Plant antimicrobial peptides

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Pathogens, like fungi, nematodes, virus and bacterial are responsible for several human and plant diseases. According FAO, around 20 to 40 percent of crops yields are lost, per year, due plant diseases and pest. Besides this, only USA spend 30 billion dollars a year with hospital infections, and the numbers are increasing as the infection are becoming more and more resistant to conventional antibiotics. Antimicrobial peptides (AMPs) are found in different species: insects, mammals, amphibians, fish, birds and plants. Plant antimicrobial peptides have an important action in plants metabolism: defense against pathogens. Some examples of this class of peptides are: defensins, cyclotides, glycine-rich proteins, thionins and lipid transfer proteins. Usually, the target for AMPs are the DNA, RNA and proteins and they should have selectivity against bacterial cells and not been effective against mammal or plant cells. The interest on AMPs is growing, especially on their structure and mechanism of action. The use as medicine of AMPs is limited and still remaining some open questions that need to be answered: the oral bioavailability improvement, peptidase degradation, before they became an antimicrobial drug.

Keywords: peptide; antimicrobial; plant; defense

1. Introduction

Phytopathogens such as bacteria, fungi, viruses, and nematodes are responsible for major agricultural losses, destroying crops and thus causing severe damage to the world economy. In addition to acute food shortages, the damage to production caused by these pathogens seriously undermines food security, which can result in malnutrition, migration, and the death of humans and livestock [1-4].

The United Nations' Food and Agriculture Organisation (FAO) estimates that 20-40% of global crop yields are lost each year due to damages caused by pests and plant diseases [5].

Representative examples of such pathogens are *Xanthomona soryzae* pv. *Oryzae* (Xoo) and *Xanthomonas oryzae* pv, *oryzicola* (Xoc), which causes diseases like bacterial blight and striated leaf spots, respectively, thus seriously affecting rice crops (*Oryza sativa*) worldwide and causing significant damage and losses of up to 75% of the grain production. In 1954, for example, Japan suffered an annual loss of 90-150 hectares of rice due to *Xanthomonas oryzae* pv. *Oryzae* [6,7].

Another pest that causes severe damage to agricultural crops is the bacterium *Ralstonia solanacearum*, which is the causative agent of bacterial wilt in more than 200 plant species including important crops such as potatos (*Solanum tuberosum*), tomatos (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), rubber (*Hevea brasiliensis*), cassava (*Manihot esculenta*), castor beans (*Ricinus communis*), eggplants (*Solanum melongena*), ginger (*Zingiber officinalis*), and bananas (*Musa* spp.). The disease exhibits a wide geographical distribution and has a major economic impact in many parts of the world. The highest economic damage has been reported for potato, tobacco, and tomato crops in the Southeast of the USA, Indonesia, Brazil, Colombia, and South Africa. In general, losses depend on the local climate, soil type, farming practices, and the virulence of the bacterial strain [7-9]

In Brazil, especially citrus (*Citrus sinensis*) crops are often endangered by pests and diseases. The diseases most feared by the industry are citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *Citri*, citrus variegated chlorosis (CVC), also known as yellowing, caused by the bacterium *Xylella fastidiosa*, and greening, a disease caused by the bacterium *Candidatus liberibacter* spp. The estimates regarding expenses and losses caused by these diseases are alarming: in 2009 and 2010, these three diseases alone were responsible for a decrease of 20% of the citrus harvest in Brazil [10].

In addition to causing agricultural losses, bacteria are also responsible for the vast majority of hospital infections. In the USA, an estimated 30 billion USD/year are spent dealing with hospital infections, and these estimates are expected to increase as more bacteria become drug resistant [11].

Among the transmissible diseases caused by bacteria, tuberculosis is one of the most dangerous. In 2013, an estimated 9 million people contracted tuberculosis and 1.5 million died from the disease. Especially the BRICS countries (Brazil, Russia, India, China and South Africa) suffer from tuberculosis and account for almost 50% of tuberculosis cases worldwide. Worldwide costs for battling tuberculosis are estimated to accumulate $ 8 billion per year [12].

Another public health problem that affects several developing countries is cholera, caused by the *Vibrio cholerae* bacterium. The World Health Organization (WHO) estimates that in 2013, 47 countries reported a total of 129,064 cholera cases including 2,102 deaths [13].

The use of antimicrobial vegetable peptides may represent a desirable alternative in the clinical treatment of bacterial infections, which has generally been handled inappropriately by both community hospitals and health professionals. The excessive and indiscriminate use of antibiotics plays a crucial part in the increased resistance of pathogens, often requiring multiple-drug treatment, extended treatment periods, and/or hospitalization, thus raising costs and increasing the generation and distribution of resistant microorganisms. Ultimately, pathogen resistance contributes to increased patient morbidity and mortality rates, making it a serious global problem [14-16].

Faced with these problems, the pharmaceutical industry is looking for new antibiotics, trying to modify existing treatment strategies or adding new alternative therapeutic approaches. Unfortunately, pathogens have demonstrated the ability to quickly develop and disseminate resistance mechanisms, which usually renders drugs less effective. This phenomenon clearly illustrates the need to develop new treatment strategies based on action mechanisms that are different from conventional antibiotics. Antimicrobial peptides derived from plants may offer a promising alternative, mostly due to their low toxicity towards humans [17].

