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Antifungal plant defensins

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Plants are exposed continuously to a multiple of potential pests and pathogens throughout their lifespan. During their evolution, plants like all multicellular organisms have developed a number of mechanisms to defend themselves against such assaults. There are two outcomes for a plant-pathogen interaction- the compatible (susceptible) or incompatible (resistant) interaction. In the compatible interaction, infection occurs. In contrast, in the incompatible interaction, sets of defense responses are elicited by plants that limit the spread and/or damage caused by pathogens. These responses include, an oxidative burst as a part of the hypersensitive response that leads to localized cell death, thus trapping the pathogen in layers of dead cells. The cell wall can also undergo restructuring and fortification in response to perception of signals either of self-origin or derived from the invading organism. Some of the changes that occur in the cell wall include the accumulation of structural proteins such as the extensins, the glycine-rich proteins, the proline-rich proteins, the solanaceous lectins and the arabinogalactan proteins.

Enzymes involved in the construction and/or modification of other wall polymers such as suberin, lignin, callose and wall-bound phenolics can also be activated. Thus, the cell wall not only poses a tough static barrier to entry by organisms into the plant cell, but can also undergo dynamic changes in its defense response.

Among the chemical defenses that are elaborated by plants, the *de novo* synthesis of defense-related proteins is of pivotal importance. The suite of defense-related proteins can either be expressed constitutively and/or be induced as a result of wounding by herbivores or by microbial invasion. As such, these proteins form pre and post-infection defensive barriers, respectively. Examples of these proteins include enzyme inhibitors such as α-amylase and proteinase inhibitors, hydrolytic enzymes such as β,1,3-glucanases and chitinases and other low molecular weight cysteine-rich antimicrobial proteins. The accumulation of antimicrobial compounds such as oxidized phenolics, tannins and other low molecular weight secondary metabolites such as phytoalexins also play an important role in the chemical defense strategy of plants. In addition to responding locally to infection, these defense-related proteins and compounds can also accumulate in more distant, yet uninfected parts of the plant (systemic response). This phenomenon is known as induced systemic resistance (ISR) or systemic acquired resistance (SAR). Collectively, these defense-related proteins constitute part of the innate immune system, an ancient system that seems to prevail in all multicellular organisms.

This review provides an overview of antifungal plant defensins, classes of plant defensins, groups of antifungal plant defensins, structure of plant defensins, distribution and location of plant defensins, plant defensins from different plant families, purification of defensins, various biological activities displayed by plant defensins, mode of action of antifungal plant defensins and plant defensins used to engineer fungal resistance in crop plants.

Small, cysteine-rich antimicrobial proteins in plants

Small cysteine-rich antimicrobial proteins in plants include the plant defensins, thionins, lipid transfer proteins, hevein and knottin-type proteins, as well as antimicrobial proteins from *Macadamia integrifolia* and *Impatiens balsamina* (Table 1). They are generally small (<10 kDa), highly basic proteins and often contain an even number of cysteine residues (typically 4, 6 or 8). Based on amino acid sequence identities, primarily with reference to the number and spacing of the cysteine residues, a number of distinct families have been defined. All these antimicrobial proteins appear to exert their activities at the level of the plasma membrane of the target microorganisms, although it is likely that the different protein families act via different mechanisms. The cyclotides are a new family of small, cysteine-rich plant peptides that are common in members of the Rubiaceae and Violaceae families (Lay and Anderson, 2005).

Table 1: List of Small, cysteine-rich antimicrobial proteins in plants

Peptide family	Representative member	No. of amino acids
Plant defensins	Rs-AFP2	51
Thionin	α-Purothionin	45
Lipid transfer protein	Ace-AMP1	93
Hevein-type	Ac-AMP2	30
Knottin-type	Mj-AMP1	36
Macadamia	MiAMP1	76
Impatiens	Ib-AMP1	20
Cyclotide	Kalata B1	29

Plant defensins

Plant defensins are small (~5 kDa, 45 to 54 amino acids), basic, cysteine-rich (typically eight cysteine residues) proteins with antimicrobial activities (Lay and Anderson, 2005; Bart *et al*., 2002; Henrik *et al*., 2009 and Willem *et al*., 1995). These defensins are cationic peptides which provide a first line of defense against potential pests and pathogens. Plant defensins are ubiquitous throughout the plant kingdom.

