Introduction
Cell wall reinforcement, phytoalexin production and the accumulation of antimicrobial properties are effectively employed in plant defense reactions to stall the infection process. Their temporal and spatial regulations are the decisive factors governing the outcome of the host-pathogen interactions. In numerous incompatible interactions, these reactions are often associated with the death of a small number of cells, at and around the site of infection, often referred to as the 'hypersensitive response' (HR). Membrane damage, necrosis, and collapse of challenged cells are the common features of the HR, but the early stages of the response remain poorly defined. HR is a best-studied form of genetically regulated programmed cell death (PCD) in plants (Greenberg et al., 1994) and shares some phenotypic features with apoptosis (Levin et al., 1996). Cell death that occurs as a part of normal developmental processes, is referred to as PCD. Gilchrist (1998) reviewed some of the fundamental characteristics of apoptosis in animals and points to a number of connections to PCD in plants that may lead to both a better understanding of the HR and novel strategies for engineering disease resistance in plants. The HR and other examples of cell death in plants, therefore, have become the focus of intensive research.

1.1 HR limits pathogen growth and activates defense genes in plants

Plant HR is a means to eliminate the microbes that have a potential to cause plant disease. The most common expression of host resistance, and a frequent expression of non-host resistance, is the HR, a rapid death of cells at the infection site that is associated with pathogen limitation as well as with defense gene activation (Goodman and Novacky, 1994). The HR serves to inhibit the growth of the invading pathogen by killing infected and uninfected cells and producing a physical barrier composed of dead plant cells. The rapid dehydration that follows the death of plant tissue may also have deleterious effects on pathogen growth by limiting the available nutrients. In addition, during the HR, dying plant cells strengthen their cell walls and accumulate certain toxic compounds such as different phenolics and phytoalexins (Dangl et al., 1996). Bacteria, fungi, and viruses induce different types of cell death with different morphological and physiological characteristics, and at a different rate. This function may be similar to the activation of apoptosis in response to infection with viruses or bacteria in animal cells (Mittler and Lam, 1996). Thus, activation of cell death as a means of preventing further infection by an invading pathogen appears to be a general theme in the biology of
multicellular organisms. Plant HR is triggered by (a) interaction of \( R-avr \) gene products or (b) pathogen-derived molecules.

1.1.1 Interaction of \( R-avr \) gene products: HR is one of the classical examples of disease resistance with a strong genetic basis, often referred as "gene-for-gene" interaction. It occurs as a result of an interaction between pathogens' avirulence (\( avr \)) gene and the hosts' resistance (\( R \)) gene (Flor, 1955) and postulated that pathogens contain a specific \( avr \) gene whose expression is recognized by a corresponding \( R \) gene in the plant. When matching \( avr-R \) genes are present, 'incompatible' interaction occurs (the plant is resistant and the pathogen is avirulent). A 'compatible' interaction occurs if either the plant lacks the appropriate \( R \) gene or the pathogen lacks the corresponding \( avr \) gene. The compatible or incompatible interaction is determined by the alleles of one specific plant \( R \) gene and a pathogen's \( avr \) gene. If the plant carries the correct \( R \) gene, and if the pathogen carries the corresponding \( avr \) alleles, recognition occurs and the HR follows. However, if the plant does not carry the \( R \) allele (but rather the non-functional allele \( r \)), or if the pathogen does not have the \( avr \) allele (recessive allele \( avr \)), there is no recognition and the result is disease (Ellingboe, 1976).

