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Plant defensins
Defense, development and application

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Abbreviations: AMP, antimicrobial peptide; DEFL, defensin-like; alfAFP, alfafa antifungal protein; DaD1, tobacco flower defensin; CTPP, C-terminal propeptide domain; RsAFP2, radish antifungal protein 2

Plant defensins are small, highly stable, cysteine-rich peptides that constitute a part of the innate immune system primarily directed against fungal pathogens. Biological activities reported for plant defensins include antifungal activity, antibacterial activity, proteinase inhibitory activity and insect amylase inhibitory activity. Plant defensins have been shown to inhibit infectious diseases of humans and to induce apoptosis in a human pathogen. Transgenic plants overexpressing defensins are strongly resistant to fungal pathogens. Based on recent studies, some plant defensins are not merely toxic to microbes but also have roles in regulating plant growth and development.

Defensins are diverse members of a large family of cationic host defence peptides (HDP), widely distributed throughout the plant and animal kingdoms.[1-3] Defensins and defensin-like peptides are functionally diverse, disrupting microbial membranes and acting as ligands for cellular recognition and signaling.[4] In the early 1990s, the first members of the family of plant defensins were isolated from wheat and barley grains.[5,6] Those proteins were originally called γ-thionins because their size (~5 kDa, 45 to 54 amino acids) and cysteine content (typically 4, 6 or 8 cysteine residues) were found to be similar to the thionins.[7] Subsequent "γ-thionins" homologous proteins were indentified and cDNAs were cloned from various monocot or dicot seeds.[8] Terras and his colleagues[9] isolated two antifungal peptides, Rs-AFP1 and Rs-AFP2, noticed that the plant peptides' structural and functional properties resemble those of insect and mammalian defensins, and therefore termed the family of peptides "plant defensins" in 1995. Sequences of more than 80 different plant defensin genes from different plant species were analyzed.[10] A query of the UniProt database (www.uniprot.org/) currently reveals publications of 371 plant defensins available for review. The Arabidopsis genome alone contains more than 300 defensin-like (DEFL) peptides, 78% of which have a cysteine-stabilized α-helix β-sheet (CSαβ) motif common to plant and invertebrate defensins.[11] In addition, over 1,000 DEFL genes have been identified from plant EST projects.[12]

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Unlike the insect and mammalian defensins, which are mainly active against bacteria,[2,3,10,13] plant defensins, with a few exceptions, do not have antibacterial activity.[14] Most plant defensins are involved in defense against a broad range of fungi.[2,3,10,15] They are not only active against phytopathogenic fungi (such as *Fusarium culmorum* and *Botrytis cinerea*), but also against baker's yeast and human pathogenic fungi (such as *Candida albicans*).[2] Plant defensins have also been shown to inhibit the growth of roots and root hairs in *Arabidopsis thaliana*[16] and alter growth of various tomato organs which can assume multiple functions related to defense and development.[4]

Plant Defensins Play Roles in Protection of Seeds and Various Organs

Most plant defensins isolated to date are seed-derived and have been characterized at the molecular, biochemical and structural levels.[2,10,14,17-19] In radish, defensin protein (Rs-AFPs) represents 0.5% of the total protein in seeds. During seed germination, the imbibed seed loses its coat protection and is more vulnerable to soil microorganism. Rs-AFPs are preferentially released after disruption of the seed coat and accounts for 30% of the proteins released from radish seed coats. The amount of released proteins is sufficient to suppress the fungal growth in the soil.[9] Defensins are relatively abundant in seed tissue could protect seed from soil fungi and thus enhance seedling survival rate.[9,20] Defensins have also been identified in other tissues, including leaves, pods, tubers, fruit, roots, bark and floral organs.[2,3,10,20] Each organ expresses at least one defensin gene, some organs express two or more in Arabidopsis.[11] They are expressed during normal plant growth and development.[20] Most of the plant defensins are concentrated mainly in peripheral cells and stomatal cells in plants which is consistent with a role of defensins in protection against pathogens.[2,9,20] The defense role is also supported by the pathogen-inducibility of some plant defensin genes. Defensin genes induced upon pathogen infection have been identified in pea, tobacco, Arabidopsis and spruce.[2,10,20]