History

The first members of this family were isolated from the endosperm of barley and wheat in the year 1990 and were proposed to form a novel subclass of the thionin family (γ- thionins) that was distinct from α and β-subclasses. Thus, these barley and wheat proteins were named γ1-hordothionin (γ^1-H) and γ^1- and γ^2-

purothionin (γ^1-P and γ^2-P), respectively (Mendez *et al.*, 1990 and Colilla *et al.*, 1990). Their original assignment as the γ-thionin subclass of the thionin family was based on similarities in size, charge and cysteine content to α and β-thionins, however the spacing of the cysteines was significantly different. In subsequent years, numerous other α-thionin-like proteins were identified, either as purified protein or deduced from cDNAs from both monocotyledonous and dicotyledonous plants. The term "Plant defensin" was coined in 1995 by Terras and his colleagues who isolated two antifungal proteins from radish seeds (Rs-AFP1 and Rs-AFP2) and noticed that these proteins were more related to insect and mammalian defensins than to the plant thionins at the level of primary and three-dimensional structure (Lay and Anderson, 2005).

Classes of plant defensins

Plant defensins can be divided into two major classes according to the structure of the precursor proteins predicted from cDNA clones. In the first and largest class, the precursor protein is composed of an endoplasmic reticulum (ER) signal sequence and a mature defensins domain. These proteins enter the secretory pathway and have no obvious signals for post-translational modification or subcellular targeting. The second class of defensins are produced as larger precursors with C-terminal prodomains of about 33 amino acids. To date, these defensins have been found only in solanaceous species where they are expressed constitutively in floral tissues and fruit. The prodomains on these solanaceous defensins have an unusually high content of acidic and hydrophobic amino acids. Interestingly, at neutral pH, the negative charge of the prodomain counter-balances the positive charge of the defensin domain. C-terminal prodomain may function as a targeting sequence for subcellular sorting (Lay and Anderson, 2005; Lay *et al.*, 2003).

Groups of antifungal plant defensins

The best characterized activity of plant defensins is their ability to inhibit a broad range of fungi. Based on their effects on the growth and morphology of the fungus, *Fusarium culmorum*, two groups of defensins can be distinguished (Lay and Anderson, 2005; Osborn *et al.*, 1995). The "morphogenic" plant defensins cause reduced hyphal elongation with a concomitant increase in hyphal branching. The "non-morphogenic" plant defensins reduce the rate of hyphal elongation, but do not induce marked morphological distortions.

Structure of plant defensins

To date, the solution structures of seven seed-derived and two flower-derived plant defensins have been determined by H^1-NMR spectroscopy. Rs-AFP1, features an α-helix and a triple-stranded antiparallel β-sheet (in a βαββ configuration) that are stabilized by four intramolecular disulfide bonds. Plant defensins form a characteristic structure known as the cysteine-stabilized αβ (CSαβ) motif (Lay and Anderson, 2005; Bart *et al.*, 2002; Henrik *et al.*, 2009; Willem *et al.*, 1995).