Plants usually defend themselves against microorganism attacks *via* a complex protection system that includes local and systemic production of secondary metabolites, proteins, peptides, and reactive oxygen species (ROS). Sometimes protection proceeds *via* physical barriers such as lignin, polysaccharides synthesis, or through the reaction type of the programmed cell death, which is a hypersensitive response. The plant immune system also depends on the production of several antimicrobial peptide classes (AMPs) [14,18-20].

AMPs differ from other antibiotic peptides such as gramicidins, bacitracins, and polymyxins, as these are synthesized by large enzymatic complexes and contain unusual amino acids and various types of modifications. In contrast, AMPs are encoded by genes, generally form small peptides of up to 100 L-amino acids present in plants, and exhibit linear or cyclic configurations [19].

2. Characterization of bacteria of medical and/or agronomical interest

Bacteria of interest in a clinical and agronomical context are characterized by their morphology, especially by their size, shape, as well as by their arrangement and the structures that comprise it. Usually, the phenotypic characteristics are common to the members of each group [21,22].

The structure of prokaryotic beings (bacteria) is greatly simplified, because it does not contain a defined nucleus that contains the DNA. Instead, the genetic material is compressed and coiled in a cytoplasmic region called the nucleoid. Bacteria are unicellular, between 2 and 5 μm in size, and usually harmless for humans. Even though some might be beneficial to humans, others are pathogens of medical interest [21,22].The reproduction of bacteria proceeds by cell division or binary fission. During this process duplication of the DNA occurs, thus allowing the division of the bacterial cell into two daughter cells identical to the original. At the beginning of the division, a septum is formed on the surface of the cell wall, which grows into the cell supporting the cell division [21,22].

One of the most important differences between eukaryotic and prokaryotic cells is the presence of a cell wall called “protective cover” in prokaryotic cells, below which the plasma membrane is located. Plant-associated bacteria, as well as those of medical interest have several morphologies, e.g. bacilli (rods), cocci (spherical), vibrios, spirals, and some pleomorphics (tendency to form irregular shapes). While most bacteria are aerobic, some are facultative anaerobic, and some rare ones are anaerobic. The bacteria of medical interest can belong to different phyla, but one characteristic of pathogenic plant bacteria is that the vast majority of Gram-positive bacteria are classified as phylum Actinobacteria, while the Gram-negative bacteria are classified as Phylum Proteobacteria [21-23].

The structural differences of bacteria of interest in a medical and agronomical context, which are generally classified into Gram-positive and Gram-negative bacteria, are important for the understanding of the mechanism of antimicrobial action. Thus, the presence of peptidoglycans, external polysaccharide membranes, and the wall of Gram-negative bacteria, impart those with more complexity, which is reflected in difficulties for substances to enter and exit the cell, usually requiring porin channels. In contrast, the ease of diffusion through the cell wall of Gram-positive bacteria is higher, which is important both for the identification of the microorganism as well as for the choice of appropriate antimicrobial agent to be used [14,16,21,24]

3. Action Mechanisms of antibacterial peptides

Some plant species produce hundreds of different AMPs, and antimicrobial plant peptides vary their size according to the number of constituent amino acids. They are subdivided into groups according to structure, type, and the occurrence of amino acids. The group containing cysteine residues is the largest group of AMPs, and can be either anionic or cationic. Other AMP groups are rich in tryptophan and contain proline, arginine, histidine, glycine, and are mostly

cationic. Linear peptides rich in aspartic and glutamic acids are usually anionic, while other groups are still under investigation [14,25].

However, it is important to emphasize that the vast majority of plant peptides are cationically charged, and therefore have the ability to initially interact with cell membranes and walls of negatively charged pathogenic bacteria. In response to the infection, they modify the overall net charge of the liquid intracellular medium [26]. This type of arrangement facilitates peptide binding and insertion into the bacterial membrane to create transmembrane pores, which results in permeability of the membrane [20,27].

Therefore, it has been proposed that the AMPs are drawn into the bilayer of the cell membrane by electrostatic interactions between the cationic amino acid residues of the peptides and the anionic phospholipid groups, which leads to permeability or lysis. Alternatively, such interaction with the membrane may also lead to the transient formation of pores, which allows the peptide to entre and interact with intracellular targets, while certain other peptides may be transported into the cell *via* channels or carrier molecules [27,28].

An important and desirable feature of AMPs is the selective toxicity of bactericidal or bacteriostatic peptides. The specific interaction with the bacterial cell requires them to be non-toxic to mammalian cells. The significant differences between mammalian cells and microbial cells, such as the composition of the membrane, the transmembrane potential, polarization and structural characteristics determine the selectivity of AMPs, and may thus be responsible for the differences in the mechanisms of action of the different AMP classes. Ongoing efforts to develop potential applications for AMPs and improved production methods, elucidation of mechanisms of action, and toxicity studies, may be the key to future therapeutic applications. [27,29].

Furthermore, several other AMPs have shown different antimicrobial properties, which renders them important tools for new therapeutic applications in biomedical fields, *e.g.* in the modulation of the immune response. When used in combination with other antibiotics, usually an improvement of the treatments with conventional antibiotics is observed, *e.g.* in anti-cancer and anti-inflammatory therapies, as well as in the regulation of blood pressure. Accordingly, these peptides may enhance the potency of existing antibiotics *in vivo*, probably by facilitating access of the antibiotics into the interior of the bacterial cell. Such phenomena have previously been studied for the cationic peptide component of polymyxin [16,17,30,31].

