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Review

Host-Defense Peptides as New Generation Phytosanitaries: Low Toxicity and Low Induction of Antimicrobial Resistance

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Abstract: Host-defense peptides (HDP) are emerging as promising phytosanitaries due to their potency, low plant, animal and environmental toxicity, and above all, low induction of antimicrobial resistance. These natural compounds, which have been used by animals and plants over millions of years to defend themselves against pathogens, are being discovered by genome mining, and then produced using biofactories. Moreover, truncated or otherwise modified peptides, including ultra-short ones, have been developed to improve their bioactivities and biodistribution, and also to reduce production costs. The synergistic combination of HDP and other antimicrobials, and the development of hybrid molecules have also given promising results. Finally, although their low induction of antimicrobial resistance is a big advantage, cautionary measures for the sustainable use of HDPs, such as the use of precision agriculture tools, were discussed.

Keywords: host-defense peptides; antimicrobial peptides; antibiotic; antibacterial; antifungal; phytosanitaries

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1. Introduction

The development of new-generation phytosanitaries, which combine potency with low toxicity and low environmental impact, is an urgent need. The FAO has claimed that the emergence of antimicrobial resistance is a major threat to food security, behind only climate change [1]. On the other hand, many effective phytosanitary products have been withdrawn or limited in their use due to their toxicity or environmental contamination [2–6]. Finally, new pathogen threats emerge continually, such as the huanglongbing (HLB) caused by the bacterium *Xanthomonas citri*, which has endangered citrus production worldwide in recent years [7].

To face this challenge, agrocompanies have been developing new products and strategies [5]. For instance, biopesticides (such as the use of *Trichomonas* sp. to fight fungal infections, and the use of natural plant or seaweed extracts) allow for more environmentally-friendly treatments [5,8]. A particular family of natural compounds has attracted much attention: the host-defense peptides (HDPs, also called antimicrobial peptides AMPs), which include plant systemins, as well as defensins, dermaseptins, cathelicidins, and temporins, among others [9–18]. These biomolecules have been used by animals and plants for millions of years to defend themselves against pathogens, [12] and remarkably, some structurally-related compounds (such as polymyxins or gramicidins) are produced by microorganisms to get rid of competitors or potential threats [19–22]. The host-defense peptides display a broad spectrum of action, induce little antimicrobial resistance due to their multiple modes of action, and moreover, often have a synergic effect with other antimicrobials [9–18].

Although HDPs present an important structural variety, they are usually short peptides (with less than 100 residues, and often from a few to 50 units) with an amphiphilic nature

and net cationic charge [12]. The right balance between cationic and hydrophobic residues is crucial for a potent antimicrobial activity and selectivity. In effect, due to their net positive charge, HDPs bind preferentially to bacterial membranes, which have a net negative charge because of their anionic lipids (cardiolipin, phosphatidylglycerol). After adsorption of the peptide to the pathogen surface, the HDP hydrophobic residues permeate the membrane, allowing the peptide to penetrate and carry out its antimicrobial action [21–26]. In contrast, eukaryotic membranes are neutral in character, due to the high content of cholesterol and zwitterionic lipids (e.g., phosphatidylcholine and sphingomyelin), and bind only weakly to HDPs [23–26]. In any case, antimicrobial peptides display a variety of mechanisms of action (Figure 1): membrane disruption, disturbance of cytoplasmic processes (such as cell wall, amino acid, protein and nucleic acid synthesis, and protein folding) [9–18] and blockade of bacterial biofilm formation [27,28]. This combination of stresses often results in microbial death. Due to the difficulty in adapting to simultaneous unrelated stresses, the emergence of resistance is much lower than with conventional antimicrobials [9–12].

