Apoptosis in plant disease response: A close encounter of the pathogen kind

S. M. Paul Khurana*, Suman K. Pandey, Debabrata Sarkar and A. Chanemougasoundharam

Central Potato Research Institute, Shimla 171 001, India

Apoptosis, a ubiquitous programmed cell death (PCD), is the essence of the biological system. Like animals, plants also endure apoptosis for growth and development and in response to environmental insults. In plant disease response, localized PCD is a characteristic phenotype, and stands for both a symptom of susceptibility and resistance. Sphingolipid and its phosphorylated derivatives, synthesized in plants through ceramide biosynthetic pathway, are the signal molecules negotiated by the pathogens to trigger the endogenous PCD during susceptible disease response. A resistant disease response in most cases is characterized by hypersensitive response (HR)-linked PCD at the infection site of an avirulent pathogen in recognition of a number of intrinsic signals such as caspases, reactive oxygen/nitrogen species, salicylic acid, mitogen-activated protein kinase, membrane ion channels, etc., cascading either alone or in combinations through a 'coordinated signal transduction' pathway. Selective inhibition or induction of apoptosis can be successfully employed to control plant diseases caused by necrotrophic or biotrophic pathogens respectively. Transgenic expression of animal anti-apoptotic genes in plants provides broad-spectrum disease resistance against compatible obligate pathogens. Similarly, plant- and animal-derived proapoptotic genes can be engineered to be expressed in plants for activating HR-linked resistant disease response against biotrophic pathogens. The promises and challenges of therapeutic modulation of apoptosis for plant disease management are also discussed.

DEATH is the default. But deliberate attempt to attain death or to initiate processes leading to self-destruction is known as suicide. Even though suicide, in human terms, is regarded as 'impulsive or irrational and not consistent with balanced behaviour'; it is, in cellular terms, 'pervasive, organized, rational, and leads to organized balance, both in development and in response to stress'[1]. Cells in multicellular organisms commit suicide to achieve and maintain homeostasis by specifically ordered metabolic changes during normal development, environmental stress, or pathogen attack. This functionally conserved and gene-directed cell suicide process is known as programmed cell death (PCD). It is a physiological death process involved in the selective elimination of unwanted cells[2], and comprises cytoplasmic shrinkage, membrane blebbing, loss of cell-to-cell contact, fragmentation of nDNA at interchromosomal sites and ordered disassembly[3,4]. This orderly process of PCD, known as apoptosis (a term originally introduced by Kerr *et al.*[5] as 'a basic biological phenomenon with wide-ranging implications in tissue kinetics'), depends on the active participation of the dying cells, and is regulated by genetically controlled, well-orchestrated cell suicide machinery. At the molecular level, the apoptotic cell death follows two pathways – the interaction of a death receptor with its ligand and the participation of mitochondria, most notably release of cytochrome *c* from the mitochondrial intermembrane space[6]. Since it plays an important role in tissue sculpting and disease, the genes and signal molecules involved in apoptosis are being used for therapeutic manipulation of both degenerative and proliferative diseases of animals and humans[7,8].

Although as early as in 1982, Barlow[9] recognized that cell death was 'an integral part of plant development', it took, in fact, nearly a decade-and-a-half after that for PCD and its biological importance to be appreciated in the plant system. Today there is ample evidence to support that cell death during plant development and environmental challenge involves PCD[10]. In plants, PCD resembles either a common form seen in animals called apoptosis or it resembles a morphologically distinct form of cell death[11]. Typical biochemical hallmarks of apoptosis in plants include calcium influx, exposure of phosphatidylserine, activation of specific proteases, nDNA fragmentation leading to nucleosomal ladders and cytochrome *c* release from mitochondria[12]. PCD occurs in numerous vegetative as well as reproductive phases of plant development[13,14], including senescence of leaves[15], development of tracheary elements[16], timely death of petals after fertilization[17], post-embryonic decay of aleurone layers[18], root cap development[19], somatic[10] as well as zygotic embryogenesis[20] and sex determination[21]. Cell death in the plant system in response to abiotic stresses such as waterlogging and hypoxia is a clear example of PCD. Hypoxia stimulates ethylene production, which in turn activates a signal transduction pathway involving phosphoinositides and Ca^{2+}, suggesting the event to be a typical

*For correspondence. (e-mail: dircpri@sancharnet.in)

process of PCD[22]. The progression of aluminum-toxicity in plants has also been shown to occur via a PCD-related signal transduction pathway[23].

In plants, the occurrence of PCD in response to pathogen infections has been legitimized even before circumstantial evidence came to support its involvement in normal plant development and in response to different abiotic stimuli. Plant cell death regulation is linked to a number of signalling pathways, and serves not only to amplify disease defence responses, but also to promote the aggressiveness and/or dissemination of some pathogens[24]. PCD occurs when the pathogen unsuccessfully parasitizes the host as well as when the pathogen successfully causes disease. Therefore, the exact role and regulation of PCD during plant–pathogen interactions are of great importance to understand the relationship between cell death and activation of defence mechanisms. Plant–pathogen interactions depend on the classical gene-for-gene resistance model, requiring an avirulence gene (*Avr*) in the pathogen and a corresponding resistance gene (*R*) in the plant[25–27]. This results in an incompatibility reaction leading to successful disease resistance. A compatibility reaction due to loss or alteration of either plant *R* gene or pathogen *Avr* gene leads to disease[28]. Avirulent pathogenic infections are usually characterized by a rapid, localized cell death known as hypersensitive response (HR), which results in the formation of necrotic lesions around the infection sites[29]. HR helps the plant defence by limiting the spread of microorganisms, and represents a form of PCD[30]. Several lines of evidence suggest that the death of host cells during HR results from the activation of an intrinsic cell death programme that is encoded by the plant genome. HR is also believed to generate a signal that activates host defence mechanisms, and in many cases, induces long-lasting systemic acquired resistance (SAR) to a broad spectrum of pathogens[31,32]. Induction of SAR is accompanied by an increase in the rate of synthesis of several pathogenesis-related proteins and the accumulation of a complex network of signalling molecules.

Over the past one decade, efforts have been directed to understand plant disease and/or defence mechanisms in relation to PCD. They have evoked many a question than resolving few riddles. If the present understanding allows us to speculate as to why and how plant cells die when encountered with a pathogenic infection, we are not so certain how precisely some of these host defence mechanisms are activated even in the absence of a pathogen. Is there a genetically defined pathway for PCD during the disease response? How are the PCD genes regulated at appropriate space and time during susceptible or resistant disease reaction? What are the exact PCD signals and how are they propagated during disease reactions leading to active plant defence response? In this review, we discuss the present understanding of apoptosis during plant disease reactions and its possible role in activating defence response.

Apoptosis in compatible plant–pathogen interactions: Perception and perceptibility of susceptible disease response

In a susceptible disease response, plant cells are killed either directly by toxins produced by the pathogens or indirectly by a metabolic catastrophe due to secretion of virulence factors, or by an endogenous cell death programme triggered in recognition of specific signal molecules[11]. Over the past one decade, great efforts have been directed to study the process of PCD in plants undergoing a susceptible response to pathogens to identify gene or gene products that may modulate the process. That cell death during susceptible interactions is genetically programmed comes from the identification of disease lesion mimic mutants (LMMs) across the plant kingdom[33,34]. The characterization of LMMs has further enabled identification of genes that might regulate PCD and dissection of the defence signalling pathway[34]. One of the systems most extensively used to study PCD during susceptible disease response is the Alternaria stem canker disease of tomato caused by *Alternaria alternaria* f. sp. *lycopersici*. This system offers unique opportunities for studying PCD during susceptible disease reaction because the pathogen expresses host-selective toxins that are primary determinants of the disease, and the host in return expresses a gene that determines susceptibility or resistance to the pathogen and to the toxins[12].

Ceramide signalling: Execution or suicide?

Ceramide signalling pathway as a critical second-messenger system has been studied in detail to understand apoptosis during both degenerative and proliferative disease expressions in animal systems[35–37]. It is also known to play multiple roles in growth regulation, stress response, membrane biology and protein sorting in animals[38]. The balance between the bioactive sphingolipid ceramide and its phosphorylated derivatives has been proposed to modulate the PCD not only in animals[37], but also in plants[39,40]. Sphingolipids are ubiquitous membrane components in bacteria and eukaryotic cells and are generated by the addition of a polar head group to ceramides[41] (Figure 1). As second messengers, sphingolipids and sphingoid bases regulate cell behaviour at many levels, including cell-to-cell communication, growth factor receptors, growth, differentiation and transformation[39]. The interplay between sphingolipid metabolite sphingosine-1-phosphate and heterotrimeric G proteins represents an evolutionary conserved signal transduction mechanism in plants[42]. Investigations of sphinganine-analogue mycotoxins (SAMs), which include fumonisins (synthesized by *Fusarium moniliforme*) and AAL toxins (synthesized by *A. alternata*), have provided evidences for involvement of sphingolipids and ceramide signalling in PCD during plant disease response. The most abundant and active

SAM congeners, AAL toxin TA and fumonisins B_1 (FB_1), are functionally potent inhibitors of ceramide synthase in plants[43] resulting in activation of PCD, which is characterized by accumulation of free long-chain bases (LCBs), TUNEL-positive cells, DNA ladders, Ca^{2+}-activated nucleosomal DNA cleavage and formation of apoptotic-like bodies[12,44]. FB_1-induced PCD in *Arabidopsis* protoplasts has been shown to be light-dependent and linked to jasmonate-, ethylene- and salicylate-dependent signal transduction pathways[45]. In *Arabidopsis*, the *fbr* mutants–resistant to PCD-inducing toxin fumonisin–also showed reduced disease symptoms and pathogen replication when challenged by virulent *Pseudomonas syringae*[46], suggesting the involvement of PCD in susceptible disease response. A recently identified *Arabidopsis* ceramide kinase (CERK) mutant, called *acd5*, accumulates CERK substrates, and shows apoptosis-like PCD dependent on defence signalling in late developmental stage followed by enhanced disease symptoms during pathogen attack[40]; the phosphorylated derivatives of ceramide partially block plant PCD, suggesting the involvement of ceramide phosphorylation in modulating cell death. Victorin, a host-specific toxin secreted by *Cochliobolus victoriae*, induces apoptotic-like PCD, including mitochondrial alterations in oat plants, supporting further the involvement of PCD in susceptible disease response[47,48].