4. Some bactericidal plant peptide classes

For decades, researchers have tried to classify bactericidal peptides. The proposed classifications have primarily taken their structures (particularly the tertiary structure) and the composition thereof into consideration. However, the lack of structural information on such plant-derived antibacterial peptides prevents a more detailed classification. Currently, there are several classes of plant-based AMPs, such as defensins (γ-thionins), α/β-thionins, lipid transfer proteins (LTPs), cyclotides, hevein-type peptides, *knottin*-type peptides (some researchers group cyclotides, hevein-type peptides, and knottin-type peptides due to similarities in their structures), *snakins*, glycine-rich peptides, MBP-1 counterparts, and proteinase inhibitors. In the following, some of the these classes are described [18,19,32-35].

4.1 Defensins

In the 1990s, the first plant defensins were characterized. As they were the first to be described in this peptide family, they are considered the oldest antimicrobial peptides with activity against bacteria and yeast present in eukaryotes. In the plants, they are usually found in abundance in seeds, but they are also contained in leaves, pods, tubers, fruit, root, bark, and floral tissue [32,33].

With a molecular weight of 5-7 kDa and containing 45-54 amino acids, defensins are among the most basic peptides. They contain eight positively charged cysteine residues that form four disulfide bonds, which are responsible for stabilizing their three-dimensional structure and the formation of the cystine knot [18,33,35,36].

As far as the primary structure is concerned, it should be noted that only the cysteine residues are involved in disulfide bridges. Few other residues are conserved, and the remaining residues have varying degrees of freedom, while the number of residues separating the cysteines may also vary. As the primary structure defines the tertiary structure, the variations in the primary structure are reflected in small spatial variations in the three dimensional structure, especially in the size of the handles, which provides the overall structural diversification and contributes to the broad spectrum of biological activities observed for defensins [18,19,33].

Defensins exhibit antifungal and/or antibacterial activity for very low concentrations (~ μM), and they are active against a broad spectrum of pathogenic fungi and human pathogens (Table 1). More importantly, they usually do not display toxicity towards mammals and/or plants [33,36-39].

In addition, their toxicity can be extended to enveloped viruses, exo- and endo-parasites, and eventually cancer cells. With the understanding of the essential role these AMPs play in the defence of the host against infections, these peptides have been proposed as a new class of antimicrobials [40-42].

Defensins also exhibit other biological activities, *e.g.* the inhibition of protein synthesis [43], and the inhibition of the protease [44] and α-amylase activity [45].

4.2 Cyclotides

Cyclotides form a second highly important class of bactericidal peptides. They consist of small cyclic peptides, which are highly expressed in leaves, stems, and roots of various plants of the rubiaceae, violaceae, cucurbitaceae, and fabaceae families [46]. They consist of "head-tail" cyclized polypeptide chains of 29-37 amino acid residues, six of which are highly conserved cysteine residues that are involved in three disulfide links. This structural motif endows cyclotides with a high resistance towards thermal, chemical, and enzymatic degradation [47-49].

Cyclotides are desirable targets for the pharmaceutical and agrochemical industry, due to their cyclic "head-tail" structure, their biological activity, and their sequence diversity. They are now one of the most studied plant peptide family, and the understanding of this natural peptide library may be very important in the search for new antibacterial agents [47-49].

They were first discovered in the context of indigenous medical applications, *i.e.* women of the Lulua tribe used the tea of the native plant *Oldenlandia affinis* or "kalata kalata" to accelerate uterus contractions during childbirth [50]. The preparation of tea by boiling plant material and the oral administration suggested that the active ingredient was both resistant to heat and bioavailable [46]. Later studies showed that the cyclotide Kalata B1 was responsible for the uterotonic activity [50]. Kalata B1 was also the first macrocyclic plant peptide that had its structure completely elucidated. Since then, others have been isolated from plant biomass and a comparison with the prototype Kalata B1 revealed structural similarities for these molecules [51].

Initially, cyclotides were considered to be a variation of plant defensins, due to the observed structural similarities. Defensins consist of 45-54 amino acid residues, of which eight are cysteine residues that form four disulfide bonds, which are responsible for stabilizing the three-dimensional structure and the formation of the cystine knot. Conversely, cyclotides contain six highly conserved cysteine residues involved in three disulfide links, of which two disulfide bonds form the cystine knot, while the third disulfide bond penetrates a macrocyclice that hides the N-terminal region and forms a cyclic head-tail structure [33,47,52].

The most abundant cyclotides in *Oldenlandia affinis* are the Kalata B1 and B2 peptides, which may accumulate in amounts of up to 2 mg/g of fresh leaves. Due to the ability of the plant to accumulate large amounts of these cyclotides, to present different isoforms in the same plant, as well as to exhibit geographic and seasonal variation in the expression of these isoforms, it was suggested that they might be involved in plant protection against pathogens [52,53].

For a variety of fungi and bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida sp.*, the antimicrobial activity of cyclotides [54,55] has been reported for a minimum inhibitory concentration of 0.2-50 μM (Table 1).

Biological activity for cyclotides has been documented for an even wider range: they inhibit digestive peptidases of insects and thus show insecticidal activity [56]; they show activity against HIV [57] and neurotensin [58], and they also act cytotoxic [59].

