Review Article

Functional Peptides: Novel Tools for Controlling Plant Diseases

Abstract

Chemical pesticides, which are today subject to stringent regulations and limits, are the primary means of controlling plant diseases. In terms of plant health, functional peptides are intriguing substances. Many novel synthetic and natural compounds have been found and employed in plant protection in recent years. Functional peptides are a good option among them to combat phytopathogens (Amso and Hayouka, 2019). Functional peptides are synthetic analogues or derived from living organisms, they offer new methods of action against plant diseases, making them potential biopesticide candidates. For a long time, functional peptides have been suggested as possible anti-fungal and anti-bacteria reagents in agriculture (Van der Biezen, 2001). Functional peptides from different origins share similar killing mechanisms when targeting bacterial and fungal pathogens. As summarized by many previous reviews (Zasloff, 2002; Brogden, 2005; Melo et al., 2009; Bocchinfuso et al., 2011; Sibel Akalın, 2014), to destroy infections, the majority of peptides target and breach the cell membrane directly. Peptides may be made available to the industry and growers on a big scale through chemical synthesis, biotechnological platforms, and natural sources. It is anticipated that a number of functional peptides may soon be offered for sale as plant disease control agents, although further research is required to confirm these peptides' effectiveness in real-world settings.

Introduction

Pesticides are a vital tool for protecting plants and are essential to agriculture and food security. The production of fruits and vegetables would decline by 78%, vegetables by 54%, and cereals by 32% if pesticides weren't used (Tudi et al.,2021).Pesticides help to boost crop yields globally, but they need to be updated to satisfy environmental safety regulations and agricultural development demands. Eco-friendly pesticides that are effective against pests and poses little threat to nontarget organisms are of paramount importance in the age of ecological agriculture, which emphasises sustainable development.“The primary tenet of crop protection remains chemical control. However, due to the requirement to produce safe food and the fact that many pesticides have nontarget environmental consequences, several countries have limited the number and types of pesticides that are permitted. For instance, the European Union has mandated a significant decrease in the active ingredients in pesticides in recent years, and governments all over the world have followed suit. This allowed for the retention of more

selective compounds with lower intrinsic toxicity and less detrimental effects on the environment. Following the restrictions' introduction, a number of pesticides were outlawed, and the absence of substances that effectively combat certain economically significant plant diseases has made management of these illnesses challenging. Several diseases may now be inadequately or completely uncontrolled as a result of the lack of adequate new chemicals, biopesticides, or effective cultural and management techniques to offset the decrease in the usage of conventional pesticides" (Zhang et al., 2023). Since there has traditionally been less bactericides than fungicides, the issue is more challenging when it comes to infections caused by bacteria than fungal diseases. Furthermore, a "number of regions have seen the establishment of new and re-emerging bacterial diseases of economic significance, such as bacterial leaf blight of rice (*Xanthomonas oryzae*pv. *oryzae*), bacterial wilt of tomato and potato (*Ralstonia solanacearum*), bacterial wilt of banana (Xanthomonas vasicolapv. musacearum), bacterial canker of kiwifruit (*Pseudomonas syringae*pv. *actini-diae*), bacterial blight of cassava (*Xanthomonas axonopodis*pv. *manihotis*), and fire blight of apple and pear (*Erwinia amylovora*) that have emerged and re-emerging bacterial diseases of economic importance"(Sundin et al., 2016).

The low return on investment (market value) and the challenges of gaining registration approval due to stringent regulatory requirements have made pesticide companies less interested in offering novel pesticides to growers, despite research efforts to identify and develop new plant-protection products. Additionally, a number of innovative disease control strategies are still being developed (such as RNA interference and defence elicitors), have safety issues (such as novel nanoparticle formulations), or have not undergone enough field testing and validation.

"In the realm of crop protection, functional peptides have been the focus of intense investigation. Peptides are considered polypeptides of up to 50–60 amino acids (upper size limit considered as big peptides or small proteins) but also comprise pseudopeptides containing peptide bonds, non-natural or modified amino acids. The majority of peptides are derived from living things, and they have an antagonistic or antibiosis effect on microorganisms. Many different kinds of species, including humans, plants, animals, and invertebrates, form functional peptides which can be antimicrobial to ward off infection. Their defense systems work against pathogens in many ways" (Brogden 2005). "They also form the first line of defense against stress in both plants and animals, as well as the immune system (Huan et al., 2020). Reviews of AMPs have been carried out in bacteria" (Jack & Jung, 2000; Cooter et al., 2005; Raaijmakers et al., 2006), fungi (Degenkolb et al., 2003; Ng, 2004), insects (Hancock, 2001; Bulet et al., 2004), marine invertebrates (Tincu & Taylor, 2004), amphibians and mammals (Andreu & Rivas, 1998; Zasloff, 2002; Toke, 2005), and plants (Garc´ıa-Olmedo et al., 1998; Lay & Anderson, 2005). Approximately 900 AMPs have been reported; these can be divided into three types based on their structural characteristics: linear peptides that often take on helical shapes, cysteine-rich open-ended peptides with disulphide bridges, and cyclopeptides that form peptide rings. Peptides, which are mostly obtained from living organisms, are essential for lowering stress levels in both plants and animals. As the initial line of defense against bacteria, they function by

either antagonistic or antibiosis.The present review summarizes the overview, mechanism of action of functional peptides along with the procedures for finding and developing new compounds and learning about their mechanisms of action. It also covers the current understanding of peptides that target plant pathogens and the diseases they cause in crops, the platforms for production that can be exploited, and the difficulties and opportunities associated with developing novel biopesticides.

