

Effects Of Antimicrobial Peptides With Emphasis On Plant Defenses

^{1*}Zarqa Hassan , ²Muhammad Hafeez Javed , ³Beenish Butt , ⁴Fareeha Akram , ⁵Rana Javaid Iqbal , ⁶Muhammad Tahir Akbar , ⁷Abdul Waheed , ⁸Saiqah Toor , ⁹Faraz Ahmed ,
 ¹⁰Muhammad Asif , ^{9*}Muhammad Ibrar Ahmed , ^{11*}Ansa Rebi

¹Department of Plant pathology, University of Agriculture Faislabad-38000, Punjab, Pakistan.

²Soil and Water Testing Laboratory Okara-56141, Punjab, Pakistan.

³Soil and water testing laboratory for research Multan-60000, Punjab, Pakistan

⁴Soil and water Testing Laboratory, Nankana Sahib-39100, Punjab, Pakistan.

⁵Soil Fertility Research Institute, Lahore-54000, Punjab, Pakistan.

⁶Office of the Senior Scientist Soil Fertility (Field), Multan-60000, Punjab

⁷Soil and Water Testing Laboratory, Rawalpindi-46000, Punjab, Pakistan.

⁸Soil and water testing laboratory, Gujrat-50700, Punjab, Pakistan.

⁹Soil and Water Testing Laboratory, Sargodha 40100, Punjab, Pakistan

¹⁰Soil and Water Testing Laboratory Bahawalnagar-62300, Punjab, Pakistan

¹¹Jianshui Research Station, School of Soil and water Conservation, Beijing Forestry University, Beijing 100083, China.

Abstract

Antimicrobial peptides of plants had been isolated from an extensive range of species. Under environmental stress, they are composed of different protein groups with different properties such as overall molecular charge, disulfide bond contents, and structural stability. Several plant peptides possessed three-dimensional structures but the mechanism of action of these peptides was unknown. Plants produced these peptides in a lot of different places, including roots, seeds, flowers, stems, and leaves, by enhancing their physiological importance. Here is an overview of what was currently known about plant-derived bioactive peptides, with such an emphasis on their antimicrobial activity and contribution in the plant signaling network, as well as potential uses.

1. Introduction

Pathogens like fungi, bacteria, viruses, even nematodes frequently damage plants. These pathogens induce severe infection and cause disease development due to the plant's constitutive and inducible defense mechanisms. When a pathogen is attacked, a cascade of a signal is stimulated in plants such as the strengthening of a cell wall, the secondary metabolites formation, and the synthesis of pathogenesis-related protein(PR) protein (Benko-Iseppon et al., 2010). These PR proteins were divided into several categories. Moreover, in all proteins certain features like net positive charge, physiological Ph, and an even number of cysteine residues (Cys)are present. PR proteins are very common because of their small size(less than 10kda) (Sels et al; 2008). Although these AMPs vary in size, amino acid composition, and structure.

As a result of these similarities, several different groups had been reported e.g. defensins, snakins, cyclotides, and hevein like protein. (Rhoads, 2016). AMPs are produced in ribosomes and present in all living organisms including bacteria plants and animals. AMPs do not contain enzymes that kill microorganisms during their hydrolytic activities but include all peptides that are capable of destroying microorganisms (Witkowska et al; 2008).

In innate immunity, AMPs represent the first defense system against invading pathogens. These AMPs were isolated first time in wheat flour (triticum aestivum) and prevent the growth of phytopathogens such as pseudomonas solanacearum, xanthomonas campestris, and corynebacterium michiganense. Other peptides secreted by plants including cyclotides, glycine-rich proteins, snakins, 2S albumins, and hevein-type proteins. They could act as immunomodulators, which improved the host's immunity (Rhoads, 2016). Other antimicrobial peptide families comprise cecropins8 and magainins9, which had antibacterial properties, and numerous cysteine-bridge-containing defensins, which had antibacterial and antifungal properties. Pro-rich AMPs had activity against bacteria and filamentous fungi while Gly-rich peptides have activity against gram-negative and gram-positive bacteria. These AMPs, which played important roles in plant defense, had a lot of potential as therapeutic agents. As a result, they could be utilized in agricultural production systems for resistance mechanisms, disease control, and management, decreasing pesticide use (Nagaraj, 2002).

These AMPs were seen to be effective against phytopathogens as well as human bacterial infections., For the further development of a wide range of key products such as medicines and plant protection, a diverse variety of AMPs was synthesized (Terras et al., 1992).

2. Important antimicrobial peptides (AMPs) in plants

2.1. Thionine

Thionine-containing (arginine, lysine, and cysteine) are low molecular weight Sulphur containing basic peptides. They produce a toxic effect on bacteria, fungi, yeast, animal, and plant cells due to their structure and sequence similarities. Purothionin-like proteins had been identified from different cereal left and endosperms, including barley, oat, and rye. In the endosperm of all Triticum and Aegilops species, this thionine is also found (Redman et al;1969).