4.3 α/β-Thionins

With minor exceptions, these are mostly cationic peptides with amphipathic properties. They differentially inhibit the growth of various bacteria and are thus potentially important in the control of plant pathogens. However, they are not active against mycellar fungi [35,60].

The common structural feature of all thionins is the presence of two antiparallel chains, whereby α-thionins exhibit an α-helical configuration and form a stem, while β-thionins exhibit a β-sheet configuration and form arms [61].

The antimicrobial biological activity of thionins (Table 1) can be feasibly explained on the basis of their structure. The primary action mechanism of these peptides affects biological membranes of pathogens, especially by connecting with phospholipids. The amino acids residues lysine and arginine are highly conserved and specifically contribute to phosphate linkages, while serine and tyrosine form a bond with glycerol. These interactions between membrane phospholipids and thionins have also been investigated by molecular modelling [35,62,63].

4.4 Lipid transfer proteins (LTPs)

This peptide class is named after their ability to transfer phospholipids between a donor and an acceptor through the membrane in *in vitro* tests [64,65].

Two families of lipid transfer proteins (LTP1 / LTP2) exist. While the members of the plant family LTP1 contain 90-95 amino acid residues and thus exhibit a molecular weight of ca. 10 kDa, LTP2 family members usually contain ca. 70 amino acid residues and display a molecular weight of ca. 7 kDa. The extracellular location of LTPs was confirmed from a variety of plants [66-69]. Both families contain eight conserved cysteine residues at similar positions in their primary structure and form four disulfide bonds to stabilize their tertiary structure [65].

Especially antifungal and antibacterial activity (Table 1) has been observed for LTPs on barley, corn, and spinach [63,67-69].

4.5 Snakins

Peptides belonging to this class were named by Segura and coworkers (1999) after the similarity of the amino acid sequences to a (desintegrin-like) hemotoxine, which is found in the venom of some snakes. They have been isolated from potatoes, a plant of the Solanaceae (*Solanum tuberosum*) family, and contain ca. 63-66 amino acid residues, of which twelve are cysteines [70,71].

They are cationic peptides with a hydrophobic core and highly polar domains in the N- and C-terminal regions. The mechanism of action of these peptides has been proposed on the basis of studies with the potato peptides StSN1 and StSN2 (Table 1). Their mechanism of action was compared to that of the defensin PTH1, which was isolated from the same potato species and exhibited synergistic antimicrobial activity. Against several agronomical bacteria, varying mechanisms of action were observed. For example, the StSN1 peptide was unable to disrupt the lipid membranes of bacteria and fungi studied in this experiment [35,71,72].

5. **Antimicrobial activity of some plant peptides classes**

Several peptide classes with antimicrobial activity play an important role in finding potential targets for antibacterial drugs in development. However, it is important to note that even though defensins and cyclotides still represent the majority of plant peptides with antibacterial activity, other classes also exhibit such activity [18,34,35,48,73].

Table 1 shows some AMPs with antimicrobial activity. Some representatives of these families demonstrate inhibitory activity against both agronomical bacteria of those of medical interest (Gram-positive and Gram-negative), as well as against fungi. But even though these peptides have shown inhibitory activity against bacteria and fungi, their mechanism of action is not yet fully understood [32,74].

Table 1 Antimicrobial activity of some peptide classes

AMP class	AMP name	Plant species	Antimicrobial activity (MIC)	Reference
Defensin	Cp-thionin-2	*V.unguiculata*	*S.Aureus* (128 μg·mL^{-1}) *E.Coli* (64 μg·mL^{-1})	[37]
Defensin	Fabatin-1	*V. faba*	*E. coli* (100 μg·mL^{-1}) and *P. aeruginosa* (30 μg·mL^{-1})	[74]
Defensin	Fabatin-2	*V. faba*	*E. coli* (100 μg·mL^{-1}) and *P. aeruginosa* (30 μg·mL^{-1})	[74]
Defensin	*StPTH1*	*Solanum tuberosum* cv Jaerla	*Clavibacter michiganensis* (7 uM) *Ralstonia solanacearum* (25 uM) *R. solanacearum* (*rfa*$^{-}$) (25 uM) EC_{50} - Effective concentration for 50% inhibition	[71]
Cyclotide	Kalata B1	*Oldenlandiaaffinis* (Roem. & Schuld.) DC.	*S. aureus* (0.75 μg·mL^{-1}) and *K. oxytoca* (158.37 μg·mL^{-1})	[55]
Cyclotide	Circulin A	*C. parvifolia*Schum.	*S. aureus* (0.59 μg·mL^{-1}) and *P. vulgaris* (172.04 μg·mL^{-1})	[55]
Cyclotide	Circulin B	*C. parvifolia*Schum.	*S. aureus* (44.32 μg·mL^{-1}) , *E. coli* (1.35 μg·mL^{-1}), *P. aeruginosa* (83.7 μg·mL^{-1}), *P. vulgaris* (22.3 μg·mL^{-1}) and *K. oxytoca* (26.92 μg·mL^{-1})	[55]
Cyclotide	Cycloviolacin O2	*Viola odorata*	*S. enterica* (8.75 μg·mL^{-1}) *E. coli* (2.2 μg·mL^{-1}) *S. aureus* (>50 μg·mL^{-1})	[75]
α/β-Thionin	Alpha-1-purothionin	*Triticum aestivum*	*Pseudomonas solanacearum* (5 μg·mL^{-1}) *Xanthomonas phaseoli* (27 μg·mL^{-1}) *Xanthomonas campestres* (56 μg·mL^{-1}) *Erwinia amylovora* (540 μg·mL^{-1})	[60]