1.1.2 R-proteins as guards of cellular machinery: The 'guard hypothesis' provides an intriguing conceptual framework for the action of disease effectors and the R-protein complex. Nucleotide-binding leucine-rich-repeats (NB-LRR) proteins constitute the major R-protein class and specify in gene-for-gene plant resistance to animal, fungal, bacterial and viral pathogens, and collectively constitute a comprehensive pathogen-detection system (Hammond-Kosack and Jones, 1997; Milligan et al., 1999; Wang et al., 1999; Rossi et al., 1998; Vos et al., 1998). This innate, genetic recognition-response apparatus resembles the animal immune system. R-proteins might detect the association of plant pathogenicity targets with pathogen virulence factors that are then destined to become Avr products. Guard hypothesis was put forward to rationalize why Pto protein kinase requires the NB-LRR protein Prf to activate defense upon recognition of AvrPto. According to this model, Pto is a general component of host defence, perhaps in a pathway for response to nonspecific elicitors of phytopathogenic bacteria (van der Biezen and Jones, 1998). Conceivably, one particular Avr product corresponds to one specific pathogenicity target, which, in turn, could be safeguarded by one matching R protein. In order to adapt rapidly to pathogen Avr modification or loss, novel
recognition specificities in R proteins are created through the generation of sequence variation in the β-sheet (functions as a ligand-binding surface) of the LRR domains (Parniske et al., 1997; Thomas et al., 1997).

The guard hypothesis provides a step beyond the previous notion that R proteins are simply direct receptors for Avr proteins. This elicitor/receptor model may still be true for some systems, but for many others, the lack of direct R/Avr interaction is sufficiently convincing that it can be excluded (Dangl and Jones, 2001).

1.1.3 Pathogen-derived molecules trigger plant HR: A variety of fungal products elicit inducible defensive plant responses in both host and non-host plants, and that such responses can also be triggered by plant products released during cell-wall degradation. These ‘non-specific elicitors’ are the prime inducers of defense responses in non-host plant-pathogen interactions. Evidence suggests that cryptogein, one family of proteinaceous elicitors produced by Phytophthora species, has binding sites on cells from both plant species that do and that do not defensively respond to the elicitor raising important questions about receptor and signal pathway differences between species (Bourque et al., 1999).

Non-specific elicitors from bacteria have been the cell-death-eliciting harpins, heat-stable proteins, encoded by members of the *hrp* (hypersensitive response pathogenicity) gene cluster of some Gram-negative bacteria (Collmer et al., 2000). The harpin binds to the lipid bilayers and forms an ion-conducting pore permeable to the cations (Lee et al., 2001).

1.2 Programmed cell death (PCD) is essential in plants’ life

In plants, PCD is essential for development and survival. Available evidences suggest that plant cell death, in some cases might be mechanistically similar to apoptosis in animal cells, since the dying plant cells appear morphologically similar to apoptotic cells (Bennetzen et al., 1988; Bent 1992). In addition, some types of plant cell death are accompanied by DNA cleavage, often with the characteristics of endonucleolytically processed DNA, one hallmark of apoptosis (Bennetzen et al., 1988; Bent 1992; Bestwick et al., 1995; Bowler et al., 1989). Typical apoptotic events include the fragmentation of DNA into internucleosomal-sized fragments with 3’OH ends indicative of
endonucleolytic cleavage (DNA ladders), membrane blebbing, nuclear condensation, and fragmentation with nucleic acids found in membrane-bound vessels (apoptotic bodies), and cytoplasmic condensation (Greenberg 1997).

1.2.1 Mitochondria play a key role in inducing disease resistance and cell death in plants: Involvement of mitochondria in pathogen-induced plant defense responses has long been implicated. Salicylic acid (SA)-induced tobacco resistance to Tobacco mosaic virus (TMV) is sensitive to salicylhydroxamic acid (SHAM), an inhibitor of the terminal oxidase of the mitochondrial alternative pathway (Chivasa et al., 1997). The respiratory inhibitors like antimycin A and cyanide induced both the accumulation of alternative oxidase transcript and resistance to TMV. Cyanide also restored \( N \) gene-mediated resistance to TMV in transgenic tobacco expressing the salicylate hydroxylase (nahG) gene (Chivasa and Carr, 1998). These findings suggest that certain functions of plant mitochondria do play an important role in SA-induced disease resistance. The mitochondrial connection to PCD in plants was suggested by Lacomme and Santa Cruz (1999). This may act via leakage of cytochrome C, which has not been demonstrated in plant PCD. Altered mitochondrial functions play an important role in harpin-induced hypersensitive cell death in tobacco (Xie and Chen, 2000).

