

The role of plant antimicrobial peptides (AMPs) in response to biotic and abiotic environmental factors

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Abstract

Plants are continuously exposed to various biotic and abiotic factors that may trigger cascade reactions aimed at maintaining homeostasis. One of the most important components of plant protection from biotic factors is the synthesis of antimicrobial peptides (AMPs). AMPs are a large group of peptides present in insects, animals and plants. Plant innate immunity is provided by AMPs from different families that are categorized according to sequence similarity, the number and order of amino acid residues, and the tertiary structure of the mature peptide. AMPs may also participate in plant response to abiotic stresses such as high salinity, drought, high or low temperature, and heavy metals. In nitrogen-fixing nodules of some members of the Fabaceae family, AMP-like molecules named NCR peptides promote the differentiation of the symbiotic bacteria into bacteroids. Thus, AMPs are used by plants for fine tuning their responses to biotic and abiotic factors alike.

Keywords: plants, antimicrobial peptides, abiotic factors, biotic factors, symbiosis, stress.

Introduction

Plants, as sessile organisms, have evolved an extensive arsenal of diverse and coordinated systems of defense against, and resistance to, adverse environmental conditions. Environmental factors are usually split into two categories: abiotic (e.g., salinity, heavy metal contamination, freezing or heat-shock temperatures, drought) and biotic (other organisms, e.g., bacteria, fungi, insects, etc.). Plant responses to biotic and abiotic stress are variable and depend on the type of stress factor; moreover, a particular factor, e.g., non-optimal temperature, may invoke different responses. In turn, a response to different types of stress may have common elements, such as expression of particular genes and/or production of secondary messengers (Zhang and Sonnewald, 2017).

In general, plant response to either biotic or abiotic stress begins with the recognition of a stressor by specific receptors (Fig. 1). Then, the signal transduction cascades are activated, leading to substantial transcriptional changes that result in biosynthesis of stress-related proteins and/or secondary metabolites that protect plant cells and participate in subsequent repair of the damage caused by stress. The most common and numerous group of stress-related proteins, characteristic mainly for biotic stress response, are antimicrobial peptides (AMPs).

Under abiotic stress, at the first stage, a stressor is recognized by specific cellular receptors. For example, in the case of drought, salinity and cold, activation of receptors — such as G-protein-coupled receptors (GPCRs), receptor-like protein

Citation: Kulaeva, O., Kliukova, M., Afonin, A., Sulima, A., Zhukov, V., and Tikhonovich, I. 2020. The role of plant antimicrobial peptides (AMPs) in response to biotic and abiotic environmental factors. *Bio. Comm.* 65(2): 187–199. <https://doi.org/10.21638/spbu03.2020.205>

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Manuscript Editor: Anton Nizhnikov, Department of Genetics and Biotechnology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

Received: November 27, 2019;

Revised: December 10, 2019;

Accepted: January 15, 2020.

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Funding: The work of OAK, AMA and IAT was supported by the Russian Science Foundation (Grant # 17-76-30016). The work of ASS and VAZ was supported by the Russian Science Foundation (Grant # 16-16-00118). The work of MSK (analysis of the role of AMPs in legume–rhizobial symbiosis) was supported by the Russian Foundation for Basic Research (Grant # 18-34-00187).

Competing interests: The authors have declared that no competing interests exist.

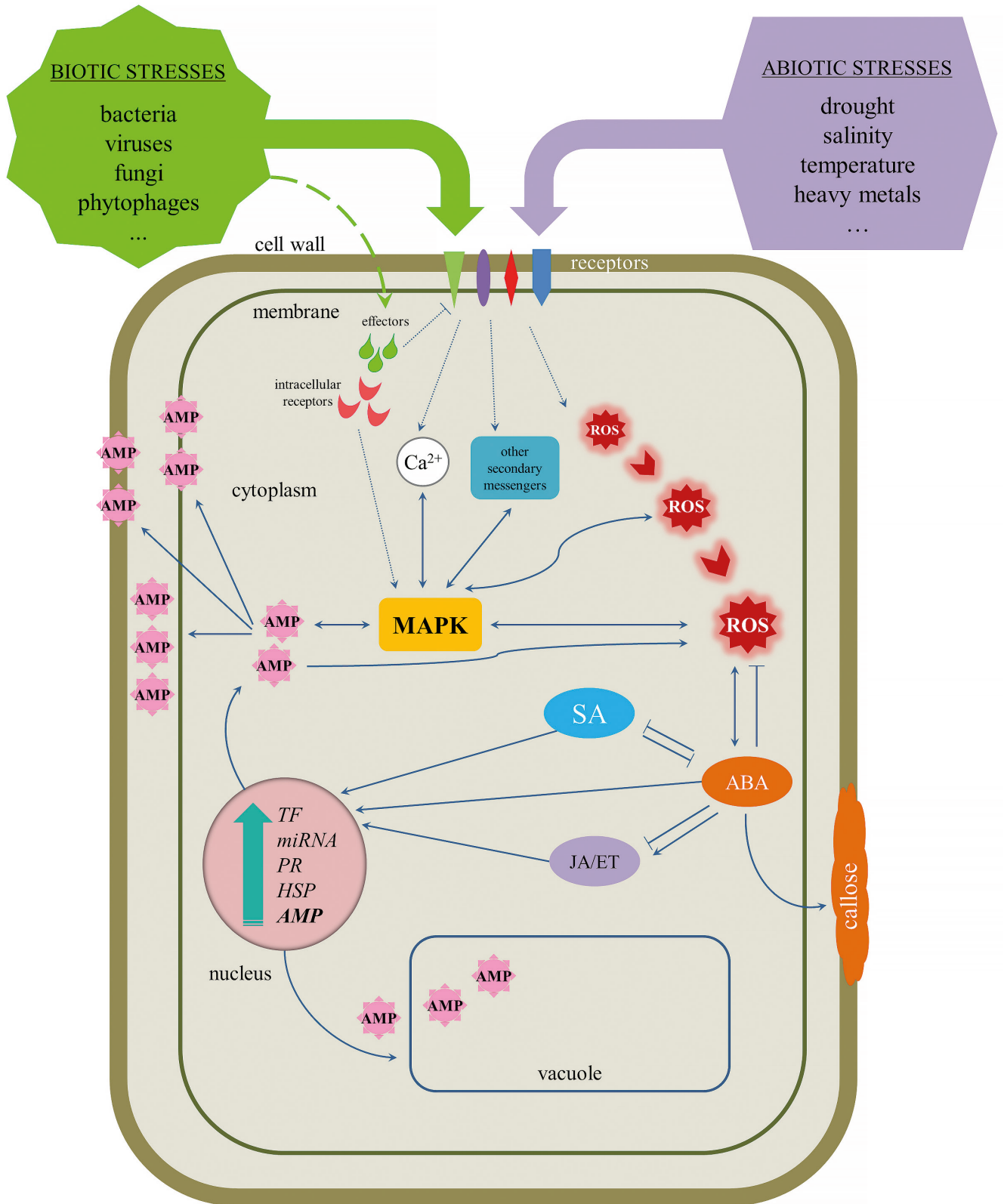


Fig. 1. A general scheme of plant cell responses to biotic and abiotic stress (adapted from Rejeb et al., 2014, with modifications).

Table 1. Functions of the main families of cysteine-rich AMPs in biotic stress

Group of AMP	Amino acid composition	Activity	Mechanism of action	Targets
Thionins	Cationic peptides, 45–48 aa in length; 6 or 8 cysteine residues in conservative positions form 3 or 4 disulfide bonds.	Active against a broad spectrum of microorganisms, also insects (De Caley et al., 1972; Kramer et al., 1979; Carrasco et al., 1981; Bohlmann et al., 1988)	Interaction with electronegative cell membranes, which may lead either to a leakage of water through the lipid bilayer or to the instability of the membrane (Stec et al., 2004; Stec, 2006; Oard, 2011)	Phospholipase A2 (Vernon and Bell, 1992) adenylate cyclase (Huang et al., 2008), protein kinase C, calmodulin, ribonucleotide reductase, β -glucuronidase DNA and RNA (Diaz et al., 1992), DNA and RNA (Woynarowski and Kono-pa, 1980; Li et al., 2002)
Defensins	Stable peptides, 45–54 aa in length; contain 8 or 10 conserved cysteine residues.	Antifungal activity, some show antibacterial activity (Terras et al., 1995; Fujimura et al., 2003; Stotz et al., 2009); in experiments some defensins exhibit inhibitory activity against α -amylase, protease, HIV1 reverse transcriptase, as well as anti-cancer activity (Carvalho and Gomes, 2009, 2011).	Interaction with the target on the fungal membrane, which can be located on the cell wall and plasma membrane (Sagaram et al., 2011; De Coninck et al., 2013); activation of the ROS response and apoptosis (Aerts et al., 2007, 2011; Weerden et al., 2008), effects on the cell cycle (Lobo et al., 2007).	Glucosylceramides (GlcCer), sphingolipids (M(IP)2C) (Sagaram et al., 2011; De Coninck et al., 2013)
Hevein-like peptides	Basic peptides of 29–45 aa in length; contain 6, 8 or 10 conservative cysteines. Conserved glycines and aromatic amino acids in the hevein domain. Chitin-binding domain (SXFGY/SXYGY, where X is any amino acid residue).	Antifungal activity, some show activity against chitin-containing and non-chitin-containing fungi alike (Koo et al., 1998).	Interaction with the fungal cellular chitin that leads to the destruction of the fungal membrane and leakage of cytoplasm content. (Koo et al., 1998, 2004).	The fungal cellular chitin (Van den Bergh et al., 2004; Slavokhotova et al., 2017).
Knottins/cyclotides	Very short peptides, about 30 aa in length; with 6 cysteines at the C-terminus. Can be present in two forms: linear (cystine-knot peptides or knottins) and cyclic (cyclotide).	Active against fungi and bacteria, but in experiments also show anti-HIV, α -amylase, carboxypeptidase A or trypsin inhibitory activity (Polanowski et al., 1980; Gustafson et al., 1994; Van den Bergh et al., 2004; Ireland et al., 2008; Campos et al., 2018).	Interaction with target cell membranes. Cyclotides can aggregate in multimeric structures for pore formation, changes in ion flow and cell death (Ireland et al., 2008; Strömstedt et al., 2010).	Lipid membranes (Svan-gård et al., 2007; Burman et al., 2011).
Hairpinins	Peptides with 4 cysteine residues, with unique tertiary structure; are enriched in Lys and Arg.	Antifungal and antibacterial activity (Nolde et al., 2011; Sousa et al., 2016).)	Not well studied. Hairpinins accumulating inside the cell without changes in membrane integrity of spore or fungal hyphae; may bind to a target on the cell wall or on the membrane of the fungus, after which it penetrates into the cells and accumulates in the vesicles and interacts with intracellular targets (Nolde et al., 2011; Vasilchenko et al., 2016).	No known targets on the cell wall or on the membrane of the fungus, may interact with intracellular targets, such as DNA (Sousa et al., 2016).
Lipid transfer proteins	70–90 aa in length; contain 8 conservative cysteines.	Active against fungi and some bacteria (Molina et al., 1993).	Insertion into the microbial membrane. Such integration leads to efflux of intracellular ions and, as a result, cell death (Selitrennikoff, 2001).	Fatty acids (C10–C14), phospholipids, prostaglandin B2, lyso-derivatives, and acyl-coenzyme A (Tam et al., 2015).

