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The hypersensitive response and its role in local and systemic disease resistance

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Abstract

A ubiquitous feature of plant/pathogen interactions is host cell death that is manifested as rapid collapse of tissue and is termed the hypersensitive response (HR). This response accompanies many but not all incompatible interactions and is considered one of the important mechanisms leading to resistance. The sites of HR, the infection sites proper, are invariably the focal points for transcriptional activation of a large variety of plant defence genes in neighbouring cells. The subsequent biosynthesis of protective secondary metabolites and inhibitory proteins around the infection sites are considered to be important for overall pathogen containment. In addition, local HR is often associated with the onset of systemic acquired resistance (SAR) in distal plant tissues. This type of resistance is generally effective against a broad range of pathogens and it is associated with the transcriptional activation of whole set of marker genes, many of which encode pathogenesis-related proteins, such as chitinases and 1,3-β-glucanases. Cell death is also a feature of disease symptoms in many compatible interactions, but in these cases it usually occurs rather late during the course of host colonisation by the pathogen. Necrotic lesions may develop but are not required for triggering SAR and systemic gene activation. Apparently, different forms of cell death and mechanisms leading to HR exist and are executed in plant/pathogen interactions. Although the importance of small molecules, such as reactive oxygen intermediates (ROI), for the establishment of HR cell death has been recognized, a functional and causal link between ROI production, initiation of HR cell death, and induced local and systemic disease resistance remains to be unequivocally demonstrated.

Introduction

Plants have evolved a large variety of sophisticated defence mechanisms to resist the colonisation by microbial pathogens and parasites. These can be divided into three major categories (Kombrink and Somssich, 1995): (i) immediate, early defence responses of the directly invaded plant cells, starting with signal recognition and transduction and frequently leading to rapid cell death, the so-called hypersensitive response (HR); (ii) local gene activation in the close vicinity of infection sites, resulting in the *de novo* synthesis of numerous secondary products, including phytoalexins, in the reinforcement of structural barriers, such as the cell wall, or in indirect inhibition of the pathogen; (iii) systemic activation of genes encoding pathogenesis-related (PR) proteins, including chitinases and 1,3-β-glucanases, which are directly or indirectly inhibitory towards pathogens and have been associated with the phenomenon of systemic acquired resistance (SAR). This classification is mainly based on the distinct temporal and spatial expression patterns of different defence responses as observed in several systems. Many of these responses have been extensively studied in elicitor-treated, cultured plant cells and have been found to be essentially the same in

this simplified system as in true plant/pathogen interactions (Hahlbrock et al., 1995; Somssich and Hahlbrock, 1998).

The initial responses of pathogen-invaded or elicitor-treated plant cells occur within a few minutes and are rapidly followed by local gene activation (Somssich and Hahlbrock, 1998). They include rapid and transient changes in inorganic ion fluxes across the plasma membrane (Nürnberger et al., 1994), the accumulation of reactive oxygen intermediates (ROI) referred to as oxidative burst (Apostol et al., 1989; Jabs et al., 1997), and changes in the phosphorylation status of various proteins (Dietrich et al., 1990), all of which have been associated with intracellular signal transduction mechanisms. As a result, an extensive reprogramming of both primary and secondary metabolism at the gene expression level is initiated (Somssich et al., 1989; Batz et al., 1998), and many of the encoded proteins are either directly or indirectly inhibitory towards invading pathogens (Kombrink and Somssich, 1995; Hammond-Kosack and Jones, 1996; Kombrink and Somssich, 1997). The majority of genes studied in this context are rapidly and transiently activated after infection or elicitor treatment, presumably reflecting, at least in part, the design of the screening strategies for particularly early events in pathogen defence (Somssich et al., 1989). In addition, more slowly and prolonged activation of gene expression has also been observed, which is frequently not restricted to the vicinity of the infection site, in contrast to many of the rapidly activated genes (Schmelzer et al., 1989; Schröder et al., 1992; Kombrink et al., 1993; Büchter et al., 1997; Ponath et al., 2000). Systemic gene activation, followed by the accumulation of a large variety of proteins, collectively referred to as PR proteins, is tightly correlated with the phenomenon of induced or systemic acquired resistance (SAR) (Ryals et al., 1996; Sticher et al., 1997; van Loon, 1997). PR proteins are considered to be ubiquitous in the plant kingdom and are presently grouped into 14 different families (Kombrink and Somssich, 1997; van Loon and van Strien, 1999). The best known and most extensively studied representatives are from families 2 and 3, which have been identified as 1,3-β-glucanases and chitinases, respectively, whereas others comprise members with unknown functions. Typically, SAR is induced in response to avirulent pathogens causing necrotic lesions. It is generally effective against subsequent infection by a broad range of pathogens, including viruses, bacteria and fungi, and it can last for several weeks or even months (Madamanchi and Kuć, 1991; Sticher et al., 1997). The induction of PR proteins is mediated via a salicylic acid dependent signalling pathway and their expression as well as the SAR phenotype can also be induced by exogenous application of salicylic acid or its synthetic functional analogues 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH) (Kessmann et al., 1994). Recently, a SA-independent pathway leading to systemic resistance has been discovered. It is induced by plant growth-promoting rhizobacteria, such as *Pseudomonas fluorescence*, independent of PR protein induction and mediated via jasmonate and ethylene signalling (Pieterse et al., 1996; van Loon et al., 1998).