A Comprehensive Overview of Peptides

Secretin was the first peptide to be identified; it was found in the gastrointestinal tracts of animals by Bayliss and Starling in 1902 (Tam JKV et al. 2014). Subsequent research revealed that functional peptides include insulin, which decreases blood sugar, and oxytocin, which promotes uterine contraction. Solid phase peptide synthesis (SPPS), which was quicker and simpler to use than traditional liquid phase synthesis, laid the groundwork for automated synthesis in 1963. For this innovation, Merrifield, the man behind SPPS, received the 1984 Chemistry Nobel Prize. Since then, there has been significant advancement in the field of peptide studies. Ten Nobel Prizes have been awarded to peptides, indicating their significant role in science and technology. The 53-amino acid peptide known as epidermal growth factor (EGF), which promotes skin and corneal cell proliferation, was discovered in 1986 and its discoverer was awarded the Nobel Prize in Physiology or Medicine. By controlling cellular transport and localization, signal peptides enable the more effective use of cells as "protein factories" for the manufacture of medications.For this discovery, they were awarded the 1999 Nobel Prize in Physiology or Medicine. The 2018 Chemistry Nobel Prize was given in recognition of the discovery of peptides manufactured by phages that can be used to treat autoimmune disorders. Peptides are particularly useful for protecting plants; this was highlighted by the 2020 Presidential Green Chemistry Challenge Award for the neuropeptide-based bio insecticide Spear®.

Microorganism-derived Antimicrobial Peptides

Microorganisms produce a wide range of antimicrobial peptides, including small bacteriocins and fungal defensins synthesised through ribosomal synthesis, as well as secondary metabolites produced by non-ribosomal synthesis such as peptaibols, cyclopeptides, and pseudopeptides.The most often used classification scheme involves considering the structures that these molecules can adopt in vivo, including a-helix, b-sheet, b-hairpin, and looping topologies, as well as linear peptides with unusual bias.

Table 1. Antimicrobial cyclic-peptides

Type	Compound	Composition	Producer microorganism
Simple	Gramicidins	C10	*Bacillusbrevis*
	Calophycin	C10	*Calothrixfusca*
	Laxaphycins	C11	*Anabaena laxa*
Tailed	Bacitracins	T5-C7	*Bacillus licheniformis*
Simple lipidic	Xanthostatin	R-C6	*Streptomyces*

			spiroverticillatus
	Echinocandins	R-C6.	*Aspergillus spp.*
	Cryptocandins	R-C6	*Cryptosporiopsisquercina*
	Fusaricidins	R-C6	*Paenibacilluspolymixa*
Tailed lipidic	Viscosins	R-T2-C7	*Pseudomonas fluorescens*
	Polymixins	R-T3-C7	*Paenibacilluspolymixa*
	Agrastatins	R-T2-C8	*Bacillus subtilis*
	Amphisins	R-T2-C9	*Pseudomonas fluorescens*
	Putisolvins	R-T8-C4	*Pseudomonas putida*

C, peptide ring size; T, peptide tail size; R, linked fatty acid

Table 2. Classification of some antimicrobial peptides.

AMPs from Animals				
Peptide	Source	Function	Species effectiveness	Refs.
Abaecin	*Apis mellifera*	Antibacterial	*Agrobacterium tumefaciens* *Erwinia salicis* *Pseudomonas syringae* *Xanthomonas campestris*	18
Apidaecins	*Apismelifera*	Antibacterial	*A. tumefaciens* *E. salicis* *P. syringae* *Rhizobium meliloti*	17,19
Cecropin B	*Hyalophora cecropia*	Antibacterial, Antifungal	*P. syringaepv. Tomato* *P. syringaepv. Syringae* *P. syringaepv. Tabaci* *X. campestris pv. Vesicatoria* *Clavibactermichiganensis subsp. Michiganensis* *Erwinia carotovora subsp. Carotovora* *E. carotovora subsp. Chrysanthemi* *A. tumefaciens* *Penicillium digitatum* *Phytophthora infestans*	2
Dermaseptin	*Rhacophorus*	Antibacterial	*Xylella fastidiosa*	48
Drosomycin	*Drosophila melanogaster*	Antifungal	*Botrytis cinerea* *Fusarium culmorum* *Fusarium oxysporum* *Nectriahaematococca* *Alternaria brassicola* *Alternaria longipes* *Trichoderma viride* *Ascochytapisi*	30