Distribution and Location of Plant defensins

Plant defensins have a widespread distribution throughout the plant kingdom and are likely to be present in most, if not all, plants (Lay and Anderson, 2005; Broekaert *et al.*, 1997; Broekaert *et al.*, 1995; Osborn *et al.*, 1999; Shewry and

Lucas, 1997). Most plant defensins have been isolated from seeds where they are abundant and have been characterized at the molecular, biochemical and structural levels (Broekaert *et al*., 1995 and Thomma *et al*., 2003). The quantity of defensins released from a single seed was sufficient to inhibit fungal growth. Therefore, plant defensins contribute to the protection of seeds or seedling against attack by soil-borne pathogens to enhance seedling survival. Defensins have also been identified in other tissues including leaves (Terras *et al*., 1995; Kragh *et al*., 1995; Yamada *et al*., 1997; Komori *et al*., 1997 and Segura *et al*., 1998), pods (Chiang and Hadwiger, 1991), tubers (Moreno *et al*., 1994), fruit (Meyer *et al*., 1996; Aluru *et al*., 1999 and Wisniewski *et al*., 2003), roots (Sharma and Lönneborg, 1996), bark (Wisniewski *et al*., 2003) and floral tissues (Lay and Anderson, 2005; Moreno *et al*., 1994; Gu *et al*., 1992, Milligan and Gasser, 1995; Karunanandaa *et al*., 1994; Li, and Gray, 1999; Urdangarin *et al*., 2000; Van den Heuvel *et al*., 2001 and Park *et al*., 2002).

Plant defensins are also expressed in vegetative tissues where they accumulate in the cell layers of cotyledons, hypocotyls, endosperms, tubers and floral structures. These locations are consistent with a role in a first line of defense against potential pathogens. Plant defensins are also expressed in peripheral cell layers (Penninckx *et al*., 1996; Thomma *et al*., 1998), the epidermal cell layer and primordial (Moreno *et al*., 1994) of plant tissues, which is consistent with defensins having an important role in the first line of defense against pathogens (Gu *et al*., 1992; Terras *et al*., 1995). Defensins are also found in stomatal cells and in the cell walls lining substomatal cavities (Kragh *et al*., 1995), which is interesting since stomata are well-known entry points for specific pathogens. Thus, cell walls lining substomatal cavities may still be the first line of defense for stomatal penetrating pathogens.

Plant defensins from different plant families

Defensins are widespread in plants and are expressed in tissues that provide a first line of defense against potential pathogens. Plant defensins have been identified from different plant species across plant kingdom. Today, it is clear that these plant defensins are ubiquitous among the plant kingdom, integrating the plant innate immune system (Andre and Valdirene, 2009). Plant defensins with regard to family, species and tissue where peptides were obtained and information about the peptide *viz.,*number of residues, mass in Da, number of cysteine residues and disulfide bridges, isoelectric point of the amino-signal peptide and the mature peptide and biological activities are described in Table 1.

Table 2: Plant defensins from different families with active compounds, isoelectric point of the amino-signal peptide and the mature peptide and biological activities

Family	**Plant species**	**Defensin name**	**Number of Residues per Da**	**Cysteine /disulfide bridge**	**Signal peptide pI/peptide pI**	**Tissue**	**Biological activity**
Amaranthaceae	*Spinacea oleracea*	So-D2	52/5803.73	8/4	nd/9.35	Leaf	F, B^+
Asteraceae	*Dhalia merckii*	Dm-AMP1	50/5525.17	8/4	nd/7.80	Seed	F
		Dm-AMP2	nd	nd	Nd		