We have studied the cultivar/race-specific interaction between potato (*Solanum tuberosum*) and the late blight pathogen, *Phytophthora infestans*, and the non-host interaction between parsley (*Petroselinum crispum*) and the soybean pathogen, *Phytophthora sojae*, at the morphological level by live video microscopy and immunohistochemistry to characterise the cytological events and early plant defence reactions related to hypersensitive cell death. Conditions were also established for the induction of SAR in potato against *P. infestans* and biochemical and molecular changes occurring in distal tissues were characterised with the aim to uncover the mechanisms responsible for the resistance phenotype as well as for the associated systemic signalling events leading to systemic gene activation. The results will be compared and discussed in relation to data obtained with other plant/pathogen systems. The focus of this paper is the HR and its importance for local and systemic disease resistance. It is not our intention to provide a comprehensive review of the extensive literature on the genetic, biochemical, and cellular control mechanisms of programmed cell death or apoptosis, which at present is an intensely studied area in plant biology. For these aspects the reader is referred to excellent recent review articles (Dangl et al., 1996; Greenberg, 1997; Morel and Dangl, 1997; Heath, 1998; Richael and Gilchrist, 1999; Shirazu and Schulze-Lefert, 2000).

Features of the hypersensitive response

The HR was first identified by Stakman in 1915 (Stakman, 1915) and since then has been observed as a general feature in numerous plant/pathogen interactions (Goodman and Novacky, 1994; Dangl et al., 1996; Greenberg, 1997). It is defined as a rapid, localised necrosis of cells at the infection site and it occurs in

resistant plants in response to pathogenic viruses, bacteria, fungi or nematodes. It has been suggested that the HR is a form of programmed cell death (PCD) in plants, and indeed, there are some similarities between HR and mammalian apoptosis, as well as many differences (Greenberg, 1996; Morel and Dangl, 1997; Heath, 1998). In particular, plant cells dying due to the HR do not clearly show all the morphological cellular changes that characterise mammalian apoptosis, such as chromatin condensation, nuclear blebbing, cell fragmentation into apoptotic bodies, or the associated biochemical markers, such as DNA cleavage into nucleosomal fragments, degradation of DNA repair enzymes, and the activation of specific proteases (Heath, 1998). Despite numerous light and electron microscopic studies of the HR, no consistent and universal morphological features have emerged that clearly define and distinguish the HR from cell death caused by other means. Instead, a diversity of cellular processes among different forms of cell death have been described (Heath, 1998).