End of Table 1

Group of AMP	Amino acid composition	Activity	Mechanism of action	Targets
Snakins	Peptides with the largest number of cysteines of all the AMPs. All 12 cysteines are located on the C-terminus of the mature peptide.	Active against fungi, gram-positive and gram-negative bacteria (Segura et al., 1993; Berrocal-Lobo et al., 2002; Daneshmand et al., 2013).	Formation of pores in the membranes of target cells. Due to their cationic charge, snakins can penetrate through the negatively charged cell walls of bacteria or fungi, forming pores (Herbel et al., 2015; Herbel and Wink, 2016).	Membrane of target cells (Herbel et al., 2015; Herbel and Wink, 2016).
NCR peptides	30–60 aa in length, have 4 or 6 cysteine residues in conservative positions.	In vivo — terminal differentiation of compatible rhizobial strain (change in morphology (size, shape, branching of bacteria), physiology (abolishment of division, inducing of nitrogen fixation), and genetics (polyploidization of genetic material)) and elimination of incompatible strains. In experimental conditions — antibacterial and antifungal activity.	Temporary or constitutive pore formation in the cell membrane (Nagy et al., 2015). After that, some NCR peptides can penetrate into the cells of a bacteria or fungus and interact with intracellular targets (Farkas et al., 2014).	NCR247 is capable of interacting with FtsZ, ribosomal proteins, GroEL, pyruvate dehydrogenase complex, transaldolase, DNA-directed RNA polymerase subunits β and β' , elongation factor Tu and G, and a Maf-like protein (Farkas et al., 2014). In bacteroids, NCR247 also binds subunits of the nitrogenase complex (Farkas et al., 2014).

kinases (RLKs), histidine kinases and ion channels — occurs, which leads to a change in the concentration of intracellular Ca^{2+} (Kacperska, 2004; Ward et al., 2009; Nongpiur et al., 2012; Ye et al., 2017).

In addition, various secondary messengers, namely phytohormones (abscisic acid (ABA), gibberellic acid, jasmonic acid, etc.), reactive oxygen species (ROS), inositol phosphates, etc., are produced (Verma et al., 2013; Khan et al., 2018).

Secondary messengers activate phosphorylation/dephosphorylation cascades that include CDPKs, CIPKs, MAP kinases, protein kinases and protein phosphatases, etc. (Khan et al., 2018). As a result of the signal transduction, a number of transcription factors are activated (Asai et al., 2002; Molina et al., 2008). Some of them are specific to a certain stress factor — for example, the transcription factors DREB2A and DREB2B are induced during drought and salinity, and OsCDPK13 is only activated by cold (Abbasi et al., 2004; Nakashima et al., 2000) — while others are nonspecifically activated in response to various abiotic factors. The activated TFs then start/suppress transcription of genes whose products regulate the stress response, thus repairing the damage caused by stress and increasing further resistance to a particular stress factor.

Plant response to biotic factors also begins with the recognition of a stressor. In the case of pathogenic microorganisms attacking a plant, the pathogen-associated molecular patterns (PAMPs) are recognized by plant membrane receptors, thereby triggering an immune response in cells to prevent the infection from spreading (Bigeard et al., 2015; “Pathogen Associated Molecular

Pattern — an overview | ScienceDirect Topics”, n.d.). However, some pathogens are able to suppress this response with special molecules, so-called effectors, and penetrate into the plant cell. During evolution, plants adapted to the existence of effectors by recognizing these molecules with intracellular receptors (Thomma et al., 2011). After pathogen recognition, the plant activates the conserved MAPK cascade (e.g., MEKK1-MKK4/MKK5-MPK3/MPK6 in Arabidopsis), which induces the activation of several specific TFs (AP2/ERF, WRKY, MYB, bZIP). The plant also activates diverse cross-communicating signaling pathways in which salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play key roles (De Vos et al., 2005). All these signal transduction pathways lead to the activation of biosynthesis of antimicrobial molecules such as secondary metabolites (phytoanticipins and phytoalexins), pathogenesis-related proteins (Karpun et al., 2015; Miller et al., 2017; “Pathogenesis-Related Protein — an overview | ScienceDirect Topics”, n.d.) and small antimicrobial peptides (AMPs) (Piasecka et al., 2015; Onaga and Wydra, 2016). Finally, ROS can also be accumulated in infected plant cells, causing the death of these cells in the affected area to prevent the spread of infection (Karpun et al., 2015).

Plant AMPs are small and very diverse peptides containing from 7 to 100 amino acids. They are divided into several main families (thionins, defensins, hevein-like peptides, etc.) that contain conserved cysteine residues (Tab. 1). However, minor groups of AMPs were also found in plants, namely Gly-rich peptide (GRP), Gly-and-His-rich peptide shepherins, and a number of unclassified peptides with antimicrobial activity (Tam et

Table 2. Participation of cysteine-rich AMPs in abiotic stress response

AMP family	AMP gene name	Plant species	Type of stress
Defensins	<i>TAD1</i>	<i>Triticum aestivum</i>	Low-temperature stress (Koike et al., 2002; Gaudet et al., 2003).
	<i>Gmdefensin</i>	<i>Glycine max</i>	Drought stress (Stolf-Moreira et al., 2010).
	<i>CADEF1</i>	<i>Capsicum annuum</i> L.	Pathogen infection, wounding, high salinity and drought stresses (Do et al., 2004).
	<i>Ca-AFP</i>	<i>Cicer arietinum</i> in <i>Arabidopsis thaliana</i>	Water-deficit stress (Kumar et al., 2019).
	AhPDF1.1	<i>Arabidopsis halleri</i>	Heavy metal stress (zinc) (Mirouze et al., 2006; Mith et al., 2015).
	<i>CAL1</i>	<i>Oryza sativa</i>	Heavy metal stress (cadmium) (Luo et al., 2018).
	<i>AtPDF2.6</i>	<i>A. thaliana</i>	Heavy metal stress (cadmium) (Luo et al., 2019).
Snakins/GASA proteins	<i>GASA4</i>	<i>A. thaliana</i> and <i>Zea mays</i>	Heat stress (Ko et al., 2007; Maruyama et al., 2014).
	<i>FsGASA4</i>	<i>Fagus sylvatica</i> in <i>A. thaliana</i>	Salt, oxidative and heat stress (Alonso-Ramirez et al., 2009).
	<i>GASA14</i>	<i>A. thaliana</i>	Abscisic acid and salt stress. Reduce ROS accumulation (Sun et al., 2013).
Hevein-like peptides	<i>Snakin -1</i>	<i>Solanum tuberosum</i>	Pathogen infection (Nahirñak et al., 2019).
	<i>WAMP-1a/b</i>	<i>Triticum kiharae</i>	Fungal and salinity stress (Andreev et al., 2012).
Lipid transfer proteins	<i>Gamma-thionin</i>	<i>Panax ginseng</i>	Stresses related with abscisic acid, for example water stress (Lee et al., 2011).
	<i>TaLt</i>	<i>T. aestivum</i>	Cold stress (Gaudet et al., 2003).
	<i>LPT3</i>	<i>A. thaliana</i> and <i>Z. mays</i>	Drought, salt and low-temperature stress (Guo et al., 2013; Zou et al., 2013).
	<i>nsLTP1</i>	<i>S. tuberosum</i> in transgenic plants	Heat, drought and salt stresses (Gangadhar et al., 2016).

al., 2015). AMPs constitute an integral part of plant immunity and play an important role in maintaining the homeostasis of plant cells. In addition, some of them are components of the plant response to abiotic environmental factors. In this review, the current knowledge on the role of plant AMPs in plant responses to biotic and abiotic stress is summarized, with the intention to highlight the unique features of these antimicrobial molecules, including NCR (nodule-specific cysteine-rich) peptides that play a key role in legume–rhizobial symbiosis.

