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Opinion Article

Can plant defensins be used to engineer durable commercially useful fungal resistance in crop plants?

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ABSTRACT

Plant defensins are cysteine-rich proteins that play an important role in defense against fungal pathogens. Because of their potent antifungal activity, they have a strong potential to be used for engineering disease resistance in crops. Significant advances have been made in elucidating their structure–activity relationships and modes of antifungal action. Their expression in transgenic plants provides resistance to fungal pathogens in crop plants. In this article, we review recent advances and offer future perspectives on the use of these proteins for engineering durable commercially useful disease resistance in transgenic crop plants.

1. Introduction

One of the major challenges in modern agriculture is achieving effective and durable control of fungal pathogens (Collinge *et al.*, 2010). Despite the continued release of resistant cultivars and chemical fungicides, estimated 10 % of crop yields are lost due to fungal diseases (Strange and Scott, 2005). Fungal diseases are often catastrophic resulting in massive destruction of crop yields and food shortage. Successful pathogens are able to cause disease because of their ability to thwart the surveillance and defense mechanisms of their host plants. Fine tuning of defense responses to fungal pathogens should allow plants to combat fungal pathogens without compromising their normal growth and development. Molecular breeding and transgenic approaches are being pursued aggressively for development of disease resistant crops. The paradigm of expressing insecticidal Bt proteins has been highly successful in commercial development of insect resistant cotton and maize. However, extension of this model to achieving commercially useful level of resistance to fungal diseases in the field has remained elusive because transgenes often had adverse effects on plant growth and development or seed yield (Stuiver and Custers, 2001; Hammond-Kosack and Parker, 2003; Gurr and Rushton, 2005). Expression of plant antimicrobial proteins (AMPs) known as defensins in crop plants is emerging as a commercially viable approach for disease control. Here we present a brief overview of the current status of our knowledge of the modes of action (MOA) of antifungal plant defensins and discuss the prospects for deploying them in transgenic crops as weapons against fungal attack.

Plant defensins are small cysteine-rich proteins of 45–54 amino acids that are closely related to insect and mammalian defensins (Thomma *et al.*, 2002; Zasloff, 2002; Lay and Anderson, 2005). They are expressed in most, if not all, plants. Although abundant in seeds, they are expressed in almost all organs of a plant. A majority of plant defensins are

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synthesized as precursor proteins and post-translational processing cleaves out the C-terminal mature defensin peptide from the secretory signal peptide. Although a majority of defensins are secreted to the extracellular space, a few floral defensins are targeted to the vacuole. Defensins have a compact shape and share an identical backbone structure stabilized by four, occasionally five, intramolecular disulfide bonds. The three-dimensional structures of several plant defensins have been determined and are each characterized by the presence of a single α-helix and three antiparallel β-strands (Bloch *et al.*, 1998; Fant *et al.*, 1998; Fant *et al.*, 1999; Almeida *et al.*, 2002; Janssen *et al.*, 2003; Lay *et al.*, 2003a,b). The α-helix is connected to the second β-strand through a cysteine-stabilized α-helix/β-sheet (α/β) motif. Despite their structural similarity, amino acid sequences of mature plant defensins are highly variable indicating a rich diversity of variants (Thomma *et al.*, 2002). This variation in primary sequences may account for different biological roles attributed to plant defensins which include antibacterial activity (Zhang and Lewis, 1997; Segura *et al.*, 1998; Chen *et al.*, 2005; Aerts *et al.*, 2008), zinc tolerance (Mirouze *et al.*, 2006), proteinase inhibitory activity (Wijaya *et al.*, 2000), α-amylase inhibitory activity (Bloch and Richardson, 1991), ion channel blocking activity (Kushmerick *et al.*, 1998; Spelbrink *et al.*, 2004; Amien *et al.*, 2010) and pollen tube growth arrest, burst and sperm discharge (Amien *et al.*, 2010).

