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Antimicrobial peptides
Modes of mechanism, modulation of defense responses

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Complicated schemes of classical breeding and their drawbacks, environmental risks imposed by agrochemicals, decrease of arable land, and coincident escalating damages of pests and pathogens have accentuated the necessity for highly efficient measures to improve crop protection. During co-evolution of host-microbe interactions, antimicrobial peptides (AMPs) have exhibited a brilliant history in protecting host organisms against devastation by invading pathogens. Since the 1980s, a plethora of AMPs has been isolated from and characterized in different organisms. Nevertheless the AMPs expressed in plants render them more resistant to diverse pathogens, a more orchestrated approach based on knowledge of their mechanisms of action and cellular targets, structural toxic principle, and possible impact on immune system of corresponding transgenic plants will considerably improve crop protection strategies against harmful plant diseases. This review outlines the current knowledge on different modes of action of AMPs and then argues the waves of AMPs' ectopic expression on transgenic plants' immune system.

Introduction

Cationic as well as anionic antimicrobial peptides (AMPs) are peptides serving as constitutive or inducible defense barriers against microbial infections in plants, insects, amphibians and mammals including human.[1-3] They might additionally have the ability to boost the host immunity by functioning as immunomodulators.[4,5] Plenty of AMPs exist to cope with practically all potential infection sources. In general, the amphipathic peptides consist of positively charged residues, predominantly arginine and lysine, or else histidine in acidic setting, and a substantial ratio of hydrophobic amino acids.[6,7] The best-known antimicrobial peptide families are (1) linear α-helical peptides comprising cecropins[8] and magainins[9] exhibiting generally antibacterial activities, (2) multiple Cystein-bridge-containing defensins showing antibacterial and antifungal activities,[10,11] (3) Pro-rich peptides with activity against bacteria and filamentous fungi,[2,12] and (4) the Gly-rich peptides active mainly against Gram-negative and occasionally Gram-positive bacteria.[13,14]

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Major environmental concerns have been leading to prohibition of a huge part of existing agrochemicals all around the world, and coincident growing demands for sustainable strategies in crop protection have inspired the idea of recruiting AMPs for improvement of plant health.[15] This review covers the diverse modes of action, by which AMPs impede assaulting pathogens and overviews the impact of expression of AMPs in corresponding transgenic plants in terms of modulating the plant's different immune pathways.

AMPs; General Aspects

Controlled by only one single gene, AMPs can be produced rather quickly upon infection with narrow energy consumption;[16] nonetheless, some are constitutively expressed. Despite the fact that AMPs in insects and mammals may modulate the innate and adaptive immune reactions,[17] common persuasion on their most important function is elimination of infectious microorganisms.[18,19] Several kinds of classification have been proposed for AMPs; however, consistent with their secondary structures AMPs are generally categorized into four clusters:[20]

(1) linear α-helical peptides containing cationic amphipathic helices that perform inhibitory activities generally against bacteria. Many peptides of this group have been classified as "pore-forming" such as alamethicin, cecropin, PGLa, magainin, melittin and mastoparan.[21-23]

(2) cyclic peptides with β-sheet structure that form predominantly β-sheets including coupled β-strands due to the presence of two or more disulfide bonds. Although being antifungal in some cases,[11,24] they are often characterized as antibacterial peptides. The β-sheets are stably assembled by either disulfide bonds, as in cases of tachyplesins,[25] defensins,[26] protegrins,[27] and gallerimycin,[28] or circling of the peptide backbone, as in cases of polymyxin B,[29] tyrocidines,[30] arenicins.[31]

(3) peptides with β-hairpin or looped configuration that include those containing a looped structure due to the presence of a single disulfide bond and/or circling of the peptide chain. Thanatin from *Podisus maculiventris* is an example of this kind of peptides.[32] The best-characterized molecules amongst this group are lantibiotics produced by Gram-positive bacteria.