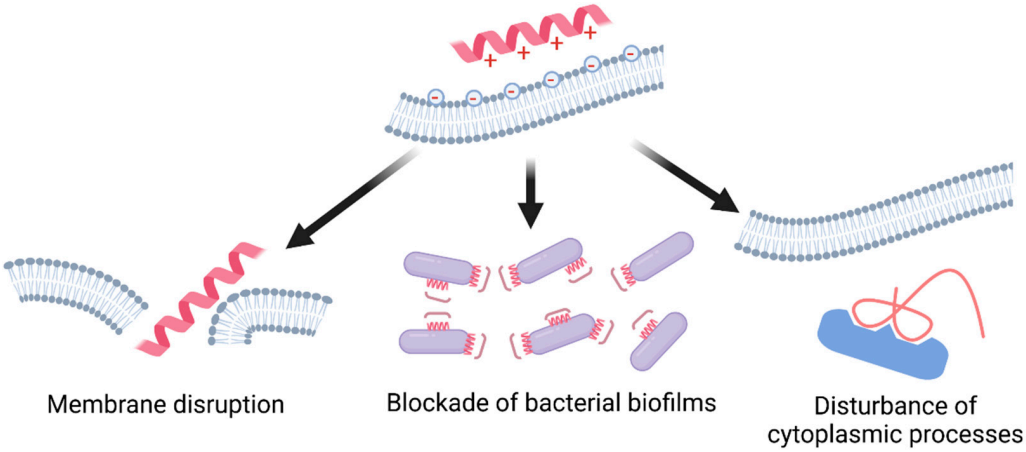

Figure 1. Multiple mechanisms of action of antimicrobial peptides. Figure created with BioRender.com (accessed on 27 June 2022).

Most applications of HDP in agriculture have been explored using transgenic plants [9–11]. In some cases, an endogenous peptide was overexpressed, as snakin-2 in tomato, which conferred protection against bacterial pathogens [29]. In others, a plant peptide was expressed in another plant, such as the radish defensin Rs-AFP2, which was used to protect tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*) from the fungus *Alternaria longipes* [30]. The defensins from petunia PhDef1 and PhDef2 were expressed in banana for protection against the fungus *Fusarium oxysporum*, which causes the lethal Panama disease [31]. A chili defensin was expressed in tomatoes, providing protection against different fungi [32].

Animal host defense peptides have also been expressed in plants. For instance, a cathelicidin derived from human LL-37 was expressed in *Brassica rapa* to protect it from bacterial and fungal diseases [33]. Cecropin A from the moth *Hyalophora cecropia* was expressed in rice (*Oryzae sativa*) to protect it against diseases caused by the fungi *Magnaporthe grisea* [34] and *Fusarium verticilloides*, and the Gram-negative bacteria *Dickeya dadantii* (the last two ones in seeds) [35]. Cecropin B from the same source was expressed in tomato (*Solanum lycopersicum*) for protection against the bacteria *Ralstonia solanacearum* and *Xanthomonas campestris* [36]. Interestingly, human-designed peptides have also been used, and thus, Company et al. have expressed the peptide BP 100 and their analogs in rice (*O. sativa*) to protect the plant against bacterial and fungal pathogens [37,38]. Many other examples including the expression of defensins, dermaseptins, magainin, heveins, temporins, and thionins among others have been reviewed [9–11].

However, the use of transgenic plants creates problems. In some cases, peptides that are effective in vitro lose their antimicrobial activity in planta [39–41], probably because

non-native peptides are rapidly degraded by the plant proteases [42,43]. For instance, the peptide from *Mirabilis jalapa* MjAMP2 inhibited *Botrytis cinerea* growth in vitro, but when the peptide was expressed in transgenic tobacco, the plant was not protected against the fungus [44], likely due to in vivo degradation. The cellular localization of the peptide also influences its effectiveness [45].

Silencing can also block AMP expression [46] and moreover, it can be transmitted to plant descendants. Thus, RNA-directed DNA methylation happened in transgenic tobacco plants, resulting in more than 200-fold down-regulation of AMP production after one plant generation [47].

Another problem is that the expression of AMPs can also consume plant resources or interfere with other cellular processes, and therefore alter plant development. Thus, when potato plants expressed the AMP msrA3 (an engineered temporin A analog), they were protected against a broad spectrum of fungi and bacteria [48,49], but the vegetative phase was extended and the floral phase retarded [49].

However, the main drawback is public concern or open rejection to transgenic plants [50] and the fact that many countries have restrictions for their use [51–54]. There is a risk that engineered genetic material can be transmitted to normal plants due to horizontal gene transfer or to cross-pollination [55]. Thus, transgenic corn developed by ProdiGene Inc. cross-pollinated nearby corn fields, whose production had to be destroyed [56]. Moreover, this corn contaminated soybean plots in Nebraska; a useful discussion is presented in a review by Oz et al. (2015) [9].