1.2.2 The HR: An example of PCD

The HR is genetically controlled and coordinately regulated with other defense-related biochemical events typical of the resistant response (Dietrich et al., 1994; Dinesh-Kumar and Baker, 2000). Plants must have active protein synthesis machinery to show an HR induced by bacteria (Croft et al., 1990; Keen et al., 1981). Purified bacterial elicitors of the HR require plants to have active metabolism to show cell death (He et al., 1993). Over production of a component of the \( R \) gene \( Pto \) signal transduction pathway of tomato expressed in tobacco caused an amplification of the HR. Mutants exist in maize, rice, tomato, barley and also in \( Arabidopsis \) that mimic the effect of infection in the absence of the pathogen (Jones and Dangl, 1996). The \( Arabidopsis \) mutant phenotypes suggest that the genes defined represent the steps along normal disease resistance response pathways.
1.3 Phytopathogenic bacteria

Phytopathogenic bacteria generally have limited host ranges, often confined to members of a single plant species or genus. This appears to result from negative factors restricting the host range rather than from positive factors, which allow the pathogen to infect its hosts. Phytopathogenic bacteria specialize in colonizing the apoplast and from this location outside the walls of living cells they incite diseases in most cultivated plants to cause rots, spots, vascular wilts, cankers, and blights (Alfano and Collmer, 1996). The majority of these pathogens are Gram-negative rod-shaped bacteria from the genera *Erwinia*, *Pseudomonas*, *Xanthomonas*, and *Ralstonia*. Two features characterize bacteria-plant relationships. First, during their parasitic life, most bacteria reside within the intercellular spaces of the various plant organs or in the xylem. Second, many cause considerable plant tissue damage by secreting toxins, extracellular polysaccharides (EPSs), or cell wall-degrading enzymes at some stage during pathogenesis.

Several bacterial genes, referred to collectively as the hypersensitive response and pathogenicity cluster (hrp cluster), are absolutely required for bacterial pathogenesis. Many *hrp* gene sequences from plant pathogenic bacteria are very similar to the genes required for pathogenesis in bacteria that infect animals, which suggest that these distinct pathogens utilize similar virulence strategies and emphasize that during the evolution of bacterial colonization of animals and plants, certain common mechanisms may have been retained (Alfano et al., 2000; Cao et al., 2001; Keen et al., 2000).

1.4 Type III secretion system (TTSS)

Genetic and molecular studies unraveled important mechanisms underlying bacterial pathogenicity. The molecular cross-talk between pathogens and their host is a specified protein delivery system, the type III secretion system (TTSS). TTSSs are present in many Gram-negative pathogens of both plants and animals (Hueck, 1998; Galan and Collmer, 1999; Cornelis and Gijsegem, 2000). These secretion systems are particularly noteworthy because they can translocate effector proteins directly into eukaryotic cells (Cornelis and Wolf-Watz, 1997). In bacterial plant pathogens belonging to the genera *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas*, TTSS (also referred to as Hrp systems) are encoded by *hrp/hrc* genes (Lindgren, 1997; He, 1998). *Pseudomonas syringae* uses a TTSS encoded by the *hrp* pathogenicity island (pai) to
translocate effector proteins into plant cells (Alfano et al., 2000). A small open reading frame (ORF), named \( shcA \), precedes the \( hopPsyA \) gene in the \( hrp \) pa of \( P. s. pv. syringae 61 \). The HopPsyA protein is secreted in culture by \( P. syringae \) and, when expressed transiently in tobacco, it elicits an HR, indicating that its site of action is inside plant cells (Alfano et al., 1997, van Dijk et al., 1999, Collmer et al., 2000). The predicted product of ORF1 shares several of the general characteristics of chaperones used in the TTSS of animal pathogens (Wattiau et al., 1996, Cornelis et al., 1998).