(4) linear peptides with unusual bias in particular amino acids, e.g., drosocin, metchnikowin, apidaecin, abaecin, formaecin, lebocin, pyrrhocoricin and metalnikowin all rich in proline,[12,33] indolicidin rich in tryptophan,[34] histatin rich in histidine,[35]

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

AMPs	Organism	Target molecule	Outcome
Native AMPs	Bacteria	Membrane phospholipids/LPS	Prevention of vital microbial homeostasis because of pore formation in membranes[64]
		DnaK; receptor/docking/ transporter molecule; GroEL	Inhibition of natural ATPase activity; Inhibition of chaperon-assisted protein folding[68-70]
		Ribosome	Protein synthesis inhibition[69]
	Fungi	Membrane glycosyl ceramide	Membrane permeabilization[79,80]
		Redox signaling cascade	Induction of ROS, membrane damage, organelle breakdown and cell death[24,82]
		MAP kinase signaling cascades	Regulation of fungal genes important for overcoming plant defense[83]
		Chitin in cell wall	Interference with chitin synthesis[85,86]
	Plants	Diverse molecules	Modification of host gene expression[88,89]
			Induction of cell death in other plants[90]
			Association with epigenetic somaclonal variation events[91]
			Conferring zinc tolerance[92]
	Mammals	Nucleus	Cell cycle impairment in rat retinal neuroblasts[81]
		L-type Ca^{2+} channel	Blockade of mammalian L-type Ca^{2+} channel[84]
		Diverse molecules	Playing as chemokines[93,94] and/or induction of chemokine production[95]
			Inhibition of pro-inflammatory cytokine production induced by LPS[96]
			Wound healing promotion[97]
			Inhibitory activities toward tumor cells and HIV-1 reverse transcriptase[98]
			Modulation of adaptive immune responses[4,5]
Transgenic AMPs in plants	Microbes	Diverse molecules	Interference with microbial fitness and virulence establishment[2,99]
	Plants	Diverse molecules	Primed status of transgenic plants due to more activated ISR and SAR and higher redox potential[2,99,100]
			Alteration of processes of synthesis, folding, and stabilization of proteins which enter to the secretory pathway[101]
			Alteration of translational machinery[101]
			Alteration of components of vesicle-associated transport machinery[101]
			Improved protection against oxidative stress[24,82,99-101]

tritrpticin rich in arginine or tryptophan,[36] and diptericins and attacins rich in glycine.[33] These peptides, predominantly found in Class Insecta, are active against bacteria and fungi.[2,37] The most recognized small Pro-rich AMPs are apidaecins that structurally consist of two domains: the conserved domain in charge of general antibacterial activity, and the variable one responsible for the antibacterial spectrum. They are lethal to many Gram-negative bacteria.[38] Of interest, several linear AMPs are amorphous in free solution and fold into their final configuration upon partitioning into biological membranes.[39]

AMPs; Modes of Action

Although mechanisms of action of antimicrobial peptides have been frequently reviewed[33, 38, 40–42], there are yet open questions regarding their heterologous functions. Generally speaking, functions of these peptides vary from membrane permeabilization to actions on an array of intracellular target molecules including immuno-modulatory activities (**Table 1**).

Pore-forming activity. Ability to interact with membranes is a classical countenance of AMPs;[43] nonetheless, membrane permeabilization is not an absolute feature. Earlier reports indicate an utter correlation between antibiotic effects of defensins and membrane permeabilization.[44,45] Notable hydrophilic positively charged domains facilitate the peptides to interact with the negatively charged microbial surfaces and head groups of bilayer phospholipids leading to cell membrane penetration. Three pore-forming mechanisms are described to explain the effects of α-helical membrane peptides.[1,40] A simplistic schematic illustration of different pore formation mechanisms is provided in **Figure 1** and a selection of peptides showing different pore-forming mechanisms is given in **Table 2**. The so-called "carpet-like" mechanism refers to destruction of membrane assembly by collaborative action of peptides.[40] Peptides self-associate onto the acidic phospholipid-rich regions of lipid bilayers and once their concentrations reach a certain threshold they permeate into the membrane by mounting the bilayer positive potential.[38] The second pore-formation mechanism "barrel-stave"[63] is symbolized in alamethicin;[60] it inserts into the membrane hydrophobic area and creates a pore by forming trans-membrane helical bundles. In the third mechanism, the "toroidal" model, the peptide builds toroidal pores in lipid bilayers. Pore construction is, intriguingly, managed by the lipid polar head groups and the helix bundles that orient vertically to the membrane exterior. More precisely, the attached peptides aggregate and induce the lipid monolayers to bend continuously through the pore so that both the inserted