2. *In vitro* antifungal activity and structure–activity relationships of plant defensins

Several plant defensins have cationic charge and the ability to interact with negatively charged fungal plasma membrane through hydrophobic amino acids. Many of the plant defensins have now been reported to inhibit the growth of a broad range of hemibiotrophic and necrotrophic fungi at micromolar concentrations *in vitro*, while others have no known antifungal activity (Thomma *et al.*, 2002; Lay and Anderson, 2005; Carvalho Ade and Gomes, 2009). It has not been possible to determine the antifungal activity of plant defensins against biotrophic fungi because of the difficulty of culturing these fungi *in vitro*. Antifungal plant defensins are divided into two different subgroups: morphogenic, which cause reduced hyphal elongation with a concomitant increase in hyphal branching and nonmorphogenic, which reduce hyphal elongation without causing significant morphological changes (Terras *et al.*, 1992; Broekaert *et al.*, 1995). For example, MsDef1 (previously referred to as AlfAFP, (Gao *et al.*, 2000)) induces prolific hyperbranching of hyphae in *Fusarium graminearum*, whereas MtDef4 does not (Ramamoorthy *et al.*, 2007a). All antifungal plant defensins contain a highly conserved γ-core motif ($GXCX_{3-9}C$), a structural motif present in the antimicrobial peptides containing disulfide bonds (Yount and Yeaman, 2004), composed of β2 and β3 sheets and the interposed loop. The structure–activity studies reported so far indicate that major determinants of the antifungal activity and morphogenicity of plant defensins reside in their γ-core motifs, although some determinants outside the γ-core motifs also contribute to their antifungal activity (De Samblanx *et al.*, 1997; Schaaper *et al.*, 2001; Lay *et al.*, 2003a,b; Sagaram *et al.*, 2011). Although more studies are needed to fully elucidate the structure–activity relationships of defensins, current knowledge will permit a rational design of more potent peptides with a wider spectrum of antifungal activity. For example, it has been possible to convert morphogenic MsDef1 to near nonmorphogenic MtDef4 by substituting its own γ-core motif with that of MtDef4 (Sagaram *et al.*, 2011).

3. Modes of action of antifungal defensins

While plant defensins inhibit the growth of plant as well as human pathogenic fungi, they are non-toxic to plant and animal cells. Evidently they have targeted unique properties of fungal membranes to provide selectivity of their action. Earlier studies established that plant defensins bind to the plasma membranes of sensitive fungi with high affinity (Thevissen *et al.*, 1997) and permeabilize them resulting in cell growth arrest (Thevissen *et al.*, 1999). However, it is also becoming increasingly evident that the ability to permeabilize plasma membranes is not a direct indicator of the antifungal potency of defensins (Sagaram *et al.*, 2011). Evidence is now accumulating rapidly that plant defensins differ in their modes of antifungal action (Fig. 1).

Two models were earlier proposed to explain the modes of action of antimicrobial peptides. The barrel-stave model involves the formation of discrete oligomeric pores which allow ions and other molecules to pass through the membrane, whereas the carpet model suggests that peptides lay across the surface of the membrane and, at certain critical concentration, insert in a detergent-like manner causing the formation of micelles and disintegration of the membrane (Brogden, 2005; Hale and Hancock, 2007). While some plant defensins use variations of these two models as part of their mechanisms of action, recent studies also suggest alternative modes of antifungal action. The early MOA studies established that some plant defensins bind to specific sphingolipids present in the plasma membrane of the sensitive fungi with high affinity. For example, DmAMP1 from *Dahlia merckii* binds to mannosyl diinositolphosphoryl ceramide, whereas RsAFP2 from radish, binds to glucosylceramide (GlcCer) of the plasma membrane (Thevissen *et al.*, 2000). GlcCer also seems essential for the antifungal action of MsDef1, but not of MtDef4. A GlcCer synthase knockout mutant of *F. graminearum* displays strong resistance to MsDef1, but remains sensitive to MtDef4 (Ramamoorthy *et al.*, 2007b). Thus, plasma membrane sphingolipids are now clearly established as receptors of some plant defensins. The resistance of certain fungi to these defensins is most likely due to the absence of these sphingolipids in their plasma membranes. How this defensin/sphingolipid interaction results in the growth arrest of the fungus remains unclear. Whether interaction of these defensins with other receptors or activation of specific signaling events is essential for the antifungal action of these peptides remains to be determined. It will be interesting to know if those defensins which bind to sphingolipids in the fungal plasma membrane are subsequently internalized by the sensitive fungal cells.

The modes of action of some defensins do not involve binding to sphingolipids. For example, using the patch clamp technique, it was demonstrated that defensins isolated from