Successful parasitism appears to require multiple TTSS effectors. Genomic searches for TTSS effector genes in genome of \( P. syringae \ pv. tomato \) DC3000 revealed 33 confirmed effectors and several effector candidates (Buell et al., 2003; Collmer et al., 2002; Guttman et al., 2002; Petnicki-Ocwieja et al., 2002; Zwiesler-Vollick et al., 2002). Some effectors can block the ability of other 'masked' effectors to trigger the HR, which suggested that they may allow subversion of the HR and lead to disease development. Indeed, several \( P. syringae \) effectors were recently shown to suppress plant defenses (Abramovitch et al., 2003; Axtell and Staskawicz 2003; Bretz et al., 2003; Espinosa et al., 2003; Mackey et al., 2003). Several of the effectors that suppress the HR can also suppress Bax-triggered PCD in yeast and plants. AcrPphE\(_{Pp}\), AvrPpiB\(_{Pp}\), HopPtoE, AvrPtoB, HopPtoF, and HopPtoG effectors possess suppressor activity (Jamir et al., 2004), which provides a global picture of the capacity of this bacterium to regulate PCD pathways in plants.

1.5 Harpins are the proteinaceous elicitors of phytopathogenic bacteria

Harpins, bacterial proteinaceous elicitors of HR, have been isolated from Gram-negative phytopathogenic bacteria (Table 1). HrpN of \( E. amylovora \) was the first Hrp protein shown to elicit an HR in tobacco (Wei et al., 1992), secreted via the TTSS system. The properties of HrpN define the characteristics of the class of HR elicitors known as harpins. They are hydrophilic, rich in glycine, heat stable, lack cysteine, and elicit an HR when infiltrated into the apoplast of certain plants (Bauer et al., 1995). The \( P. syringae \ pv. syringae 61 \) \( hrpZ \) gene encodes harpin\(_{Pss}\), a 34.7 kD extracellular protein that elicits HR in tobacco and other plants. Interestingly, four truncated harpin peptides, of different sizes, elicit HR that is indistinguishable from that of full-length harpin (Alfano et al., 1996).
Table 1 Harpins of phytopathogenic bacteria.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Protein Size (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erwinia amylovora</td>
<td>hrpW</td>
<td>45</td>
</tr>
<tr>
<td>Erwinia carotovora</td>
<td>hrpN</td>
<td>36.3</td>
</tr>
<tr>
<td>Erwinia chrysanthemi</td>
<td>hrpN</td>
<td>34.7</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. syringae</td>
<td>hrpZ</td>
<td>34.7</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. phaseolicola</td>
<td>hrpZ</td>
<td>35</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. tomato</td>
<td>hrpZ</td>
<td>38.4</td>
</tr>
<tr>
<td>Ralstonia solanacearum</td>
<td>popA</td>
<td>34.5</td>
</tr>
</tbody>
</table>
As a characteristic feature of proteins secreted by TTSS, harpins lack N-terminal signal peptide (Wei et al., 1992). Detailed studies revealed that harpins differ substantially in their primary structure and their contribution to Hrp phenotypes, and their actual function is unknown (Arlat et al., 1994; Bauer et al., 1995; Cui et al., 1996; He et al., 1993). Harpin<sub>Pss</sub>, a 34.7kDa extracellular protein, is glycine-rich, dissimilar in amino acid sequence to any known protein, and is produced only in apoplastic fluid-mimicking minimal media (Alfano et al., 1996).