An alternative to the use of transgenic plants is the development of AMPs as the active principle of phytosanitary products. However, although much research has been devoted to in vitro screenings of AMPs as potential antibacterial or antifungal agents (as shown in several reviews [9–11]), the reports on their use to protect crop plants are scarce. In part, this may be due to commercial concerns about their cost of production and stability in field, and in some cases to other problems, such as insufficient hydrosolubility or target selectivity [9–12]. Therefore, some modifications have been implemented to overcome these problems, in many cases inspired in AMP research for veterinary or human biomedicine. Thus, short or ultrashort analogs of HDPs have been prepared to decrease production costs, and the half-life has been increased using non proteinogenic units such as D-aminoacids, or using cyclized peptides [12]. The hydrosolubility and selectivity can be tuned by attaching the peptides to other groups [9–11]. This minireview tries to cover the most recent applications of HDPs and their analogs to crop plants and postharvest protection.

2. HDP as the Active Principle of Phytosanitary Products for Crop Protection

Some HDPs for use in agriculture have been obtained from natural sources, in some cases using bioinformatics tools to detect "cryptic" peptides codified in animal or plant genoma. For instance, Kishi, Machado et al. (2018) screened databases of citrus sequences to identify putative antimicrobial peptides, and also reviewed the literature to select other AMP candidates against citrus canker produced by *Xanthomonas citri* subsp. citri (*X. citri*) [57]. Six peptides from citrus, amphibian and mammal (pig) sources were selected for the preliminary in vitro screenings. The in vivo assays were performed on one-year *Citrus sinensis* (L.) Osbeck grafted on *Citrus limonia*. The leaves were infected with *X. citri* and some were treated with the peptides. After seven days, only the positive control plants showed canker symptoms, and although at 14 and 21 dpi the symptoms (and the bacteria) appeared in the plants treated with citrus-amp1 and the amphibian K0-W6-Hy-a1, the damage was less severe than in the positive control. With other peptides, such as the amphibian ocellatin4-analogue and Hylin-a1, and the pig HDP tritrpticin, no canker symptoms were observed, and no bacterial growth was detected either (X.citri::GFP assays). Unfortunately, ocellatin and Hylin-a1 displayed significative hemolytic activity, although triptrpticin, citrus-amp1 and citrus-amp2 presented much lower hemolytic properties. In fact, these peptides were not toxic to the animal model *Galleria mellotia* (wax moth) [57].

A cationic alpha-helix antimicrobial peptide (ZM-804) from maize (*Zea mays*, line B73) was discovered by in silico screening a cDNA library, and then synthesized and tested against eleven pathogens of Gram-negative and Gram-positive species, displaying a high antimicrobial activity [58]. Thus, it was observed by SEM and TEM microcopy that the peptide disrupted the cell membrane of *Clavibacter michiganensis subsp. michiganensis* and *Pseudomonas syringae* pv. *tomato* (Pst) DC3000. Besides, when the peptide was sprayed on tomato leaves, it prevented the infection by Pst DC3000. Moreover, low concentrations of ZM-804 (2–0.5 μM) blocked the hypersensitive response (HR) in tobacco leaves under attack from virulent bacteria such as *Ralstonia solanacearum*, *Pseudomonas syringae* pv. *syringae* and pv. *tomato*, and *Erwnia amylovora*. In addition, ZM-804 displayed low hemolytic activity against mouse red blood cells, making it a promising lead for new phytosanitaries.

Recently, the 3D-structure of a 36-amino acid antimicrobial peptide found in the nodules of model legume *Medicago truncatula*, the nodule-specific cysteine-rich NCR044 [59] was reported. This NCR peptide was then produced biotechnologically in the fungus *Pichia pastoris*. It presented a highly dynamic structure composed of four alpha-helices and one beta-sheet, stabilized by two disulfide bonds. NCR044 displayed a potent activity against many fungal phytopathogens, including *Botrytis cinerea* and three *Fusarium* spp. The peptide inhibited spore germination of *B. cinerea*, and in the germlings, it penetrated the cell membrane and accumulated in the cytoplasm and nucleoli, inducing ROS generation. When the peptide was sprayed on tomato and tobacco plants infected by *B. cinerea*, it significantly decreased symptoms of gray mold disease. It also conferred resistance to *B. cinerea* in lettuce leaves, even at doses of 6 and 12 μM NCR044. Remarkably, it was also useful in postharvest products. For instance, in a rose petal infection assay, it reduced virulence of *B. cinerea* with respect to non-treated petals. An almost total suppression of disease symptoms was reported at a concentration of 1.5 μM peptide.