The structure of AMPs and its role in response to biotic stress

Plant AMPs are synthesized in the form of a precursor containing a signal sequence, which is cleaved upon maturation in the endoplasmic reticulum. After that, AMPs are transported to various cell compartments. Most AMPs are secreted into the extracellular space (apoplast), but for a number of AMPs, localization in

the cell wall, plasma membrane, vacuole, cytoplasm and nucleus has been shown (Segura et al., 1993; Lay et al., 2003; Oomen et al., 2011; García et al., 2012; Nahirñak et al., 2012; Slazak et al., 2016).

There are several approaches to classification of AMPs. Some authors classify these peptides into families according to their similarity in primary, secondary and tertiary amino acid sequence. Furthermore, AMPs can be categorized based on the amino acid motifs. The largest group of AMPs — cysteine-rich peptides — includes the main families of AMPs: thionins, defensins, knotins, hevein-like proteins, etc. Other groups include peptides that are enriched with other amino acids, for example, Gly-rich peptides (GRP) and Gly- and His-rich peptide shepherins (Tam et al., 2015). Enrichment in specific amino acids determines the function of AMPs and their biological role, mainly in defense against pathogens. Information about the function of the main families of plant AMPs is summarized in Table 1.

Despite the fact that the main function of AMPs is protection against pathogens, some peptides are charac-

terized by non-trivial functions, such as participation in response to abiotic factors and regulation of mutualistic (i.e., non-pathogenic) interactions between plants and microorganisms.

Participation of AMPs in abiotic stress mitigation

For most AMPs, participation in the response to abiotic stress is not a major, but an additional function. However, for some groups of AMPs, especially for defensins, such additional functions have been clearly demonstrated. Studies of various members of the defensin family have shown that they are involved in the response to stress associated with salt, drought, heavy metals and temperature (Tab. 2).

Indeed, salinity stress leads to increased expression of many protective genes, including defensins (Taji et al., 2004; Nishiyama et al., 2012; Sui et al., 2016), yet the specific role of these peptides in salinity stress is not known. Some defensins were shown to be induced under low-temperature stress in winter wheat (Koike et al., 2002; Gaudet et al., 2003) and under drought stress in soybeans (Stolf-Moreira et al., 2010). Expression of the defensin gene *CADEF1* in *Capsicum annuum* plants was found to be induced not only by pathogen infection, but also by wounding, high salinity, drought stress, as well as by excessive treatment with exogenous plant hormones (Do et al., 2004). Also, recent studies have shown some defensins to be associated with resistance to heavy metals, namely zinc and cadmium (Mirouze et al., 2006; Luo et al., 2018, 2019).

The expression of AMP genes in transgenic plants may increase stress tolerance. For example, overexpression of the defensin gene *AtPDF2.6* in *Arabidopsis thaliana* enhanced resistance to cadmium of transgenic plants (Luo et al., 2019). Similarly, the transgene expression of the *Cicer arietinum* defensin gene *Ca-AFP* in *Arabidopsis* led to increased drought tolerance via the regulation of superoxide dismutase, ascorbate peroxidase, catalase and proline content (Kumar et al., 2019).

Interestingly, the opposite reaction was observed in experiments with *GASA 5*: the overexpression of *GASA5* increased the sensitivity of *A. thaliana* plants to heat stress, together with decreased expression of some genes encoding heat-shock proteins and elevated accumulation of hydrogen peroxide (Zhang and Wang, 2011).

In general, AMPs may act both as positive and negative regulators to abiotic stress responses associated with ROS, hormone and heat shock protein synthesis. However, the causal relationship between the presence of AMPs and abiotic stress reactions is not clear and may be indirect. AMPs acting as chelating molecules demonstrate a simple case of heavy metal stress mitigation, but transgene expression experiments point towards the

presence of regulatory feedbacks in plant cells that link the hormone level, transcription/translation processes and biosynthesis of secondary metabolites and other active compounds. Undoubtedly, the induction of AMPs under abiotic stress conditions may indeed play an important role, but the details of their action are possibly different in different species, and for particular AMPs.

AMPs in mutualistic plant–microbe symbiosis

Since AMPs have evolved as a component of plant–microbe interactions, it is not surprising that several groups of AMPs participate in mutualistic symbiotic relationships of plants with bacteria or fungi. It is possible that several antifungal defensins and defensin-like peptides may participate in arbuscular mycorrhiza (AM) symbiosis, since their expression has been detected in mycorrhized roots (Liu et al., 2007); however, their direct effect on mycorrhization is questionable (Turrini et al., 2004). The role of AMPs is significantly more pronounced in legume–rhizobial and actinorhizal nitrogen-fixing symbioses, in which new organs (root nodules) are formed.

AMPs in legume–rhizobial symbiosis

During establishment of legume–rhizobial symbiosis, the plant forms root nodules, the specific organs where bacteria are hosted inside the plant cells (Oldroyd, 2013). It is important for the plant to discriminate the mutualistic bacteria from neutral (not useful for plant) or harmful (pathogenic) ones (Zipfel and Oldroyd, 2017). Observably, members of several classes of AMPs may participate in this discrimination. Indeed, it was shown that the expression of genes encoding defensins, defensin-like peptides, and glycine-rich peptides (GRPs) is increased in nodule tissue of *Medicago truncatula*, the model object for studying legume–rhizobial symbiosis (Hanks et al., 2005; Mergaert et al., 2006; Guefrachi et al., 2014; Maróti et al., 2015; Kereszt et al., 2018). However, the percentage of defensin genes that are expressed in the root nodules is low: of about 63 defensin genes encoded in the *M. truncatula* genome only 8 are expressed during symbiosis.

About 15 years ago, a new protein family — Nodule-Specific Cysteine-Rich Peptides (NCRs) — was discovered (Mergaert et al., 2003; Alunni et al., 2007; Maróti and Kondorosi, 2014). This family was first described in *M. truncatula*; later they were identified in other IRLC legumes (Inverted Repeat-lacking clade) (Mergaert et al., 2006; Alunni et al., 2007). To date, the NCR gene family contains about 700 members in the *M. truncatula* genome and more than 600 members in *Pisum sativum* (Zorin et al., 2019). NCR peptides resemble defensins (so the NCR genes are considered to have arisen from

the ancestral defensin genes (Maróti et al., 2015), but they are shorter than defensins (30–60 amino acids) and have 4 or 6 cysteine residues in conservative positions (instead of 8 or 10 cysteines present in defensins) (Tab. 1). The expression of NCR genes is extremely specific to nodules (Guefrachi et al., 2014), implying that they have a specific role in legume–rhizobial symbiosis. Indeed, recently it has been demonstrated that NCR peptides in *M. truncatula* govern the terminal (i.e., irreversible) differentiation of nodule bacteria into a symbiotic form called bacteroids (Van de Velde et al., 2010). Bacteroids are 10 times larger than free-living rhizobial cells, contain up to 24C genome copies of DNA, have permeabilized cell membranes, and possess an ability to fix atmospheric nitrogen (Mergaert et al., 2006; Van de Velde et al., 2010; Farkas et al., 2014; Alunni and Gourion, 2016).

Interestingly, NCR peptides can kill bacteria that are not compatible with the plant, thus demonstrating antimicrobial activity (in fact, the irreversible differentiation of bacteroids may also be viewed as the result of antimicrobial activity, since bacteroids lose the ability to procreate). On the other hand, mutations in specific NCR genes also lead to preliminary elimination of bacteria in nodule cells, so another role of NCRs may be maintenance of bacteria inside the nodule cells (Wang et al., 2017, 2018; Yang et al., 2017).

In experiments, NCR peptides also showed variable antimicrobial activities against bacteria and fungi (Van de Velde et al., 2010; Ördögh et al., 2014; Nagy et al., 2015; Farkas et al., 2017, 2018); thus, they can be considered potential antibiotics. When considering NCR peptides as components of antibiotic preparations it is important to remember that the defining feature of NCR peptides is not to cause lysis of bacterial cells, but to start the change in their morphology, physiology and genetics, and their antibiotic activity is inconsistent (Farkas et al., 2018).

The family of NCR-like peptides was discovered in other legume plants, *Aeschynomene* spp. (about 80 members in *A. evenia*) and *C. arietinum* (15 members) (Czernic et al., 2015; Montiel et al., 2015). The nodules of *Aeschynomene* species contain polyploid and elongated bacteroids, but less differentiated in comparison to that of *M. truncatula* (Czernic et al., 2015). Apparently, NCR-like peptides of *Aeschynomene* may perform the same function as in *Medicago*, but their number is an order of magnitude lower than in *M. truncatula*. They are referred to as NCR-like genes on the basis of low similarity of nucleotide and amino acid sequences with the NCR peptides of the IRLC legumes.

Besides NCR peptides, other AMPs may have an impact on establishment of legume–rhizobial symbiosis. It was demonstrated that during penetration of *Sinorhizobium meliloti* into *M. truncatula* roots, the expres-

sion level of the lipid transfer protein *MtN5* is increased in plant roots. It is assumed that *MtN5* modulates the perception or molecular activity of rhizobial signal molecules (Pii et al., 2009, p. 200, 2012). In symbiosis of *Astragalus sinicus* (Chinese milk vetch) with *Mesorhizobium huakuii* 7653R, a lipid transfer protein Ase246 is apparently involved in the transport of lipids synthesized by the plant to the symbiotic compartments (Lei et al., 2014).