peptides and the lipid head groups line the water core.[1] Pores act as non-selective channels for ions, toxins and metabolites, thus preventing the microbe from maintaining the vital homeostasis.[64] Conditional on experimental settings, α-helical membrane peptides can take on different pore-forming mechanisms. For instance, the pore-forming mechanism differs appropriate to the type of membranes and pH, which indicates the essence of experimental settings in the studies on AMPs' modes of action.[65] On the other hand, making the Magainin2 tetravalent and octavalent largely increases the pore-forming capability of peptide leading to decreasing the peptide minimal inhibitory concentration to low nanomolar ranges.[66]

Inhibition of DNA and protein functions. It is postulated that the positive charge in the short Pro-rich AMPs boosts bacterial cell access[67] and the existing prolines may perhaps inhibit helix formation and hence, toxicity to the host. Nevertheless, apidaecins belonging to short Pro-rich peptides are of distinction by lacking the pore-inducing action.[38] Eradication of bacteria by apidaecins commences by an ambiguous interaction of the peptide with an outer membrane component like LPS and DnaK,[68] and consequently, its entrance into the periplasmic space. Next, peptide traverses inner membrane specifically by an irrevocable band with either a receptor/docking/transporter molecule[69] or the 60-kDa bacterial chaperone GroEL.[68] Ultimately, the peptide is displaced into the cell where it runs into its certain target that is either ribosome leading to protein synthesis inhibition[69] or DnaK leading to protein folding inhibition.[68,70] Evidently, pyrrhocoricin, a relative of apidaecins, inhibits natural ATPase activity and chaperon-assisted protein folding of *E. coli*'s DnaK, whereas it lacks any activity on human HSP70.[68,70] In contrast, PR-39 kills bacteria by stopping their DNA and protein syntheses and gives rise to degradation of these components.[71] Interestingly, PR-39 does pass through membranes without any apparent damage. The molecule can induce the synthesis of syndecans involved in wound healing[72] and hamper the NADPH-dependent redox reactions.[73]

Prokaryotic DnaK recognizes extended peptide constituents as well as positively charged residues inside and outside of its substrate-binding furrow.[74] This might also occur to similar sequence motifs in typical members of Pro-rich peptides family, i.e., pyrrhocoricin, drosocin, apidaecin and metchnikowin. It is evident that metchnikowin can be triggered by both major pathways of fruit fly immune system, *imd* and *Toll*,[75,76] which makes it unique in terms of extreme immune capacity to almost all potential microbes, e.g., fungi, bacteria and even viruses. As well, obstruction of chaperone-assisted protein folding by Pro-rich cationic peptides[70] prospects a gallant approach to combating microbial infections.

