Role of Antimicrobial Peptides in Plant Defense Mechanisms

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ABSTRACT

Antimicrobial peptides (AMPs) are short peptide sequences of about 50 aminoacid residue which are considered as first line of defense in plants. These peptides contain 4-12 cysteine residues forming disulphide bonds which can make them exceptionally stable to chemical, thermal and enzymatic degradation. Antimicrobial peptides exhibit a broad-spectrum activity against pathogenic bacteria, fungi and also viruses. AMPs have a wide range of inhibitory affects against fungi, bacteria, viruses and nematodes. Despite their vast diversity, most AMPs work directly against microbes through a mechanism involving membrane disruption and pore formation, allowing efflux of essential ions and nutrients. Various antimicrobial peptides which contribute major role in plant defense mechanisms are Thionin, Defensin, Lipid Transfer Protein, Hevein, Knotting type peptides, Cyclopeptides, Snakins, Puroindolins etc. Antimicrobial peptides are highly efficient and safe hence beneficial for plant protection in agriculture.

INTRODUCTION

Plant diseases are mainly caused by various pathogenic microorganisms and their management mainly relies on chemical approaches which leads to emergence of resistant isolates of pathogens and also the tremendous use of chemicals create negative impacts on environment. Hence, new approaches are being adapted and practiced which can substitute the use of chemicals in plant disease management. Antimicrobial peptides have been the object of attention in past few years for plant protection. Antimicrobial peptides are short peptide sequences of about 50 amino acid residue which are considered as first line of defense in plants. Plant AMPs are considered not only to play a key function in plant defence against pathogens but also significantly improve plant growth and development.

What are Anti-Microbial Peptides (AMPs)

During the evaluation of plants, to protect themselves from the biotic stresses, plants have developed certain defense mechanisms and among the plant defense molecules AMPs are of the most common and effective chemical barriers. AMPs are part of innate immune system, which contributes greatly to the host defense against pathogens. These are small polypeptides, synthesized by ribosomes where as some AMPs can be synthesized through non ribosomal peptide synthetases. Antimicrobial peptides exhibit a broad-spectrum activity against pathogenic bacteria, fungi and also viruses. Near about 96 % of AMPs are cationic whereas few anionic peptides are also existed which rich in glutamic acid and aspartic acid. Most of the cationic antimicrobial peptides have a net charge of + 2 at neutral pH because of the presence of arginine or lysine residues in aminoacid sequences.AMPs have been isolated from microorganisms, insects, amphibians, plants as well as from mammals. First report of the plant AMPs is Thionin which was found in Triticum aestivum.

Structures of Antimicrobial peptides

Antimicrobial peptides contain 4-12 cysteine residues forming disulphide bonds which can make them exceptionally stable to chemical, thermal and enzymatic degradation. The smallest known AMP comprising 7 amino acids was isolated from Jatropha curcas. Key features of AMPs are high constituents of cysteine and or glycine and the presence of disulphide bridges and on this basis, the AMP structure is classified as helical(where α helix are arranged in a right-handed helical structure), β sheet(2 or more polypeptide chains run alongside each other and linked in a regular manner), Hairpin or loop(presence of a single disulphide bonds and cyclization of peptide chains) and Extended (Glycine, arginine or histidine rich peptides. It's a linear antimicrobial peptide but gives extended boat shaped conformation when the AMPs bind with Sodium dodecyl sulphate).

Mechanisms of Anti-microbial peptides (AMPs)

AMPs have a wide range of inhibitory affects mostly against fungi, bacteria and viruses. Target site of mode of action of AMPs such as inhibition of cell wall synthesis, inhibition of DNA, RNA, protein synthesis, bursting of hyphal tip, cell lysis etc vary on the basis of their target microorganism.

I) Antibacterial: When the AMPs interact with the bacterial cell, it binds to the cell surface which leads to permeabilization of the outer membrane (gram negative bacteria) and cytoplasmic membrane and due to this cell lysis and damage of DNA occurs. In gram negative bacteria, positively charged domain of the cationic peptides bind to the divalent cation binding sites of Lipopolysaccharides followed by displacement of the native Ca+2 and Mg+2 ions that disrupts the structure of bacterial outer membrane. In gram positive bacteria, cell wall contains negatively charged teichoic acid which are probably initial binding site of AMPs.The AMPs then interact with cytoplasmic membrane in the presence of lipid composition. After binding of positively charged cationic peptides with negatively charged lipid head groups, the peptides insert into the membrane and undergoes conformational changes which forms channels or pores in the cell leads to leakage and cell death.

II) II) Antifungal: In fungal cell, the AMPs predominantly may target both cell wall as well as nucleic acid. These also causes mitochondrial disruption and induce osmotic stress by formation of reactive oxygen species. AMPs also inhibit spore germination and germ tube formation in certain fungal genera such as Rhizopus, Fusarium.