	Helianthus annuus	SD2	47/5347.06	8/4	5.90/9.14	Flower	F
Brassicaceae	*Arabidopsis thaliana*	At-AFP1	nd/nd	nd/nd	nd/nd	Seed	F
	Brassica napus	Bn-AMP1 Bn-AMP2	nd/nd	nd/nd	nd/nd	Seed	F
	Brassica rapa	Br-AMP1 Br-AMP2	nd/nd	nd/nd	nd/nd	Seed	F, B^+
	Lepidium meyenii	Lm-def	51/5742.51	8/4	8.35/8.73	Leaf	F
	Raphanus sativus	Rs-AFP1	51/5751.60	8/4	4.25/8.72	Seed	F
		Rs-AFP2	51/5792.70	8/4	4.53/9.08		F
		Rs-AFP3	50/5499.28	8/4	4.53/8.51	Leaf	F
		Rs-AFP4	51/5747.53	8/4	4.53/8.51		F
	Sinapis alba	Sa-AMP1 Sa-AMP2	nd/nd	nd/nd	nd/nd	Seed	F
Convolvulaceae	*Ipomoea batatas*	SPD1	nd/nd	8/4	nd/nd	Tuber	F, B^+
Chenopodiaceae	*Beta vulgaris*	AX1	46/5085.89	8/4	nd/8.21	Leaf	F
		AX2	46/5184.96		nd/8.51		
Curcubitaceae	*Trichosanthes kirilowii*	TDEF1	47/5613.57	8/4	4.49/9. 58	Leaf	F
Fabaceae	*Clitoria ternatae*	Ct-AMP1	49/5613.27	8/4	nd/8.51	Seed	F
	Pisum sativum	PsD1	46/5208.88	8/4	nd/7.73	Seed	F
		PsD2	47/5404.10	8/4	nd/8.52		F
	Vigna unguiculata	VUDEF	47/5413.06	8/4	nd/7.72	Seed	F
	Vigna sesquipedalis	sesquin	nd	nd	nd/nd	Seed	F, B^-, B^+
	Vigna radiata	VrD1	46/5122.09	8/4	4.87/9.06	Developing seed	F
	Vigna angularis	VaD1	46/5209.17	8/4	4.87/9.20	Developing seed	F
	Phaseolus vulgaris	PBAFP	nd	nd	Nd	Seed	F
		PvD1	47/5448.11	8/4	nd/8.20		F
		WCBAF	47/5472.13	8/4	nd/7.72		F, B^+

		P M					
		vulgarinin	nd	nd	Nd		F
	Medicago sativa	alfAFP	45/5194.85	8/4	4.78/8.51	Seed	F
	Mendicago trucatula	MtDEF2	45/5152.77	8/4	4.78/8.21	Seed	F
	Lens culinaris	Lc-def	47/5449.18	8/4	4.78/8.20	Germinated seed	F
	Phaseolus limensis	BLBAMP	48/5502.12	8/4	nd/6.86	Seed	F
		limyin	nd	nd	Nd	Seed	F,B^+
	Tephrosia villosa	TvD1	47/5475.18	8/4	4.78/8.20	Leaf	F
	Adzuchia angularis	RBAFP	nd	nd	nd	Seed	F
	Trigonella foenum-graecum	Tfgd1	47/5446.18	8/4	4.78/8.53	Leaf	F
	Delandia unbellata	RBAFP	nd/nd	nd	nd/nd	Seed	F
Hippocastanaceae	*Aesculus hippocastanum*	Ha-AMP1	50/5863.48	8/4	nd/7.73	Seed	F
Poaceae	*Triticum aestivum*	TAD1	49/5529.31	8/4	8.50/8.51	Crown	F, B^-
	Echinochloa crusgalli	Ec-AMP-D1	46/5050.72	8/4	nd/8.74	Seed	F
		Ec-AMP-D2	46/5116.79		nd/8.74		
Rosaceae	*Prunus persica*	PpDfn1	47/5234.92	8/4	5.90/9.17	Bark	F
Saxifragaceae	*Heuchera sanguinea*	Hs-AMP1	54/5948.71	8/4	nd/8.49	Seed	F
Solanaceae	*Capsicum annuum*	J1-1	48/5196.05	8/4	4.37/8.52	Red fruit	F
	Nicotiana alata	NaD1	47/5304.32	8/4	5.75/9.08	Flower bud	F
	Petunia hybrida	Ph1	47/5211.27	10/5	4.37/8.90	Petal	F
		Ph2	49/5403.48		4.53/8.76		
	Solanum tuberosum	StSN2	66/7037.14	10/nd	4.14/9.16	Tuber	F
Vitaceae	*Vitis vinifera*	Vv-AMP1	47/5355.08	8/4	5.90/9.37	Berry	F

Molecular weight (Da) and pI were calculated from computer pI/Mw tool at expasy; B^-: inhibitory activity against Gram-negative bacteria; B^+: inhibitory activity against Gram positive bacteria; F: inhibitory activity against fungi; nd: not determined