Disturbance of other intracellular targets. It is documented that plant defensins, contrary to their mammalian and insect orthologs, neither induce ion permeable pores in artificial phospholipidic membranes nor change their electrical status, making evident that these defensins do not interact directly with plasma membrane phospholipids.[77,78] Though it was reported that the antifungal defensin *Na*D1 from *Nicotiana alata* induces membrane permeabilization, its activity may not only be restricted to the hyphal membrane, but it also enters cells and affects intracellular targets.[24]

Defensins of different origins exhibit no clear similarities in their modes of mechanism; nevertheless, fungal membrane sphingolipid glycosyl ceramide is the most common key target of a number of defensin-called peptides. Glycosyl ceramide was identified as specific target for the antifungal plant defensin *Rs*AFP2 and the insect defensin-like peptide heliomicin.[79]

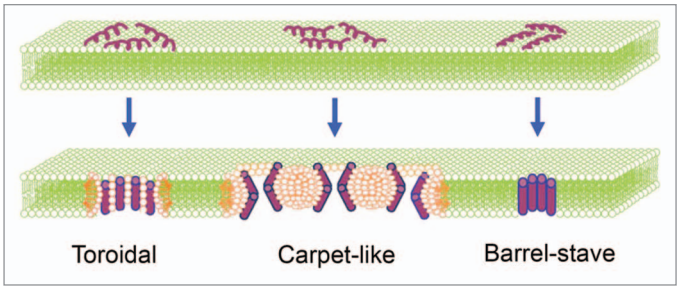

Figure 1. Schematic illustration of three pore-forming mechanisms to explain the α-helical membrane peptides. In "toroidal" model, the peptide builds toroidal pores in lipid bilayers. Pore construction is managed by the lipid polar head groups and the helix bundles that orient vertically to the membrane exterior. In other words, the attached peptides aggregate and tempt the lipid monolayers to bend continuously through the pore so that both the inserted peptides and the lipid head groups line the water core. "Carpet-like" mechanism refers to destruction of membrane assembly by collaborative action of peptides. Peptides self-associate onto the acidic phospholipids-rich regions of lipid bilayers, and as soon as their concentrations reach to a certain threshold, they permeate into the membrane. This is assisted by escalating the positive potential of bilayer. Via "barrel-stave" mechanism, peptide inserts into the membrane hydrophobic substance, flips inward and creates a pore by forming transmembrane helical bundles. (Scheme is modified after refs. 1 and 46).

Table 2. Classification of different α-helical AMPs according to their membrane permeabilization mechanisms

Pore-forming mechanism	Peptide	Origin	Reference
Carpet-like (detergent-like)	Cecropins	*Hyalophora cecropia*	47
	PGLa	*Xenopus laevis*	48
	Dermaseptins	*Phyllomedusa spp.*	49
	Ovispirin	*Ovis aries*	50
	Latarcins	*Lachesana tarabaevi*	51
	RL-37	*Macaca mulatta*	52
Toroidal (wormhole)	Mastoparan X	*Vespa xanthoptera*	53
	Magainin 2	*Xenopus laevis*	54, 55
	LL-37	*Homo sapiens*	52, 56
	Melittin	*Xenopus laevis*	57
	Piscidin	*Morone saxatilis*	58
Barrel-Stave	Pardaxin	*Pardarchirus marmoratus*	59
	Alamethicin	*Trichoderma viride*	60
	Amphotricin B	*Streptomyces nodosus*	61
	Ceratotoxin	*Ceratitis capitata*	62