In addition to pathogen-induction, defensins can also be induced by environmental stress, such as drought, salt and cold,[2] and the signaling molecules, such as methyl jasmonate, ethylene and salicylic acid.[21] The expression of the defensins induced in response to a variety of biotic and abiotic stimuli provides examples of cross-talk between signal transduction pathways and gene expression programs.[2]

A Role for Antimicrobial Activity

The mature transcript of most plant defensins can be divided into two classes.[2,20] The first and largest class, the precursor protein is composed of an amino-signal peptide that targets the peptide to the extracellular space. The second class of defensins is produced as larger precursors with C-terminal prodomains. Comparison of a series of sequences of plant defensins from various plant species reveals that the plant defensin family shows very limited sequence conservation except for eight cysteine, two glycine and one glutamate acid residues.[2] With the exception of defensins that have antibacterial activity[22,23] or insecticide activity,[24] most plant defensins have a powerful fungicide action. Antimicrobial subfamilies usually exhibit distinct antifungal traits such as those that alter the morphology of the target fungal hyphae, distorting their growth and thus impeding cellular penetration.[9,14] Structural determinants of plant defensins that govern their antifungal activity and the molecular mechanism by which they inhibit fungal growth remain unclear.[2,3,20,25]

Unlike mammalian and insect defensins, plant defensins do not interact directly with plasma membrane phospholipids.[2,3,20] RsAFP2 interacts with glucosylceramides (GlcCer) in membranes of susceptible yeast and fungi and induces membrane permeabilization and fungal cell death.[26-28] Aerts et al.[29] reported that the RsAFP2 is involved the induction of reactive oxygen species (ROS) in *Candida albicans* resulting in membrane permeabilization and leading to fungal cell growth arrest. Calcium signaling, which is vitally important for tip growth of fungi, can be altered by defensins. For example, the alfafa defensin (MsDef1) can block the L-type Ca^{2+} channel and inhibit hyphal growth and induce hyperbranching of fungal hyphae.[25]

Certain plant defensins also display other biological activities, including proteinase,[30] α-amylase inhibitory activity, and inhibition of protein translation that may contribute to their role in defense.[5,6,20,31]

Regulation of Plant Growth and Development

To date, only two studies reported that the plant defensins may have additional roles in plant growth and development beyond their presumed role in plant defense against fungal pathogens. For example, the *Medicago sativa* defensins MsDef1 and MtDef2 are not expressed in the roots of Medicago spp. Exogenously treated MsDef1, MtDef2, KP4 and RsAFP2 inhibit the growth of Arabidopsis root and root hair.[16] However, transgenic Arabidopsis plants expressing MsDef1 constitutively did not exhibit any growth alterations at the macroscopic level (unpublished results).[16] Our data indicated that antisense repression and overexpression of tomato defensin DEF2 reduces pollen viability and seed production. DEF2 overexpression in tomato plant showed that growth was initially retarded. The transgenic plants leaves were smaller and growth was more upright, resulting in a more open architecture.[4] So far, the developmental process is still poorly understood. These findings provided new insights that plant defensins may play a role in regulating plant growth and development.

Potential for Applied Biotechnology

Transgenic plants have the potential to provide broad resistance against different pathogens, and are likely to reduce dependence on chemical pesticides. Various defensins genes have been successfully transformed into tobacco, tomato, oilseed rape, rice and papaya.[2,4,10,20,32] Overexpression of wasabi defensin (WT1) in rice, potato and orchid has resulted in increased resistance against *Magnaporthe grisea*, *Erwinia carotovora* and *Botrytis cinerea*.[2] The generation of transgenic tomato plants constitutively expressing the chili defensin (*cdef1*) gene resulted in enhanced resistance against *Phytophthora infestans* and Fusarium sp.[33] Expression of Dahlia defensin, Dm-AMP1, in rice directly inhibits the pathogen, *Magnaporthe oryzae* and *Rhizoctonia solani*. It was observed that constitutive expression of Dm-AMP1 suppresses the growth of *M. oryzae* and *R. solani* by 84% and 72%, respectively.[34] Results from greenhouse inoculation experiments demonstrate that expressing the *DmAMP*1 gene in papaya plants increased resistance against *P. palmivora* and that this increased resistance was associated with reduced hyphae growth of *P. palmivora* at the infection sites.[32] Gao and his colleagues showed that the transgenic plants accumulated high levels of the alfalfa defensin in both roots and leaves. The plants performed well both in greenhouse and field trials, where they did at least as well as commercial fumigants. Results suggest that defensin genes have important commercial potential for effective fungal control in economically important crops.[35]