AMPs in actinorhizal symbiosis

Actinorhizal symbiosis is formed between actinobacteria *Frankia* sp. and dicotyledon trees (Boonkerd, 1998; Pawlowski and Sirrenberg, 2003). The best-known example of this symbiosis is the association of *Frankia* with *Alnus* sp. that results in the formation of nodules containing actinobacteria on the tree roots. During the screening of Expressed Sequence Tags (ESTs) from the nodule database of *A. glutinosa*, 15 defensin-like transcripts have been identified as nodule-specific. One of them, Ag5, encodes a peptide that is similar in structure to the A3 class of defensins, and is able to interact with the *Frankia* cell membrane in a way similar to defensins. In experiments, addition of the peptide led to permeabilization of the bacterial membrane and slowing down of cell respiration, while the level of nitrogen fixation remained stable and even increased. It was also shown that the content of several amino acids increased in the supernatant of Ag5-treated cells, which may indicate the participation of this peptide in the metabolite exchange between partners (Carro et al., 2015). In another plant able to participate in actinorhizal symbiosis, *Datisca glomerata*, two transcripts highly expressed in nodules, DgDEF1 and DgDEF2, also similar to class 3 defensins, were identified. Interestingly, the two peptides were classified as a separate subfamily based on a unique amino acid pattern at their C-terminal region. This fact indicates that these peptides might play a role specific to actinorhizal symbioses. (Demina et al., 2013).

Evolution of AMPs

Despite the discovery of more than a thousand sequences encoding AMPs in plants, their origin and evolution remain unclear. Defensin-like proteins with similar sequences, structures, and functions were found in the plant, fungal and animal kingdoms, indicating their ancient origin (Hughes, 1999; Aerts et al., 2008); thus, the emergence of antimicrobial molecules in eukaryotes can be roughly dated to one billion years ago (Mygind et al., 2005; Zhu, 2008). One theory postulates that the defensin-like AMPs found in myxobacteria *Anaeromyxobacter dehalogenans* and *Stigmatella aurantiaca* might have served as the progenitors to all the AMPs found in

eukaryotes (Zhu, 2007). There is also a hypothesis about the independent origin of (some groups of) plant AMPs, which probably occurred before the plants assumed a terrestrial lifestyle.

It is assumed that the ancestral defense genes in plants were subjected to alternating multiple duplication events (resulting from unequal crossing over and/or retroposition) and directional (positive) selection (Hughes, 2008; Magadum et al., 2013). This evolution pattern may be possible due to special features of these genes (for example, high tolerance to changes in the copy number in the genome, functional redundancy (multiple genes serve a similar function) or location of the genes in between similar transposons). Thus, the gene sequence duplications, the mutual exchange of gene regions (mainly cysteine-containing), and the subsequent positive selection led to the emergence of new specialized families of AMPs in plants (Tiffin and Moeller, 2006). Some evolved AMP families are taxon-specific, such as the NCR peptides of legumes belonging to inverted-repeat lacking clade (IRLC), which points towards their recent appearance and quick evolution (Mergaert et al., 2003; Montiel et al., 2015). The rapid evolution rate of plant AMPs is also evidenced by the low sequence similarity percentage of mature peptides, which is consistent with their main function — being the best weapon in the arms race with rapidly evolving microorganisms.

Conclusion

Biotic and abiotic stresses are the major factors that affect crop yields. In light of this, AMPs are an important object for study, because they participate in the fine-tuning of plant responses to biotic and abiotic factors. AMPs not only provide defense against pathogens, but are also involved in plant response to abiotic factors. The activation of AMPs under any type of stress may serve to enhance its primary function, i.e., protection against pathogens: since abiotic stress increases the risk of pathogen attack, preventive activation of the immune system can increase plant resistance. On the other hand, a number of AMPs have more specific effects, such as providing resistance to heavy metals due to their ability to chelate metal ions, or regulating the development of symbiosis, especially the formation of symbiotic nodules in the case of legume–rhizobial and actinorhizal symbioses. Hence, further study of the molecular mechanisms of the participation of AMPs in biotic and abiotic interactions is important for achieving the goal of developing crops tolerant to multiple stresses.

References

- Abbasi, F., Onodera, H., Toki, S., Tanaka, H., and Komatsu, S. 2004. *OscDPK13*, a calcium-dependent protein kinase gene from rice, is induced by cold and gibberellin in rice leaf sheath. *Plant Molecular Biology* 55:541–552. <https://doi.org/10.1007/s11103-004-1178-y>
- Aerts, A. M., Bammens, L., Govaert, G., Carmona-Gutierrez, D., Madeo, F., Cammue, B. P. A., and Thevissen, K. 2011. The antifungal plant defensin HsAFP1 from *Heuchera sanguinea* induces apoptosis in *Candida albicans*. *Frontiers in Microbiology* 2:47. <https://doi.org/10.3389/fmicb.2011.00047>
- Aerts, A. M., François, I. E. J. A., Cammue, B. P. A., and Thevissen, K. 2008. The mode of antifungal action of plant, insect and human defensins. *Cellular and Molecular Life Sciences* 65:2069–2079. <https://doi.org/10.1007/s00018-008-8035-0>
- Aerts, A. M., François, I. E. J. A., Meert, E. M. K., Li, Q.-T., Cammue, B. P. A., and Thevissen, K. 2007. The antifungal activity of RsAFP2, a plant defensin from *Raphanus sativus*, involves the induction of reactive oxygen species in *Candida albicans*. *Journal of Molecular Microbiology and Biotechnology* 13:243–247. <https://doi.org/10.1159/000104753>
- Alonso-Ramirez, A., Rodriguez, D., Reyes, D., Jimenez, J. A., Nicolas, G., Lopez-Climent, M., Gomez-Cadenas, A., and Nicolas, C. 2009. Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in *Arabidopsis* seeds. *Plant Physiology* 150:1335–1344. <https://doi.org/10.1104/pp.109.139352>
- Alunni, B. and Gourion, B. 2016. Terminal bacteroid differentiation in the legume-rhizobium symbiosis: nodule-specific cysteine-rich peptides and beyond. *New Phytologist* 211:411–417. <https://doi.org/10.1111/nph.14025>
- Alunni, B., Kevei, Z., Redondo-Nieto, M., Kondorosi, A., Mergaert, P., and Kondorosi, E. 2007. Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in *Medicago truncatula*. *Molecular Plant-Microbe Interactions* 20:1138–1148. <https://doi.org/10.1094/MPMI-20-9-1138>
- Andreev, Y. A., Korostyleva, T. V., Slavokhotova, A. A., Rogozhin, E. A., Utkina, L. L., Vassilevski, A. A., Grishin, E. V., Egorov, T. A., and Odintsova, T. I. 2012. Genes encoding hevein-like defense peptides in wheat: distribution, evolution, and role in stress response. *Biochimie* 94:1009–1016. <https://doi.org/10.1016/j.biochi.2011.12.023>
- Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., and Sheen, J. 2002. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* 415:977–983. <https://doi.org/10.1038/415977a>
- Berrocal-Lobo, M., Segura, A., Moreno, M., López, G., García-Olmedo, F., and Molina, A. 2002. Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. *Plant Physiology* 128:951–961. <https://doi.org/10.1104/pp.010685>
- Bigeard, J., Colcombet, J., and Hirt, H. 2015. Signaling mechanisms in pattern-triggered immunity (PTI). *Molecular Plant* 8:521–539. <https://doi.org/10.1016/j.molp.2014.12.022>
- Bohlmann, H., Clausen, S., Behnke, S., Giese, H., Hiller, C., Reimann-Philipp, U., Schrader, G., Barkholt, V., and Apel, K. 1988. Leaf-specific thionins of barley — a novel class of cell wall proteins toxic to plant-pathogenic fungi and possibly involved in the defence mechanism of plants. *The EMBO Journal* 7:1559–1565. <https://doi.org/10.1002/j.1460-2075.1988.tb02980.x>
- Boonkerd, N. 1998. Symbiotic association between *Frankia* and actinorhizal plants, in: Malik, K. A., Mirza, M. S., and Ladha, J. K. (Eds.), Nitrogen Fixation with Non-Legumes: Proceedings of the 7th International Symposium on Nitrogen Fixation with Non-Legumes, Held 16–21 October 1996 in Faisalabad, Pakistan, Developments in Plant and