AMPs targeting cell wall

B glucan is major polysaccharide of fungal cell wall due to presence of β 1,3 glycosidic bonds which confers strengthen to cell wall. Few AMPs recognize the fungal pathogens by specific receptor for β 1,3 glucan and induce immune response. AMPs also act as chitin inhibitors. Few AMPs are mannan binding peptides which recognize D- Mannose and leads to cell death.

Nucleic acid inhibitors

AMPs also target nucleic acid biosynthesis and metabolism. They bind with DNA and inhibit functions of DNA processing enzymes hence affects repair mechanisms.

III) Antiviral: The AMPs interact with the membranes of the enveloped viruses which are composed of anionic phospholipids and disrupt membrane structure. These not only lyse the viral envelop but also affect the stability of the nucleocapsid. In case of TMV, the viral gene expression is inhibited by AMP Melittin. Binding of AMP with TMV causes conformational changes in the structure of RNA thus affects the level of gene expression. Antiviral peptides also interact with aminoglycan present on the cell surface hence competes with virus for cellular binding sites. These AMPs bind with the viral receptor required for entry.

Types of Antimicrobial peptides (AMPs)

A total of 3425 AMPs has been reported so far. Natural AMPs are generated from animals, plants and microorganisms. Plant antimicrobial peptides are classified generally on the basis of sequences and structures as thionins, defensins, hevein like peptides, knottins, lipid transfer proteins, snakins and cyclotides.

Defensins

In 1990, Mendez et al. isolated from barley and wheat and named as Defensin which are Small, cystine rich antimicrobial peptides ranging from 45-54 amino acids and consist of 3-5 disulfide bonds. In growing seeds, defensins are expressed to prevent the newly formed radicle tissues from fungal invasion.Defensin interacts with the cell membrane and increases ion permeability. Membrane permeability and ion leakage from the membrane occurs due to the interaction of serine residue with glycosyl part of fungal cell membrane. Interaction of defensin also accelerates production of ROS hence activates programmed cell death.

Thionin

Thionins consist of 45-48 amino acids, 6 or 8 cysteine and 3 or 4 disulfide bonds. Cambrin, viscotoxin are thionin peptides with six cysteine residue and three disulphide bridgeswhereasα/β-purothionins, α/β-hordothionin are peptides with eight cysteine residue and four disulphide bridges.Glucosylceramide molecules present in the cell membrane interacts with γ thionin peptide which causes repulsion of γ thionin into cell membrane leads to membrane disruption and ion efflux.

Lipid Transfer Protein (LTP)

Small cysteine rich peptides having molecular masses of lower than 10KDa. It consists of 70-

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100 amino acid. Cationic peptides with a conserved pattern of four to five disulfidebridges having eight to ten cys-cysbonds.LTPs having synergistic activity with thionins against Clavibacter spp. When plant cell wall is degraded by cutinase activity of pathogens, LTP interacts with cutin monomer and form complex which initiates cutin synthesis and thus repairs the cell wall of plants.

Hevein like peptides

Heveins are small antimicrobial proteins of 42-45 amino acids and of 4.7 Kda with conserved residues of glycine and aromatic acids.They are cationic proteins having 3-5 disulfide bonds. To overcome fungal infection, plants produce chitinases. Fungi in turn secrete fungalysin which cleaves fungal chitinases. However, Hevein like WAMPs inhibit the activity of fungalysin and protect chitinase cleavage.

Table 1: Classification ofmajor antimicrobial peptides (AMPs)

Peptides	Classification	Target organism/ Function	References
Cp-thionin ii	**Thionin**	**Antifungal**	**Schmidt *et al.*, 2019**
WRKY	**Thionin**	**Capsicum chlorosis virus**	**Naito *et al.*,(2022)**
NaD1	**Plant defensin**	***Candida albicans***	**Hayes *et al.*,(2018)**
ZmD32	**Plant defensin**	***Helminthosporium, Fusariumspp***	**Kerenga *et al.*,(2019)**
LCTP1	**Lipid transfer protein**	**Antifungal, antibacterial**	**Bogdanov *et al.*,(2015)**
Gt-LTP2	**Lipid transfer protein**	***Botrytis cinerea***	**kiba *et al.*, (2012)**
M-hevein	**Hevein like peptides**	***Trichoderma viride***	**Zhao *et al.*, (2011)**
PINA and PINB	**Puroindoline**	***Botrytis cinerea, Verticillium dahliae, Cochliobolushetero strophus***	**Zhang *et al.*, (2011)**
bevuTI	**Knottin type peptides**	**Trypsin inhibitory prolyl oligopeptidase**	**Retz *et al.*,(2020)**

CONCLUSION

Most of the antimicrobial peptides are highly efficient and safe hence beneficial for plant protection in agriculture. The AMPs have high target affinity which means a small amount is efficient to control pathogens, insects, weeds. Further to enhance broad spectrum resistance in host, a combination of different AMP molecules with complementary mode of action should be applied which can act at different stages of disease development. The successful application of AMP in plant protection will likely help eradicate certain plant diseases, reduce the environmental degradation of intensive agriculture, and improve the quality and safety of our food.

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