However, despite published reports of successful expression of certain functional plant defensins in certain transgenic plants (Arabidopsis, tomato and cotton), it has now been discovered that some defensins have toxic effects when continuously expressed. The effects can include, for example, reduced cell growth, reduced efficiency of regeneration, reduced fertility and abnormal morphology of regenerated transgenic plants.[4,16,36] The toxic effects can vary depending on the level of defensin expression, tissue specificity of expression, and the developmental stage of the host plant when expression occurs. Anderson et al.[36] modified a "chimeric defensin" to reduce or eliminate a toxic effect of transgenic defensin expression in a host plant. The modified defensin has a mature defensin domain NaD1 combined with a C-terminal propeptide domain (CTPP). The presence of mature NaD1 lacking a CTPP causes abnormal growth of transgenic cotton resulting in distorted leaves and short internodes. Plants that had been transformed with NaD1 gene containing the CTPP do not exhibit this abnormal phenotype, suggesting the CTPP either protects the plant from a toxic part of the molecule or it targets the protein to the vacuole where it is sequestered.[36]

It has been demonstrated that plants expressing human,[37] rabbit[38] or insect[39] defensin acquire resistance against fungal pathogens, indicating a functional homology next to the already known structural homology, between defensins originating from different eukaryotic kingdoms. Aert et al.[37] showed that that the antifungal plant defensin RsAFP2 from radish induces apoptosis and concomitantly triggers activation of caspases or caspase-like proteases in the human pathogen *Candida albicans.* The plant defensin PSD1 can interact with cyclin F and inhibit human cancer cell cycle.[40] Ground bean AMP peptide exerted antiproliferative activity toward breast cancer (MCF-7) cells and leukemia M1 cells. It also exhibited some inhibitory activity toward human immunodeficiency virus-type 1 reverse transcriptase[41] and the molecular mechanism is still unknown. All the results indicate a possibility of medical applications involving plant defensins to treat human diseases.

Conclusion

Plant defensins are expressed in various organs and provide a first line of defense against pathogen attack. Although interactions of plant defensins with pathogens are not well understood, these peptides can be overexpressed in transgenic crops, often resulting in improved resistance to pathogen. Since there is functional and structural homology among plant and human defensins, the plant system can be used to express the defensins for human needs. Defensin functions are not limited to antimicrobial activity; they also appear to be involved in cellular signaling, and growth regulation.[4,42,43] More detailed studies of receptor-ligand interactions are needed to understand the functional diversity of this class of antimicrobial peptides.