In potato cultivars carrying known resistance (*R*) genes, hypersensitive cell death appears to be the major defence response to infection by *P. infestans*. The HR is always observed in resistant plants (incompatible interactions) and it occurs rapidly, usually within 24 h after inoculation, resulting in death of one to three cells, and is frequently restricted to the epidermis. However, even in the resistant case, hyphae are occasionally able to escape from the HR area and establish a biotrophic interaction, often associated with a trailing HR (Cuypers and Hahlbrock, 1988; Freytag et al., 1994; Vleeshouwers et al., 2000).

In susceptible potato plants (compatible or biotrophic interactions), likewise, the penetrated epidermal cells occasionally show the characteristics of the HR, such as granular, brownish cytoplasm, thickened cell walls, autofluorescence under UV light and condensed nuclei near penetration sites (Schröder et al., 1992; Freytag et al., 1994; Vleeshouwers et al., 2000). Thus, the defence response to *P. infestans* infection is ambiguous, even on the same plant. These and other results (see below) indicate that the difference between resistance and susceptibility is quantitative rather than qualitative despite the presence of major resistance (*R*) genes (Schröder et al., 1992; Freytag et al., 1994; Vleeshouwers et al., 2000).

Since the HR is the major defence response that is associated with all forms of resistance, i.e., race-specific, race-nonspecific, and nonhost, and it is found in all tested plant species (Vleeshouwers et al., 2000), the question arises whether or not *R* genes are involved in all types of resistance to *Phytophthora* or other oomycetes. The observation that the HR also correlates with partial resistance can be explained by the classical (*R*)gene-for-(*Avr*)gene model with the assumption that 'strong' and 'weak' *R*-gene/*Avr*-gene interactions exist resulting from different receptor strength or ligand affinity if the receptor–ligand model of the gene-for-gene model is accepted. The differential HR effectiveness has lead to the proposal that differential thresholds are responsible for activating HR and different resistance-related genes. With the barley *mlo* and *Arabidopsis lsd1* mutants two examples exist that exhibit a lowered sensitivity threshold for triggering the HR (Büschges et al., 1997; Dietrich et al., 1997; Vleeshouwers et al., 2000).

Cellular and morphological changes associated with HR

The highly dynamic, morphological changes occurring in the first, directly penetrated cell in the interaction between potato and *P. infestans* were intensively studied by live video microscopy. By this *in vivo* analysis of the infection process of single cells within the intact leaf, we were able to uncover and record various stages of the single cell HR, including the final step, the actual incident of cell death (Freytag et al., 1994). Immediately after attempted penetration by the pathogen, the plant cell formed directly at the penetration site a local barrier (papilla) by apposition of material to the cell wall. This process was associated with increased cytoplasmic streaming and translocation of the nucleus to this site. Nuclear migration and accumulation of cytoplasm underneath the pathogen's appressorium was completed within 1.5–2 h after appearance of the intracellular infection hyphae and subsequently the increasing collar-like wall apposition surrounded and frequently encased the invading fungal hyphae. The dark brown colour of the wall apposition and its autofluorescence under UV light indicated the presence of phenolic material and at the same time callose deposition was also found in collars and papillae which is in accordance with previous results reported for potato/*P. infestans* interactions (Cuypers and Hahlbrock, 1988; Gees and Hohl, 1988; Vleeshouwers et al., 2000). The initiated cellular alterations turned out to be reversible once pathogen invasion had been successfully stopped

by papilla formation: the cytoplasmic aggregation disappeared and the nucleus left its position at the penetration site.

However, when this local defence response failed and *P. infestans* succeeded to develop an intracellular vesicle, the cellular HR was executed to prevent further pathogen growth. The first symptom indicative of irreversible changes leading to cell death was a more granular appearance of the cytoplasm and its aggregation around the invading pathogen. Cytoplasmic streaming ceased and subsequently the conglomerate of plant cytoplasm and nucleus expanded and collapsed abruptly within about 20 s, followed by a collapse of the intracellular vesicle of the pathogen. Subsequently, the cell wall and the cell interior turned dark and became autofluorescent within the next few hours (Freytag et al., 1994). In conclusion, only small quantitative differences between the compatible and incompatible interactions of two *P. infestans* races were observed for these early responses of epidermal cells.