2.2. Plant defenses

In wheat, barley, sorghum, radishes, and other Brassicaceae plants, plant defenses are present. These are tiny basic peptides. It was most likely g-thionine, a novel thionine family, based on its amino acid sequence analysis. G-thionine shows little genetic similarities with wheat and barley according to later studies. These groups differ from g-thionine in several ways, along with the location of the basic residue and the structure of disulfide bridges (Colella et al; 1990).

2.3. Lipid transfer protein

In the endoplasmic reticulum and other organelles, lipid synthesis takes place. In various plant species search discovery of lipid carrier protein required. With a relative molecular mass of 9kda and 7kda, plant LTPs were classified into two subfamilies. They could transfer a variety of polar lipids, such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, in addition to galactolipids. Because of their limited specificity, plant LTPs are also known as "nonspecific lipid transfer proteins. They also can link with fatty acids and acyl-COA (Arondel et al; 1990).

2.4. Puroindolines

Puroindolines are tiny basic proteins that which consist of a tryptophan-rich domain that distinguishes them from other protein. Wheat endosperm was used to isolate these proteins. They consist of five disulfide bridges and a molecular mass is about 13(kDa). They have two main isoforms were puroindoline (pin-a and pin-b) respectively. Puroindolines were antifungal and govern kernel texture. In wheat grain hardness locus. puroinodolines are a functional component (Bhave et al; 2008).

2.5. Snakins

Snakins were isolated from potato tubers. Plant cell walls consist of peptide snakin-1. Snakin-2 (StSN2) and snakin-3 are antimicrobial peptides with 63 amino acid residues (StSN3). These peptides demonstrate equivalent antibacterial action against bacterial and fungal diseases from different plant species despite having just 38% sequence similarity. In all snakins, 12 cysteine residues were conserved, as well as six disulfide connections. The mechanism of snakin action was unknown (Segura et al;1999).

2.6. Cyclotides

Cyclotides were circular proteins found in bacteria, plants, and animals that were spontaneously occurring. Cyclotides exhibit a lot of structural and sequence similarities. Plant cyclotides consist of 28–37 amino acids. Cyclotides consist of three intramolecular disulfide linkages with cysteine backbone knot shape. (Pelegrini et al; 2007).

2.7. Hevein-like proteins

In the lucid bodies of rubber trees (Hevea brasiliensis latex) hevein is present. It is a chitin-binding peptide. Their molecular mass is (47) kda with cysteine-rich protein. By binding to chitin in plant cell walls, this protein inhibits fungus hyphal development. The antimicrobial activity of other hevein-like proteins had been discovered in a variety of plants. Hevein-like peptides were chitin-binding peptides that were short (43 amino acid residues) (Koo et al., 1998).

AMPs	Organism	Target molecule	Function
Native	Bacteria	Membrane	Natural ATPase activity is
		Phospholipids,	inhibited.
		Ribosomes	
Native	Fungi	Chitin in cell wall	Fungal gene control is crucial for
			overcoming plant defenses.
Native	Plants	Diverse molecules,	Changes in the expression of host
		Nucleus	genes
Native	Mammals	Diverse molecules	Wounds may heal. Overwhelming
			plant defenses involve the control
			of fungal gene expression.
Native	Microbes	Diverse molecule	Interfering with microorganism
			ability to grow
Transgenic	Plants	Diverse molecule	Translational machinery is
AMPs			switched on and off.

Table 1.Effect of native and transgenic AMPs in different organisms

3. Mode of action of antimicrobial peptides

Plants have developed toxic substances called AMPs as part of their defense response, which interacts with phospholipids and membrane permeabilization to kill pathogens. CCPs (cell-penetrating peptides) were another type of AMP that could enter a cell without the requirement of receptor sites by interacting with phospholipids on the cell membrane (Barbosa et al; 2008). AMPs and CCPs were both parts of the nonspecific host defense system, which fought a wide range of microorganisms. AMPs interact with the microbial membrane in two ways, depending on the kind and microbial species (Marlow et al., 2009. These peptides are active in membrane rupture during cell lysis and membrane contact, which causes temporary holes to form and aids in the transport of peptides inside the cell to intracellular destinations (Brogden, 2005). Transporters also processed some peptides. DNA, RNA, and protein are intracellular targets for these AMPs. DNA, RNA, protein synthesis and cell wall production were all inhibited by them. Peptides' antibacterial activity was related to their amphipathic nature and the presence of motifs in their structure. Peptide adsorption and insertion into the bacterial membrane is enabled by this structure, results in the establishment of transmembrane holes and membrane permeabilization (Marlow et al., 2009).