Purification of defensins

Over the last two decades, numerous plant defensins have been purified, particularly from seeds where the proteins are relatively abundant (Osborn *et al*., 1995 and Terras *et al*., 1992). While several different methods have been reported for defensin purification, many of these rely on the intrinsic physio-biochemical properties of the protein such as their small size, overall net positive charge, tolerance to acids and organic solvents, and their thermo stability. This is reflected in the use of mild acids or organic solvents in the initial extraction (Craik *et al*., 1999), heating of the samples to remove heat labile proteins (Lay *et al*., 2003; Ozaki *et al*., 1980; Zhang *et al*., 1997; Zhang and Lewis, 1997) and a combination of various chromatographic steps including gel filtration, size exclusion, ion-exchange and reverse-phase high performance liquid chromatography (Lay and Anderson, 2005). Gel filtration chromatography was performed on a Sephadex G-50 column and purity of the Tfgd1 fractions was checked by SDS-PAGE. Tfgd1 was a new legume defensin isolated from *Trigonella foenum-graecum* L. The protein concentration was determined using Lowry method and the protein was used in the antifungal assay (Sudar Olli and Kirti, 2006). Abre de Beer and Melane (2011) purified Hc-AFPs from *Heliophila coronopifolia* by using affinity chromatography. Purification of defensins from *Nigella sativa* seeds (Ns-D1 and Ns-D2) included several chromatographic procedures. At each stage the molecular masses of the obtained fractions were measured by MALDI-TOF-MS. By affinity chromatography, fractions were obtained and fractions were further separated by ion-exchange chromatography and RP-HPLC (Eugene *et al*., 2011).

Various biological activities displayed by plant defensins

A wide range of biological activities have been attributed to plant defensins including growth inhibitory effects on a broad range of fungi and Gram-positive and Gram-negative bacteria. Some defensins are also effective inhibitors of digestive enzymes such α-amylases and serine proteinases, two functions consistent with a role in protection against insect herbivory.

Some defensins also inhibit protein translation or bind to ion channels (Table 3). Intriguingly, individual defensins exhibit one or two, but not all of these properties (Lay and Anderson, 2005).

Table 3: Biological activities of plant defensins

Biological activity	Examples	Plant source
Antifungal	Rs-AFP1-4 Ah-AMP1 AlfAFP	*Raphanus sativus* *Aesculus hippocatanum* *Medicago sativa*
Antibacterial	Pth-St1 Fabatin-1 and -2 SoD1-7	*Solanum tuberosum* *Vicia faba* *Spinacia oleracea*
Insecticidal	VrCRP	*Vigna radiata*

Protein synthesis inhibitor	γ1-H γ1-P ω-H HvAMP1	*Hordeum vulgare* *Triticum turgidium* *Hordeum vulgare* *Hardenbergia violacea*
α-amylase inhibitor	SIα1-3	*Sorghum bicolor*
Proteinase inhibitor	CfD2 Cp-thionin	*Cassia fistula* *Vigna unguiculata*
Sodium channel inhibitor	γ1-Z and γ2-Z	*Zea mays*

Mode of action of antifungal defensins

The precise mechanism of action that is employed by plant defensins to inhibit the growth of fungi is not completely understood, although it is generally accepted that they act at the level of the plasma membrane. The molecular basis for the antifungal inhibitory activity of most plant defensins has not been elucidated except in the cases of Dm-AMP1 and Rs-AFP2 where their targets are known.

The radish (Rs- AFP2) and dahlia (Dm-AMP1) defensins induce rapid Ca^{2+} influx and K^{+} efflux that are inhibitory for growth of the fungus. Fungi grow from the tip and require the maintenance of an intracellular Ca^{2+} concentration gradient to drive polarized growth, it has been suggested that the growth inhibition may be due to dissipation of this gradient.