AMP class	AMP name	Plant species	Antimicrobial activity (MIC)	Reference
			Corynebacterium flaccumfaciens (110 μg·mL^{-1}) *C. michiganense* (450 μg·mL^{-1}) *C. poinsettiae* (56 μg·mL^{-1}) *C. sepedonicun* (1 μg·mL^{-1})	
α/β-Thionin	PR-13 thionins	*Nicotiana attenuate*	*Pseudomonas syringae* pv. *Tomato* (0,25 μg·mL^{-1})	[76]
Snakin	Snakin-1	*S. tuberosum*	*L. monocytogenes* (10 μg·mL^{-1})	[77]
Snakin	StSN1	*Solanum tuberosum* cv Jaerla	*Clavibacter michiganensis* (4 uM) *R. solanacearum* (*rfa* $^{-}$) (15 uM) EC_{50} - Effective concentration for 50% inhibition	[70,71]
Snakin	StSN2	*Solanum tuberosum* cv Jaerla	*Clavibacter michiganensis* (1uM) *R. solanacearum* (*rfa* $^{-}$) (30 uM) *Rhizobium meliloti* (8 uM) EC_{50} - Effective concentration for 50% inhibition	[70,71]
LTP	LTP-s1 LTP-s2	*Spinacia oleracea*	*Clavibacter michiganensis* subsp. *Sepedonicus* (100 μg·mL^{-1})	[70]

5. Perspectives - Use of peptides with bactericidal action

Establishing a relationship between structure and antibacterial activity is hampered by the lack of any structural or sequential similarities of the peptides within the families. Also, it is important to note that these classes of plant defense compounds also demonstrate insecticidal, antifungal, and/or hemolytic activity in addition to the antimicrobial activity [18,73].

Interest in examining structure-activity relationships of such peptides has recently risen, especially by using computer modeling techniques and nuclear magnetic resonance (NMR) spectroscopy. Ultimately, these studies aim to facilitate a better understanding of the structural characteristics necessary to induce antimicrobial activity [18,34,78,79].

However, the use of such peptides as drugs is still limited, because these types of molecules suffer from various drawbacks, such as peptidase degradation, low oral bioavailability, and short duration of action. Other approaches to reduce the lability of AMPs include peptidomimetics, the use of peptide compounds of unusual or D-amino acids, as well as the use of formulations, *e.g.* by incorporation into liposomes. Defensins have not yet been investigated in advanced clinical trials, but it has been suggested that these peptides should be more resistant to protease degradation due to the structure stabilization by disulfide bonds and therefore, may have longer serum half-life times compared to other AMPs [18,34,79].

Another factor that restricts the enormous potential of these AMPs is the limited amount that can be extracted *in vivo*. However, the use of biotechnology as a tool to make these molecules available in the development of new drugs against human and plant pathogens, or as anticancer agents may form part of new clinical and therapeutic strategies [31,34,80,81].

Many drugs and vaccines are made from substances extracted from natural environments [82]. Brazil is the country with the highest biodiversity on the planet, with an estimated 170,000-210,000 known species. Moreover, Brazil also exhibits the greatest wealth of floral species. In total, 45,835 species have been documented, of which 4,680 are algae, 32,715 angiosperms, 1,519 bryophytes, 5,652 fungi, 30 gymnosperms, and 1,239 ferns and lycophytes [82]. Accordingly, the biodiversity of Brazil should offer great potential in the development of future drugs derived from antimicrobial plant peptides.

The great diversity of structurally different cysteine-rich antimicrobial peptide classes in nature suggests that these peptides are extremely important and fulfil different biological functions, thus providing clear evidence that AMPs are part of the complex plant immune system and not just executors of a defense program to eliminate pathogens. However, it should also be noted that the molecular evolution of different classes of plant AMPs is not yet fully understood.

Further research on the mechanisms of action is therefore required, in order to better understand the interactions of plants with pathogenic and non-pathogenic agents, as well as insects and other herbivores [35].