Another example is the protection provided to geranium plants and leaves by the HDP cecropin and an *Aspergillus* antifungal protein (AFP) against the pathogenic fungi *Botrytis cinerea* [60]. AFPs are small, cationic, and cysteine-rich proteins, and therefore resemble some antimicrobial peptides. While the authors mostly described the promising results obtained with AFP, they also commented that cecropin A was active as well, and that a synergistic effect between AFP and cecropin was observed. Garriguea et al. also reported antifungal proteins from *Penicillium expansum* [61]. PeAfpA was the most effective, protecting tomato leaves against the infection caused by *B. cinerea*, and protecting oranges against postharvest decay caused by *Penicillium digitatum*. In addition, this peptide was not cytotoxic, and did not produce hemolysis. Therefore, it could be useful for crop protection and food preservation.

The natural peptides have also been modified in different ways, either to reduce production costs, or to improve some properties, such as solubility, potency or field stability. The simplest modification is truncation, where a section of the peptide is detached, retaining its antimicrobial properties.

For instance, several truncated lactoferricin derivatives were prepared: LfcinB (20–25), which contains six amino acid residues believed to be the active core, and LfcinB (17–31), which contains 15 amino acids [62]. Both peptides were active against several phytopathogenic fungi in the in vitro tests, with LfcinB (20–25) killing conidia more efficiently and Lfcin (17–31) inhibiting growth better. These truncated HDPs were assayed as postharvest protectors, using wounded mandarin fruits (*Citrus clementina Hort. Ex Tan*) infected with *Penicillium digitatum*. A significant decrease in the percentage of infected wounds when mandarins were treated with the peptides was observed. In general, both peptides displayed similar activity, although in some experiments, LfcinB (20–25) had lower disease incidence.

In other cases, synthetic peptides are prepared de novo. Interestingly, some small or even ultra-short peptides have displayed a potent antibacterial or antifungal activity.

In a library of amphipathic helical peptides, presenting different spatial distributions of the positive charges, the peptides with a pattern "BBHBBHHBBH" (where B was a

cationic residue and H was a hydrophobic unit) possessed potent bactericidal and fungicidal activities for many plant pathogens [63]. When a long acyl chain (fatty acid) was attached to the N-terminus, plant protection was increased. The most active peptides had an *N*-terminal *N*-myristoyl tryptophan unit, while the remaining sequence contained lysine and leucine residues. The *N*-acylated peptides were applied on detached leaves of tomato and leaves of intact Arabidopsis plants infected by *Pectobacterium carotovorum subsp. carotovorum* or *Botrytis cinerea*, displaying a promising protection against these pathogens.

Another example is the use of ultrashort cationic lipopeptides for the inhibition of fungal and bacterial plant pathogens in vitro and in planta [64]. In a preliminary work the authors reported new ultrashort lipopeptides (fatty acid-KXXK-NH_2, where X = L, A, G or K) with antimicrobial activity. Their mode of action against bacterial and fungal phytopathogens was studied. At low micromolar concentrations, the peptides caused the lysis of the microbial membrane. The best results in vitro were obtained with C14-K-L-l-K-NH_2 which was selected for in planta studies. A potent antifungal activity in planta was observed for cucumber fruits and leaves infected with the fungus *Botrytis cinerea*, and for corn seedlings infected with *Cochliobolus heterostrophus*. Necrotic lesions were avoided spraying cucumber and corn tissues with the peptide. A fast, effective antibacterial effect against *Pseudomonas syringae* infecting the leaves of *Arabidopsis thaliana* was also reported. The authors pointed out that unlike many native lipopeptides, these ultra-short ones did not generate phytotoxicity.