Antimicrobial peptides have the amphipathic character required for hydrophobic motifs that directly involve membrane lipid components, whereas hydrophilic cationic groups engage with membrane phospholipid groups. AMPs exhibited an antifungal impact via two mechanisms: the destruction of fungal cells and interference with fungal cell wall construction. Antifungal peptides had a specific structural arrangement that includes polar and neutral amino acid sequences. As with antibacterial peptides, there were no clear conserved structural motifs directly associated with antifungal activity. Chitin, a component of the cell wall, had been proposed as a fungal target for bioactive peptides. Antiviral AMPs interact with

the membrane via electrostatic interactions with the negative charges of glycosaminoglycan, which aid in the binding of AMPs and virus competition (Stotz et al; 2009).

4. Antimicrobial peptides in plant defenses

During plant-pathogen interactions, pathogens utilized a variety of chemical substances, such as poisons and hydrolytic enzymes, whereas plants used a variety of defensive chemicals in addition to physical barriers. Pathogens utilized chemicals to facilitate their passage by establishing that they may obtain nutrients from the host and promoting the neutralizing of the host's defense responses using the physical barriers provided by plants.

Pathogens released chemicals in host tissues are as follows:

4.1. Hydrolytic enzymes

Hydrolytic enzymes degrade the host cell wall, the structural component of a plant (Benko-Iseppon et al., 2010). There are the following enzymes involved in this degradation. Cutinase are enzymes that degrade cutin, a key component of a plant cuticle. Fungi also synthesize cutinases which are involved in the degradation of host cells. Pectinases are enzymes that degrade pectin, a component of the plant cell wall. Poly-galacturanses, pectin lyases, and pectin methyl asterases are all pectinases. Other enzymes like cellulases, hemicellulases, and lignans are also involved in the breakdown of the plant cell wall (Sels et al., 2008).

4.2. Toxins

Toxins disturb the host's metabolic activities by modifying cell membrane permeability or inactivating enzymes, and other circumstances by disrupting plant metabolic pathways(Odintsova et al; 2012).

4.3. Growth regulators

In the host, hormonal system growth regulators like auxins, ethylene, and gibberellins caused an imbalance in plant development patterns (Nagaraj et al; 2008).

4.4. Polysaccharide

Polysaccharides were found to be the cause of water transfer blockage in plant vascular systems.

By generating membrane pores, these AMPs cause ion and metabolite leakage, depolarization, and cell death. They interfere with membrane lipids due to their amphipathic structure, as well as positive charge at physiological Ph. The negatively charged molecules such as (anionic phospholipids, lipopolysaccharides, and teichoic acid) interfere with cationic residues. This phenomenon was explained by three models the barrel-stave model, the wormhole (or toroid pore) model, and the carpet model. in cell membrane penetration, Peptides with hydrophilic positively charged domains interact with negatively charged microbial surfaces and bilayer phospholipid head groups. (Pelegrini et al; 2005). Once their concentrations reach a certain threshold, peptides self-associate onto the acidic phospholipid-rich

portions of lipid bilayers and are transferred into the membrane by raising the bilayer positive potential. The "barrel-stave" mechanism is also known as the "pore-formation "mechanism. In the third process, known as the "toroidal" transmembrane helical bundles generate ore in a membrane. Pore formation was controlled by lipid polar head groups and helix bundles oriented vertically to the membrane outside, which was remarkable.

Conclusion

Plant AMPs are peptides that consist of diverse amino acid compositions and structures. These AMPs were known for their fast killing and broad-spectrum antimicrobial properties. In healthcare and agriculture, plant AMPs had a variety of applications. However, despite their many positive characteristics, no single AMP has been recognized for therapeutic use. As a result, future areas of commercial plant AMP application include developing less toxic and more stable compounds, as well as lowering production costs, mostly through enhanced biotechnological approaches or preparative peptide synthesis. Numerous research had been carried out by analyzing the genuine participation of these peptides' classes especially in a restriction to pests and pathogens. Biotechnology was expected to have a key role in increasing agricultural productivity.