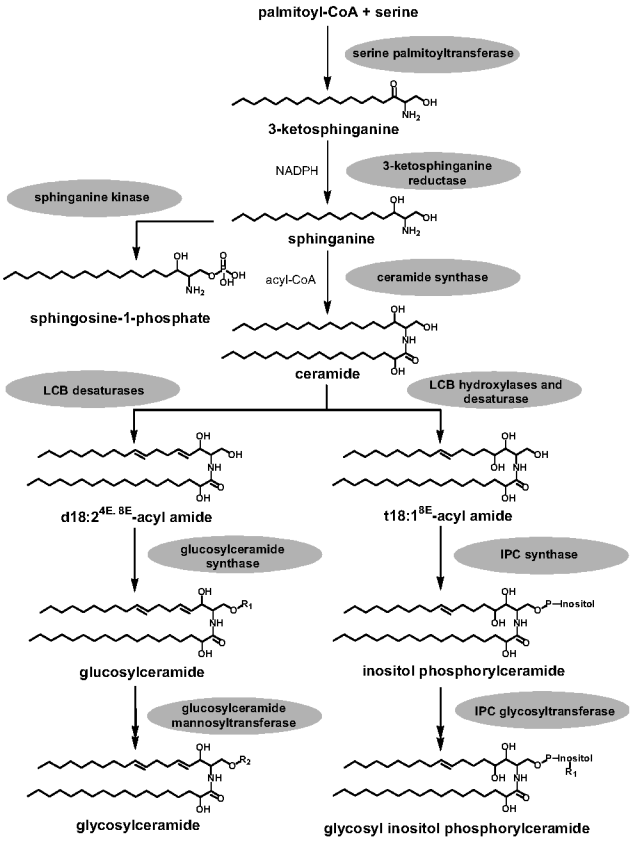

Figure 1. A tentative pathway for *de novo* sphingolipid biosynthesis in plants. Letters bold-faced and within grey ovals represent metabolites and enzymes respectively. Steps involving LCB desaturations, substrate specificities of the enzymes involved and formation of complex sphingolipids are hypothetical. Glucosylceramide and glycosylceramide (cerebroside) are formed by glycosylation of the C1-OH group of ceramide by glucose (R_1) and mannose (R_2) respectively. Glycosyl inositol phosphorylceramide carries a 1-phospho-inositol residue linked via C2 and/or C6 to C1 of an α-glucuronosyl residue (R_1).

The genetic basis for susceptible disease response to SAMs has been mapped to the co-dominant *Asc* locus of the tomato plants[1,12,49]. The fungus *A. alternata* f. sp. *lycopersici* infects tomato plants of the homozygous recessive genotype *asc/asc* by utilizing AAL toxin that kills the host cells by inducing PCD. The gene *Asc-1* has been found to confer resistance to AAL toxin in homozygous condition (*Asc/Asc*). A schematic model of ceramide signalling pathway in activating PCD *vis-à-vis* susceptible disease response in tomato plants in relation to co-dominant *Asc* locus is shown in Figure 2. The *Asc* locus, which is homologous to the yeast longevity assurance (*LAG1*) and *LAC1* genes essential for ceramide synthase activity[50], is conserved in most eukaryotes[51]. By labelling tomato leaf discs with tritiated serine, Spassieva *et al.*[52] showed that AAL toxin blocked sphingolipid biosynthesis in *asc/asc* leaf discs, and the presence of *Asc-1* was able to relieve this AAL toxin-induced block that would otherwise lead to PCD. Myriocin, a toxin known to inhibit the first enzyme of *de novo* sphingolipid biosynthesis (serine-palmitoyltransferase), did not cause PCD, but blocked *de novo* sphingolipid biosynthesis[52]. This strongly indicates that the LCBs could be the cell death signal in AAL toxin-induced PCD. However, exogenously applied ceramide could not prevent the accumulation of LCBs from inhibition of ceramide synthase[51]. Therefore, it appears that an interaction between the levels of ceramide and LCBs controls the decision to enter the PCD with no single factor taking the decision alone[52].

The genetic dissection of *Asc* locus in tomato showed that *Asc-1* produced a protein, Asc-1p, believed to be a component of or is associated with ceramide synthase enzyme in the sphingolipid biosynthesis pathway. *asc-1* is a recessive allele which carries a two-base-pair deletion in exon 2 that leads to a premature stop codon, resulting in production of a non-functional protein[51], asc-1p. As a result, the *asc/asc* tomato plants lacking Asc-1p protein become sensitive to AAL toxin, while the presence of Asc-1p protein makes *Asc/Asc* plants insensitive. However, the changes in sphingolipid profile in *lag1 lac1* yeast mutant expres sing the *Asc-1* gene[51] indicate that Asc-1p protein enables the sphingolipid pathway to utilize different substrates[52]. Accordingly, Spassieva *et al.*[52] have proposed a ceramide-transduction model for PCD based on 'change in substrate utilization' in relation to *Asc* locus of tomato plants showing susceptible disease response (Figure 3). Endoplasmic reticulum-to-Golgi transport of glycosylphosphatidylinositol (GPI)-anchored proteins (EGGAP transport) has also a role in apoptosis *vis-à-vis* susceptible disease response in plants. In *Saccharomyces*, deletion of both *LAG1* and *DGT1* (delayed GPI-anchored protein transport) genes led to delayed EGGAP transport and abnormal multilayered cell walls[53];

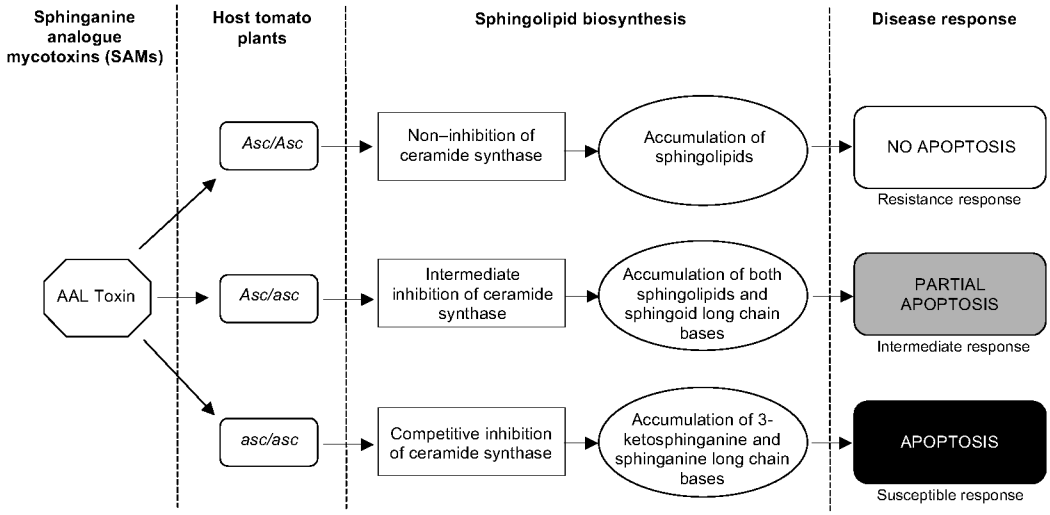

Figure 2. Ceramide signalling pathway leading to susceptible disease response through activation of apoptosis in *Lycopersicon esculentum* following infection with *Alternaria alternata* f. sp. *lycopersici*. AAL toxin secreted by fungus activates apoptosis through competitive inhibition of ceramide synthase, a key enzyme in sphingolipid biosynthesis, and this effect is modulated by co-dominant *Asc* locus of tomato plants.