References

[1] Strange RN, Scott PR. Plant disease: A threat to global food security. Annu. Rev. Phytopathol. 2005; 43: 83-116.
[2] Oerke EC. Crop losses to pests. Journal of Agricultural Science. 2006; 144: 31-43.
[3] Ellis SD, Boehm MJ, Coplin D. Bacterial diseases of plants. Agriculture and Natural Resources. The Ohio State University extension. [internet] 2008 [cited 2015 may 30]. Available from: http://ohioline.osu.edu/hyg-fact/3000/pdf/PP401_06.pdf
[4] Chakraborty S, Newton AC. Climate change, plant disease and food security: an overview. Plant Pathol. 2011; 60: 2-14.
[5] Food and agriculture organization of the United Nations [internet]. Keeping plant pests and diseases at bay: experts focus on global measures. [cited 2015 may 30]. Available from: http://www.fao.org/news/story/en/item/280489/icode/
[6] Niño-liu DO, Ronald PC, Bogdanove AJ. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Mol Plant Pathol. 2006; 7(5): 303-24.
[7] European and Mediterranean Plant Protection Organization [internet]. [cited 2015 may 30]. Available from: https://gd.eppo.int/.
[8] Álvares B, Biosca EG, López MM. On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. In: A. Méndez-Vilas editor. Current research,technology and education topics in applied microbiology and microbial biotechnology. Spain: Formatex; 2010. p. 267-279.
[9] Meng F. *Ralstonia solanacearum* species complex and bacterial wilt disease. J. Bacteriol. Parasitol. 2013; 4:2.
[10] Neves MF, Trombin VG, Milan P, Lopes FF, Cressoni F, Kalaki F. O retrato da citricultura brasileira. Universidade de São Paulo, Campus Ribeirão Preto, Centro de Pesquisa e Projetos em Marketing e estratégia. [internet] 2010 [cited 2015 may 30]. Available from: http://www.citrusbr.com.br/download/Retrato_Citricultura_Brasileira_Marcos_Fava.pdf.
[11] Committee to reduce infection deaths [internet]. The cost of infection. [cited 2015 may 29]. Available from: http://www.hospitalinfection.org/cost_of_infection.shtml.
[12] World Health Organization. Global tuberculosis report. France; 2014. 171 p.
[13] World Health Organization. Cholera,2013. Wkly epidemiol rec. 2014; 89 (31):345-55.
[14] Maróti G, Kereszt A, Kondorosi E, Mergaert. Natural roles of antimicrobial peptides in microbes, plant and animals. Res Microbiol. 2011; 162: 363-374.
[15] Agencia Nacional de Vigilância Sanitária. Microbiologia clínica para o controle de infecção relacionada à assistência à saúde. Detecção e identificação de bactérias de importância médica. Brasilia: Ed. ANVISA; 2013. Mod. 1, 6.
[16] Guzmán-Rodríguez JJ, López-Gómez R, Suárez-Rodríguez LM, Salgado-Garciglia R, Rodríguez-Zapata LC, Ochoa-Zarzosa A, et al. Antibacterial activity of defensing PaDef from avocado fruit (*Persea americana* var. *drymifolia*) expressed in endothelial cells against *Escherichia coli* and *Staphylococcus aureus*. Biomed Res Int. 2013. PubMed PMID: 24319695.
[17] López-Meza JE, Ochoa-Zarzosa A, Aguilar JA, Loeza-Lara PD. Antimicrobial peptides: Diversity and perspectives for their biomedical application. In: Sylwia Olsztynska editor. Biomedical engineering, trends, research and technologies. Croatia: Intech; 2011. p. 275-304.
[18] Pelegrini PB, Franco OL. Plant γ-thionins: Novel insights on the mechanism of action of a multi-functional class of defense proteins. Int J Biochem Cell Biol. 2005; 37911): 2239–53.
[19] Carvalho AO, Gomes VM. Plant defensin and defensin like peptides – Biological activities and biotechnological applications. Curr Pharm Des. 2011; 17 (38): 4270-93.
[20] Salas CE, Badillo-Corona JA, Ramírez-Sotelo G, Oliver-Salvador C. Biologically active and antimicrobial peptides from plants. Biomed Res Int. 2015. PubMed PMID: 25815307.
[21] Trabulsi LR, Alterthum F, Gompertz OF, Candeias JAN. Microbiology. 3th ed. São Paulo: Atheneu; 1999.
[22] Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Cell Biology. 5th ed. Porto Alegre: Artmed; 2010.
[23] Vidaver AK, Lambrecht PA. Bacteria as plant pathogens. The plant health instructor. 2004. Doi: 10.1094/phi-i-2004-0809-01.
[24] Rang HP, Dale MM, Ritter JM. Farmacology. 3th ed. Rio de Janeiro: Guanabara Kooagan; 1997.
[25] Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol. 2005; 3: 238-50.
[26] Peschel A, Sahl HG. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat Rev Microbiol. 2006; 4 (7): 529-36.
[27] Wang G, Mishra B, Lau K, Lushnikova T, Golla R, Wang X. Antimicrobial peptides in 2014. Pharmaceuticals. 2015; 8 (1): 123-50.
[28] Onaizi SA,Leong SS. Tethering antimicrobial peptides: current status and potential challenges. Biotechnol Adv. 2011; 29 (1): 67-74.
[29] Peters BM, Shirliff ME, Jabra-Rizk MA. Antimicrobial peptides: primeval molecules or future drugs? PLOS Pathogens. 2010; 6(10): 1-4.
[30] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002; 415: 389-95.
[31] Al-Rayahi IAM, Sanyi RHH. The overlapping roles of antimicrobial peptides and complement in recruitment and activation of tumor-associated inflammatory cells. Front Immunol. 2015; 6:1-5. PubMed PMID: 25657649.
[32] Zhang Y, Lewis K. Fabatins: New antimicrobial plant peptides. FEMS Microbiol Lett. 1997; 149: 59-64.
[33] Thomma BPHJ, Cammue BPA. Plant defensis. Planta. 2002; 216: 193-202.
[34] Cândido ES, Porto WF, Amaro DS, Viana JC, Dias SC, Franco OL. Structural and functional insights into plant bactericidal peptides. In: A. Méndez-Vilas editor. Science against microbial pathogens: communicating current research and technological advances. Spain: Formatex; 2011. p.951-60.
[35] Stotz HU, Waller F, Wang K. Innate immunity in plants: The role of antimicrobial peptides. In: Hiemstra PS, Zaat SAJ editors. Antimicrobial peptides and innate immunity. USA: Springer; 2013: 29-51.