The harpin genes of <i>E. amylovora</i> (hrpN) (Wei et al., 1992), <i>E. chrysanthemi</i> (hrp<sub>N<sub>Ec</sub></sub>) (Bauer et al., 1994) and <i>R. solanacearum</i> (PopA) (Arlat et al., 1994) are located adjacent to or near their respective hrp clusters, whereas the <i>P. syringae</i> hrpZ gene resides within a hrp operon (He et al., 1993). <i>E. chrysanthemi</i> hrpN mutants are reduced in infectivity at low inoculum levels and are unable to elicit the HR (Bauer et al., 1994), but harpin gene mutations in <i>E. amylovora</i> CFBP1430 (a highly virulent strain) (Baray, 1995), <i>R. solanacearum</i> (Arlat et al., 1994), and <i>P. syringae</i> (Alfano et al., 1996) produce weak phenotype or no phenotype. The potential role of harpins in determining host specificity is uncertain. <i>PopA</i> may be a host specificity factor because the isolated protein elicits the HR selectively in those plants in which <i>R. solanacearum</i> also elicits the HR, whereas the isolated harpin from <i>E. amylovora</i> and three <i>P. syringae</i> pathovars trigger the HR in various plants in a manner that shows no relationship to bacterial host range (He et al., 1994; He et al., 1993; Wei and Beer, 1993). The harpin<sub>Pss</sub> resembles that hrpZ gene products identified in <i>P. syringae</i> pvs. glycinea, syringae, or tomato (Preston et al., 1995).

Harpin<sub>Ea</sub>-induced responses in tobacco suspension cells such as oxidative burst (Baker et al., 1993), extracellular alkalization, active oxygen species production, and membrane depolarization are blocked by lanthanum chloride, a Ca<sup>++</sup> channel blocker and K252a, a protein kinase inhibitor (Baker et al., 1993, He et al., 1993, Popham et al., 1995). Though structurally different, both cause immediate K<sup>+</sup> efflux and extracellular alkalization in tobacco suspension-cultured cells (Wei et al., 1992, He et al., 1993, Popham et al., 1995). These events suggest that harpin<sub>Pss</sub> triggers a signal transduction pathway that involves active oxygen species production, protein phosphorylation, and Ca<sup>++</sup> influx. Alkalization was induced immediately after addition of different concentrations of full-length harpin<sub>Pss</sub>. The pH change caused by the same concentrations of truncated harpin<sub>Pss</sub> is similar in magnitude to the changes caused by the full-length protein (Alfano et al., 1996). 

Introduction
The hrpZ gene product from the bean halo-blight pathogen, Pseudomonas syringae pv. phaseolicola (HrpZ_{psph}), is secreted in an hrp-dependent manner and exported by the TTSS. HrpZ_{psph} associates stably with liposomes and synthetic bilayer membranes (Lee et al., 2001). HrpZ_{psph}-related proteins from P. syringae pv. tomato or syringae triggered ion current similar to those stimulated by HrpZ_{psph}. The HrpZ_{psph}-mediated ion-conducting pore was permeable for cations but did not mediate fluxes of Cl. Such pore-forming activity may allow nutrient release and/or delivery of virulence factors during bacterial colonization of host plants.

1.5.1 Harpin function is intriguing: The function of harpin_{ps} is particularly puzzling. Several observations suggest a simple, direct role for HrpZ in HR elicitation. HrpZ is the predominant protein secreted by the P. syringae Hrp system in culture (He et al., 1993; Yuan and He, 1996), the hrpZ gene is conserved in divergent P. syringae pathovars (Preston et al., 1995), and the isolated protein elicits an apparent PCD in plants that is indistinguishable from HR elicited by living bacteria (He et al., 1993). Furthermore, hrpZ deletion mutations in the cosmid pHIR11 functional cluster of P. syringae pv. syringae hrp genes strongly reduce the HR elicitation activity of E. coli cells carrying only pHIR11. The same mutation only slightly reduces the HR in P. syringae pv. syringae, postulating that possibly due to a second harpin encoded elsewhere in the bacterial genome (Alfano et al., 1996).

However, other observations show that the relationship of HrpZ to HR elicitation is more complex. Mutation of hrmA (Hue and Hutchenson, 1993), which is in a variable region flanking the conserved hrp cluster in pHIR11, abolishes HR activity in tobacco without diminishing HrpZ synthesis or secretion (Alfano et al., 1996). Thus, isolated HrpZ was sufficient to elicit a HR in tobacco leaves but HrpZ produced by bacteria in plants is not. Instead, HrmA, with no apparent function in the Hrp secretion apparatus, is necessary for bacterial elicitation of the HR, and thus, HrmA appears to be the actual elicitor of the HR produced by bacteria carrying pHIR11. HrmA has several characteristics of an Avr protein (Alfano and Collmer, 1997).