Indolicidin	*Bovine*	Antibacterial	*X. fastidiosa*	48
LfcinB	*Bovine*	Antifungal	*P. digitatum* *Penicillium italicum* *Penicillium expansum* *Penicillium sp.* *Alternaria sp.* *Aspergillus nidulans* *B. cinerea* *F. oxysporum*	68
Magainin II	*Xenopus laevis*	Antibacterial, Antifungal	*P. syringaepv. Tomato* *P. syringaepv. Syringae* *P. syringaepv. Tabaci* *X. campestris pv. Vesicatoria* *C. michiganensis subsp. Michiganensis* *P. digitatum* *X. fastidiosa*	2
Penetratin	*Drosophilid*	Antibacterial	*Bacillus megaterium*	75
PGQ	*X. laevis*	Antibacterial	*X. fastidiosa*	48
pVEC	*Mammalian*	Antibacterial	*B. megaterium*	75
SpodopsinIa	*Spodopteralitura*	Antibacterial	*B. megaterium*	22
AMPs from Plants				
α1-purothionin	*Triticum aestivum*	Antibacterial	*Xanthomonas* *Erwinia*	15
BLAD	*Lupinus albus*	Antifungal	*B. cinerea* *Erysiphales*	81
Ca-AFP	*Capsicum annuum*	Antifungal	*F. oxysporum* *Phytophthora capsici*	16
Ca-LTP1	*C. annuum L.*	Antifungal	*F. oxysporum* *Colletotrichum lindemuthianum*	24
J1	*C. annuum*	Antifungal	*Colletotrichum gloeosporioide* *Colletotrichum musae* *F. oxysporum*	27,94
NaD1	*Nicotiana alata*	Antibacterial, Antifungal	*B. cinerea* *F. oxysporum* *F. oxysporum f. Sp. Vasinfectum* *Thielaviopsisbasicola* *Verticillium dahlia* *Leptosphaeria maculans* *A. nidulans*	44,109,110
Pa-AFP1	*Passiflora alata Curtis*	Antifungal	*C. gloeosporioide*	88
Pe-AFP1	*Passiflora edulis*	Antifungal	*Aspergillus fumigatus*	79

			F. oxysporum	
Peptide-1	*Oryza sativa*	Antifungal	*Magnaportheoryzae*	89
Pf2	*Passiflora edulis f. Flavicarpa*	Antifungal	*F. oxysporum* *C. musae* *C. lindemuthianum*	1
PhD1	*Petunia hybrida*	Antifungal	*B. cinerea* *F. oxysporum*	53,41
PhD2	*P. hybrida*	Antifungal	*B. cinerea*	53,41
PvD1	*Phaseolus vulgaris*	Antifungal	*F. oxysporum* *Fusarium solani* *Fusarium laterithium*	60
Snakin-1	*Solanum tuberosum*	Antibacterial, Antifungal	*B. cinerea* *F. solani* *F. culmorum* *F. oxysporum* *Plectosphaerellacucumerina* *Colletotrichum lagenarium* *Colletotrichum graminicola* *Bipolaris maydis* *Aspergillus flavus* *C. michiganensis* *Ralstonia solanacearum*	11,93
Snakin-2	*S. tuberosum*	Antibacterial, Antifungal	*C. michiganensis* *R. solanacearum (rfa-)* *R. meliloti* *B. cinerea* *F. solani* *F. culmorum* *F. oxysporum f. Sp. Conglutinans* *F. oxysporum f. Sp. Lycopersici* *P. cucumerina* *C. graminicola* *C. lagenarium* *B. maydis* *A. flavus*	11
ZmPep1	*Z. mays*	Antifungal	*Pythiumspp*. *Fusarium*	58
AMPs from microorganism				
AFP	*Aspergillus giganteus*	Antifungal	*F. culmorum* *Fusarium equiseti* *Fusarium lini* *Fusarium moniliforme* *F. oxysporum* *Fusarium poae*	9

			Fusarium proliferatum *F. solani* *Fusarium sporotrichoides* *Fusarium vasinfectum* *Magnaporthegrisea* *P. infestans*	
ANAFP	*A. niger*	Antifungal	*A. fumigatus* *A. flavus* *F. oxysporum* *F. solani*	9
NAF	*Penicillium nalgiovense*	Antifungal	*A. flavus* *F. solani* *P. italicum*	9
PAF	*Penicillium chrysogenum*	Antifungal	*A. fumigatus* *A. flavus* *A. niger* *B. cinerea* *Cochlioboluscarbonum* *F. oxysporum* *Blumeriagraminis f. Sp. Hordei* *Puccinia reconditaf.sp. Tritici*	9,43

Antimicrobial Peptides in the Management of Plant Diseases

One of the things that fascinates AMPs as antimicrobial agents for managing plant diseases is their method of action on the target bacterium. Most AMPs are cationic and reach the cytoplasmic membrane because they bind to the surfaces of bacteria through receptor-mediated interaction. Certain AMPs cause membrane disruption, while others do not; they can pass through cell membranes to interact with intracellular targets and stop the creation of proteins, nucleic acids, or enzymes. (Powers & Hancock, 2003; Brogden, 2005).