[36] Thevissen K, François IEJA, Takemoto JY, Ferket KKA, Meert EMK, Cammue BPA. DmAMP1, an antifungal plant defensis from dahlia *(Dahliamerckii*), interacts with sphingolipids from *Saccharomyces cerevisiae*. FEMS Microbiol Lett. 2003; 229: 169-73.
[37] Franco OL, Murad AM, Leite JR, Mendes PAM, Prates MV, Bloch C. Identification of a cowpea y-thionin with bactericidal activity. FEBS J. 2006; 273 (15): 3489-97.
[38] Osborn RW, De Samblanx GW, Thevissen K, Goderis I, Torrekens S, Van Leuven F, et al. Isolation and characterisation of plant defensis from seed of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae. FEBS Lett. 1995; 368 (2): 257-62.
[39] Sagaram US, Pandurangi R, Kaur J, Smith TJ, Shah DM. Structure-activity determinants in antifungal plant defensis MsDef1 and MtDef4 with different modes of action against *Fusarium graminearum*. Plos One. 2011; 6(4): 1-13. PubMed PMID: 21533249.
[40] Hilpert K, Volkmer-Engert R, Walter T, Hancock REW. High-throughput generation of small antibacterial peptides with improved activity. Nat Biotechnol. 2005; 23(8): 1008-12.
[41] Parachin NS, Mulder KC, Viana AAB, Dias SC, Franco OL. Expression systems for heterologous production of antimicrobial peptides. Peptides. 2012; 38: 446-456.
[42] Silva BR, Freitas VAA, Carneiro VA, Arruda FVS, Lorenzón EN, Aguiar ASW, et al. Antimicrobial activity of the synthetic peptide Lys-a1 against oral streptococci. Peptides. 2013; 42: 78-83.
[43] Mendez E, Moreno A, Colilla F, Pelaez F, Limas GG, Mendez R, et al. Primary structure and inhibition of protein synthesis in eukaryotic cell-free system of a novel thionin, gamma-hordothionin, from barley endosperm. Eur J Biochem. 1990; 194(2): 533-39.
[44] Wijaya R, Neumann GM, Condron R, Hughes AB, Polya GM. Defense proteins from seed of Cassia fistula include a lipid transfer protein homologue and a protease inhibitory plant defensin. Plant Sci. 2000;159 (2): 243-55.
[45] Pelegrini PB, Murad AM, Silva LP, Dos Santos RC, Costa FT, Tagliari PD, et al. Identification of a novel storage glycine-rich peptide from guava (*Psidium guajava*) seed with activity against Gram-negative bacteria. Peptides. 2008; 29 (8): 1271-79.
[46] Poth AG, Colgrave ML, Lyons RE, Daly NL, Craik DJ. Discovery of an unusual biosynthetic origin for circular proteins in legumes. PNAS. 2011; 108 (25): 10127–132.
[47] Craik DJ, Daly NL, Bond T, Waine C. Plant cyclotides: a unique family of cyclic and knotted proteins that defines the cyclic cystine-knot structural motif. J. Mol. Biol. 1999; 294: 1327-36.
[48] Daly NL, Rosengren KJ, Craik DJ. Discovery, structure and biological activities of cyclotides. Adv Drug Deliv Rev. 2009; 61: 918-30.
[49] Schroeder CI, Swedberg JE, Craik DJ. Recent progress toward pharmaceutical applications of disulphide-rich cyclic peptides. Curr Protein Pept Sci. 2013;14: 532-42.
[50] Gran, L. On the effect of a polypeptide isolated from "Kalata-Kalata" (*Oldenlandia affin*is DC) on the oestrogen dominated uterus. Acta Pharmacol Toxicol. 1973; 33: 400-408.
[51] Picchi DG, Altei WF, Saito MS, Bolzani VS, Cilli EM. Peptídeos cíclicos de biomassa vegetal: características, diversidade, biossíntese e atividades biológicas. Química Nova. 2009; 32(5): 1262-77. Brasil.
[52] Schroeder BO, Wu Z, Nuding S, Groscurth S, Marcinowski M, Beisner J, et al. Reduction of disulphide bonds unmasks potent antimicrobial activity of human β-defensin 1. Nature. 2011; 469 (7330): 419-23.
[53] Trabi M, Svangard E, Herrman A, Garansson U, Claeson P, Craik DJ, et al. Variations in cyclotide expression in Viola species. J.Nat. 2004; 67: 806-810.
[54] Tam JP, Lu Y, Yang J, Chiu K. An unusual structure motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides. Proc Natl Acad Sci USA. 1999; 96 (16): 8913-18.
[55] Gruber CW. Global cyclotide adventure: a journey dedicated to the discovery of circular peptides from flowering plants. Biopolymers. 2010; 94 (5): 565-72.
[56] Jenning C, West J, Waine C, Craik D, Anderson M. Biosynthesis and insecticidal properties of plant cyclotides: The cyclic knotted proteins from *Oldenlandia affinis*. PNAS. 2001; 98(19): 10614-619.
[57] Gustafson KR, McKee TC, Bokesch HR. Anti-HIV cyclotides. Curr Protein Pept Sci. 2004; 5: 331-40.
[58] Witherup KM, Bogusky MJ, Anderson PS, Ramjit H, Ransom RW, Wood T, et al. Cylopsychotride A, a biologically active, 31 residue cyclic peptide isolated from Psychotria longipes. J. Nat. Prod. 1994; 57: 1619-25.
[59] Lindholm P, Goransson U, Johansson S, Claeson P, Gullbo J, Larsson R,Bohlin L, Backlund A. Cyclotides: A novel type of cytotoxic agents. Mol Cancer Ther. 2002; 1: 365-69.
[60] de Caleya RF, Gonzalez-Pascual B, García-Olmedo F, Carbonero P. Susceptibility of phytopathogenic bactéria towheat purothionins in vitro. ApplMicrobiol. 1971; 23 (5): 998-1000.
[61] Padovan L, Segat L, Tossi A, CalsaTJr, Ederson AK, Brandao L, et al. Characterization of a new defensis from cowpea (*Vigna unguiculata* (L) Walp). Protein Pept Lett. 2010;17 (3): 297-304.
[62] Stec B, Markman O, Rao U, Heffron G, Henderson S, Vernon LP, et al. Proposal for molecular mechanism of thionins deduced from physic-chemical studies of plant toxins. J Pept Res. 2004; 64(6): 210-24.
[63] Nawrot R, Barylski J, Nowicki G, Broniarczyk J, Buchwald W. Plant antimicrobial peptides. Folia Microbiol. 2014; 59: 181-96.
[64] Bloj B, Zilversmit DB. Rat liver proteins capable of transferring phosphatidylethanolamine. Purification and transfer activity for other phospholipids and cholesterol. J Biol Chem. 1977; 252 (5): 1613-19.
[65] Kader JC, Julienne M,Vergnolle C. Purification and characterization of a spinach-leafprotein capable of transferring phospholipids from liposomesto mitochondria or chloroplasts. Eur J Biochem. 1984; 139: 411-16.
[66] Sterk P, Booij H, Schellekens GA, Van Kammen A, De Vries SC. Cell-specific expression of the carrot EP2 lipid transfer protein gene. Plant Cell. 1991; 3 (9): 907-21.
[67] Terras FR, Goderis IJ, Van Leuven F, Vanderleyden J, Cammue BP, Broekaert WF. In vitro antifungal activity of a radish (*Raphanus sativus* L.) seed protein homologous to nonspecific lipid transfer proteins. 1992; 100 (2): 1055-58.
[68] Molina A, García-Olmedo F. Developmental and pathogen-induced expression of three barley genes encoding lipid transfer proteins. Plant J. 1993; 4 (6): 983-91.