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References

1. Taylor K, Barran PE, Dorin JR. Structure-activity relationships in beta-defensin peptides. Biopolymers 2008; 90:1-7.
2. Lay FT, Anderson MA. Defensins—components of the innate immune system in plants. Curr Prot Pept Sci 2005; 6:85-101.
3. Wong JH, Xia L, Ng TB. A review of defensins of diverse origins. Curr Protein Peptide Sci 2007; 8:446-59.
4. Stotz HU, Spence B, Wang Y. A defensin from tomato with dual function in defense and development. Plant Mol Biol 2009; 71:131-43.
5. Colilla FJ, Rocher A, Mendez E. Gamma-Purothionins: amino acid sequence of two polypeptides of a new family of thionins from wheat endosperm. FEBS Lett 1990; 270:191-4.
6. Mendez E, Moreno A, Colilla F, Pelaez R, Limas GG, Mendez R, et al. Primary structure and inhibition of protein synthesis in eukaryotic cell-free system of a novel thionin, gamma-thionin, from barley endosperm. Europ J Biochem 1990; 194:533-9.
7. Bruix M, Jime nez MA, Santoro J, Gonzalez C, Colilla FJ, Mendez E, et al. Solution structure of gamma1-H and gamma1-P thionins from barley and wheat endosperm determined by ^{1}H-NMR: a structural motif common to toxic arthropod proteins. Biochemistry 1993; 32:715-24.
8. Broekaert WF, Terras FR, Cammue BP, Osborn RW. Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol 1995; 108:1353-8.
9. Terras FRG, Eggermont K, Kovaleva V, Raikhel NV, Osborn RW, Kester A, et al. Small cysteine-rich antifungal proteins from radish: Their role in host defense. Plant Cell 1995; 7:573-88.
10. Thomma BPHJ, Cammue BPA, Thevissen K. Plant defensins. Planta 2002; 216:193-202.
11. Silverstein KAT, Graham MA, Paape TD, VandenBosch KA. Genome organization of more than 300 defensin-like genes in Arabidopsis. Plant Physiol 2005; 138:600-10.
12. Graham MA, Silverstein KAT, VandenBosch KA. Defensin-like genes: Genomic perspectives on a diverse superfamily in plants. Crop Sci 2008; 48:3-11.
13. Wong JH, Ng TB. Sesquin, a potent defensin-like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. Peptides 2005; 26:1120-6.
14. Osborn RW, De Samblanx GW, Thevissen K, Goderis I, Torrekens S, Van Leuven F, et al. Isolation and characterisation of plant defensins from seeds of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae. FEBS Lett 1995; 368:257-62.
15. Thevissen K, Cammue BPA, Lemaire K, Winderickx J, Dickson RC, Lester RL, et al. A gene encoding a sphingolipid biosynthesis enzyme determines the sensitivity of *Saccharomyces cerevisiae* to an antifungal plant defensin from dahlia (*Dahlia merckii*). Proc Natl Acad Sci USA 2000; 97:9531-6.
16. Allan A, Snyder AK, Preuss M, NIelsen EE, Shah DM, Smith TJ. Plant defensins and virally encoded fungal toxin KP4 inhibit plant root growth. Planta 2008; 227:331-9.
17. Terras FRG, Torrekens S, Van Leuven F, Osborn RW, Vanderleyden J, Cammue BPA, et al. A new family of basic cysteine-rich plant antifungal proteins from Brassicaceae species. FEBS 1993; 316:233-40.
18. Liu Y, Luo J, Xu C, Ren F, Peng C, Wu G, Zhao J. Purification, characterization and molecular cloning of the gene of a seed-specific antimicrobial protein from Pokeweed. Plant Physiol 2000; 122:1015-24.
19. Song X, Wang J, Wu F, Li X, Teng M, Gong W. cDNA cloning, functional expression and antifungal activities of a dimeric plant defensin SPE10 from *Pachyrrhizus erosus* seeds. Plant Mol Biol 2005; 57:13-20.
20. Carvalho AO, Gomesa VM. Plant defensins—Prospects for the biological functions and biotechnological properties. Peptides 2009; 30:1007-20.
21. Hanks JN, Snyder AK, Graham MA, Shah RK, Blaylock LA, Harrison MJ, et al. Defensin gene family in *Medicago truncatula*: structure, expression and induction by signal molecules. Plant Mol Biol 2005; 58:385-99.
22. Moreno M, Segura A, Garcia-Olmedo F. Pseudothionin-St1, a potato peptide active against potato pathogens. Eur J Biochem 1994; 223:135-9.
23. Segura A, Moreno M, Molina A, Garcia-Olmedo F. Defensin subfamily from spinach (*Spinacia oleracea*). FEBS Lett 1998; 435:159-62.