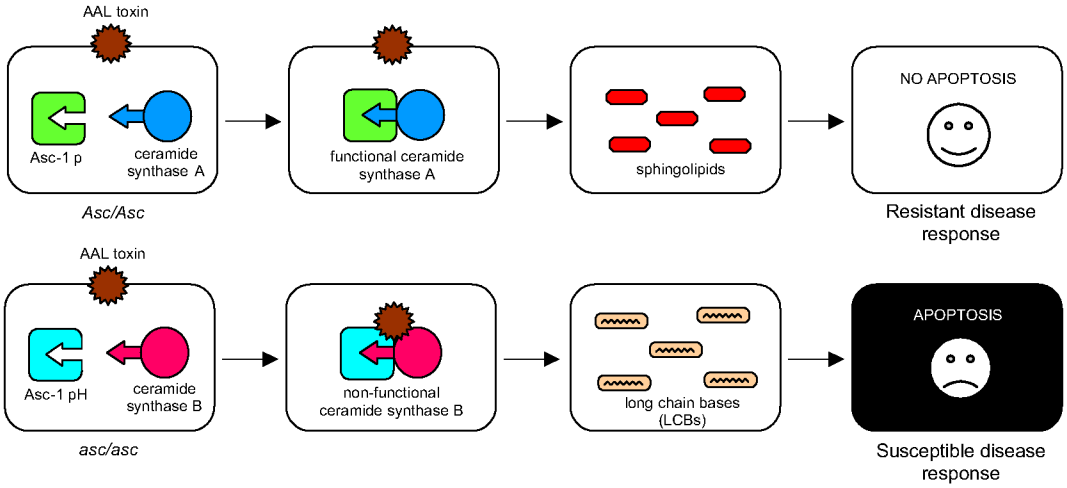

Figure 3. A 'change in substrate utilization' model of ceramide signalling for susceptible disease response through activation of apoptosis in tomato plants in relation to co-dominant *Asc* locus[52]. It is presumed that ceramide synthase exists in two forms – one utilizing Asc-1p, a product of *Asc-1* (labelled as ceramide synthase A) and another utilizing Asc-1pH, a homologue of Asc-1p (labelled as ceramide synthase B). AAL toxin secreted by *A. alternata* f. sp. *lycopersici* does not inhibit ceramide synthase A, but inhibits ceramide synthase B. In *asc/asc* tomato plants, which produce only Asc-1pH, ceramide synthase B is inhibited by AAL toxin leading to apoptosis followed by susceptible disease response. However, in *Asc/Asc* tomato plants, ceramide synthase A utilizing Asc-1p is not inhibited by AAL toxin. As a result, sphingolipid biosynthesis continues leading to inactivation of apoptosis and elicitation of resistant disease response.

two cDNA homologues of *LAG1* (AF 198179 and AF 198180) have been identified to encode two putative ceramide synthases in *Arabidopsis*[51]. A schematic model of EGGAP transport mechanism for activation or inactivation of apoptosis leading to susceptible or resistant disease responses respectively, in tomato plants in relation to *Asc* locus is shown in Figure 4. That PCD during susceptible disease response results from host-encoded functions has been supported by recent findings in other plant systems too. *Arabidopsis* LMM mutant *acd5*, exhibited increased disease symptoms and modestly increased growth of *P. syringae* when infected before spontaneous lesion formation[54]. As has been mentioned earlier, the mutant *acd5*, encodes a ceramide kinase (CERK) that is induced during virulent *P. syringae* infection[40]. Ceramide induces PCD, but CERK-reaction products, i.e. phosphorylated derivatives of ceramide

partially block PCD. The accumulation of increased amounts of acd5 CERK substrate in *acd5* LMM mutants suggests that ceramide-activated PCD function is responsible for *P. syringae* virulence during susceptible disease response.

Taken together, the current lines of evidence suggest that cell deaths in many susceptible interactions are caused by pathogen-derived factors, taking advantage of endogenous PCD to effect their functions. The ability of a pathogen to interdict and disrupt evolutionarily conserved signal transduction pathways of plant development would fulfil the functional requisites of pathogenicity factors and expose a fundamental genetic vulnerability of plants to pathogens[12,55]. Since diverse pathogens may interdict conserved developmental pathways in plants leading to PCD, molecular dissection of the signal transduction pathways could identify genes common to plant development and disease response. For example, the current state of information indicates that ceramide biosynthetic pathway in plants is a target of pathogens for initiating one of the apoptotic routes involved in compatible disease reaction. Since the enzyme ceramide synthase is known to utilize a variety of substrates, it appears over-simplistic to explain PCD in tomato plants (*Asc* locus) in terms of only 'alteration in substrate utilization' model[52] as proposed. Neither do we know how precisely ceramide and its different precursors or derivatives coordinate with the gene(s) at a particular time and space to initiate PCD following compatible pathogen interaction. In this context, the identification of several LMMs that misregulate cell death constitutes a powerful tool to unravel PCD pathways in plants and to characterize the cross-talk between multiple signal transduction pathways involved in plant development *vis-à-vis* disease response[34].

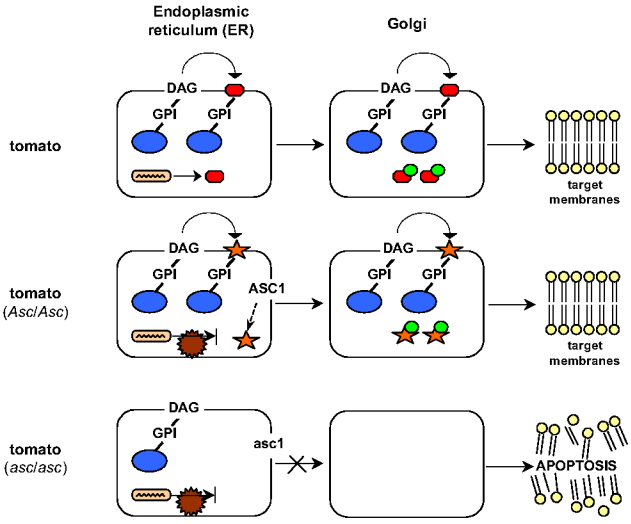

Figure 4. A working hypothesis of endoplasmic reticulum (ER)-to-Golgi transport of glycosylphosphatidylinositol (GPI)-anchored proteins (EGGAP transport) for elicitation of resistant disease response (to AAL toxin) through inactivation of apoptosis in tomato plants in relation to *Asc* locus[51]. Ceramides ( ) are synthesized on the ER membrane from LCBs ( ), while GPI-anchors are synthesized in the ER and covalently linked to certain proteins ( ). The diacylglycerol (DAG) moiety of GPI-anchored proteins is then remodelled to ceramide to facilitate EGGAP transport. In resistant tomato plants (*Asc/Asc*), AAL toxin ( ) inhibits *de novo* ceramide biosynthesis, but ASC1 could restore EGGAP transport by production of alternate ceramides ( ). In susceptible tomato plants (*asc/asc*), the dysfunctional ASC1 protein does not produce alternate ceramides and as a result EGGAP transport is arrested by AAL toxin leading to apoptosis. Complex ceramides ( ) formed downstream of sphingolipid biosynthetic pathway are also shown.

Apoptosis in incompatible plant–pathogen interactions: Sense and sensitivity of hypersensitive response

One of the hallmarks of the resistance disease response in plants is the HR – a rapid, localized plant cell death at the infection site, first identified by Stakman[56] in 1915, and still poses a long-standing enigma of plant pathology[57]. HR is a characteristic phenotype of specific resistance by which pathogen invasion is halted, and can be induced by viruses, bacteria, fungi and nematodes. However, only for pathogens that do not immediately kill cells in susceptible tissue is this response clearly separable from adverse cellular changes induced by successful pathogenesis[55]. HR-linked cell death occurs in host tissue at the infection site of an avirulent pathogen and results in the formation of a distinct 'dry lesion'; virulent pathogens, which do not trigger HR, cause diseases. It requires an active plant metabolism depending on the activity of host transcriptional machinery[58]. Perhaps the first evidence that HR-resistance to microbial pathogens may involve a PCD with some of the characteristics of animal apoptosis came from studies in cowpea leaf cells exhibiting HR to cowpea rust fungus, *Uromyces vignae*[55]. Cell death triggered in intact leaves of two resistant cowpea cultivars by cowpea rust fungus was accompanied by the endonucleolytic cleavage of nuclear DNA into oligonucleosomal fragments (DNA laddering), a typical hallmark of apoptosis in animal cells. However, striking dissimilarities in the execution processes of HR-linked cell death have been observed between cell death caused by *Uromyces vignae* in cowpea leaf cells[55] and by *Phytophthora infestans* in potato leaf mid-rib cells[59]. Recent findings have shown that the morphological trademark of a typical HR–PCD involves membrane dysfunction, progressive vacuolization of the cytoplasm, vacuolar disruption (oncosis) and changes in gross mitochondrial morphology characterized by swelling and cristae disorganization[24]. Analysing the interactions between oats and fungal pathogen *Puccinia coronata* and between *Arabidopsis* and avirulent *P. syringae*, Greenberg's group (University of Chicago, Chicago, IL) has suggested that PCD at the infection zone in HR may be mediated by multiple mechanisms, and different plant–pathogen interactions may adopt different PCD mechanisms. Many studies have confirmed that HR-linked cell death is subject to genetic control, and factors

important for its positive and negative regulation have been identified[60]. The observations that some of the defence genes activated during plant developmental PCD (for example, leaf senescence) are also expressed during HR[61], may well argue for the existence of a significant cross-talk between developmental PCD and the HR, with active participation of endogenously programmed signalling cascades. Over the past few years, different signalling requirements being operated under equally diverse transduction pathways have been identified to be involved in HR-linked cell death control in plants[60–63]. Despite the fact that we are still far behind in identifying a 'consensus signalling pathway' for PCD mechanism underlying the HR in plants, if at all there exists one, the sum of observations and data may have enabled us at least to identify few HR signalling components and to determine the relationship between apoptosis and HR.