- Soil Sciences. Springer Netherlands, Dordrecht, pp. 327–331. https://doi.org/10.1007/978-94-011-5232-7_38
- Burman, R., Strömstedt, A. A., Malmsten, M., and Göransson, U. 2011. Cyclotide-membrane interactions: defining factors of membrane binding, depletion and disruption. *Biochimica et Biophysica Acta (BBA) — Biomembranes* 1808:2665–2673. <https://doi.org/10.1016/j.bbmem.2011.07.004>
- Campos, M. L., Lião, L. M., Alves, E. S. F., Migliolo, L., Dias, S. C., and Franco, O. L. 2018. A structural perspective of plant antimicrobial peptides. *Biochemical Journal* 475:3359–3375. <https://doi.org/10.1042/BCJ20180213>
- Carrasco, L., Vázquez, D., Hernández-Lucas, C., Carbonero, P., and García-Olmedo, F. 1981. Thionins: plant peptides that modify membrane permeability in cultured mammalian cells. *European Journal of Biochemistry* 116:185–189. <https://doi.org/10.1111/j.1432-1033.1981.tb05317.x>
- Carro, L., Pujic, P., Alloisio, N., Fournier, P., Boubakri, H., Hay, A. E., Poly, F., François, P., Hocher, V., Mergaert, P., Balmand, S., Rey, M., Heddi, A., and Normand, P. 2015. *Alnus* peptides modify membrane porosity and induce the release of nitrogen-rich metabolites from nitrogen-fixing *Frankia*. *The ISME Journal* 9:1723–1733. <https://doi.org/10.1038/ismej.2014.257>
- Carvalho, A. de O. and Gomes, V. M. 2011. Plant defensins and defensin-like peptides — biological activities and biotechnological applications. *Current Pharmaceutical Design* 17:4270–4293. <https://doi.org/10.2174/138161211798999447>
- Carvalho, A. de O. and Gomes, V. M. 2009. Plant defensins — prospects for the biological functions and biotechnological properties. *Peptides* 30:1007–1020. <https://doi.org/10.1016/j.peptides.2009.01.018>
- Czernic, P., Gully, D., Cartieaux, F., Moulin, L., Guefrachi, I., Patrel, D., Pierre, O., Fardoux, J., Chaintreuil, C., Nguyen, P., Gressent, F., Silva, C. D., Poulain, J., Wincker, P., Rofidal, V., Hem, S., Barrière, Q., Arrighi, J.-F., Mergaert, P., and Giraud, E. 2015. Convergent evolution of endosymbiont differentiation in dalbergioid and inverted repeat-lacking clade legumes mediated by nodule-specific cysteine-rich peptides. *Plant Physiology* 169:1254–1265. <https://doi.org/10.1104/pp.15.00584>
- Daneshmand, F., Zare-Zardini, H., and Ebrahimi, L. 2013. Investigation of the antimicrobial activities of Snakin-Z, a new cationic peptide derived from *Zizyphus jujuba* fruits. *Natural Product Research* 27:2292–2296. <https://doi.org/10.1080/14786419.2013.827192>
- De Caleyra, R. F., Gonzalez-Pascual, B., García-Olmedo, F., and Carbonero, P. 1972. Susceptibility of phytopathogenic bacteria to wheat purothionins *in vitro*. *Applied and Environmental Microbiology* 23:998–1000. <https://doi.org/10.1128/AEM.23.5.998-1000.1972>
- De Coninck, B., Cammue, B. P. A., and Thevissen, K. 2013. Modes of antifungal action and in planta functions of plant defensins and defensin-like peptides. *Fungal Biology Reviews* 26:109–120. <https://doi.org/10.1016/j.fbr.2012.10.002>
- De Vos, M., Van Oosten, V. R., Van Poecke, R. M. P., Van Pelt, J. A., Pozo, M. J., Mueller, M. J., Buchala, A. J., Métraux, J.-P., Van Loon, L. C., Dicke, M., and Pieterse, C. M. J. 2005. Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Molecular Plant-Microbe Interactions* 18:923–937. <https://doi.org/10.1094/MPMI-18-0923>
- Demina, I. V., Persson, T., Santos, P., Plaszczyca, M., and Pawlowski, K. 2013. Comparison of the nodule vs. root transcriptome of the actinorhizal plant *Datisca glomerata*: actinorhizal nodules contain a specific class of defensins. *PLoS ONE* 8(8):e72442. <https://doi.org/10.1371/journal.pone.0072442>
- Diaz, I., Carmona, M. J., and García-Olmedo, F. 1992. Effects of thionins on beta-glucuronidase *in vitro* and in plant protoplasts. *FEBS Letters* 296:279–282. [https://doi.org/10.1016/0014-5793\(92\)80304-y](https://doi.org/10.1016/0014-5793(92)80304-y)
- Do, H. M., Lee, S. C., Jung, H. W., Sohn, K. H., and Hwang, B. K. 2004. Differential expression and *in situ* localization of a pepper defensin (*CADEF1*) gene in response to pathogen infection, abiotic elicitors and environmental stresses in *Capsicum annum*. *Plant Science* 166:1297–1305. <https://doi.org/10.1016/j.plantsci.2004.01.008>
- Farkas, A., Maróti, G., Dürögő, H., Györgypál, Z., Lima, R. M., Medzihradszky, K. F., Kereszt, A., Mergaert, P., and Kondorosi, É. 2014. *Medicago truncatula* symbiotic peptide NCR247 contributes to bacteroid differentiation through multiple mechanisms. *Proceedings of the National Academy of Sciences USA* 111:5183–5188. <https://doi.org/10.1073/pnas.1404169111>
- Farkas, A., Maróti, G., Kereszt, A., and Kondorosi, É. 2017. Comparative analysis of the bacterial membrane disruption effect of two natural plant antimicrobial peptides. *Frontiers in Microbiology* 8:51. <https://doi.org/10.3389/fmicb.2017.00051>
- Farkas, A., Pap, B., Kondorosi, É., and Maróti, G. 2018. Antimicrobial activity of NCR plant peptides strongly depends on the test assays. *Frontiers in Microbiology* 9:2600. <https://doi.org/10.3389/fmicb.2018.02600>
- Fujimura, M., Minami, Y., Watanabe, K., and Tadera, K. 2003. Purification, characterization, and sequencing of a novel type of antimicrobial peptides, Fa-AMP1 and Fa-AMP2, from seeds of buckwheat (*Fagopyrum esculentum* Moench.). *Bioscience, Biotechnology, and Biochemistry* 67:1636–1642. <https://doi.org/10.1271/bbb.67.1636>
- Gangadhar, B. H., Sajeesh, K., Venkatesh, J., Baskar, V., Abhinandan, K., Yu, J. W., Prasad, R., and Mishra, R. K. 2016. Enhanced tolerance of transgenic potato plants over-expressing non-specific lipid transfer protein-1 (StnsLTP1) against multiple abiotic stresses. *Frontiers in Plant Science* 7:1228. <https://doi.org/10.3389/fpls.2016.01228>
- García, B. L., Segundo, B. S., and Coca, M. 2012. Antimicrobial peptides as a promising alternative for plant disease protection. *Small Wonders: Peptides for Disease Control* 263–294. <https://doi.org/10.1021/bk-2012-1095.ch013>
- Gaudet, D. A., Laroche, A., Frick, M., Huel, R., and Puchalski, B. 2003. Cold induced expression of plant defensin and lipid transfer protein transcripts in winter wheat. *Physiologia Plantarum* 117:195–205. <https://doi.org/10.1034/j.1399-3054.2003.00041.x>
- Guefrachi, I., Nagymihaly, M., Pislariu, C. I., Van de Velde, W., Ratet, P., Mars, M., Udvardi, M. K., Kondorosi, E., Mergaert, P., and Alunni, B. 2014. Extreme specificity of NCR gene expression in *Medicago truncatula*. *BMC Genomics* 15:712. <https://doi.org/10.1186/1471-2164-15-712>
- Guo, L., Yang, H., Zhang, X., and Yang, S. 2013. Lipid transfer protein 3 as a target of MYB96 mediates freezing and drought stress in *Arabidopsis*. *Journal of Experimental Botany* 64:1755–1767. <https://doi.org/10.1093/jxb/ert040>
- Gustafson, K. R., Sowder, R. C., Henderson, L. E., Parsons, I. C., Kashman, Y., Cardellina, J. H., McMahon, J. B., Buckheit, R. W., Pannell, L. K., and Boyd, M. R. 1994. Circulins A and B. Novel human immunodeficiency virus (HIV)-inhibitory macrocyclic peptides from the tropical tree *Chassalia parvifolia*. *Journal of the American Chemical Society* 116:9337–9338. <https://doi.org/10.1021/ja00099a064>
- Hanks, J. N., Snyder, A. K., Graham, M. A., Shah, R. K., Blylock, L. A., Harrison, M. J., and Shah, D. M. 2005. Defensin