Bacteriocins and fungal defensins

Major bacterial groups secrete a form of protein and peptide called bacteriocins, which are capable of killing closely related species. Although small bacteriocins have not been investigated, examples of some bacteriocins that suppress plant pathogenic bacteria have been reported from bacteria associated with plants (Ishimaru et al., 1988; Jabrane et al., 2002; Lavermicocca et al., 2002; Pham et al., 2004; Parret et al., 2005). Similar to plant and animal defensins, AMPs consisting of 51–58 amino acid residues with a compact structure of antiparallel strands held together by disulphide bridges are secreted by a number of filamentous fungus. Antifungal action is exhibited by the peptides Anafp from Aspergillus niger (Lee et al., 1999), PAF from Penicillium chrysogenum and Penicillium nalgiovense (Kaiserer et al., 2003), and AFP from Aspergillus giganteus (Lacadena et al., 1995).

Synthetic AMPs

Synthetic molecules containing six to 47 amino acid residues have been developed or analogues from plants and animals have been used. Solid-phase techniques have been used to produce

synthetic AMPs. (Andreu et al., 1983). Combinatorial chemistry is a potent method for designing novel compounds that diverge from better leader compounds and concentrate their activity on specific target pathogens while reducing their toxicity to plants and animals and their susceptibility to protease digestion (Powell et al., 1995; Reed et al., 1997; Oh et al., 1999; Monroc et al., 2006a).

Table 3. Peptides used to regulate plant pathogens

Peptides	**Origin**	**Targeting pathogens**	**Methods of testing**	**Refs**
Thionin	*Arabidopsis thaliana*	*Ralstonia solanacearum, Fusarium oxysporum*	Transgenic expression	(Chan *et a*l., 2005)
Snakin-1	Potato	*Clavibactermichiganensis, Botrytis cinerea*	Transgenic expression	(Segura *et al*., 1999)
alfAFP	Alfalfa	*Verticillium dahliae*	Transgenic expression	(Gao *et al*., 2000)
Melittin	*Apis mellifera*	*Xanthomonas oryzae*	In-vitro killing assay	(Shi *et al*., 2016)

Multifunctional Peptides

Several examples of multifunctional peptides have been developed in various ways, such as by searching the genome, transcriptome, or proteome of a disease-resistant plant or by engineering from sequences of other peptides. Peptides with simultaneous mechanisms of action are interesting in plant protection because they counteract possible resistance in the pathogen and improve its activity.*Phakopsorapachyrhizi,* the causative agent of Asian soybean rust, is inhibited from germination and infections by the engineered peptide DS01-THA, a chimaera of dermaseptin and thanatin that adheres to the wax layer of soybean, barley and maize (Schwinges*et al.*, 2019). Among the various natural defence genes that may be in charge of HLB tolerance, the peptide MaSAMP was found in the huanglongbing (HLB)-tolerant *Microcitrusaustralasica* (Huang *et al.*, 2021). The antimicrobial peptide MaSAMP, which is present in the plant's phloem, was one of the potential gene product regulators. Similar to BP178, the peptide's anticipated structure consists of two amphipathic a-helices joined by a hinge, with the helix-2 domain serving as the bactericidal motif.MaSAMP is bactericidal, inhibits Ca. *Liberibacterasiaticus* infections, and strengthens the citrus host's defences.

Plant growth-regulating peptides

Plant hormones that facilitate intercellular communication during development, such as auxin, cytokinin, and gibberellin, have an impact on plant growth and development. But according to

recent research, peptide signal molecules are also crucial for a variety of plant development processes and environmental reactions, including meristematic stem cell differentiation, tissue and organ formation, fruit maturation, abscission, and biotic and abiotic stress adaptation (Chen *et al*., 2020). These peptides' precursors undergo processing in plants to become mature peptides, which subsequently interact with plant receptors and trigger downstream signal pathways to produce growth responses. PGRPs have a variety of roles in the growth and development of plants.
For instance, TIBO Crop Science discovered the functional peptide PY91 in 2021, which hinders crop growth. Meristem size is regulated by the CLAVATA3 peptide (Lay *et al.,* 2005). Cruciferous pollen's self-incompatibility is recognised by the SCR peptide (Fletcher *et al.,* 1999). A family of peptides known as RALFs is involved in the proliferation of plant cells (Okuda *et al.,* 2006).

Peptides of four different kinds have been employed as commercial plant growth regulators. The KEYLAN range of natural products, which includes KEYLAN Ca, KEYLAN Combi, KEYLAN Fe, KEYLAN Max, KEYLAN Mn, and KEYLAN Zn, has been developed by Italy Hello Nature (https://www.hello-nature.com/us/). These goods function as biostimulants and offer micronutrients in a biochelated form. KEYLANS are employed in hydroponic farming or soil fertilisation to prevent and treat malnutrition. These products can be used across a wide range of soil pH levels, have good stability and water solubility, and can be safely combined with other calcium foliar fertilisers, growth regulators, adjuvants, insecticides, fungicides, and biocontrol protectants.