[69] Segura A, Moreno M, García-Olmedo F. Purification and antipathogenic activity of lipid transfer protein (LTPs) from the leaves of *Arabidopsis* and spinach. FEBS Lett. 1993; 332(3): 243-6.
[70] Segura A, Moreno M, Madueño F,Molina A, García-Olmedo F. Snakin-1, apeptide from potato that is active against plant pathogens. Mol Plant Microbe Interact. 1999; 12 (1): 16-23.
[71] Berrocal-Lobo M, Segura A, Moreno M, López G, García-Olmedo F, Molina A. Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to phatogen infection. Plant Physiol. 2002; 128 (3): 951-61.
[72] Caaveiro JM, Molina A, González-Manãs JM, Rodríguez-Palenzuela P, Garcia-Olmedo F, Goñi FM. Differential effects of five types of antipathogenic plant peptides on model membranes. FEBS Lett.1997; 410(2-3): 338-42.
[73] Castro MS, Fontes W. Plant defense and antimicrobial peptides. Protein and Peptide Letters. 2005; 12:13-18.
[74] Carvalho AO, Gomes VM. Plant defensis – prospects for the biological functions and biotechnological properties. Peptides. 2009; 30 (5): 1007-20.
[75] Pranting M, Lööv C, Burman R, Göransson U,Andersson DI. The cyclotide cycloviolacin O2 from *Viola odorata* has potent bactericidal activity against Gram-negative bacteria. J Antimicrob Chemother. 2010; 65: 1964-71.
[76] Rayapuram C, Wu J, Haas C,Baldwin IT. PR-13/Thionin but not PR-1 mediates bacterial resistance in *Nicotiana attenuate* in nature, and neither influences herbivore resistance. Mol Plant Microbe Interact. 2008; 21 (7): 988-1000.
[77] Yount NY, Yeaman MR.Multidimensional signatures in antimicrobial peptides. Proc Natl Acad Sci USA. 2004; 101 (19): 7363-68.
[78] Quartara L, Pavone V, Pedone C, Lombardi A, Renzetti AR, Maggi CA. A review of the design, synthesis and biological activity of the bicyclic hexapeptide tachykinin NK2 antagonist MEN 10627. Regul Pept. 1996; 65:55-59.
[79] Ulm H, Wilmes M, Shai Y, Sahl H. Antimicrobial host defensis – specific antibiotic and innate defense modulation. Front Immunol. 2012; 3(249):1-4.
[80] Marshall SH, Arenas G. Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. Eletron J Biotechnol. 2003; 6 (2): 271- 84.
[81] Guzmán-Rodriguez JJ, Ochoa-Zarzosa A, López-Gómez R, López-Meza JE. Plant antimicrobial peptides as potential anticâncer agentes. Biomed Res Int. 2015. PubMed PMID: 25815333.
[82] Sistema de Informação sobre a Biodiversidade Brasileira [Internet]. [cited 2015 june 04]. Available from: http://www.sibbr.gov.br/.