- gene family in *Medicago truncatula*: structure, expression and induction by signal molecules. *Plant Molecular Biology* 58:385–399. <https://doi.org/10.1007/s11103-005-5567-7>
- Herbel, V., Schäfer, H., and Wink, M. 2015. Recombinant production of Snakin-2 (an antimicrobial peptide from tomato) in *E. coli* and analysis of its bioactivity. *Molecules* 20:14889–14901. <https://doi.org/10.3390/molecules200814889>
- Herbel, V. and Wink, M. 2016. Mode of action and membrane specificity of the antimicrobial peptide snakin-2. *PeerJ* 4:e1987. <https://doi.org/10.7717/peerj.1987>
- Huang, G.-J., Lai, H.-C., Chang, Y.-S., Sheu, M.-J., Lu, T.-L., Huang, S.-S., and Lin, Y.-H. 2008. Antimicrobial, dehydroascorbate reductase, and monodehydroascorbate reductase activities of defensin from sweet potato [*Ipomoea batatas* (L.) Lam. 'tainong 57'] storage roots. *Journal of Agricultural and Food Chemistry* 56:2989–2995. <https://doi.org/10.1021/jf072994j>
- Hughes, A. L. 2008. Defensins: Evolution, in: ELS. American Cancer Society. <https://doi.org/10.1002/9780470015902.a0006136.pub2>
- Hughes, A. L. 1999. Evolutionary diversification of the mammalian defensins. *Cellular and Molecular Life Sciences* 56:94–103. <https://doi.org/10.1007/s000180050010>
- Ireland, D. C., Wang, C. K. L., Wilson, J. A., Gustafson, K. R., and Craik, D. J. 2008. Cyclotides as natural anti-HIV agents. *Biopolymers* 90:51–60. <https://doi.org/10.1002/bip.20886>
- Kacperska, A. 2004. Sensor types in signal transduction pathways in plant cells responding to abiotic stressors: do they depend on stress intensity? *Physiologia Plantarum* 122:159–168. <https://doi.org/10.1111/j.0031-9317.2004.00388.x>
- Karpun, N. N., Yanushevskaya, E. B., and Mikhailova, Ye. V. 2015. Formation of plants nonspecific induced immunity at the biogenous stress (review). *Sel'skokhozyaistvennaya Biologiya [Agricultural Biology]* 50:540–549. <https://doi.org/10.15389/agrobiologia.2015.5.540eng>
- Kereszt, A., Mergaert, P., Montiel, J., Endre, G., and Kondorosi, É. 2018. Impact of plant peptides on symbiotic nodule development and functioning. *Frontiers in Plant Science* 9:1026. <https://doi.org/10.3389/fpls.2018.01026>
- Khan, S.-A., Li, M.-Z., Wang, S.-M., and Yin, H.-J. 2018. Revisiting the role of plant transcription factors in the battle against abiotic stress. *International Journal of Molecular Sciences* 19:1634. <https://doi.org/10.3390/ijms19061634>
- Ko, C.-B., Woo, Y.-M., Lee, D.-J., Lee, M.-C., and Kim, C. S. 2007. Enhanced tolerance to heat stress in transgenic plants expressing the *GASA4* gene. *Plant Physiology and Biochemistry* 45:722–728. <https://doi.org/10.1016/j.plaphy.2007.07.010>
- Koike, M., Okamoto, T., Tsuda, S., and Imai, R. 2002. A novel plant defensin-like gene of winter wheat is specifically induced during cold acclimation. *Biochemical and Biophysical Research Communications* 298:46–53. [https://doi.org/10.1016/S0006-291X\(02\)02391-4](https://doi.org/10.1016/S0006-291X(02)02391-4)
- Koo, J. C., Lee, B., Young, M. E., Koo, S. C., Cooper, J. A., Baek, D., Lim, C. O., Lee, S. Y., Yun, D.-J., and Cho, M. J. 2004. Pn-AMP1, a plant defense protein, induces actin depolarization in yeasts. *Plant and Cell Physiology* 45:1669–1680. <https://doi.org/10.1093/pcp/pch189>
- Koo, J. C., Lee, S. Y., Chun, H. J., Cheong, Y. H., Choi, J. S., Kawabata, S., Miyagi, M., Tsunasawa, S., Ha, K. S., Bae, D. W., Han, C. D., Lee, B. L., and Cho, M. J. 1998. Two hev-ein homologs isolated from the seed of *Pharbitis nil* L. exhibit potent antifungal activity. *Biochimica et Biophysica Acta (BBA) — Protein Structure and Molecular Enzymology* 1382:80–90. [https://doi.org/10.1016/S0167-4838\(97\)00148-9](https://doi.org/10.1016/S0167-4838(97)00148-9)
- Kramer, K. J., Klassen, L. W., Jones, B. L., Speirs, R. D., and Kammer, A. E. 1979. Toxicity of purothionin and its homologues to the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae). *Toxicology and Applied Pharmacology* 48:179–183. [https://doi.org/10.1016/S0041-008X\(79\)80020-4](https://doi.org/10.1016/S0041-008X(79)80020-4)
- Kumar, M., Yusuf, M. A., Yadav, P., Narayan, S., and Kumar, M. 2019. Overexpression of chickpea defensin gene confers tolerance to water-deficit stress in *Arabidopsis thaliana*. *Frontiers in Plant Science* 10:290. <https://doi.org/10.3389/fpls.2019.00290>
- Lay, F. T., Schirra, H. J., Scanlon, M. J., Anderson, M. A., and Craik, D. J. 2003. The three-dimensional solution structure of NaD1, a new floral defensin from *Nicotiana glauca* and its application to a homology model of the crop defense protein alfAFP. *Journal of Molecular Biology* 325:175–188. [https://doi.org/10.1016/S0022-2836\(02\)01103-8](https://doi.org/10.1016/S0022-2836(02)01103-8)
- Lee, O. R., Kim, Y.-J., Devi Balusamy, S. R., Kim, M.-K., Sathiyamoorthy, S., and Yang, D.-C. 2011. Ginseng γ -thionin is localized to cell wall-bound extracellular spaces and responsive to biotic and abiotic stresses. *Physiological and Molecular Plant Pathology* 76:82–89. <https://doi.org/10.1016/j.pmpp.2011.05.004>
- Lei, L., Chen, L., Shi, X., Li, Yixing, Wang, J., Chen, D., Xie, F., and Li, Y. 2014. A nodule-specific lipid transfer protein AsE246 participates in transport of plant-synthesized lipids to symbiosome membrane and is essential for nodule organogenesis in Chinese Milk vetch. *Plant Physiology* 164:1045–1058. <https://doi.org/10.1104/pp.113.232637>
- Li, S.-S., Gullbo, J., Lindholm, P., Larsson, R., Thunberg, E., Samuelsson, G., Bohlin, L., Claesson, P., 2002. Ligatoxin B, a new cytotoxic protein with a novel helix-turn-helix DNA-binding domain from the mistletoe *Phoradendron liga*. *Biochemical Journal* 366:405–413. <https://doi.org/10.1042/BJ20020221>
- Liu, J., Maldonado-Mendoza, I., Lopez-Meyer, M., Cheung, F., Town, C. D., and Harrison, M. J. 2007. Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *The Plant Journal* 50:529–544. <https://doi.org/10.1111/j.1365-313X.2007.03069.x>
- Lobo, D. S., Pereira, I. B., Frangel-Madeira, L., Medeiros, L. N., Cabral, L. M., Faria, J., Bellio, M., Campos, R. C., Linden, R., and Kurtenbach, E. 2007. Antifungal *Pisum sativum* defensin 1 interacts with *Neurospora crassa* cyclin F related to the cell cycle. *Biochemistry* 46:987–996. <https://doi.org/10.1021/bi061441j>
- Luo, J.-S., Gu, T., Yang, Y., and Zhang, Z. 2019. A non-secreted plant defensin AtPDF2.6 conferred cadmium tolerance via its chelation in *Arabidopsis*. *Plant Molecular Biology* 100:561–569. <https://doi.org/10.1007/s11103-019-00878-y>
- Luo, J.-S., Huang, J., Zeng, D.-L., Peng, J.-S., Zhang, G.-B., Ma, H.-L., Guan, Y., Yi, H.-Y., Fu, Y.-L., Han, B., Lin, H.-X., Qian, Q., and Gong, J.-M. 2018. A defensin-like protein drives cadmium efflux and allocation in rice. *Nature Communications* 9:645. <https://doi.org/10.1038/s41467-018-03088-0>
- Magadum, S., Banerjee, U., Murugan, P., Gangapur, D., and Ravikesavan, R. 2013. Gene duplication as a major force in evolution. *Journal of Genetics* 92:155–161. <https://doi.org/10.1007/s12041-013-0212-8>
- Maróti, G., Downie, J. A., and Kondorosi, É. 2015. Plant cysteine-rich peptides that inhibit pathogen growth and control rhizobial differentiation in legume nodules.