Role of caspases in apoptosis

A family of cysteine proteases known as caspases (so called because they cleave after an aspartic acid), are the key enzymes in the regulation of animal apoptosis, and constitute a critical point in the PCD pathway of animal cells. Although the existence of caspases in plants is controversial, they have been shown to be also induced in plant systems undergoing PCD[64]. Recent studies have revealed the presence of a gene family encoding proteins with distant homology to mammalian caspases (metacaspases) in *Arabidopsis thaliana* and *Lycopersicon esculentum* (a type-II metacaspases gene *LeMCA1*)[65]. Inhibition of the caspases by ectopic expression of cystatin, an endogenous caspases inhibitor gene, blocked PCD in soybean cells triggered either by an avirulent strain of *P. syringae* pv. *glucinea* or oxidative stress[66]. This study showed that PCD could be regulated in plants by activity poised between the caspases and the caspases inhibitors. Specific animal caspase inhibitors for caspase-1 (Ac-YVAD-cmk) and caspase-3 (Ac-DEVD-CHO) have been shown to attenuate HR in tobacco leaves induced by bacteria and TMV[67]. The same animal caspase inhibitors reduced the apoptosis of tobacco cells induced by isopentyladenosine[68] and menadione[69]. The baculovirus antiapoptotic protein p35, an inhibitor of apoptosis (IAP), which is known to inhibit animal caspases, was also effective in preventing PCD induced by bacterial, fungal and viral infections[70]. Tobacco plants expressing the baculovirus Op–IAP were reported to acquire resistance to several fungal pathogens and tomato spotted wilt virus[71], whereas those expressing p35 showed partial inhibition of HR-linked cell death when challenged similarly[72]. Lincoln *et al.*[70] showed that transgenic tomato plants expressing a p35 binding site mutant (DQMD to DRIL), inactive against animal caspases-3, did not protect against AAL toxin, indicating that plants possess a caspase with substrate-site specificity that is functionally equivalent to certain animal caspases. Using an approach based on the exploitation of the *Agrobacterium tumefaciens*-encoded VirD2 protein (a possible substrate for caspases), Chichkova *et al.*[73] have recently shown a plant caspase-like protease activity during the HR reactions in tobacco. Targetting the human caspase-3, which specifically cleaves VirD2 protein at two sites, viz. TATD and GEQD, they showed that specific proteolytic processing of the ectopically produced VirD2 derivatives at these sites occurred early in the course of HR induced by TMV in tobacco leaves. The noteworthy observation in this study that the suppression of the induction of TMV-induced PCD by biotinyl-TATD-CHO was correlated with a delay in the activation of the *HSR203J* gene (an early HR-specific molecular marker), indicates that plant caspase regulates an early step in the HR reaction preceding the activation of *HSR203J*[73]. However, the fact that the expression of the *PR-1a* gene, a marker of the host defence response during HR, was affected only slightly by biotinyl-TATD-CHO, suggests that host defence mechanisms and HR-linked cell death may be regulated by different pathways that are expressed coordinately[64,67]. Although it is not clear whether *A. tumefaciens*-induced apoptosis results from plant caspase-mediated fragmentation of VirD2, the involvement of caspases in *Agrobacterium*-induced PCD has also been reported in maize cells[74].

Role of reactive oxygen/nitrogen species in apoptosis

The involvement of reactive oxygen species (ROS) and nitric oxide (NO) in HR-triggered PCD is well characterized in plants[1,61,75,76]. Extracellularly secreted plant peroxidases catalyse the generation of ROS coupled to oxidation of IAA and defence-related compounds like salicylic acid (SA), aromatic mono-amines and chitooligosaccharides[77]. Inter- and intra-cellular generation of ROS (O_2^- and H_2O_2) resulting in oxidative burst is widely accepted to trigger HR reactions[61]. Signalling responses of ROS include the activation of mitogen-activated protein kinases and the up- and down-regulation of gene expression leading to PCD characteristic of HR[78]. In plants, ROS production is closely linked to the expression of [l-] ascorbate peroxidase (APX), an important H_2O_2 detoxifying enzyme. It has been shown that virus-induced PCD in tobacco is accompanied by the post-transcriptional suppression of cytosolic APX (cAPX) expression, and this suppression contributes to a reduction in the capability of cells to scavenge H_2O_2 leading to PCD[79]. Expression of cAPX is under the control of the HR signal transduction pathway and results in signalling events such as changes in protein phosphorylation and induction of ion fluxes[80]. Many recent studies have confirmed the involvement of cellular antioxidant metabolism in ROS-triggered signal transduction, which leads to apoptosis in plants. In general, ROS and NO interfere in phenylalanine ammonia-lyase (PAL) activity and ascorbate (ASC)

and glutathione (GSH) metabolisms, and their simultaneous increase activates a process typical of hypersensitive PCD[81]. In animals, NO cooperates with ROS to kill tumour cells possibly through unregulated NO levels, driving a diffusion-limited reaction with O_2^- to generate peroxynitrite ($ONOO^-$), a mediator of cell injury in many biological systems. In comparison, HR-linked cell death is triggered only by balanced production of NO and ROS in plants[75]. Hypersensitive cell death is activated following the interaction of NO with H_2O_2 (but not with O_2^-) generated from O_2^- by superoxide dismutase. In contrast to the animal system, $ONOO^-$ is not a mediator in cell death reaction[75]. NO has been suggested to have a role in cell–cell signalling and the spreading of cell death during the course of infection[82]. As has been mentioned earlier, H_2O_2 is believed to initiate the cell death pathway by acting as a signal molecule to induce the expression of defence genes encoding enzymes such as PAL and glutathione S-transferase (GST)[83]. However, O_2^- rather than H_2O_2 had been later identified as the primary signal molecule for pathogen induction of GST, and the rates of production and dismutation of O_2^- generated during the oxidative burst play a crucial role in the modulation and integration of NO/H_2O_2 signalling in HR[75].

Role of salicylic acid and mitogen-activated protein kinase in apoptosis

In contrast to animals, plants synthesize salicylic acid (SA) and activate SA-dependent physiological programmes. It plays a central role in many types of plant PCDs and activates disease resistance associated with HR[11,61,84]. The role of salicylhydroxamic acid (SHAM)-sensitive pathway in virus localization during HR in plants is well characterized[84]. Besides promoting the *PR* gene expression and activation of early steps in the HR cascade, SA also signals mitochondrial processes in defence reactions[85]. The involvement of SA in HR-cell death has been linked to the alternative respiratory pathway; the inhibition of alternative oxidase (AOX), a terminal enzyme in the alternative respiratory pathway, leads to a loss of SA-induced viral resistance[85]. However, a recent report has shown in tobacco that lack of AOX compromises neither the ability of SA to heighten the resistance of susceptible plants, nor the ability of HR to restrict the virus in the plant harbouring the *N* gene[86]. This study unequivocally establishes that AOX is not a critical component of the previously characterized SHAM-sensitive pathway important in viral resistance. A constitutively active kinase mitogen-activated protein kinase (MAPK), called NtMEK2DD, is capable of eliciting HR-linked cell death in plants. In tobacco, two MAPKs, viz. SA-induced protein kinase (SIPK) and wounding-induced protein (WIPK) activated by NtMEK2 are responsible for HR-linked cell death. Using potato virus X-induced gene silencing, Jin *et al.*[87] showed that the suppression of the three above components in the NtMEK2–SIPK/WIPK pathway attenuated *N* gene-mediated resistance against tobacco mosaic virus (TMV), thereby establishing their positive role in *N* gene-mediated resistance, possibly through regulation of PCD during HR. Further, based on both gain-of-function and loss-of-function analyses, SIPK has been identified as the kinase involved in regulating WIPK gene expression, which accelerates pathogen-induced HR-linked cell death[88].

Role of ion fluxes in apoptosis

Emerging body of evidence indicates that membrane channel-related proteins are intimately involved in mediating HR-linked cell death. Ion channels play an important role in regulating PCD in HR[61]. The role of cytosolic free Ca ($[Ca^{2+}]_i$) has been investigated in *U. vignae*–cowpea HR reactions involving PCD[89]. This study showed that the elevation of $[Ca^{2+}_i]$ was involved in signal transduction leading to HR during rust fungal infection. Current line of evidence also supports that plasma membrane anion channels are essential components of early signal transduction processes resulting in PCD triggered by HR[61,76]. The function of these anion channels is assumed to initiate or amplify plasma membrane depolarization, which in turn may activate Ca^{2+} voltage-dependent channels or K^+ channels. Mitochondria voltage-dependent-gated anion channels, a family of channels involved in the release of cytochrome *c* during apoptosis in mammals, are also presumed to operate in early stages of HR in plants[90]. Further evidence for the involvement of anion channels in regulating HR-linked cell death came from studies on cryptogein-induced anion channel activation, which constitutes an early branch point in the signalling pathway leading to the activation of inducible defence responses. Cryptogein belongs to a family of homologous proteinaceous elicitors, known as elicitins, that are secreted by all *Phytophthora* species. It induces a fast NO_3^- efflux that is sensitive to anion channel blockers and regulated by phosphorylation events and Ca^{2+} influx. This NO_3^- efflux is an essential component of the cryptogein signalling pathway leading to HR cell death[76]. Recently, Balague *et al.*[91] characterized a gene (*HLM1*) in *Arabidopsis*, which encodes a cyclic nucleotide-gated channel (CNGC4; gene bank accession number AB0100695). The expression of this *HLM1* gene is induced in response to pathogen infection, indicating that it might constitute a common downstream component of the signalling pathways leading to HR cell death *vis-à-vis* disease response[91]. The above observations suggest that ion channel activity modulates the threshold of activation of the multiple resistant disease responses, including the several defence genes and the HR.