Mutations in the hrpN gene abolish the ability of E. amylovora to elicit the HR in non-host tobacco to elicit disease in highly susceptible pear fruit (Wei et al., 1992). In contrast, the P. solanacearum PopA1 protein also elicits the HR in tobacco but PopA mutants retain their ability to elicit the HR in this and other non-host plants and

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1 Introduction
to incite disease in tomato (Arlat et al., 1994). Mutants in hrpNEd have an intermediate phenotype of abolished HR elicitation and reduced frequency of infection (Bauer et al., 1994). Therefore, the relative contribution of these elicitors to plant-bacterium interactions varies.

1.6 Structure-function relationship

Comparative or homology protein structure modeling builds a three-dimensional model for a protein of unknown structure (the target) based on one or more related proteins of known structure (the templates) (Blundell et al., 1987; Greer, 1981; Johnson et al., 1994; Sali and Blundell, 1993; Sali, 1995, Sanchez and Sali, 1997; Marti-Renom et al., 2000; Fiser et al., 2001, Fiser et al., 2002, Sanchez and Sali, 2000). The necessary conditions for calculating a useful model are (1) detectable similarity between the target sequence and the template structures and (2) availability of a correct alignment between them. The comparative approach to protein structure prediction is possible because a small change in the protein sequence usually results in a small change in its 3D structure (Chothia and Lesk, 1986). It is also facilitated by the fact the 3D structure of proteins from the same family is more conserved than their primary sequences (Lesk and Chothia, 1980). Therefore, if the similarity between two proteins is detectable at the sequence level, structural similarity can usually be assumed. Moreover proteins that share low or even non-detectable sequence similarity many times also have similar structures. Ab initio structure prediction (Bonneau and Baker, 2001), another method of protein structure prediction, where the structure of the protein's native state is predicted from the protein's amino acid sequence. It is generally assumed that a protein sequence folds to a native conformation or ensemble of conformations that is at or near the global free-energy minimum. Thus, the problem of finding native-like conformations for a given sequence can be decomposed into two subproblems: a) developing an accurate potential and (b) developing an efficient protocol of searching the resultant energy landscape. Several methods have made good predictions in the ab initio category, and some ab initio methods outperformed fold recognition methods for certain proteins in the fold recognition category (Murzin, 1999; Orengo et al., 1999; Orengo et al., 1999). Despite progress in ab initio protein structure prediction, comparative modeling remains the only method that can reliably
predict the 3D structure of a protein with accuracy comparable to a low-resolution experimentally determined structure (Marti-Renom et al., 2000).

He et al. (1993) analysed the DNA sequence and predicted its product harpin_{Pss}, to be a glycine-rich protein with no extensive similarity to known proteins. Harpin_{Pss} has no significant sequence similarity with sequences deposited in major sequence databases accessible with the Blast search program (Altschul et al., 1990), nor were motifs of known biological significance detected for harpin_{Pss} using the MOTIF program. However, they found an intriguing, albeit limited, significance similarity was detected between harpin_{Pss} and harpin_{Pa} over a stretch of 22 amino acids He et al. (1993). Because the gene encoding harpin_{Pss} showed little relationship with the hrpN gene of E. amylovora and encodes the apparent end product of the P. s. pv. syringae 61 hrp cluster, it was designated hrpZ. He et al. (1993) also reported that carboxy-terminal 148 amino acid portion of harpin_{Pss} contains two directly repeated sequences of GGGLGTP and QTGT and is sufficient and necessary for elicitor activity. The same group showed that all four HrpZ fragments elicit an HR that is indistinguishable from HrpZ-elicited HR in tobacco (Alfano et al., 1996). Since these HrpZ fragments represent non-overlapping fragments, it was concluded that the elicitor activity of HrpZ is not confined to one region on the protein.