Mode of action of Dm-AMP1, an antifungal plant defensin from Dahlia in *Saccharomyces cerevisiae*

Membrane patches enriched in sphingolipids (known as rafts) act as binding sites for Dm-AMP1. The interaction of Dm-AMP1 and sphingolipids facilitates the insertion of the defensin into the fungal plasma membrane. This in turn leads to membrane destabilization /permeabilization resulting in arrest of fungal growth.

Plant defensins bind to rafts composed of sphingolipids in the fungal plasma membrane, where after permeability of the membrane is altered, resulting in increased Ca^{2+} uptake and K^{+} efflux. Whether plant defensins are internalized and interact with intracellular targets is currently not known (Karin Thevissen *et al*., 2003).

Mode of action of RsAFP2, an antifungal plant defensin from radish in *Pichia pastoris*

RsAFP2, an antifungal plant defensin from radish interacts with the structurally related membrane lipid glucosylceramides (GlcCer) in the plasma membrane of susceptible fungi. Upon this initial interaction, membranes are permeabilized, leading to fungal cell death. Additionally, RsAFP2 induces toxic reactive oxygen species (ROS) after its initial interaction with glucosylceramides (Karin Thevissen *et al*., 2007). It is currently not clear how ROS in susceptible fungi are generated, and whether there is a functional link between ROS generation and membrane permeabilization.

Model for antifungal action of plant defensins

Steps in the mechanism of antifungal action of defensins have been confirmed experimentally. Plant defensins either bind to sphingolipids of plasma membrane or are internalized into fungal cells. Receptor mediated signals are either transmitted through MAP kinases or directly to unidentified molecular factors eventually affecting the downstream processes. It is not clearly known if the interaction of plant defensins with fungal cell wall components and/or plasma membrane components (other than sphingolipids) is required for entry into the fungal cells. It remains to be determined if plant defensins have specific organelle and/or other subcellular targets inside the fungal cell(Jagdeep Kau r *et al.*, 2011). Pea defensin, Psd1 was shown to co-localize with cyclin F in the nucleus of fungus (Lobo *et al.*, 2007). A few plant defensins are likely to be internalized into vacuole and others are likely to affect fungal mitochondria thus resulting in the production of ROS (reactive oxygen species). It is unclear if permeabilization caused by plant defensins results in leakage of cell contents.

Engineering crop plants for resistance to fungal pathogens using antifungal defensins

The potential of antifungal defensins to provide resistance to various fungal pathogens has been examined in a number of plants by several labs.

Constitutive overexpression of a plant defensin significantly enhances disease resistance against pathogens. Constitutive promoters such as the cauliflower mosaic virus 35S promoter in dicots and maize ubiquitin promoter in monocots have been widely used for expression of defensin genes.

Another strategy for expression of a defensin gene in transgenic plants is to use tissue-specific promoters. The choice of appropriate tissue-specific promoter is dependent on the infection biology of the pathogen and knowledge of host tissues affected by the initial infection. Extracellularly targeted expression of a defensin is sufficient for robust resistance to a biotroph, whereas coexpression of extra and intracellularly targeted defensins is required for robust resistance to a hemibiotroph.