- Current Opinion in Plant Biology* 26:57–63. <https://doi.org/10.1016/j.pbi.2015.05.031>
- Maróti, G. and Kondorosi, E. 2014. Nitrogen-fixing *Rhizobium*-legume symbiosis: are polyploidy and host peptide-governed symbiont differentiation general principles of endosymbiosis? *Frontiers in Microbiology* 5:326. <https://doi.org/10.3389/fmicb.2014.00326>
- Maruyama, D., Sugiyama, T., Endo, T., and Nishikawa, S.-I. 2014. Multiple *BiP* genes of *Arabidopsis thaliana* are required for male gametogenesis and pollen competitiveness. *Plant and Cell Physiology* 55:801–810. <https://doi.org/10.1093/pcp/pcu018>
- Mergaert, P., Nikovics, K., Kelemen, Z., Maunoury, N., Vauvert, D., Kondorosi, A., and Kondorosi, E. 2003. A novel family in *Medicago truncatula* consisting of more than 300 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs. *Plant Physiology* 132:161–173. <https://doi.org/10.1104/pp.102.018192>
- Mergaert, P., Uchiumi, T., Alunni, B., Evanno, G., Cheron, A., Catrice, O., Mausset, A.-E., Barloy-Hubler, F., Galibert, F., Kondorosi, A., and Kondorosi, E. 2006. Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume symbiosis. *Proceedings of the National Academy of Sciences USA* 103:5230–5235. <https://doi.org/10.1073/pnas.0600912103>
- Miller, R. N. G., Costa Alves, G. S., and Van Sluys, M.-A. 2017. Plant immunity: unravelling the complexity of plant responses to biotic stresses. *Annals of Botany* 119:681–687. <https://doi.org/10.1093/aob/mcw284>
- Mirouze, M., Sels, J., Richard, O., Czernic, P., Loubet, S., Jacquier, A., François, I. E. J. A., Cammue, B. P. A., Lebrun, M., Berthomieu, P., and Marquès, L. 2006. A putative novel role for plant defensins: a defensin from the zinc hyperaccumulating plant, *Arabidopsis halleri*, confers zinc tolerance. *The Plant Journal* 47:329–342. <https://doi.org/10.1111/j.1365-313X.2006.02788.x>
- Mith, O., Benhamdi, A., Castillo, T., Bergé, M., MacDiarmid, C. W., Steffen, J., Eide, D. J., Perrier, V., Subileau, M., Gosti, F., Berthomieu, P., and Marquès, L. 2015. The antifungal plant defensin AhPDF1.1b is a beneficial factor involved in adaptive response to zinc overload when it is expressed in yeast cells. *MicrobiologyOpen* 4:409–422. <https://doi.org/10.1002/mbo3.248>
- Molina, A., Segura, A., and García-Olmedo, F. 1993. Lipid transfer proteins (nsLTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens. *FEBS Letters* 316:119–122. [https://doi.org/10.1016/0014-5793\(93\)81198-9](https://doi.org/10.1016/0014-5793(93)81198-9)
- Molina, C., Rotter, B., Horres, R., Udupa, S. M., Besser, B., Bellarmino, L., Baum, M., Matsumura, H., Terauchi, R., Kahl, G., and Winter, P. 2008. SuperSAGE: the drought stress-responsive transcriptome of chickpea roots. *BMC Genomics* 9:553. <https://doi.org/10.1186/1471-2164-9-553>
- Montiel, J., Szűcs, A., Boboescu, I. Z., Gherman, V. D., Kondorosi, É., and Kereszt, A. 2015. Terminal bacteroid differentiation is associated with variable morphological changes in legume species belonging to the inverted repeat-lacking clade. *Molecular Plant-Microbe Interactions* 29:210–219. <https://doi.org/10.1094/MPMI-09-15-0213-R>
- Mygind, P. H., Fischer, R. L., Schnorr, K. M., Hansen, M. T., Sönksen, C. P., Ludvigsen, S., Raventós, D., Buskov, S., Christensen, B., De Maria, L., Taboureau, O., Yaver, D., Elvig-Jørgensen, S. G., Sørensen, M. V., Christensen, B. E., Kjaerulf, S., Frimodt-Møller, N., Lehrer, R. I., Zasloff, M., and Kristensen, H.-H. 2005. Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature* 437:975–980. <https://doi.org/10.1038/nature04051>
- Nagy, K., Mikuláss, K. R., Végh, A. G., Kereszt, A., Kondorosi, É., Váró, G., and Szegletes, Z. 2015. Interaction of cysteine-rich cationic antimicrobial peptides with intact bacteria and model membranes. *General Physiology and Biophysics* 34:135–144. https://doi.org/10.4149/gpb_2015002
- Nahirňak, V., Almasia, N. I., Hopp, H. E., and Vazquez-Rovere, C. 2012. Snakin/GASA proteins: Involvement in hormone crosstalk and redox homeostasis. *Plant Signaling & Behavior* 7:1004–1008. <https://doi.org/10.4161/psb.20813>
- Nahirňak, V., Rivarola, M., Almasia, N. I., Barón, M. P. B., Hopp, H. E., Vile, D., Paniego, N., and Rovere, C. V. 2019. Snakin-1 affects reactive oxygen species and ascorbic acid levels and hormone balance in potato. *PLoS ONE* 14:e0214165. <https://doi.org/10.1371/journal.pone.0214165>
- Nakashima, K., Shinwari, Z. K., Sakuma, Y., Seki, M., Miura, S., Shinozaki, K., and Yamaguchi-Shinozaki, K. 2000. Organization and expression of two *Arabidopsis DREB2* genes encoding DRE-binding proteins involved in dehydration and high-salinity-responsive gene expression. *Plant Molecular Biology* 42:657–665. <https://doi.org/10.1023/a:1006321900483>
- Nishiyama, R., Le, D. T., Watanabe, Y., Matsui, A., Tanaka, M., Seki, M., Yamaguchi-Shinozaki, K., Shinozaki, K., and Tran, L.-S. P. 2012. Transcriptome analyses of a salt-tolerant cytokinin-deficient mutant reveal differential regulation of salt stress response by cytokinin deficiency. *PLoS ONE* 7:e32124. <https://doi.org/10.1371/journal.pone.0032124>
- Nolde, S. B., Vassilevski, A. A., Rogozhin, E. A., Barinov, N. A., Balashova, T. A., Samsonova, O. V., Baranov, Y. V., Feofanov, A. V., Egorov, T. A., Arseniev, A. S., and Grishin, E. V. 2011. Disulfide-stabilized helical hairpin structure and activity of a novel antifungal peptide EcAMP1 from seeds of barnyard grass (*Echinochloa crus-galli*). *The Journal of Biological Chemistry* 286:25145–25153. <https://doi.org/10.1074/jbc.M110.200378>
- Nongpiur, R., Soni, P., Karan, R., Singla-Pareek, S. L., and Pareek, A. 2012. Histidine kinases in plants. *Plant Signaling & Behavior* 7:1230–1237. <https://doi.org/10.4161/psb.21516>
- Oard, S. V. 2011. Deciphering a mechanism of membrane permeabilization by α -hordothionin peptide. *Biochimica et Biophysica Acta (BBA) — Biomembranes* 1808:1737–1745. <https://doi.org/10.1016/j.bbamem.2011.02.003>
- Oldroyd, G. E. D. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* 11:252–263. <https://doi.org/10.1038/nrmicro2990>
- Oliveira-Lima, M., Benko-Iseppon, A. M., Neto, J. R. C. F., Rodriguez-Decuadro, S., Kido, E. A., Crovella, S., and Pandolfi, V. 2017. Snakin: structure, roles and applications of a plant antimicrobial peptide. *Current Protein & Peptide Science* 18:368–374. <https://doi.org/10.2174/1389203717666160619183140>
- Onaga, G. and Wydra, K. 2016. Advances in plant tolerance to biotic stresses. *IntechOpen Plant Genomics*. <https://doi.org/10.5772/64351>
- Oomen, R. J., Séveno-Carpentier, E., Ricodeau, N., Bournaud, C., Conéjéro, G., Paris, N., Berthomieu, P., and Marquès, L. 2011. Plant defensin AhPDF1.1 is not secreted in leaves but it accumulates in intracellular compartments. *New Phytologist* 192:140–150. <https://doi.org/10.1111/j.1469-8137.2011.03792.x>
- Ördögh, L., Vörös, A., Nagy, I., Kondorosi, É., and Kereszt, A. 2014. Symbiotic plant peptides eliminate *Candida al-*

- bicans* both *in vitro* and in an epithelial infection model and inhibit the proliferation of immortalized human cells. *BioMed Research International*. <https://doi.org/10.1155/2014/320796>
- Pathogen Associated Molecular Pattern — an overview. ScienceDirect Topics [WWW Document], <https://www.sciencedirect.com/topics/medicine-and-dentistry/pathogen-associated-molecular-pattern>
- Pathogenesis-Related Protein — an overview. ScienceDirect Topics [WWW Document], <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pathogenesis-related-protein>
- Pawlowski, K. and Sirrenberg, A. 2003. Symbiosis between *Frankia* and actinorhizal plants: root nodules of non-legumes. *Indian Journal of Experimental Biology* 41:1165–1183.
- Piasecka, A., Jedrzejczak-Rey, N., and Bednarek, P. 2015. Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. *New Phytologist* 206:948–964. <https://doi.org/10.1111/nph.13325>
- Pii, Y., Astegno, A., Peroni, E., Zaccardelli, M., Pandolfini, T., and Crimi, M. 2009. The *Medicago truncatula* N5 gene encoding a root-specific lipid transfer protein is required for the symbiotic interaction with *Sinorhizobium meliloti*. *Molecular Plant-Microbe Interactions* 22:1577–1587. <https://doi.org/10.1094/MPMI-22-12-1577>
- Pii, Y., Molesini, B., Masiero, S., and Pandolfini, T. 2012. The non-specific lipid transfer protein N5 of *Medicago truncatula* is implicated in epidermal stages of rhizobium-host interaction. *BMC Plant Biology* 12:233. <https://doi.org/10.1186/1471-2229-12-233>
- Polanowski, A., Wilusz, T., Nienartowicz, B., Cieślak, E., Słomińska, A., and Nowak, K. 1980. Isolation and partial amino acid sequence of the trypsin inhibitor from the seeds of *Cucurbita maxima*. *Acta Biochimica Polonica* 27:371–382.
- Rejeb, I., Pastor, V., and Mauch-Mani, B. 2014. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants* 3(4):458–475. <https://doi.org/10.3390/plants3040458>
- Sagaram, U.S., Pandurang, R., Kaur, J., Smith, T.J., and Shah, D.M. 2011. Structure-activity determinants in antifungal plant defensins MsDef1 and MtDef4 with different modes of action against *Fusarium graminearum*. *PLoS ONE* 6:e18550. <https://doi.org/10.1371/journal.pone.0018550>
- Segura, A., Moreno, M., and García-Olmedo, F. 1993. Purification and antipathogenic activity of lipid transfer proteins (LTPs) from the leaves of *Arabidopsis* and spinach. *FEBS Letters* 332:243–246. [https://doi.org/10.1016/0014-5793\(93\)80641-7](https://doi.org/10.1016/0014-5793(93)80641-7)
- Selitrennikoff, C. P. 2001. Antifungal proteins. *Applied and Environmental Microbiology* 67:2883–2894. <https://doi.org/10.1128/AEM.67.7.2883-2894.2001>
- Slavokhotova, A. A., Shelenkova, A. A., Andreev, Y. A., and Odintsova, T. I. 2017. Hevein-like antimicrobial peptides of plants. *Biochemistry (Moscow)* 82:1659–1674. <https://doi.org/10.1134/S0006297917130065>
- Slazak, B., Kapusta, M., Malik, S., Bohdanowicz, J., Kuta, E., Malec, P., and Göransson, U. 2016. Immunolocalization of cyclotides in plant cells, tissues and organ supports their role in host defense. *Planta* 244:1029–1040. <https://doi.org/10.1007/s00425-016-2562-y>
- Sousa, D. A., Porto, W. F., Silva, M. Z., Da Silva, T. R., and Franco, O. L. 2016. Influence of cysteine and tryptophan substitution on DNA-binding activity on maize α -hairpinin antimicrobial peptide. *Molecules* 21:1062. <https://doi.org/10.3390/molecules21081062>
- Stec, B. 2006. Plant thionins — the structural perspective. *Cellular and Molecular Life Sciences* 63:1370–1385. <https://doi.org/10.1007/s00018-005-5574-5>
- Stec, B., Markman, O., Rao, U., Heffron, G., Henderson, S., Vernon, L. P., Brumfeld, V., and Teeter, M. M. 2004. Proposal for molecular mechanism of thionins deduced from physico-chemical studies of plant toxins. *The Journal of Peptide Research* 64:210–224. <https://doi.org/10.1111/j.1399-3011.2004.00187.x>
- Stolf-Moreira, R., Medri, M. E., Neumaier, N., Lemos, N. G., Pimenta, J. A., Tobita, S., Brogin, R. L., Marcelino-Guimarães, F. C., Oliveira, M. C. N., Farias, J. R. B., Abdelnoor, R. V., and Nepomuceno, A. L. 2010. Soybean physiology and gene expression during drought. *Genetics and Molecular Research* 9:1946–1956. <https://doi.org/10.4238/vol9-4gmr851>
- Stotz, H. U., Thomson, J., and Wang, Y. 2009. Plant defensins: defense, development and application. *Plant Signaling & Behavior* 4:1010–1012. <https://doi.org/10.4161/psb.4.11.9755>
- Strömstedt, A. A., Ringstad, L., Schmidtchen, A., and Malmsten, M. 2010. Interaction between amphiphilic peptides and phospholipid membranes. *Current Opinion in Colloid & Interface Science* 15:467–478. <https://doi.org/10.1016/j.cocis.2010.05.006>
- Sui, J., Jiang, D., Zhang, D., Song, X., Wang, J., Zhao, M., and Qiao, L. 2016. The salinity responsive mechanism of a hydroxyproline-tolerant mutant of peanut based on digital gene expression profiling analysis. *PLoS ONE* 11:e0162556. <https://doi.org/10.1371/journal.pone.0162556>
- Sun, S., Wang, H., Yu, H., Zhong, C., Zhang, X., Peng, J., and Wang, X. 2013. *GASA14* regulates leaf expansion and abiotic stress resistance by modulating reactive oxygen species accumulation. *Journal of Experimental Botany* 64:1637–1647. <https://doi.org/10.1093/jxb/ert021>
- Svangård, E., Burman, R., Gunasekera, S., Lövborg, H., Gullbo, J., and Göransson, U. 2007. Mechanism of action of cytotoxic cyclotides: cycloviolacin O₂ disrupts lipid membranes. *Journal of Natural Products* 70:643–647. <https://doi.org/10.1021/np070007v>
- Taji, T., Seki, M., Satou, M., Sakurai, T., Kobayashi, M., Ishiyama, K., Narusaka, Y., Narusaka, M., Zhu, J.-K., and Shinozaki, K. 2004. Comparative genomics in salt tolerance between *Arabidopsis* and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. *Plant Physiology* 135:1697–1709. <https://doi.org/10.1104/pp.104.039909>
- Tam, J. P., Wang, S., Wong, K. H., and Tan, W. L. 2015. Antimicrobial peptides from plants. *Pharmaceuticals* 8:711–757. <https://doi.org/10.3390/ph8040711>
- Terras, F. R., Eggermont, K., Kovaleva, V., Raikhel, N. V., Osborn, R. W., Kester, A., Rees, S. B., Torrekens, S., Van Leuven, F., and Vanderleyden, J. 1995. Small cysteine-rich antifungal proteins from radish: their role in host defense. *The Plant Cell* 7:573–588. <https://doi.org/10.1105/tpc.7.5.573>
- Thomma, B. P. H. J., Nürnberger, T., and Joosten, M. H. A. J. 2011. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *The Plant Cell* 23:4–15. <https://doi.org/10.1105/tpc.110.082602>
- Tiffin, P. and Moeller, D. A. 2006. Molecular evolution of plant immune system genes. *Trends in Genetics* 22:662–670. <https://doi.org/10.1016/j.tig.2006.09.011>
- Turrini, A., Sbrana, C., Pitto, L., Castiglione, M. R., Giorgetti, L., Briganti, R., Bracci, T., Evangelista, M., Nuti, M. P., and Giovannetti, M. 2004. The antifungal Dm-AMP1 protein from *Dahlia merckii* expressed in *Solanum melongena* is released in root exudates and differentially affects