It is apparent from the above discussions that a multitude of signals are involved in perception, initiation, regulation and execution of apoptosis during HR, when the host plants are challenged by avirulent pathogens (Figure 5). Although the precise role of these signal molecules is still the sub-

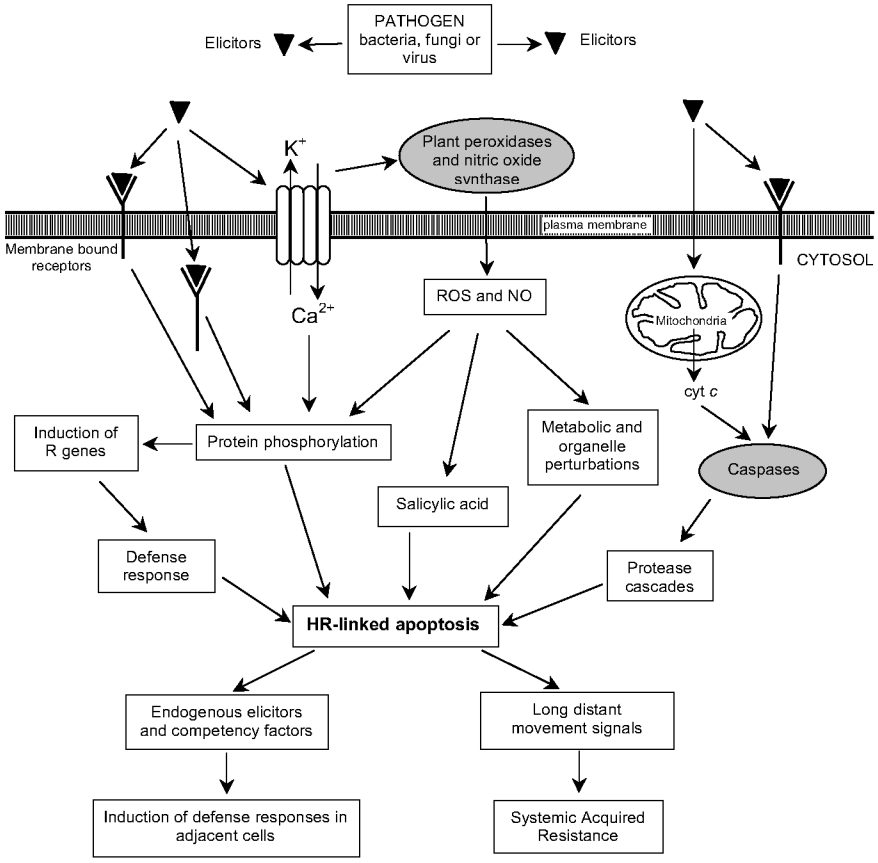

Figure 5. Different signal transduction pathways contributing to activation of apoptosis during HR in incompatible plant–pathogen interactions. Single arrows do not preclude the existence of parallel multiple signalling pathways.

ject of intense debate and investigations, it is gradually becoming clear that the interactions between some of these signals may constitute a 'complex signal transduction' pathway leading to appropriate balance between apoptosis and HR in a coordinated resistant disease response and defence framework. In animal cells, transition in mitochondrial permeability leading to release of cytochrome *c* is one of the important components of the apoptotic mechanism[6]. Recently, in plants, mitochondrial permeability transition has also been suggested to be involved in HR[24]. But, whether mitochondrial cytochrome *c* release activates the induction of HR is a challenge to the future. Furthermore, isolation and characterization of genes regulating cell death in HR would substantiate the determinative role of PCD in disease resistance. Identification of a tobacco gene (*hsr203J*) responsible for activation of incompatible reaction against bacterial pathogen *Ralstonia solanacearum*[92] and the recent discovery of a gene, *hlm1*, in *A. thaliana*, which causes aberrant regulation of cell death and is involved in HR[91], are significant steps towards elucidation of the definitive role of PCD in plant disease resistance. In this context, it is to be emphasized that 'from the standpoint of the pathogen, it seems counter evolutionary that factors leading to death elicitation would be preserved through time if death were responsible for resistance'[60]. Recent results indicate that bacterial pathogen *P. syringae* acts in the plant cells by secreting type III effector proteins, AvrPtoB[93] and HopPtoD2[94], known for antiapoptotic activities in addition to secretion of proapoptotic Avr proteins (Figure 6). This study has further identified distinct N- and C-terminal domains of AvrPtoB that are sufficient for host recognition and PCD inhibition respectively; a *P. syringae* pv. tomato DC 3000 strain with a chromosomal mutation in the AvrPtoB C-terminus elicited immunity in susceptible tomato plants and the disease was restored when full-length AvrPtoB was expressed in *trans*. Interestingly, the place of activity of proapoptotic and antiapoptotic effectors is different in the cell death pathway[93]. In this direction, future experimentations with *P. syringae* system may enable unravelling the exact cross-talk between the proapoptotic and antiapoptotic signals, and how these signals coordinate with the endogenous host PCD genes to elicit disease resistance via HR.

Apoptosis and disease resistance: Therapeutic modulation of PCD?

Therapeutic modulation of the genes and signal molecules involved in initiation and execution of apoptosis has become

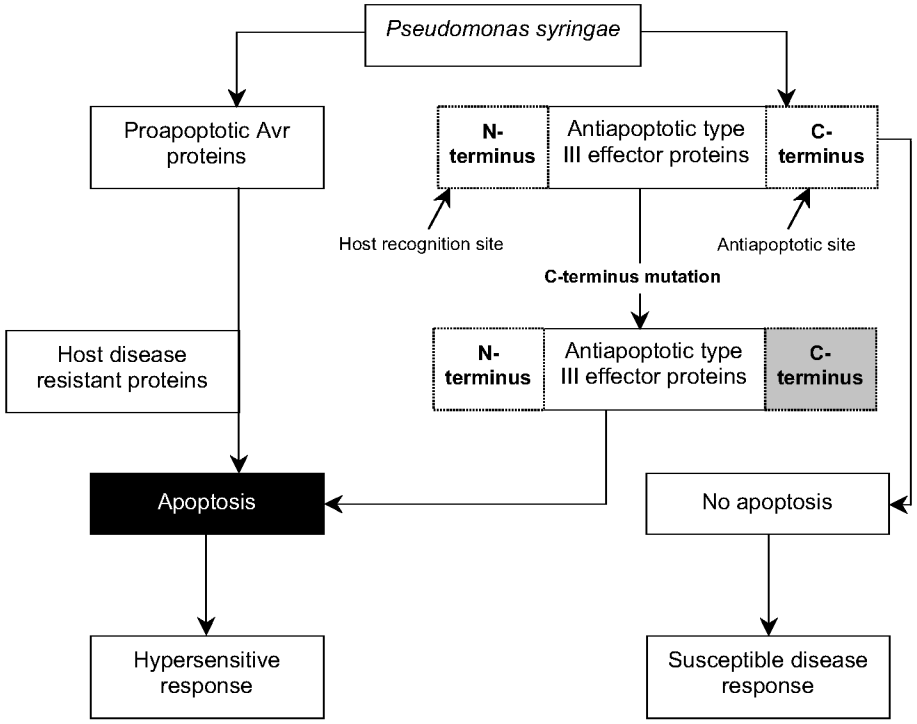

Figure 6. Interplay between proapoptotic Avr proteins and antiapoptotic type III effector proteins of *Pseudomonas syringae* acting at different places in the programmed cell death pathway during plant disease response.