24. Bloch CJ, Richardson M. A new family of small (5 kDa) protein inhibitors of insect a-amylases from seeds of sorghum (*Sorghum bicolor (L) Moench*) have sequence homologies with wheat g-purothionins. FEBS 1991; 279:101-4.
25. Spelbrink RG, Dilmac N, Allen A, Smith TJ, Shah DM, Hockerman GH. Differential antifungal and calcium channel-blocking activity among structurally related plant defensins. Plant Physiol 2004; 135:2055-67.
26. Thevissen K, Ghazi A, De Samblanx GW, Brownlee C, Osborn RW, Broekaert WF. Fungal membrane responses induced by plant defensins and thionins. J Biol Chem 1996; 271:15018-25.
27. Thevissen K, Terras FR, Broekaert WF. Permeabilization of fungal membranes by plant defensins inhibits fungal growth. Appl Environ Microbiol 1999; 65:5451-8.
28. Thevissen K, Warnecke DC, François IEJA, Leipelt M, Heinz E, Ott C, et al. Defensins from insects and plants interact with fungal glucosylceramides. J Biol Chem 2004; 279:3900-5.
29. Aerts AM, François IE, Meert EMK, Li QT, Cammue BPA, Thevissen K. The Antifungal Activity of RsAFP2, a plant defensin from *Raphanus sativus*, involves the induction of reactive oxygen species in *Candida albicans.* J Mol Microbiol Biotech 2007; 13:243-7.
30. Wijaya R, Neumann GM, Condron R, Hughes AB, Poly GM. Defense proteins from seed of Cassia fistula include a lipid transfer protein homologue and a protease inhibitory plant defensin. Plant Sci 2000; 159:243-55.
31. Mendez E, Rocher A, Calero M, Girbes T, Citores L, Soriano F. Primary structure of v-hordothionin, a member of a novel family of thionis from barley endosperm, and its inhibition of protein synthesis in eukaryotic and prokaryotic cell-free systems. Eur J Biochem 1996; 239:67-73.
32. Zhu YJ, Agbayani R, Moore PH. Ectopic expression of *Dahlia merckii* defensin Dm-AMP1 improves papaya resistance to *Phytophthora palmivora* by reducing pathogen vigor. Planta 2007; 226:87-97.
33. Zainal Z, Marouf E, Ismail I, Fei CK. Expression of the *Capsicuum annuum* (Chili) defensin gene in transgenic tomatoes confers enhanced resistance to fungal pathogens. Amer J Plant Physiol 2009; 4:70-9.
34. Jha S, Tank HG, Prasad BD, Chattoo BB. Expression of Dm-AMP1 in rice confers resistance to *Magnaporthe oryzae* and *Rhizoctonia solani.* Transgenic Res 2009; 18:59-69.
35. Gao A-G, Hakimi SM, Mittanck CA, Wu Y, Woerner BM, Stark DM, et al. Fungal pathogen protection in potato by expression of a plant defensin peptide. Nature Biotechnol 2000; 18:1307-10.
36. Anderson MA, Heath RL, Lay FT, Poon S. Modified plant defensin. US patent application 2009; application 20090083880.
37. Aerts AM, Carmona-Gutierrez D, Lefevre S, Govaert G, François I, Madeo F, et al. The antifungal plant defensin RsAFP2 from radish induces apoptosis in a metacaspase independent way in *Candida albicans*. FEBS 2009; 15:2513-6
38. Fu R, Peng Y, Cao G, Ma J, Chen C, Zhang L, et al. Expression of rabbit defensin NP-1 gene in transgenic tobacco plants and its activity against bacterial wilt. Chinese Sci Bull 1998; 43:1540-4.
39. Langen G, Imani J, Altincicek B, Kieseritzky G, Kogel KH, Vilcinskas A. Transgenic expression of gallerimycin, a novel antifungal insect defensin from the greater wax moth *Galleria mellonella*, confers resistance to pathogenic fungi in tobacco. Biol Chem 2006; 387:549-57.
40. Lobo DS, Pereira IB, Fragel-Madeira L, Medeiros LN, Cabral LM, Faria J, et al. Antifungal *Pisum sativum* defensin 1 interacts with *Neurospora crassa* cyclin F related to the cell cycle. Biochemistry 2007; 46:987-96.
41. Wong JH, Ng TB. Sesquin, a potent defensin-like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. Peptides 2005; 26:1120-6.
42. Takayama S, Shimosato H, Shiba H, Funato M, Che F-S, Watanabe M, et al. Direct ligand-receptor complex interaction controls Brassica self-incompatibility. Nature 2001; 413:534-8.
43. Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, et al. Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. Nature 2009; 458:357-61.