Consistently, a *Fusarium graminearum* mutant deficient in glycosyl ceramide was resistant to both radish *Rs*AFP2 and alfalfa *Ms*Def1 defensins, corroborating the idea that glycosyl ceramide is also the target of *Ms*Def1.[80] On the contrary, pea defensin, *Ps*d1, directs cell cycle impairment and causes *Neurospora crassa* conidia to undergo endoreduplication. Furthermore, *Ps*d1 regulates interkinetic nuclear migration from Synthesis (S) to Mitosis (M) phase of cell cycle in rat retinal neuroblasts.[81] Additionally, some defensins such as *Rs*AFP2[82] and *Na*D1[24] modulate the intracellular signaling cascades, specifically, induction of reactive oxygen species (ROS) that upon accumulation may cause membrane damage, organelle breakdown and eventually cell death. Pertinent support for this modulatory activity was found in certain mutants of *F. graminearum* disrupted in some MAPKKK(s) genes that were hypersensitive to alfalfa *Ms*Def1, barrel clover *Mt*Def2, and radish *Rs*AFP2. MAP kinase signaling cascades in *F. graminearum* regulate the fungus sensitivity to plant defensins.[83] It is giving proof that plant defensins can act as stimuli to launch MAP kinase signaling cascades involved in regulating the fungal genes important for overcoming the plant defense. It would seem, upon binding of defensins to their receptors, activation of MAPKKK(s) occurs owing to physical interaction and/or phosphorylation by either the receptor itself or intermediary factors or an interlinking kinase.[83] Moreover, *Ms*Def1 has been characterized to block the mammalian L-type Ca^{2+} channel in a manner akin to structurally unrelated antifungal toxin KP4 from *Ustilago maydis*.[84] It is, as well, documented that bamboo defensins-like AMPs, *Pp*AMP1 and *Pp*AMP2, bind to chitin in microbial cell walls,[85] and *Aspergillus giganteus* defensin, AFP, inhibits the chitin biosynthesis in susceptible fungi.[86] Consistently, chitin synthase mutants of *Fusarium oxysporum* and *Aspergillus oryzae* are less susceptible to AFP. Presumably, AFP causes cell wall stress by interfering with the chitin synthesis and disturbing the cell integrity in sensitive fungi.[86]

Several lines of evidence from different studies indicate that microbial membrane permeabilization or/and cell wall disruption are within modes of mechanism of defensins.[87] However, the issue whether the lytic action (membrane disturbance) is a phenomenon actively processed by AMPs to kill the microbes remains an enigma.

Besides direct elimination of microbes, AMPs have been shown to possess several immuno-modulatory functions such as modification of host gene expression,[88,89] induction of cell death in other plants,[90] association with epigenetic somaclonal variation events,[91] conferring zinc tolerance,[92] playing as chemokines,[93,94] and/or induction of chemokine production,[95] inhibition of pro-inflammatory cytokine production induced by LPS,[96] wound healing promotion,[97] inhibitory activities toward tumor cells and HIV-1 reverse transcriptase,[98] and modulation of adaptive immune responses, e.g., by activation of human plasmacytoid dendritic cells in some auto-immune diseases.[4,5]

Modulation of different cascades of immune system in relevant transgenic plants. There are some reports demonstrating that the host gene profiling alters after introgression of antimicrobial peptides.[2,99,100,101] Expression of metchnikowin gene from *Drosophila melanogaster* in barley to codify a peptide with antimicrobial activity was recruited to improve plant defense against microbial attacks.[2] Assessment of metchnikowin effects on powdery mildew fungus during its interaction with transgenic barley provided evidence that the antifungal peptide improves the resistance of plant as if it impedes the development of functional haustorium due to increased rate of hypersensitive response (HR) and development of cell wall apposition (CWA).[2] Comprehensive study on possible latent influence of metchnikowin on the defense system of plant revealed that the SAR and ISR pathways as well as redox status of metchnikowin-expressing barley plants are potentiated during interaction with powdery mildew fungus.[99] In Phenylpropanoid pathway, the *PAL-1* gene expression profile demonstrated that in *Bgh* challenge the activity of phenylalanine ammonia-lyase is elevated in metchnikowin transgenic plants.[99] Similar observation was reported by Distefano et al. (2008) for *PAL* gene,[100] which suggests that highly activated ISR may be one of the causes of higher resistance in these transgenic plants. This suggestion is supported by elevated expression of *PR-6* in metchnikowin plants compared with that in wild type individuals.[99] The higher level of reactive oxygen species down to expression of antimicrobial peptides[24,82,99,100] supports the notion that these peptides play some part of their roles by modulating the redox milieu, which might ultimately lead to cell death.