The active component of the commercial product Tandem, created by Italy Hello Nature, is the plant-derived peptide LRPP (https://www.hello-natur e.com/us/), which is also a biostimulant. It is a potent biostimulant that increases resilience to environmental stressors such poor soil, drought, and extremes in temperature. In order to establish a more intimate and advantageous interaction with seeds, this product is utilised at the sowing stage.

The active component of PHC-91398, which was created by PHC (https://www.planthealthcare.com/), is the peptide 91,938. As a growth regulator, it promotes growth, metabolism, and natural plant defences, protecting against nematodes and bacterial and fungal infections.Foliar spraying and seed treatment are suggested applications.

It has been demonstrated that Hicure® (https://www.syngenta.com/en), a natural biostimulant with exceptional efficacy and adaptability that contains readily absorbed peptides and amino acids, improves plant quality and increases resilience to environmental stress. To get the greatest results, this product is used as a traditional spray or maceration solution prior to important developmental stages, pot changes and transplanting, environmental stress, or transportation. Hicure® works with the majority of fertiliser and plant protection products and doesn't require specialised equipment.

Mechanism of action
Internal cell functions are affected by a class of antimicrobial peptides (Le et al., 2017) that can enter the target cell, sometimes rupturing the membrane, and disrupt the synthesis of proteins or nucleic acids, cell division, or proteinases. This is true for the antifungal PAF26 (Munoz et al., 2013), cathelicidins (which block translation, replication, and ion channels), and magainins (McMillan & Coombs., 2020), which impact DNA synthesis and metabolic activities in bacteria

and fungus. However, some cell-penetrating peptides (CPPs) have been used to deliver cargo molecules to the cytoplasm of eukaryotic cells (plant, fungal, and human), such as BP100 to BY2 tobacco cells (Eggenberger et al., 2011) and BP16 to human tumour cells (Soler et al., 2014), or enhance the uptake of RNAi in plant cell transformation (Numata et al., 2014). These CPPs do not interfere with intracellular processes or break down cell membranes. A 10-nucleotide oligomer that targets the regulator gene acpP involved in the fatty acid biosynthesis in E. amylovora is one example of how other CPP, such as peptide nucleic acid (PNA) conjugates, enter plant pathogen cells and target specific genes (Patel et al., 2017).

Plant pathogens are impacted by functional peptides through a number of ways.

- They disorganize cell membranes and promote cell lysis by causing holes in cell membranes.
- Interfere with the production of nucleic acids, cell division, and cell-penetrating peptides, among each other's internal biological functions. This is the case with magainins, which have an impact on the synthesis of DNA and the metabolic activities of fungi and bacteria.
- Interact with extracellular structures like chitin in fungus and lipopolysaccharides, fimbriae, or flagella in bacteria.
- Prevent the production of biofilms and other bacterial colonisation structures.
- Alter the outer coat or behaviour of plant-pathogenic nematodes.
- Prevent the attachment or replication of viruses.

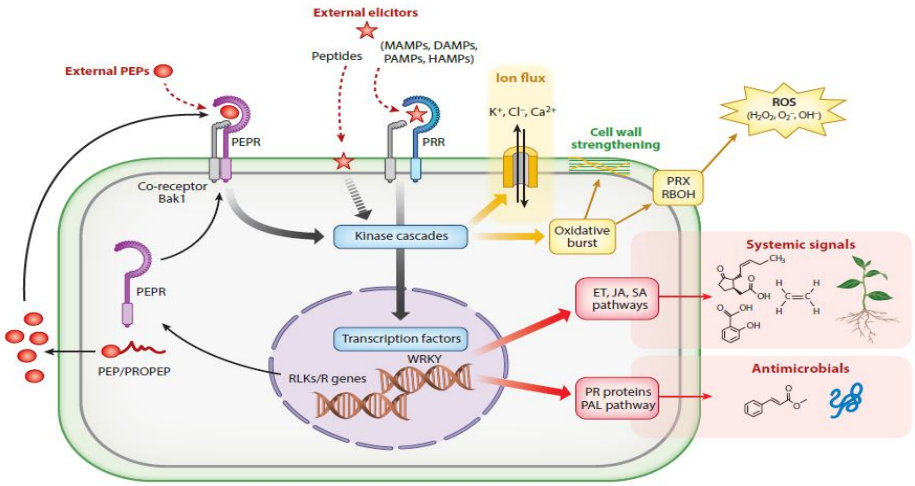

Fig 1: Impact of functional peptides on plant pathogens
Source: Emilio Montesinos ,Annu. Rev. Phytopathol. 2023. 61:301–24

AMPs in Biocontrol Agents

The ability of certain microorganisms to prevent bacterial and fungal plant diseases has been linked to the generation of AMPs. Nevertheless, the papers only show compelling evidence linking them to the genetically engineered biocontrol mechanism in a small number of cases. By analysing defective mutants incapable of producing fengycin and bacillomycin D, as well as structural and functional characterisations of gene clusters involved in their production, cyclic lipopeptides have been implicated in providing Bacillus amyloliquefaciens FZB42 with the ability to control F. oxysporum(Koumoutsi et al., 2004).The antimicrobial metabolites known as

lipopeptides are made up of fengycin, iturin, lichenycin, and surfactin. These compounds can damage the fungal membrane and compromise cellular integrity (Romero et al., 2007). Bacillus subtilis 155 produces cyclic lipopeptides and fengycin, which can harm rice membranes and prevent M. grisea hyphal development (Zhang and Sun, 2018).