An interesting application used nanoparticles of a novel cationic peptide P5VP5 (Ac-RLIRKVKRILR-NH_2) against citrus pathogenic bacteria [65]. The peptide underwent self-assembly, generating nanoparticles with a high thermal stability. The P5VP5 nanoparticles displayed a potent antibacterial activity against *Xanthomonas axonopodis* pv. *citri* with a MIC value of 20 μM. Meanwhile, freshly detached citrus leaves were treated with the peptide nanoparticles and then inoculated with the pathogen. Citrus canker lesions were greatly reduced with respect to control. In addition, the nanoparticles could disrupt biofilm formation, and then act on the free bacteria damaging their membranes.

An important advantage of HDPs is their ability to synergically interact with other antimicrobials. These combinations can reduce the required doses of both compounds and also render results that are not achieved separately.

For instance, the synthetic linear peptide BP100 (KKLFKKILKYL-NH2) was used to treat *Erwinia amylovora*, the causal agent of fire blight, an important disease of rosaceus plants [66]. The peptide displayed a promising activity in vitro and low toxicity. However, when the peptide was tested in planta (e.g., pear and apple flowers), a relatively high concentration was required due to inactivation by plant tissues or epiphytic microorganisms. This significantly increased application costs and rendered the product non-competitive. To overcome this problem, BP100 was combined with lysozyme, and as expected, a synergistic effect was observed. The combination increased cell membrane damage and reduced cell metabolism, decreased the time for cell death and the minimal inhibitory concentration (MIC). The in vitro results were supported by pear leaf infection studies. Thus, wounded pear leaves were treated with the peptide mixture, and after 1 h, with the pathogen. While individual lysozyme or BP-100 (at 100 μM) did not show differences with the control after 5 days, a combination of 25 uM BP100 and lysozyme greatly reduced disease severity. Therefore, peptide combinations could be the answer for economic treatments.

The same group also discovered other short peptides using combinatorial approaches, which have displayed activity not only against *Erwinia amylovora* but also against other bacteria (*Pseudomonas syringae* pv. *syringae*, *Xanthomonas axonopodis* pv. *vesicatoria*) and fungi (*Penicillium expansum*) [66,67]. Their combination with lysozyme and other peptides could boost their activity and provide new phystosanitary candidates.

A recent report described the activity of three peptide mixtures against three strains of *Erwinia amylovora* with different genotypes and virulence (LMG 2024, Ea 630 and Ea 680) [68]. This pathogen causes the fire blight disease that affects different plants, including fruit (pear, apple) trees. The AMP mixtures gave better results than the individual AMPs.

Assays with tobacco plants showed that the most promising AMP mixtures caused low or negligible hypersensitive response. When CFU plate counting was performed after the HR assay, and 24 h after pathogen inoculation, it was observed that for peptide mixture R:C no viable cells of strains LMG 2024 and Ea 680 were isolated. In the other cases, a significant decrease of viable cells was observed.

A previous, related work studied two mixtures formed by 20-mer-peptides generated by random combination of L-phenylalanine and L- or D-lysine (FK-20 and FdK-20, respectively) [69]. Both mixtures displayed potent bacteriostatic and bactericidal activities against bacteria from the genus *Xanthomonas*, *Clavibacter* and *Pseudomonas*. In studies carried out in glasshouse, the RPMs significantly decreased disease severity of tomato plants infected with *Xanthomonas perforans*. Similar protection was also observed for kohlrabi plants infected with and *Xanthomonas campestris pv. campestris*. Moreover, RPM had similar protective effects as commercial Kocide 2000, a copper-based bactericide which needed a concentration 12-fold higher than the RPM treatments. In addition, both RPMs were not toxic to bees or Caco-2 mammalian cells. This study demonstrates the potential of peptide mixtures as crop protection agents against bacterial phytopathogens.