- pathogenic fungi and mycorrhizal symbiosis. *New Phytologist* 163:393–403. <https://doi.org/10.1111/j.1469-8137.2004.01107.x>
- Van de Velde, W., Zehirov, G., Szatmari, A., Debreczeny, M., Ishihara, H., Kevei, Z., Farkas, A., Mikulass, K., Nagy, A., Tiricz, H., Satiat-Jeunemaître, B., Alunni, B., Bourge, M., Kucho, K., Abe, M., Kereszt, A., Maroti, G., Uchiyumi, T., Kondorosi, E., and Mergaert, P. 2010. Plant peptides govern terminal differentiation of bacteria in symbiosis. *Science* 327:1122–1126. <https://doi.org/10.1126/science.1184057>
- Van den Bergh, K. P. B., Rougé, P., Proost, P., Coosemans, J., Krouglova, T., Engelborghs, Y., Peumans, W. J., and Van Damme, E. J. M. 2004. Synergistic antifungal activity of two chitin-binding proteins from spindle tree (*Euonymus europaeus* L.). *Planta* 219:221–232. <https://doi.org/10.1007/s00425-004-1238-1>
- Vasilchenko, A. S., Yuryev, M., Ryazantsev, D. Y., Zavriev, S. K., Feofanov, A. V., Grishin, E. V., and Rogozhin, E. A. 2016. Studying of cellular interaction of hairpin-like peptide EcAMP1 from barnyard grass (*Echinochloa crusgalli* L.) seeds with plant pathogenic fungus *Fusarium solani* using microscopy techniques. *Scanning* 38:591–598. <https://doi.org/10.1002/sca.21305>
- Verma, S., Nizam, S., and Verma, P. K. 2013. Biotic and abiotic stress signaling in plants, in: Sarwat, M., Ahmad, A., and Abdin, M. (Eds.), *Stress signaling in plants: Genomics and proteomics perspective*, Volume 1. Springer New York, New York, NY, pp. 25–49. https://doi.org/10.1007/978-1-4614-6372-6_2
- Vernon, L. P. and Bell, J. D. 1992. Membrane structure, toxins and phospholipase A2 activity. *Pharmacology & Therapeutics* 54:269–295. [https://doi.org/10.1016/0163-7258\(92\)90003-i](https://doi.org/10.1016/0163-7258(92)90003-i)
- Wang, Q., Liu, J., Li, H., Yang, S., Körmöczy, P., Kereszt, A., and Zhu, H. 2018. Nodule-specific cysteine-rich peptides negatively regulate nitrogen-fixing symbiosis in a strain-specific manner in *Medicago truncatula*. *Molecular Plant-Microbe Interactions* 31:240–248. <https://doi.org/10.1094/MPMI-08-17-0207-R>
- Wang, Q., Yang, S., Liu, J., Terecskei, K., Ábrahám, E., Gombár, A., Domonkos, Á., Szűcs, A., Körmöczy, P., Wang, T., Fodor, L., Mao, L., Fei, Z., Kondorosi, É., Kaló, P., Kereszt, A., and Zhu, H. 2017. Host-secreted antimicrobial peptide enforces symbiotic selectivity in *Medicago truncatula*. *Proceedings of the National Academy of Sciences USA* 114:6854–6859. <https://doi.org/10.1073/pnas.1700715114>
- Ward, J. M., Mäser, P., and Schroeder, J. I. 2009. Plant ion channels: gene families, physiology, and functional genomics analyses. *Annual Review of Physiology* 71:59–82. <https://doi.org/10.1146/annurev.physiol.010908.163204>
- Weerden, N. L. van der, Lay, F. T., and Anderson, M. A. 2008. The plant defensin, NaD1, enters the cytoplasm of *Fusarium oxysporum* hyphae. *The Journal of Biological Chemistry* 283:14445–14452. <https://doi.org/10.1074/jbc.M709867200>
- Woynarowski, J. M. and Konopa, J. 1980. Interaction between DNA and viscotoxins. Cytotoxic basic polypeptides from *Viscum album* L. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* 361:1535–1545. <https://doi.org/10.1515/bchm2.1980.361.2.1535>
- Yang, S., Wang, Q., Fedorova, E., Liu, J., Qin, Q., Zheng, Q., Price, P. A., Pan, H., Wang, D., Griffiths, J. S., Bisseling, T., and Zhu, H. 2017. Microsymbiont discrimination mediated by a host-secreted peptide in *Medicago truncatula*. *Proceedings of the National Academy of Sciences USA* 114:6848–6853. <https://doi.org/10.1073/pnas.1700460114>
- Ye, Y., Ding, Y., Jiang, Q., Wang, F., Sun, J., and Zhu, C. 2017. The role of receptor-like protein kinases (RLKs) in abiotic stress response in plants. *Plant Cell Reports* 36:235–242. <https://doi.org/10.1007/s00299-016-2084-x>
- Zhang, H. and Sonnewald, U. 2017. Differences and commonalities of plant responses to single and combined stresses. *The Plant Journal* 90:839–855. <https://doi.org/10.1111/tpj.13557>
- Zhang, S. and Wang, X. 2011. Overexpression of GAS45 increases the sensitivity of *Arabidopsis* to heat stress. *Journal of Plant Physiology* 168:2093–2101. <https://doi.org/10.1016/j.jplph.2011.06.010>
- Zhu, S. 2008. Discovery of six families of fungal defensin-like peptides provides insights into origin and evolution of the CSaβ defensins. *Molecular Immunology* 45:828–838. <https://doi.org/10.1016/j.molimm.2007.06.354>
- Zhu, S. 2007. Evidence for myxobacterial origin of eukaryotic defensins. *Immunogenetics* 59:949–954. <https://doi.org/10.1007/s00251-007-0259-x>
- Zipfel, C. and Oldroyd, G. E. D. 2017. Plant signalling in symbiosis and immunity. *Nature* 543:328–336. <https://doi.org/10.1038/nature22009>
- Zou, H.-W., Tian, X.-H., Ma, G.-H., and Li, Z.-X. 2013. Isolation and functional analysis of ZmLTP3, a homologue to *Arabidopsis* LTP3. *International Journal of Molecular Sciences* 14:5025–5035. <https://doi.org/10.3390/ijms14035025>