Transgenic plants expressing AntimicrobialPeptides

Gene constructs containing AMP-coding sequences have been expressed in model or agricultural plants, offering varying levels of defense against plant diseases. Several plants express the genes encoding animal defensin. Rice-expressed cecropins A and B provide defense against *Magneporthegrisea*(Coca et al., 2004) and *Xanthomonas oryzae* (Sharma et al., 2000), Magainin, which is found in tobacco, offers defense against a variety of bacteria and fungus (De Gray et al., 2001), and potato-expressed tachyplesin from crab proved helpful against *E. carotovora*infections (Allefs et al., 1996). Tobacco-expressed insect defensins, heliomicin and drosomycin, provide defence against *B. cinerea*. (Banzet et al., 2002), and the fruit fly sarcotoxin found in tobacco offered protection against *E. carotovora ssp. carotovora* and *Pseudomonas syringaepv. Tabaci*(Ohshima et al., 1999). Plants have also been shown to express plant defensins. Tobacco and tomato express the radish defensin Rs-AFP2, which provides defense against *Alternaria longipes*(Terras et al., 1995), Alf-AFP Potato-expressed lucerne defensin guards against *V. dahliae*(Gao et al., 2000), Tobacco-expressed SPI1 spruce defensin guards against *Heterobasidiumannosum*. (Elfstrand et al., 2001)

Exploring and developing peptides to control plant diseases

Living organisms present excellent opportunities for the discovery of peptides, such as lactoferricin B, which is produced by acidic-pepsin hydrolysis of the lactoferrin found in cow's milk, or native chemicals, which are acquired by further hydrolysis from functional proteins (Tomita *et al.*, 1991). The foundation for creating analogues or newly created compounds that can be chemically synthesised to create peptide libraries is the understanding of the chemical structure, physico-chemical characteristics, and biological characteristics of natural peptides. The methods for producing analogues include using specific motifs (at the end, in chain, or repetitive sequences) such the ATCUN, Rana box, and LPS binding gamma-core motifs, as well as chemically altering existing compounds (e.g., halogenation, cyclisation, capping, conjugation) (Mueller *et al*., 2020 and Thayer 2011). Tandem repeating sequences, cyclisation, or the addition of certain end sequences or amino acids (such D-amino acids) are examples of de novo peptide design. For the purpose of controlling plant diseases, a wide range of de novo designed cyclic peptides have been created . One such cyclic peptide, BPC194, which belongs to a cyclic decapeptide library, has strong antibacterial activity (Monroc*et al.*, 2006). Following this phase of producing peptide libraries, the compounds are put through an in vitro screening platform that evaluates their preliminary toxicity (haemolytic activity, phytotoxicity), stability under harsh physico-chemical conditions, susceptibility to protease hydrolysis, and antimicrobial activity (growth inhibition, killing assays). Microbial growth analysers or viability methods (e.g., SYTOX green, resazurin, v-qPCR) can be used to examine fungicidal or bactericidal characteristics or to perform inhibition experiments that target plant-pathogenic bacteria or fungi (Baro *et al.*, 2020).