1.7 Protein crystallization

The crystallization of proteins currently has three major applications: (1) structural biology and drug design, (2) bioseparations, and (3) controlled drug delivery. In the first application, the protein crystals are used with the techniques of protein crystallography to ascertain the three-dimensional structure of the molecule. This structure is indispensable for correctly determining the often complex biological functions of these macromolecules. The design/drugs is related to this, and involves designing a molecule that can exactly fit into a binding site of a macromolecule and block its function in the disease pathway. Producing better quality crystals will result in more accurate 3D protein structures, which in turn means its biological function can be known more precisely, also resulting in improved drug design.

Modern crystallography is intimately linked with the ability of crystals to diffract X-rays. The resulting diffraction profile can be used to determine the structure of the crystal, as well as the 3D molecular structure of the crystalline material. The ability to
know the precise molecular structure of biological macromolecules has revolutionized the study of their functions in many fields of biology. The process of determining this structure begins with the crystallization of the macromolecule.

Many plant proteins have been crystallized, to mention a couple of them; the banana lectin, 29.4kD from *Musa paradisiaca* has been isolated, purified and crystallized. The structure of the subunit was found to be similar to that of jacalin-like lectins (Singh *et al.*, 2004). Mexicain, a 23kD papain-like cystein protease from the tropical plant *Jacaratia mexicana*, was purified and crystallized (Oliver-Salvador *et al.*, 2004).

1.8 Yeast serves as a useful model to study the cell death machinery of eukaryotes

*Caenorhabditis elegans* has provided information from a genetic screen for genes that regulate and execute PCD in basal metazoan cell death machinery, which includes *ced-9, ced-3*, and *ced-4*. Yeasts, both fission and budding, have been used as tools to examine the functions of bonafide regulators/effectors of metazoan apoptosis. This approach has proved valuable in shedding light on the obscure functions of the proapoptotic Bcl-2 family homologues of the CED-9 of *C. elegans*. Expression of either of the two mammalian proapoptotic Bcl-2 family members, BAX and BAK, in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* results in cytotoxicity with similar phenotypes in each case (Greenhalf *et al.*, 1996; Ink *et al.*, 1997).

Some features of PCD appear to be conserved from bacteria to fungi to plants and animals (Ameisen, 1996). The accumulated evidence strongly suggests that the cytotoxic effects of the expression in the yeast of mammalian BAX or BAK are relevant to the mechanism of their proapoptotic action in mammalian cells (Fraser and James, 1998) and yeast may become an important model to investigate the conserved steps of apoptosis. Similar properties of Bax or Bak are required to kill both mammalian and yeast cells (Shaham *et al.*, 1998), and a Bax inhibitor-1 (BI-1) blocks the cell death induced by Bax-over expression (Xu and Reed, 1998). An evolutionarily conserved plant homologue of the BI-1 gene has been detected that is capable of the suppression of Bax-induced cell death in yeast (Kawai *et al.*, 1999). Mitochondria can play a central role in apoptosis (Desagher and Martinan, 2000) and also appear to be involved in harpin-induced HR (Xie and Chen, 2000). Boccara *et al.* (2001) by
adapting infra-red thermography, revealed a role for mitochondria in pre-symptomatic cooling during harpin-induced hypersensitive response. They showed the affect in complex I structure and function and over-expressing alternative oxidase, indicating that they are directly or indirectly mediated by mitochondrial function. Recently it was reported by Krause and Durner (2004) that treatment of Arabidopsis cells with harpin protein induced a rapid release of cytochrome C from mitochondria into the cytosol, which is regarded as a hallmark of apoptosis.

Conditional expression of harpinPss caused yeast cell death (YCD) indicating that yeast might share, with plants conserved components in cell death pathway (Podile et al., 2001). In the harpinPss-induced plant HR and YCD, oxidative burst plays a role and a protein kinase inhibitor (K252a) suppresses the cell death.

**Objectives of the present study**

The present study is focused on characterization of the conditional expression of harpinPss-mediated YCD and the structure-function relationship of harpinPss, an unusual peptide.