a distinct possibility for combating both degenerative and proliferative diseases of animals and humans, including diseases of the immune system[7,8,95]. Helper T cells (in AIDS) and neurons (in Alzheimer's/Parkinson's diseases), which degenerate through apoptosis, are recent targets for selective manipulation of PCD. Apoptin, an apoptosis-inducer and *Bax*, a proapoptotic gene are being targetted to human cancer cells to selectively induce apoptosis[7,8]. Promises and challenges are both high in this frontier of biomedical science. What about plants? Is it possible to selectively modify signal transduction pathways involved in apoptosis in plants? Is it possible to selectively target 'competent plant cells' involved in plant–pathogen interactions and disease expression? Is it feasible to selectively modulate PCD in plants for combating infections and diseases? Since PCD mechanisms are believed to have evolved from unicellular organisms, some of the regulatory PCD-machineries have been conserved between plants and animals. The plant cells appear to default to PCD in the absence of antiapoptotic signals, which suggests that the mechanisms regulating PCD in plants and animals may be similar[10]. Although the present state of knowledge in plant science may affirm its tremendous potential, the prospect of immediate practical application (therapeutic control of diseases), as expected to be realized in the animal system, is, in fact, a possibility which needs to be addressed with caution and prudence. Complete suppression of apoptosis in animals or plants is developmentally unacceptable[1]. But still the animal system offers better flexibility for combating diseases through therapeutic modulation of apoptosis than the plant system. The foundation of 'disease-defence' mechanism in vertebrate is based on the immune system, which can be distinguished from the plant system where every cell possesses preformed and/or induced defence capabilities (innate immune system). This makes plants unique in the sense that there are no specialized cells as in mammals dedicated exclusively to defence regulation. Therefore, modulation of PCD to alter development and progression of diseases in plants must be site-specific at the location of infection. Interestingly, however, particular tissue-specific cell types have been found to be more competent for PCD in plants. For example, localized bundle sheath cells surrounding the veins are more susceptible to cell-death signals than mesophyll cells during HR-linked cell death[96], possibly as a measure to prevent systemic distribution of pathogens inside the plant. This may imply that the bundle sheath cells are predisposed to undergo PCD during HR. Numerous other results[10] have also confirmed the existence of this type of 'cellular competence system' for PCD during plant growth, development and differentiation. However, the cellular basis of this 'competence system' is largely unknown in plants, and future research in this direction may broaden the scope for therapeutic manipulation of PCD in plants for disease resistance and/or immunity.

Modulation of apoptosis for disease control in plants can be achieved by either inhibition or induction of PCD. For compatible obligate pathogens (necrotrophic), broad-

spectrum disease resistance can be attained through inhibition of apoptosis. As has been discussed earlier, many IAPs are known to exist in animals; Op and p35 from baculovirus and CrmA from cowpox virus are examples of IAPs involved in viral replication. Although the potentiality of IAPs (in plants) has been speculated for the past several years[71], conclusive evidences for their effectiveness in conferring disease resistance in plants have come only recently[70]. Expression of the antiapoptotic gene p35 from baculovirus in transgenic tomato plants provided broad-spectrum resistance to *A. alternata*, *Colletotrichum coccodes* and *P. syringae* pv. tomato[70]. Thus transgenic manipulation of pathways regulating apoptosis has become a potential strategy for engineering broad-spectrum disease resistance in plants. However, this approach, howsoever provocative and encouraging it might be, should be applied with caution[97]. For example, p35, which is also an anticaspase protein, has been shown to affect PCD progression and compromise *N* gene-mediated HR to TMV in transgenic tobacco plants expressing this protein[72]. This categorically argues for the involvement of IAP-p35 in both susceptible and HR disease reactions, and warrants the existence of a cell death target common to both these phenomena[24]. Identification and characterization of IAPs having selective regulatory role in cell death pathway resulting in susceptible disease response may have a logistic advantage. In many susceptible disease responses, pathogen-derived toxins are known to block 'biosynthetic pathways' to initiate a specific 'signal transduction route' (mediated by signal molecules) leading to induction of apoptosis (e.g. ceramide biosynthetic and signalling pathways). Isolation and characterization of genes having the abilities to relieve these toxin-induced blocks in biosynthetic pathways (e.g. *Asc* locus of tomato), and their successful integration into host genome through transgenic approach may be a novel strategy for controlling compatible obligate pathogens. Direct modulation of signals transduced in response to pathogenic stimuli (e.g. sphinganine or LCBs in ceramide signal transduction pathway) for regulating apoptosis *vis-à-vis* controlling compatible host–pathogen interactions, although sounds promising, awaits better understanding of biochemical and genetic mechanisms underlying the transduction pathways of susceptible disease response mechanism.

For incompatible obligate pathogens (biotrophic), disease resistance can be achieved through induction of apoptosis; HR-linked cell death restricts their development and progression, thereby limiting the infection. The processes, mechanisms and signal transduction pathways of apoptosis-induction during HR have been a subject of intense investigation over the last couple of years. Genes with distinct roles in the induction of apoptosis during HR have been identified and characterized. For example, an *A. thaliana* gene, *AtMYB30*, has been identified to be an inducer of apoptosis and a positive regulator of HR-linked cell death following incompatible interactions in response to bacterial pathogens[98]. Identification of such proapoptotic genes, their stable integration into host plants and controlled expression may be an effective means for obtaining durable HR-related resistant disease response in plants. Expression of animal apoptotic genes in plants is another possibility for inducing PCD *vis-à-vis* HR-linked cell death. The *Bcl* family of genes (*Bcl-2* and *Bax*) are important regulators of cellular apoptosis in animals. However, these genes or their homologues do not exist in plants. The proapoptotic member of this *Bcl* family, most notably *Bax*, has tremendous potential for regulating disease response in plants. Transgenic tobacco plants expressing *Bax* elicited HR-linked cell death response synonymous with that induced by TMV[99]. Many other apoptotic genes have also been identified and characterized in nematode *Caenorhabditis elegans* (*egl1*, *ced3* and *ced4*) and fruit fly *Drosophila melanogaster* (*reaper*, *hid* and *grim*). The preliminary results are not very encouraging when these animal apoptotic genes have been cloned in plant expression cassettes, and introduced into tobacco plants (unpublished observations by A. Calderon-Urrea, Department of Biology, California State University, Fresno). Future research in this direction with improved biochemical, cellular and genetical understanding of HR-linked PCD would be useful in formulating strategies for selective expression of animal apoptotic genes in the plant system.

Conclusion

With advances in sophisticated imaging, gene cloning and microarray techniques at the disposal of the present-day researchers, it has become possible not only to monitor temporal changes at the cellular level during induction and regulation of apoptosis/PCD involved in plant disease response, but also to identify genes that regulate the signal transduction pathways participating in the overall process, from signal perception and interception to disease response elicitation. Despite significant recent developments in this field, as discussed above, the number of questions unanswered outweighs those that have been answered. The degree of similarities in apoptotic steps common to both susceptible and resistant disease responses is still not clearly known. Why are some types of plant cells more competent (or predisposed) to receive apoptotic signals, while their neighbours fail to do so? Is there really a 'consensus target' in the apoptotic pathway being negotiated by the cells during both susceptible and HR disease responses? Or, is it so that many of these results are being interpreted based on different and equally diverse experimental approaches? In biological science, it is a common practice to thread together conflicting reports and make a consensus hypothesis. But, in apoptosis research, extreme caution should be exercised before forming any firm conclusion based on different studies. Furthermore, the determinative role of apoptosis in plant growth and development is increasingly being supported by numerous evidences. It is quite plausible

that there must be a cross-talk between developmental and pathogen- (biotic stress-) induced apoptosis. But it is still not known to what extent and at what level this cross-talk operates inside the cell. Cellular and molecular understanding of this cross-talk would enable effective uncoupling of pathogen-induced apoptosis during host–pathogen interactions from developmental apoptosis. Then it may be possible to exercise selective control over pathogen-induced apoptosis with desired disease response function. These are few of the challenges expected to be unfolded in future.