Examination of susceptibility factors, i.e., *MLO* and *Bax inhibitor-1* in metchnikowin barley concluded that the susceptibility/resistance of those plants is independent of these factors.[99] Comparative analysis of gene expression between *cecropin A* transgenic and wild-type rice plants grown under optimal conditions and during infection of rice with the rice blast fungus *Magnaporthe oryzae* revealed the overexpression of diverse genes involved in (1) protection against oxidative stress, (2) processes of synthesis, folding and stabilization of proteins that enter into the secretory pathway, (3) translational machinery, and (4) genes encoding components of the vesicle-associated transport machinery in *cecropin A* rice.[101] Together, these reports imply the altered immune status of AMPs-expressing plants.

Using AMPs for Plant Disease Control

As demands for a better control of plant diseases increase, AMPs come into focus.[15] To date, a multitude of gene constructions with coding sequences of AMPs have been expressed in planta leading to various extents of protection against fungal and bacterial pathogens (see ref. 99). Insect peptides seem especially suitable owing to their exceptionally broad antimicrobial potential for protecting their hosts against various biotic challenges. Growing knowledge on structure-function relationships and thus elucidation of essential peptide domains will press forward the use of synthetic AMPs in transgenic crop plants. Synthetic analogues of cecropins facilitated their ectopic expression for improvement of plant fitness in biotic stress circumstances.[102] Interestingly, early attempts to express cecropin in tobacco for resistance induction against *Pseudomonas syringae* pv. *tabaci* were barely successful.[103] Short persistence of cecropin B has been ascribed to proteinases in the cytosol mediating proteolysis through an initial endopeptidase cleavage.[104] To put off such an interfering process,

cecropin B-derived peptides were manipulated to be shorter in length. Alternatively, targeting the mature peptides using signal sequences from different origins into the intercellular spaces, in which proteinases are seldom present, could lead to relatively higher accomplishment.[105] Accordingly, the antimicrobial activity of intercellular fluid of metchnikowin expressing barley plants[2] and the green fluorescing background of intercellular spaces surrounding the faintly fluorescing barley epidermal cells that transiently express the GFP-fused metchnikowin peptide[99] confirm the functionality of the fruit fly-origin signal sequence of metchnikowin peptide, in planta, to secrete the produced peptide into the intercellular space. As another strategy, molecular modeling for engineering the AMPs offers a dominant tool to engender peer synthetic and chimeric peptides with potentially superior properties.

Prospective for Future Endeavors

Dose-effect and synergistic activity. Though it has been reported that the degree of antimicrobial activity is not dependent on the AMP production level in transgenic plants,[106] most of the publications furnish clear evidence for peptides' dose-effect activity.[82,100,107,108] Some investigators emphasize the possibility of synergism among different AMPs.[109-111] In vivo data for different antibacterial peptides show that defensins and linear peptides work in-synergy,[112,113] as exemplified by synergism between *LL-37* and human β-defensin *HBD-2*.[114] Consistently, concomitant expression of AMPs in plants usually leads to higher levels of induced resistance than their individual expression.[115,116] This finding is reminiscing of the effect known from combining disease resistance genes (gene pyramiding) in traditional breeding, which often results in long-lasting, durable plant protection against pathogens.

Recruiting inducible promoters. General propensity toward reducing fitness costs[117] as well as downgrading the co-evolutionary collapse of resistance to microbes has weighted the generation of plants expressing AMPs on-demand, by exploiting synthetic or native inducible promoters activated upon pathogen attack.[2,11,107,118,119] Employment of wounding and/or pathogen-inducible promoters ensures high expression level of the peptide upon mechanical wounding and/or microbial infections. This may assist to avoid the development of pathogenic microbes capable of circumventing induced disease resistance, by e.g., mutation and/or synthesis of proteolytic agents.