Coexpression of two defensins or coexpression of one of pathogenesis related proteins and a plant defensin which exhibit different modes of action may afford synergistic enhancement of disease resistance against specific plant pathogens in transgenic crops. Thus, it is well documented that the constitutive overexpression of a plant defensin significantly enhances disease resistance in the growth chamber or greenhouse tests (Table 4) (Terras *et al.*, 1995; Wang *et al.*, 1999; Gao *et al.*, 2000; Kanzaki *et al.*, 2002; Turrini *et al.*, 2004; Schaefer *et al.*, 2005; Jha and Chattoo, 2009 and Roxana *et al.*, 2010). However, efficacy of a defensin to confer resistance to fungal and oomycete pathogens in the field has been demonstrated only in a few cases (Gao *et al.*, 2000 and Portieles *et al.*, 2010). It was shown a decade ago that constitutive expression of an alfalfa seed defensins MsDef1 in potato conferred strong resistance to *Verticillium dahliae* in the field (Gao *et al.*, 2000). Recently, constitutive expression of NmDef02 defensin in transgenic potato has been shown to provide strong resistance to *Phytophthora infestans* under greenhouse and field conditions (Roxana *et al.*, 2010). The current status of the defensin technology promises its commercial potential for disease control. However, in order to deploy this technology commercially, transgenic crops expressing defensins must display durable non race-specific resistance to a fungal pathogen in the field, exhibit normal growth and development, and not be compromised in their responses to other biotic as well as abiotic stress stimuli. Moreover, they must clear all regulatory and public acceptance hurdles (Collinge *et al*., 2010). With greater understanding of the modes of antifungal action of defensins in recent years, the availability of tools for their pathogen-inducible expression and subcellular localization and of other antifungal pathogenesis-related plant proteins, there is an excellent position to engineer durable, agronomically useful level of fungal resistance in transgenic crops. Furthermore, recent success in using host-induced gene silencing (HIGS) to confer fungal resistance in plants (Nowara *et al*., 2010) has now generated the possibility of using multiple approaches to achieve commercially useful fungal resistance in transgenic crops. These strategies use either defensins alone or in combination with other antifungal proteins or HIGS to control fungal plant pathogens (Table 4).

Table 4: Plant defensins and other antifungal proteins (AFPs) used to engineer fungal resistance in crop plants

Defensins/AFPs	**Source plant**	**Promoter and subcellular targeting**	**Transgenic plant**	**Targeted fungal pathogen**	**Reference**
Single gene					
RsAFP2	*Raphanus sativus*	CaMV 35S, Maize ubiquitin 1 Extracellular	Rice	*Rhizoctonia solani, Magnaporthe oryzae*	Jha and Chattoo, 2009
AlAFP	*Medicago sativa*	Figwort mosaic virus 35S Extracellular	Potato	*V. dahliae*	Gao *et al*., 2000
DRR230-a	*Pisum sativum*	CaMV 35S Extracellular	Canola	*Leptosphaeria maculans*	Wang *et al*., 1999
WT1	*Wasabia japonica*	Maize ubiquitin 1 Extracellular	Rice	*Magnaporthe grisea*	Kanzaki et al., 2002

NmDef02	*Nicotiana megalosiphon*	CaMV 35S Extracellular	Potato	*P. infestans*	Roxana *et al.*, 2010
Mj-AMP1	*Mirabilis jalapa*	CaMV 35S Extracellular	Tomato	*A. solani*	Schaefer *et al.*, 2005
DmAMP1	*Dahlia merckii*	CaMV 35S Extracellular	Aubergine	*Botrytis cinerea* *V. albo-atrum*	Turrini *et al.*, 2004
Two genes					
AlfAFP and CHI	*M. sativa* *Oryza sativa*	CaMV 35S Extracellular Tomato		*B. cinerea*	Chen *et al.*, 2009

CONCLUSION

Plants express a rich diversity of defensins that provide a first line of defense against potential pathogens. They have a wide range of biological activities ranging from enzyme inhibition to the blocking of ion channels. Some of these defensins clearly exhibit different modes of antifungal action. Most defensins have antimicrobial activity that is mediated at the level of the plasma membrane and possibly by a secondary activity in the cell. While the molecular basis for the antifungal activity has been unravelled for the defensins from dahlia and radish, the others have not been studied in detail. The diversity and widespread occurrence of defensins in the plant kingdom suggests they will be a rich source of proteins with antimicrobial activities.

Coexpression of two defensins or coexpression of one of the pathogenesis related proteins and a plant defensin which exhibit different modes of action may afford synergistic enhancement of disease resistance against specific plant pathogens in transgenic crops. Molecular tools can be deployed to develop transgenic crops that not only exhibit effective long-term resistance to plant pathogens, but also provide normal yields when grown under different environmental conditions.

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