مولکولی مرتبط با پاتوژن‌ها که اصطلاحاً PAMPs نامیده می‌شوند، توسط گیرنده‌های شناساگر الگوی (PRRs) میزبان درک می‌شوند تا ایمنی خود القایی (PTI) را آغاز کنند. سیگنال‌های درون‌زای گیاهی، که اصطلاحاً الگوهای مولکولی مرتبط با آسیب (DAMPs) نامیده می‌شوند، تحت شرایط مختلف مانند زخم، تنش‌های زنده و غیرزنده تولید می‌شوند. DAMP ها می‌توانند PTI را فعال کرده و در نتیجه باعث تحریک سیستم ایمنی در گیاهان شوند. این سیگنال‌های پپتیدی کوچک، الیسیتورهای پپتیدی گیاهی (Peps)، ابتدا در گیاه آرابیدوپسیس کشف شدند و بعداً ارتولوگ آنها در گونه‌های مختلف گیاهی شناسایی شد. Pep ها در سیستم ایمنی در برابر تنش‌های مختلف زنده و غیرزنده نقش دارند و می‌توانند مسیرهای سیگنال‌دهی سیستم ایمنی را به خوبی تنظیم کنند. تا کنون، هشت سیگنال درون‌زا (AtPep1 تا AtPep8) در گیاه مدل آرابیدوپسیس کشف شده است. مطالعات اخیر نشان داده است که اعضای Pep اضافی نیستند و هر یک از آنها عملکردی کاملاً اختصاصی در پاسخ به محرک‌های مختلف دارد. ایمنی ایجاد شده توسط AtPeps به عنوان یک فرآیند بسیار پیچیده و پویا، در آبشارهای سیگنالینگ سیستم ایمنی نقش هماهنگ کننده را دارد و در نتیجه باعث ایجاد پاسخ‌های دفاعی مناسب در آرابیدوپسیس می‌شود. بنابراین، نظر به قابلیت Peps در القاء سیستم ایمنی، می‌توان با سنتز مصنوعی Pep ها در القای سیستم ایمنی در برابر آلودگی‌های میکروبی در گیاهان استفاده نمود. در تحقیق حاضر، یافته‌ها و پیشرفت‌های اخیر در سیگنال‌دهی با تحریک Pep ها از زمان اولین کشف تا کنون ارائه شده است. علاوه بر این، گیرنده‌های سلولی مرتبط با آنها شامل گیرنده‌های AtPEPR1 و AtPEPR2 به تفصیل توضیح داده شده است. همچنین، رویدادهای بعدی در سلول‌ها که در نتیجه درک AtPeps ایجاد می‌شوند، برجسته شده است.

کلمات کلیدی: سیگنال‌های داخلی AtPeps، گیرنده‌های سلولی AtPEPR1 و AtPEPR2، سیگنال‌های درون زا، ایمونولوژی گیاهی، ایمنی خودالقایی