Approaching different strategies to alleviate existing drawbacks. Undesirably, application of AMPs to engineer pathogen resistance in plants suffers from some limitations, namely species- and race-specificity of the peptides,[120] slight enhanced resistance,[106] induction of infertility,[121] and leakage of conferred resistance after a while due to resiliency of disease-causing microbes.[38] To alleviate these drawbacks as well as to increase the antimicrobial potency of existing peptides, several approaches have been proposed to follow. Engineering crop plants for disease resistance via chloroplast genome instead of nuclear genome is proposed to achieve high levels of expression and to prevent pollen-mediated escape of transgenes.[122] In addition, synthesis/manipulation of peptides for base(s) substitution/deletion and AMPs' chimeric hybridization result in improved disease resistance in plants.[120,123,124] Expression of antibody-AMP fusion proteins has been shown to control microbial pathogens, more efficiently and durably.[125,126] Also, targeting the AMPs into endoplasmic reticulum instead of intercellular spaces dramatically drops the probability of infertility.[121] Moreover, expression of a cocktail of AMPs with different modes of action demotes the possibility of resistance depletion attributable to microbial escape. Notably, insects synthesize concurrently a continuum of low-molecular-mass inhibitors against microbial proteases in company with AMPs.[127] It is anticipated that these inhibitors annihilate the digestive action of proteolytic enzymes secreted from plant pathogenic fungi.[128] Consequently, coincident transmission/expression of insect AMPs and inhibitors of microbial proteases will possibly avert the selection of pathogens, which can negate the foreign peptides in transgenic plants.

Biosafety remarks. From the ecological point of view, the issue whether or not transfer of AMPs into plants imperils the mutualistic interactions between plants and beneficial microbes should be addressed. There are reports stating target specificity for AMPs among the kingdom of fungi. The phyla Glomeromycota and Basidiomycota that accommodate many symbiotic fungi might be less sensitive to AMPs than Ascomycota.[2,129-131] However some AMPs affect Basidiomycota rather well, this might not be taken as a rule.[108,115,119,124,132] Regarding specificity, the activity of metchnikowin on orchid mycorrhiza *Piriformospora indica*[133] has been studied in detail.[2] Growth and development of this fungus was not demolished in transgenic plants producing the metchnikowin, whilst ascomycete fungi were impaired.[2] Despite the fact that the definite cause for specificity has not been elucidated yet, these observations do prospect a promising approach: utilizing AMPs to diminish the devastating consequences of diseases and pests without affecting the plant's essential mutualistic interactions with beneficial symbionts. Clearly, these symbionts are of vital importance for plants in terms of presenting a better fitness via supporting water and mineral uptake as well as strengthening disease resistance.[134] However, it must be stressed that more research is needed to identify differential targets of AMPs in fungi in order to explain AMP's specificity on the molecular level.

AMPs potential for combating viruses and pests. Since antiviral activities are within the panel of AMP's properties,[135-137] it will be interesting to assess the potential of AMPs for controlling countless viral diseases in plants. However, since it is known that *Toll* pathway, one of the major pathways in immune response in Drosophila, is required for efficient inhibition of virus replication in infected flies,[138] expectation to restrain viruses in plants should be considered, critically. Finally, in order to manage the pest damages on plants, one might think of expressing AMPs under the control of plant tissue specific promoters, for instance, those for in-phloem expression to construct some lethal peptides to devastating pests, though the biosafety aspects must be, yet again, well thought-out.

Overall, the provided data on endogenous genes expression in AMPs transgenic plants suggest that antimicrobial genes play their roles in disease resistance in part via modulation of different

resistance mechanisms. They might also join forces of various plant immune pathways culminating in a primed status. The provided evidence on the involved pathway(s) in AMPs-induced resistance will improve our knowledge concerning the impacts of antimicrobial peptides, expressed in diverse plant species, on the immune system of transgenic plants. This helps going beyond the current notion that antimicrobial peptides-derived resistance refers solely to the direct noxious effects of these peptides on pathogenic microbes. Certainly, entire clarity of this important issue demands elaborate and comprehensive experiments, namely the use of transcriptomics to explore the impact of transgene expression in plants.

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