1. Gilchrist, D. G., Programmed cell death in plant disease: The purpose and promise of cellular suicide. *Annu. Rev. Phytopathol.*, 1998, **36**, 393–414.
2. Ellis, R. R., Yuan, J. and Horvitz, H. R., Mechanisms and functions of cell death. *Annu. Rev. Cell Biol.*, 1991, **7**, 663–698.
3. Martins, L. M. and Earnshaw, W. C., Apoptosis: Alive and kicking in 1997. *Trends Cell Biol.*, 1997, **7**, 111–114.
4. Green, D. R., Apoptotic pathways: The roads to ruin. *Cell*, 1998, **94**, 695–698.
5. Kerr, J. F. R., Wyllie, A. H. and Currie, A. R., Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer*, 1972, **26**, 239–257.
6. Green, D. R. and Reed, J. C., Mitochondria and apoptosis. *Science*, 1998, **281**, 1309–1312.
7. Pirocanac, E. C. *et al.*, Bax-induced gene therapy of pancreatic cancer. *J. Surg. Res.*, 2002, **106**, 346–351,
8. Guelen, L., Paterson, H., Gaken, J., Meyers, M., Farzaneh, F. and Tavassoli, M., TAT-apoptin is efficiently delivered and induces apoptosis in cancer cells. *Oncogene*, 2004, **23**, 1153–1165.
9. Barlow, P. W., Cell death – An integral part of plant development. In *Growth Regulators in Plant Senescence* (eds Jackson, M. B., Grout, B. and Mackenzie, I. A.), British Plant Growth Regulator Group, Wantage, 1982, pp. 27–45.
10. Pennell, R. I. and Lamb, C., Programmed cell death in plants. *Plant Cell*, 1997, **9**, 1157–1168.
11. Greenberg, J. T., Programmed cell death in plant–pathogen interactions. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1997, **48**, 525–545.
12. Wang, H., Li, J., Bostock, R. M. and Gilchrist, D. G., Apoptosis: A functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. *Plant Cell*, 1996, **8**, 375–391.
13. Danon, A., Delorme, V., Mailhac, N. and Gallois, P., Plant programmed cell death: A common way to die. *Plant Physiol. Biochem.*, 2000, **38**, 647–655.
14. Kuriyama, H. and Fukuda, H., Developmental programmed cell death in plants. *Curr. Opin. Plant Biol.*, 2002, **5**, 568–573.
15. Gan, S. and Amasino, R. M., Making sense of senescence: Molecular genetic regulation and manipulation of leaf senescence. *Plant Physiol.*, 1997, **113**, 313–319.
16. Zhang, X. G., Cote, G. G. and Crain, R. C., Involvement of phosphoinositide turnover in tracheary element differentiation in *Zinnia elegans* L. cells. *Planta*, 2002, **215**, 312–318.
17. Havel, L. and Durzan, D. J., Apoptosis during diploid parthenogenesis and early somatic embryogenesis of Norway spruce. *Int. J. Plant Sci.*, 1996, **157**, 8–16.
18. Wang, M., Oppedijk, B. J., Lu, X., Van Dujin, V. and Schilperoort, R. A., Apoptosis in barley aleurone during germination and its inhibition by abscisic acid. *Plant Mol. Biol.*, 1996, **32**, 1125–1134.
19. Moller, S. G. and McPherson, M. J., Developmental expression and biochemical analysis of the *Arabidopsis atao1* gene encoding an H_2O_2-generating diamine oxidase. *Plant J.*, 1998, **13**, 781–791.
20. Giuliani, C., Consonni, G., Gavazzi, G., Colombo, M. and Dolfini, S., Programmed cell death during embryogenesis in maize. *Ann. Bot.*, 2002, **90**, 287–292.
21. DeLong, A., Calderon-Urrea, A. and Dellaporta, S. L., Sex determination gene TASSELSEED2 of maize encodes a short–chain alcohol dehydrogenase required for stage-specific floral organ abortion. *Cell*, 1993, **74**, 757–768.
22. Drew, M. C., He, C. J., Morgan, P. W. and He, C. J., Programmed cell death and aerenchyma formation in roots. *Trends Plant Sci.*, 2000, **5**, 123–127.
23. Pan, J. W., Zhu, M. Y., Chen, H. and Han, N., Inhibition of cell growth by aluminum toxicity results from aluminum-induced cell death in barley suspension cells. *J. Plant Nutr.*, 2002, **25**, 1063–1073.
24. Greenberg, J. T. and Yao, N., The role and regulation of programmmed cell death in plant–pathogen interactions. *Cell. Microbiol.*, 2004, **6**, 201–211.
25. Hammond-Kosack, K. E. and Jones, J. D. G., Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1997, **48**, 575–607.
26. Hutcheson, S. W., Current concepts of active defense in plants. *Annu. Rev. Phytopathol.*, 1998, **36**, 59–90.
27. Ritter, C. and Dangl, J. L., Interference between two specific pathogen recognition events mediated by distinct plant disease resistance genes. *Plant Cell*, 1996, **8**, 251–257.
28. Shao, F., Golstein, C., Ade, J., Stoutemyer, M., Dixon, J. E. and Innes, R. W., Cleavage of *Arabidopsis* PBS1 by a bacterial type III effector. *Science*, 2003, **301**, 1230–1233.
29. Goodman, R. N. and Novacky, A. J., *The Hypersensitive Reaction in Plants to Pathogens. A Resistance Phenomena*, APS Press, St. Paul, MN, 1994.
30. Lam, E., Kato, N. and Lawton, M., Programmed cell death, mitochondria and the plant hypersensitive response. *Nature*, 2001, **411**, 848–853.
31. Dong, X., Genetic dissection of systemic acquired resistance. *Curr. Opin. Plant Biol.*, 2001, **4**, 309–314.
32. Kovalchuk, I., Kovalchuk, O., Kalck, V., Boyko, V., Filkowski, J., Heiniein, M. and Hohn, B., Pathogen-induced systemic plant signal triggers DNA rearrangements. *Nature*, 2003, **423**, 760–762.
33. Chung, J., Staswick, P. E., Graef, G. L., Wysong, D. S. and Specht, J. E., Inheritance of a disease lesion mimic mutant in soybean. *J. Hered.*, 1998, **89**, 363–365.
34. Lorrain, S., Vailleau, F., Balague, C. and Roby, D., Lesion mimic mutants: Keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci.*, 2003, **8**, 263–271.
35. Kroesen, B. J., Pettus, B., Luberto, C., Busman, M., Sietsma, H., de Leij, L. and Hannun, Y. A., Induction of apoptosis through B-cell receptor cross-linking occurs via *de novo* generated C16-ceramide and involves mitochondria. *J. Biol. Chem.*, 2001, **276**, 13606–13614.
36. Yu, C. H., Lee, Y. M., Yun, Y. P. and Yoo, H. S., Differential effects of fumonisin B1 on cell death in cultured cells: The significance of the elevated sphinganine. *Arch. Pharm. Res.*, 2001, **24**, 136–143.
37. Hannun, Y. A. and Obeid, L. M., The ceramide-centric universe of lipid-mediating cell regulation: Stress encounters of the lipid kind. *J. Biol. Chem.*, 2002, **277**, 25847–25850.
38. Hannun, Y. A., Luberto, C. and Angraves, K. M., Enzymes of sphingolipid metabolism: From modular to integrative signalling. *Biochemistry*, 2001, **40**, 4893–4903.
39. Ng, C. K.-Y. and Hetherington, A. M., Sphingolipid-mediated signalling in plants. *Ann. Bot.*, 2001, **88**, 957–965.
40. Liang, H., Yao, N., Song, J. T., Luo, S., Lu, H. and Greenberg, J. T., Ceramides modulate programmed cell death in plants. *Genes Dev.*, 2003, **17**, 2636–2641.