Synergies between peptides and other antimicrobials have also been reported. Fragments of a wheat hevein-like peptide increased the inhibitory effect of the triazole fungicide Folicur® on spore germination of pathogenic fungi [70]. In a preliminary work, it was reported that short peptides derived from the central, *N*- and *C*-terminal regions of the peptide WAMP-2 worked sinergically with fungicide Folicur EC250® to inhibit spore germination of *Fusarium ssp* and *Alternaria alternata* [71]. Then they synthesized other WAMP-2 derivatives and studied their combination with Folicur® to increase the fungicide action against *Fusarium oxysporum* and *Alternaria solani*, which cause wilt an early blight of tomato, respectively. The synergic action was confirmed, inhibiting conidial germination at much lower doses than required for the fungicide alone. The inhibition was studied on tomato leaves and seedlings, showing that the C-terminal oligopeptide WAMP-C was the most efficient sensitizer of *F. oxysporum*, while the central peptide WAMP-G1 gave promising results against *A. solani*. No phytotoxicity was observed for the selected peptides.

An alternative strategy to the use of peptide/antimicrobial mixtures is the use of peptide hybrids. The hybrids combine the structural features of several antimicrobial peptides, selecting the most active fragments for binding.

Thus BP21 (Ac-FKLFKKILKVL-NH_2), a Cecropin A-melittin hybrid peptide, was used for the post-harvest control of green and blue molds in citrus fruits, as well as sour rot [72]. With MICs in the micromolar range (8 μM for *Penicillium digitatum* and *P. italicum*, and 4 μM for *Geotrichum candidum*), BP21 caused irreversible damage to the membranes and cytoplasm and made the mycelia collapse. However, BP21 also displayed dose-dependent hemolytic activity, which should be taken into account for potential use to control citrus post-harvest diseases.

Finally, in the chimeric protein Hcm1, the hypersensitive response (HR)-elicitor Hpa1 of *Xanthomonas oryzae pv oryzicola* was attached to the active domains of antimicrobial peptides cecropin A and melitin [73]. This protein was expressed in engineered *Escherichia coli*, and was able to retain both the HR-induction effect and the antimicrobial properties. The compound was tested in tobacco (against tobacco mosaic virus), rice (against *Magnaporthe oryzae*, which causes rice blast) and tomato (against *Ralstonia solanacearum*, that causes bacterial wilt). In the first case, the number of necrotic spots in leaves was measured, while in the second case the necrotic area per leaf was determined. In addition, plant wilting was measured in tomato plants. In all the cases, Hcm-1 induced resistance against the infections. In tobacco and rice, the necrotic area was halved, and the pore number was reduced to a third (inhibition ratio 46% for tobacco leaves and 47% for rice leaves). In tomato stems, the inhibition ratio was about 39%. It is clear that the production of this hybrid antimicrobials would be a promising study topic in the next future.

A summary of the antimicrobial peptides and their in vivo applications is shown in Table S1 in the Supporting Information.

3. Induction of Antimicrobial Resistance: Is It Really Negligible? Cautionary Measures

As commented before, one of the great advantages of HDP as phytosanitaries is their multiple mechanism of action, which results in a very low induction of antimicrobial resistance compared to that induced by traditional phytosanitaries. Many HDPs act by disruption of the cell membrane; for the microorganisms, changing the composition of the cell wall is very costly in evolutionary terms. Due to this, many HDPs have remained unchanged –and unchallenged- for millions of years. But we must not forget that particular HDPs are located in limited places, and not distributed widely in the environment. Could a massive use of HDPs in agriculture or farming create resistances? [74]. Studies to address this subject have been developed mainly in the biomedicine field using animal models. However, these studies show that microorganisms can indeed develop some mechanisms of resistance [74,75]. As shown in Figure 2, a possible mechanism is to secrete extracellular proteases, as done by *Salmonella enterica* to resist helical cationic AMPs [76]. Other strategies are to release binding molecules as 'decoys' (such as negatively-charged membrane mimics, or hydrophilic bacterial polymers) [75]. Some microorganisms are even reducing their membrane anionic charge to decrease recognition by cationic AMPs, or using efflux pumps to drive them outside [77–80].