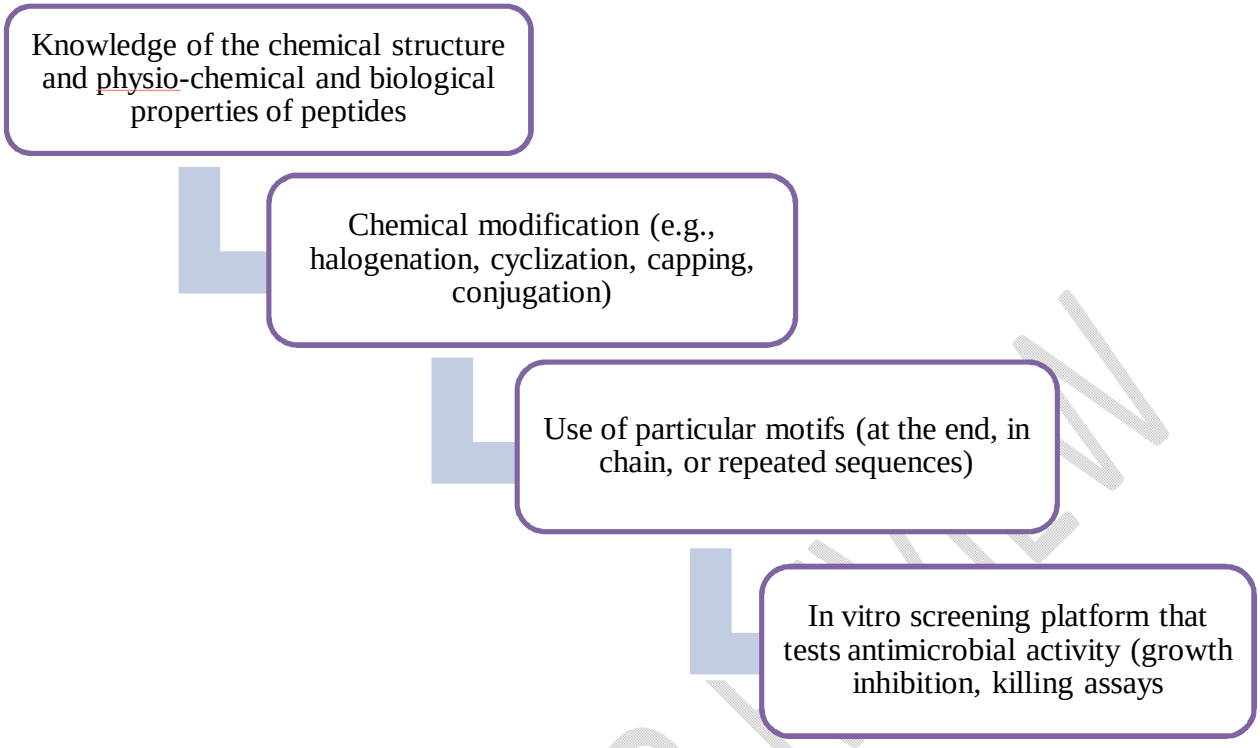

Fig 2: Steps showing exploration and development of peptides to control plant diseases

Large – scale Production of Functional Peptides

For in vitro screening, small amounts of peptides (milligrammes, for example) are needed; whereas, moderate-to-high quantities (grammes, for example) are needed for plant assays or even field testing. Functional peptides' potential as plant-protection compounds mostly relay on their ability to be produced in large numbers using industrial platforms. Peptides can be synthesised chemically, acquired directly from natural sources, or expressed heterologously in live biofactories.

Natural Sources

Natural sources often include low amounts of peptides. Because it produces a significant number of by-products (such as blood, whey, etc.) that include peptides and proteins that can be processed either directly or by enzymatic digestion, the food sector can be a valuable source of peptides.(Sánchez A et al., 2017, Meneguetti BT et al., 2017). Peptides can be more prevalent in microbial fermentations; for example, nisin produced at 100–300 mg/L in fed-batch or batch fermentation reactors by enhanced strains of *Lactococcus lactis* (Klelissa S et al., 2021, Klausmann P et al., 2021) or surfactin in B. subtilis 3NA, which produced yields that were exceptionally high at 26.5 g/L.(Cheng Q et al., 2018)

Chemical Synthesis

For the purpose of producing many peptides for medical use, large-scale chemical synthesis based on O-ring solid phase or liquid phase synthesis has been established (Andersson L et al., 2020 , Mueller LK et al., 2020 , Thayer A. et al., 2011). Chemical synthesis works better in the

pharmaceutical industry, where high-value goods are more dependable, than it does in agriculture, where plant protection calls for less costly products.

Biotechnological Production

The pharmaceutical industry has made extensive use of the relatively well-developed method of producing peptides by heterologous expression in biological systems (biofactories). This method yields linear peptides made of proteinogenic amino acids by ribosome synthesis.(Parachin NS, Mulder KC, Viana AA, Dias SC, Franco OL. 2012.) Though progress has been made in cloning biosynthetic gene clusters, biotechnological production of nonribosomallysynthesised peptides (e.g., CLPs, peptaibols) is less advanced (165). One such instance is the cloned and inserted bacillomycin NRPS cluster from B. amyloliquefaciens FZB42 for heterologous expression in B. subtilis.(Liu Q, Shen Q, Bian X, Chen H, Fu J, et al. 2016)

Peptide-based Agrochemicals: Prospects

Optimising performance for formulation and structure

Enhancing the bioavailability and stability of naturally occurring peptides is crucial for the development of novel peptide-based medications and agrochemicals. Optimising the structure and formulation of natural peptides can result in more palatable peptides or their mimics. Enhancing the delivery method can potentially produce peptide products with increased bioavailability.

Optimisation of structures

Natural peptides have poor stability and limited activity, hence several structural optimisation techniques, such as amino acid replacement, cyclisation tactics, mimic design, etc., have been developed to get around these problems.(Mora I, Cabrefiga J, Montesinos E. 2015.)(Badosa E, Moiset G, Montesinos L, Talleda M, Bardají E, et al. 2013.). Genetic engineering can be used to alter naturally occurring peptides to create new peptides with desired characteristics. For instance, the natural spider venom peptide ω/κ-HXTX-Hv1a was genetically engineered to include a glycine-serine dipeptide, leading to the development of the bioinsecticide Spear®. This product is regarded as a sustainable and efficient green tool for pest control in agriculture and public health since it has greater activity, lower risk, and more persistence than the natural product.