41. Sperling, P. and Heinz, E., Plant sphingolipids: structural diversity, biosynthesis, first genes and functions. *Biochim. Biophys. Acta*, 2003, **1632**, 1–15.
42. Coursol, S., Fan, L.-M., Le Stunff, H., Spiegel, S., Gilroy, S. and Assmann, S. M., Sphingolipid signalling in *Arabidopsis* guard cells involves heterotrimeric G proteins. *Nature*, 2003, **423**, 651–654.
43. Lynch, D. V., Enzymes of sphingolipid metabolism in plants. In *Sphingolipid Metabolism* (eds Merrill, A. H. and Hannun, Y. A.), Academic Press, San Diego, 2000, pp. 130–149.
44. Moore, T., Martineau, B., Bostock, R. M., Lincoln, J. E. and Gilchrist, G. D., Molecular and genetic characterization of ethylene involvement in mycotoxins-induced plant cell death. *Physiol. Mol. Plant Pathol.*, 1999, **54**, 73–85.
45. Asai, T., Stone, J. M., Heard, J. E., Kovtun, Y., Yorgey, P., Sheen, J. and Ausubel, F. M., Fumonisin B_1-induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signalling pathways. *Plant Cell*, 2000, **12**, 1823–1835.
46. Stone, J. M., Heard, J. E., Asai, T. and Ausubel, F. M., Simulation of fungal-mediated cell death by fumonisin B1 and selection of fumonisin B_1-resistant (*fbr*) *Arabidopsis* mutants. *Plant Cell*, 2000, **12**, 1811–1822.
47. Wolpert, T. J., Dunkle, L. D. and Ciuffetti, L. M., Host-selective toxins and avirulence determinants: What's in a name? *Annu. Rev. Phytopathol.*, 2002, **40**, 251–285.
48. Curtis, M. J. and Wolpert, T. J., The oat mitochondrial permeability transition and its implication in victorin binding and induced cell death. *Plant J.*, 2002, **29**, 295–312.
49. Mesbah, L. A., Kneppers, T. J., Takken, F. L., Laurent, P., Hille, J. and Nijkamp, H. J., Genetic and physical analysis of YAC contig spanning the fungal disease resistance locus *Asc* of tomato (*Lycopersicon esculentum*). *Mol. Gen. Genet.*, 1999, **261**, 50–57.
50. Guillas, I., Kirchman, P. A., Churad, R., Pfefferli, M., Jiang, J. C., Jazwinski, S. M. and Conzelmann, A., C26-CoA-dependent ceramide synthesis of *Saccharomyces cerevisiae* is operated by Lag 1p and Lac 1p. *EMBO J.*, 2001, **20**, 2655–2665.
51. Brandwagt, B. F., Mesbah, L. A., Takken, F. L., Laurent, P. L., Kneppers, T. J., Hille, J. and Nijkamp, H. J., A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B_1. *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 4961–4966.
52. Spassieva, S. D., Markham, J. E. and Hille, J., The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin-induced programmed cell death. *Plant J.*, 2002, **32**, 561–562.
53. Barz, W. P. and Walter, P., Two endoplasmic reticulum (ER) membrane proteins that facilitate ER-to-Golgi transport of glycosylphosphatidylinositol-anchored proteins. *Mol. Biol. Cell*, 1999, **10**, 1043–1059.
54. Pilloff, R. K., Devadas, S. K., Enyedi, A. and Raina, R., The *Arabidopsis* gain-of-function mutant *dll1* spontaneously develops lesions mimicking cell death associated with disease. *Plant J.*, 2002, **30**, 61–70.
55. Ryerson, D. E. and Heath, M. C., Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. *Plant Cell*, 1996, **8**, 393–402.
56. Stakman, E. C., Relation between *Puccinia graminis* and plants highly resistant to its attack. *J. Agric. Res.*, 1915, **4**, 193–200.
57. Heath, M. C., The enigmatic hypersensitive response: Induction, execution, and role. *Physiol. Mol. Plant Pathol.*, 1999, **55**, 1–3.
58. Heath, M. C., Apoptosis, programmed cell death and the hypersensitive response. *Eur. J. Plant Pathol.*, 1998, **104**, 117–124.
59. Freytag, S., Arabatzis, N., Hahlbrock, K. and Schmelzer, E., Reversible cytoplasmic rearrangements precede wall aposition, hypersensitive cell death and defense-related gene activation in potato/*Phytophthora infestans* interactions. *Planta*, 1994, **194**, 123–135.
60. Richael, C. and Gilchrist, D. G., The hypersensitive response: A case of hold or fold. *Physiol. Mol. Plant Pathol.*, 1999, **55**, 5–12.
61. Heath, M., Hypersensitive response-related cell death. *Plant Mol. Biol.*, 2000, **44**, 321–334.
62. Morel, J. B. and Dangl, J. L., The hypersensitive response and the induction of cell death in plants. *Cell Death Differ.*, 1997, **4**, 1318–1328.
63. Graham, T. L. and Graham, M. Y., Role of hypersensitive cell death in conditioning elicitation competency and defense potentiation. *Physiol. Mol. Plant Physiol.*, 1999, **55**, 13–20.
64. Lam, E. and del Pozo, O., Caspase-like protease involvement in the control of plant cell death. *Plant Mol. Biol.*, 2000, **44**, 417–428.
65. Hoeberichts, F. A., Ten Have, A. and Woltering, E. J., A tomato metacaspase gene is upregulated during programmed cell death in *Botrytis cinerea*-infected leaves. *Planta*, 2003, **217**, 517–522.
66. Solomon, M., Belenghi, B., Delledonne, M., Menachem, E. and Levine, A., The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. *Plant Cell*, 1999, **11**, 431–443.
67. del Pozo, O. and Lam, E., Caspases and programmed cell death in the hypersensitive response of plants to pathogens. *Curr. Biol.*, 1998, **8**, 1129–1132.
68. Mlejnek, P. and Prochazka, S., Activation of caspase-like proteases and induction of apoptosis by isopentenyladenosine in tobacco BY-2 cells. *Planta*, 2002, **215**, 158–166.
69. Sun, Y. L., Zhao, Y., Hong, X. and Zhai, Z. H., Cytochrome *c* release and caspase activation during menadione-induced apoptosis in plants. *FEBS Lett.*, 1999, **462**, 317–321.
70. Lincoln, J. E., Richael, C., Overduin, B., Smith, K., Bostock, R. and Gilchrist, D. G., Expression of the antiapoptotic baculovirus *p35* gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 15217–15221.
71. Dickman, M. B., Park, Y. K., Oltersdorf, T., Li, W., Clemente, T. and French, R., Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc. Natl. Acad. Sci. USA*, 2001, **245**, 389–399.
72. del Pozo, O. and Lam, E., Expression of the baculovirus p35 protein in tobacco affects cell death progression and compromises *N* gene-mediated disease resistance response to tobacco mosaic virus. *Mol. Plant–Microb. Interact.*, 2003, **16**, 485–494
73. Chichkova, N. V. *et al.*, A plant caspase-like protease activated during the hypersensitive response. *Plant Cell*, 2004, **16**, 157–171.
74. Hansen, G., Evidence for *Agrobacterium*-induced apoptosis in maize cells. *Mol. Plant–Microb. Interact.*, 2000, **13**, 649–657.
75. Delledonne, M., Zeier, J., Marocco, A. and Lamb, C., Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 13454–13459.
76. Wendehenne, D., Lamotte, O., Frachisse, J-M., Barbier-Brygoo, H. and Pugin, A., Nitrate efflux is an essential component of the cryptogein signalling pathway leading to defense responses and hypersensitive cell death in tobacco. *Plant Cell*, 2002, **14**, 1937–1951.
77. Kawano, T., Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. *Plant Cell Rep.*, 2003, **21**, 829–837.
78. Hancock, J. T., Desikan, R., Clarke, A., Hurst, R. D. and Neill, S. J., Cell signalling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. *Plant Physiol. Biochem.*, 2002, **40**, 611–617.
79. Mittler, R., Feng, X. and Cohen, M., Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. *Plant Cell*, 1998, **10**, 461–473.
80. Mittler, R., Herr, E. H., Orvar, B. L., van Camp, W., Willekens, H., Inze, D. and Ellis, B. E., Transgenic tobacco plants with redu-

ced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 14165–14170.
81. de Pinto, M. C., Tommasi, F. and de Gara, L., Changes in the antioxidant systems as part of the signalling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells. *Plant Physiol.*, 2002, **130**, 698–708.
82. Zhang, C., Czymmek, K. J. and Shapiro, A. D., Nitric oxide does not trigger early programmed cell death events but may contribute to cell-to-cell signalling governing progression of the *Arabidopsis* hypersensitive response. *Mol. Plant–Microb. Interact.*, 2003, **16**, 962–972.
83. Desikan, R., Reynolds, A., Hancock, J. T. and Neill, S. J., Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in *Arabidopsis* suspension cultures. *Biochem. J.*, 1998, **330**, 115–120.
84. Murphy, A. M., Chivasa, S., Singh, D. P. and Carr, J. P., Salicylic acid-induced resistance to viruses and other pathogens: A parting of the ways? *Trends Plant Sci.*, 1999, **4**, 155–160.
85. Alvarez, M. E., Salicylic acid in the machinery of hypersensitive cell death and disease resistance. *Plant Mol. Biol.*, 2000, **44**, 429–442.
86. Ordog, S. H., Higgins, V. J. and Vanlerberghe, G. C., Mitochondrial alternative oxidase is not a critical component of plant viral resistance but may play a role in the hypersensitive response. *Plant Physiol.*, 2002, **129**, 1858–1865.
87. Jin, H. L., Liu, Y. D., Yang, K. Y., Kim, C. Y., Baker, B. and Zhang, S. Q., Function of mitogen-activated protein kinase pathway in *N* gene-mediated resistance in tobacco. *Plant J.*, 2003, **33**, 719–731.
88. Liu, Y. D., Jin, H. L., Yang, K. Y., Kim, C. Y., Baker, B. and Zhang, S. Q., Interaction between two mitogen-activated protein kinases during tobacco defense signalling. *Plant J.*, 2003, **34**, 149–160.
89. Xu, H. X. and Heath, M. C., Role of calcium in signal transduction during the hypersensitive response caused by basidiospore-derived infection of the cowpea rust fungus. *Plant Cell*, 1998, **10**, 585–597.
90. Lacomme, C. and Roby, D., Identification of new early markers of the hypersensitive response in *Arabidopsis thaliana*. *FEBS Lett.*, 1999, **459**, 149–153.
91. Balague, C. *et al.*, HLM1, an essential signaling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel ion family. *Plant Cell*, 2003, **15**, 365–379.
92. Pontier, D., Tronchet, M., Rogowsky, P., Lam, E. and Roby, D., Activation of *hsr203*, a plant gene expressed during incompatible plant–pathogen interactions, is correlated with programmed cell death. *Mol. Plant–Microb. Interact.*, 1998, **11**, 544–554.
93. Abramovitch, R. B., Kim, Y. J., Chen, S. R., Dickman, M. B. and Martin, G. B., *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. *EMBO J.*, 2003, **22**, 60–69.
94. Espinosa, A., Guo, M., Tam, V. C., Fu, Z. Q. and Alfano, J. R., The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. *Mol. Microbiol.*, 2003, **49**, 377–387.
95. Duke, R. C., Ojcius, D. M. and Young, J. D., Cell suicide in health and disease. *Sci. Am.*, 1996, **275**, 80–87.
96. Hammond-Kosack, E. E., Silverman, P., Raskin, I. and Jones, J. D. G., Race-specific elicitors of *Cladosporium fulvum* induce changes in cell morphology and salicylic acid in tomato plants carrying the corresponding *Cf* disease resistance gene. *Plant Physiol.*, 1996, **110**, 1381–1394.
97. Dickman, M. B., Park, Y. K., Oltersdorf, T., Li, W., Clemente, T. and French, R., Correction for abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 11816.
98. Vailleau, F., Daniel, X., Tronchet, M., Montillet, J. L., Triantaphylides, C. and Roby, D., A R2R3-MYB gene, *AtMYB30*, acts as a positive regulator of the hypersensitive cell death program in plants in response to pathogen attack. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 10179–10184.
99. Lacomme, C. and Santa Cruz, S., *Bax*-induced cell death in tobacco is similar to the hypersensitive response. *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 7956–7961.

Received 12 April 2004; accepted 30 September 2004