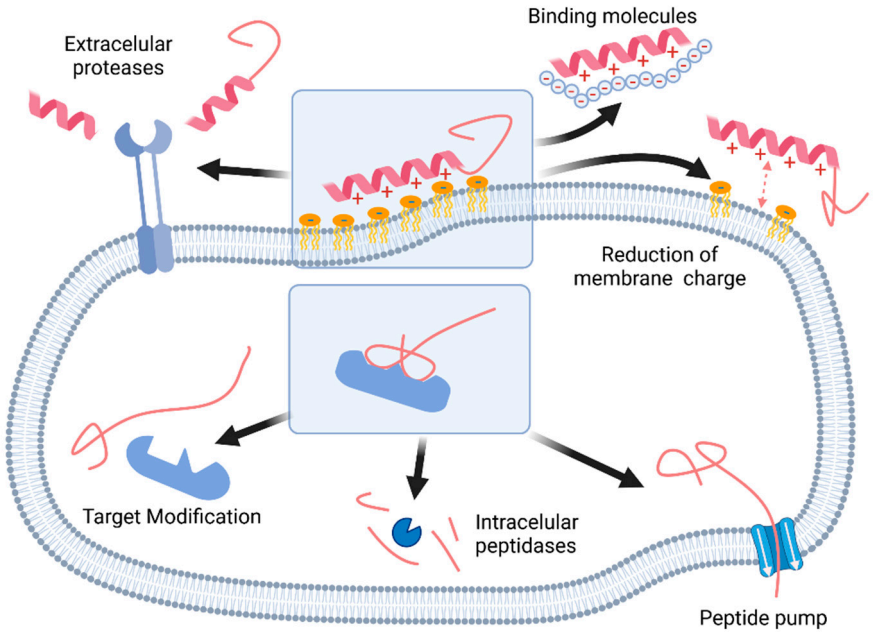

Figure 2. Mechanisms of resistance to host-defense peptides. Created with BioRender.com (accessed on 27 June 2022).

When the AMP penetrates into the cell, other resistance mechanisms may appear. The first is the production of intracellular peptidases (as done by *E. coli* to cleave proline-rich AMPs) [81]. The change of intracellular targets is sometimes possible [75].

Although these mechanisms of resistance take a long time to appear, it is sensible to prevent their apparition taking some measures. In order to render proteases inefficient, the AMPs may contain unusual or D-amino acids [82,83]. Although this may increase production costs, they would still be suitable if ultra-short peptides are effective at low doses, or if the peptide is produced by biotechnology and needed in low amounts. Other simple low-cost peptidomimetics (e.g., peptoids) could also be considered for development [12].

The combination of several HDPs which act in a synergistic way, or combinations of HDP and existing antimicrobials as seen before, can reduce the required active doses for these compounds and production costs. The use of nanoparticles to protect and slowly release the AMPs (and their combinations) is another possible measure.

But the most promising way to prevent the apparition of resistances, is using the tools currently available in precision agriculture. For instance, multi- and hyperspectral

cameras and other sensors are able to detect a disease in its very first stages, when it only affects a few plants [84–86]. In that case, it is unnecessary to treat all the field –just the contaminated section. This not only greatly reduces treatment costs but also avoids a widespread presence of the phytosanitary in the environment, and therefore, decreases the risk of resistance emergence.

4. Conclusions

Host-defense peptides have a broad antibacterial and/or antifungal spectrum against phytopathogens, and in addition, their multiple way of action allows a very low induction of resistance, compared to the traditional antimicrobials. In addition, they present low toxicity to animal and plant cells. HDPs are biodegradable and therefore, do not cause persistent environmental contamination. Finally, they are able to interact synergistically with other antimicrobials and with the host own defense system. Therefore, this class of compounds has elicited much interest. In Agriculture, most studies have been carried out with transgenic plants overexpressing their native HDPs or expressing a non-native peptide, but this approach, although useful to explore the scope of these compounds, presents some drawbacks. For instance, newly produced HDPs may undergo in vivo inactivation; besides, growth and production may decrease in some cases, and finally, there is public concern about transgenic crops, which translates to administrative limitations for their use. This review explores an alternative: the use of HDPs (or their mixtures with other antimicrobials) as the active principle of phytosanitary products. Although the area is still beginning, some promising results have been obtained. Both natural and modified HDPs have shown potential to reduce or suppress bacterial and fungal infections, and the synergistic use of HDP and other antimicrobials is also promising.

Although their low induction of resistance is a big advantage, cautionary measures are suggested to keep microorganism sensitivity for long periods of time, and therefore, to maximize the potential of this extraordinary new generation of phytosanitaries.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071614/s1. Table S1: Host-defense peptides and in planta studies.

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