References

- Arondel, V., Vergnolle, C., Tchang, F., & Kader, J. C. (1990). Bifunctional lipid-transfer: fatty acid-binding proteins in plants. Molecular and Cellular Biochemistry, 98(1–2), 49–56. https://doi.org/10.1007/BF00231367
- Barbosa Pelegrini, P., Del Sarto, R. P., Silva, O. N., Franco, O. L., & Grossi-De-Sa, M. F. (2011). Antibacterial peptides from plants: What they are and how they probably work. Biochemistry Research International, 2011. https://doi.org/10.1155/2011/250349
- Benko-Iseppon, A. M., Lins Galdino, S., Calsa Jr., T., Akio Kido, E., Tossi, A., Carlos Belarmino, L., & Crovella,
 S. (2010). Overview on Plant Antimicrobial Peptides. Current Protein & Peptide Science, 11(3), 181–
 188. https://doi.org/10.2174/138920310791112075
- Bhave, M., & Morris, C. F. (2008). Molecular genetics of puroindolines and related genes: Regulation of expression, membrane binding properties and applications. Plant Molecular Biology, 66(3), 221–231. https://doi.org/10.1007/s11103-007-9264-6
- Brogden, K. A. (2005). Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? Nature Reviews Microbiology, 3(3), 238–250. https://doi.org/10.1038/nrmicro1098
- Colilla, F. J., Rocher, A., & Mendez, E. (1990). γ-Purothionins: amino acid sequence of two polypeptides of a new family of thionins from wheat endosperm. FEBS Letters, 270(1–2), 191–194. https://doi.org/10.1016/0014-5793(90)81265-P
- Eudes, F., & Chugh, A. (2008). Cell-penetrating peptides: From mammalian to plant cells. Plant Signaling and Behavior, 3(8), 549–550. https://doi.org/10.4161/psb.3.8.5696

- Koo, J. C., Lee, S. Y., Chun, H. J., Cheong, Y. H., Choi, J. S., Kawabata, S. I., ... Cho, M. J. (1998). Two hevein homologs isolated from the seed of Pharbitis nil L. exhibit potent antifungal activity. Biochimica et Biophysica Acta Protein Structure and Molecular Enzymology, 1382(1), 80–90. https://doi.org/10.1016/S0167-4838(97)00148-9
- Marlow, V. L., Haag, A. F., Kobayashi, H., Fletcher, V., Scocchi, M., Walker, G. C., & Ferguson, G. P. (2009). Essential role for the BacA protein in the uptake of a truncated eukaryotic peptide in Sinorhizobium meliloti. Journal of Bacteriology, 191(5), 1519–1527. https://doi.org/10.1128/JB.01661-08
- Nagaraj, N. S. and R. (2002). Host-defense Antimicrobial Peptides: Importance of Structure for Activity.CurrentPharmaceuticalDesign,Vol.8,pp.727–742.https://doi.org/http://dx.doi.org/10.2174/1381612023395358
- Odintsova, T., & Egorov, T. (2012). Plant Antimicrobial Peptides BT Plant Signaling Peptides (H. R. Irving & C. Gehring, Eds.). https://doi.org/10.1007/978-3-642-27603-3_7
- Pelegrini, P. B., & Franco, O. L. (2005). Plant γ-thionins: Novel insights on the mechanism of action of a multi-functional class of defense proteins. International Journal of Biochemistry and Cell Biology, 37(11), 2239–2253. https://doi.org/10.1016/j.biocel.2005.06.011
- Pelegrini, P. B., Quirino, B. F., & Franco, O. L. (2007). Plant cyclotides: An unusual class of defense compounds. Peptides, 28(7), 1475–1481. https://doi.org/10.1016/j.peptides.2007.04.025
- Redman, D.G. and Fisher, N. (1969). Purothionin analogues from barley flour. Journal of the Science of Food and Agriculture, 20, 427–432.
- Rhoads, R. E. (2016). Synthetic mRNA: Production, introduction into cells, and physiological consequences. Methods in Molecular Biology, 1428, 3–27. https://doi.org/10.1007/978-1-4939-3625-0_1
- Segura, A., Moreno, M., Madueño, F., Molina, A., & García-Olmedo, F. (1999). Snakin-1, a peptide from potato that is active against plant pathogens. Molecular Plant-Microbe Interactions, 12(1), 16–23. https://doi.org/10.1094/MPMI.1999.12.1.16
- Sels, J., Mathys, J., De Coninck, B. M. A., Cammue, B. P. A., & De Bolle, M. F. C. (2008). Plant pathogenesisrelated (PR) proteins: A focus on PR peptides. Plant Physiology and Biochemistry, 46(11), 941–950. https://doi.org/10.1016/j.plaphy.2008.06.011
- Stotz, H. U., Thomson, J. G., & Wang, Y. (2009). Plant defensins: defense, development and application. Plant Signaling & Behavior, 4(11), 1010–1012. https://doi.org/10.4161/psb.4.11.9755
- Terras, F. R. G., Schoofs, H. M. E., De Bolle, M. F. C., Van Leuven, F., Rees, S. B., Vanderleyden, J., ... Broekaert, W. F. (1992). Analysis of two novel classes of plant antifungal proteins from radish (Raphanus sativus L.) seeds. Journal of Biological Chemistry, 267(22), 15301–15309. https://doi.org/10.1016/s0021-9258(19)49534-3
- Witkowska, D., Bartyś, A., & Gamian, A. (2008). [Defensins and cathelicidins as natural peptide antibiotics].
 Postepy Higieny i Medycyny Doswiadczalnej (Online), 62, 694–707. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19188885