Formulation

The generation of distinct formulations, such as microemulsions, suspension agents, and capsule suspensions, can shield peptide molecules from environmental deterioration caused by elements including water, sunlight, temperature, and metabolic enzymes. This will also improve stability of functional peptides.

Challenges

“The effectiveness of functional peptides as plant-protection products is hampered by a number of challenges. Plant pathogen populations' resistance to antimicrobial peptides is a significant problem. The peptide's interaction with the target plant-pathogen cell may be hampered by a number of mechanisms, such as adsorption by envelopes or external structures (biofilm barriers,

exopolysaccharides, capsules), active removal from cells (e.g., efflux pumps, secretion of outer membrane vesicles), protease degradation, or enzymatic chemical modification" (Lima et al., 2021). A number of physicochemical factors, including as cations, pH, and phenolics, might decrease activity; these factors are especially significant for cationic amphipathic peptides.

"Peptides have been effectively employed in plant protection, however due to drawbacks such poor oral efficacy, limited systemic stability, and expensive production costs, they continue to confront a number of difficulties. Natural peptides often have low stability and low bioavailability because they are quickly broken down by the body's enzymes and impacted by external environmental factors like pH and light. Peptide insecticides that are too expensive will not be widely accepted in the commercial sector, in contrast to peptide-based medications. Undoubtedly, these challenges can be lessened by altering the peptide to include non-natural amino acids (like D-amino acids)" (Ng-Choi et al., 2014) or by using a suitable formulation (like nanoencapsulation), but these solutions always make the process of development and manufacturing more difficult.

Another significant obstacle is how to express or distribute the peptides into the plants. It appears that this method is more dependable than topical treatments given the large number of reports addressing the heterologous expression of peptides in plant crops; however, further research is needed to determine its effects on food safety and the environment, and its application to genetically modified self-protected plants may be restricted in some countries. High concentrations of peptides (e.g., kg/ha) are needed for the traditional spray or soil drench techniques of applying plant protection agents in agriculture. Endotherapy could be a viable option for trees, particularly when it comes to vascular system disorders like those brought on by *Xylella fastidiosa* (citrus variegated chlorosis, sudden death syndrome of olives, and leaf scorch of almonds) and Candidatus*Liberibacterasiaticus* (Citrus HLB).

"One of the primary concerns is the cost of producing peptides for plant protection. Solid-phase chemical synthesis, which is frequently used for research or high-value products (pharmaceutical, for example), is too costly. Using mixtures of randomly synthesised peptides has been suggested as a way to lower the cost of chemical synthesis for agriculture" (Topman et al., 2008); however, this method produces combinations in which not all of the components may be active. A crude undecapeptide production in chemical synthesis currently costs several hundred dollars per gramme, and the price goes up for larger peptides. Evaluation by the regulatory framework (such as the FDA in the US and the EFSA in the EU) is the final obstacle for peptides to be unique active compounds for creating plant-protection products. Given what we already know about peptides, it stands to reason that some of the more sophisticated ones will be able to satisfy the requirements for low-risk substances.Therefore, improved stability, increased bioactivity, and reduced cost are necessary for peptide-based agrochemicals to be considered acceptable.

Summary

Potential biopesticides for use in next plant protection products are functional peptides. Peptides work against plant diseases and pathogens through a variety of methods of action, such as inducing plant defence and antibacterial activity through many routes. It is possible to synthesise

functional peptides with many mechanisms of action at once, or to employ them as cell-penetrating peptides to help pathogens and plant cells reach their intracellular targets. Functional peptides produced by ribosomal synthesis are expressed heterologously in plants, providing excellent defence against pathogen infections. Large-scale peptides can be produced chemically, naturally (from food industry by-products, for example), or through microbial fermentations and heterologous expression in living biofactories (plants, algae, and microbes).

Concluding remarks and future prospects

Similar to the pharmaceutical industry, functional peptides have the potential to be very important plant protection products in agriculture. Commercial development of functional peptides as biopesticides derived from various microbes secreting these chemicals has led to the successful usage of these compounds. Despite the development of many transgenic plants producing AMPs that offer varying degrees of disease resistance, commercial cultivars have not been released into the market due to social and legal constraints. Strong tools to optimise molecules generated from natural chemicals with enhanced activity against specific target pathogens, such as lower cytotoxicity and increased protease stability, are provided by synthetic procedures to synthesise functional peptides led by combinatorial chemical methods. Nevertheless, it has not yet been possible to utilise the large number of peptides as pesticide active components. Only a small number of functional peptides with potential applications are commercially available, and the bulk have only been investigated in vitro. Fewer molecules have been examined in plant pathosystems. There are various obstacles in the way of developing compounds that are ideal for use as pesticide ingredients in agriculture. These include the inherent toxicity and low stability of certain compounds, the necessity to create appropriate formulations, and the demand for low-cost plant protection solutions. Thus, future research priorities include creating chemicals that are less hazardous and more stable as well as lowering production costs through enhanced biotechnological processes and preparative synthesis that makes use of microbial systems or transgenic crops as plant factories.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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