For detailed cytochemical investigations of particular steps of this cellular defence program, a model system of reduced complexity was established, consisting of cultured parsley cells infected by *P. infestans*. Microscopic observations revealed that in this system all features of the defence response resembled the *in planta* situation as described above, and the ease of visualisation of either the microtubule or actin filament network allowed an insight into the role of the cytoskeleton in the drastic cytoplasmic rearrangements. In this system, a rapid translocation of the plant cell cytoplasm and nucleus to the site of attempted penetration was again observed, which was associated with the rapid and local depolymerisation of the microtubular and reorganisation of actin filament networks (Gross et al., 1993; Naton et al., 1996). Application of specific inhibitors revealed that the directed cytoplasmic streaming and nuclear translocation is apparently an actin filament dependent process whereas the microtubular network is not involved (Gross et al., 1993; Schmelzer et al., 1995). At present, it is not clear whether or not the translocation process is a prerequisite for the increased local metabolic activities leading to cell wall apposition and papillae formation at the penetration site. A similar correlation between cytoplasmic rearrangement, changes in cytoskeleton architecture, especially of the actin filaments, and the proper formation of cell wall appositions and papillae has also been observed in other plant–pathogen interactions (Heath et al., 1997; Kobayashi et al., 1997a; Kobayashi et al., 1997b; Skalamera et al., 1997; Skalamera and Heath, 1998).

Correlation of HR cell death with metabolic changes

HR-linked cell death in plants requires active plant metabolism and depends on the activity of the host transcriptional machinery. It has also long been recognised that the HR can generate signals that cause local and systemic changes in the plant. One of the most rapid plant responses engaged following pathogen recognition is the oxidative burst, which constitutes the production of reactive oxygen intermediates (ROI), primarily superoxide (O_2^-) and hydrogen peroxide (H_2O_2), at the site of attempted invasion (Lamb and Dixon, 1997; Wojtaszek, 1997; Grant and Loake, 2000). It has been suggested that the oxidative burst and cognate redox signalling play a central role in integration and co-ordination the multitude of plant defence responses (Lamb and Dixon, 1997).

Superoxide anion generation in relation to HR was first reported for potato tuber slices inoculated with an avirulent race of *P. infestans* (Doke, 1983). Subsequently, the oxidative burst has been identified in numerous plant/pathogen interactions involving different kinds of pathogen. The origin of the ROI generated during the oxidative burst is not unequivocally established, but candidate reactions are the action of a plasma membrane located NADPH-dependent oxidase complex and a cell wall peroxidase (Bolwell and Wojitaszek, 1997; Wojtaszek, 1997). The cytotoxicity and reactive nature of O_2^- requires its cellular concentration to be carefully controlled, which can be achieved by induction of antioxidant enzymes, such as glutathion *S*-transferase or glutathion peroxidase (Levine et al., 1994; Wojtaszek, 1997). Accumulation of ROI is a rapid event that precedes HR in many plant–pathogen interactions showing *R* gene-triggered resistance (Doke, 1985; Levine et al., 1994). Rapid and biphasic ROI accumulation has been observed in several cultured plant cell systems in response to bacterial or fungal elicitors, i.e., *Avr* gene products (Levine et al., 1994; Baker et al., 1997; Wojtaszek, 1997). While the first peak was considered non-specific, the second sustained ROI burst was dependent on the pathogen race and only occurred with avirulent bacteria. Collectively, these data suggested a dual function for ROI in disease resistance: (i) direct participation in the development of host cell death during HR as well as direct inhibition of the pathogen, and (ii) a role as a diffusable signal for induction of cellular protectants and defence responses in neighbouring cells. Thus, the strict spatial limitation of cell death may be the result of a dose-dependent

antagonistic action of ROI (Lamb and Dixon, 1997).

The physiological and metabolic changes of individual cells undergoing HR could also be recorded in our model system, *P. infestans*-infected parsley cells. Using cytochemical staining methods, we monitored a drastic increase of the membrane potential of mitochondria of infected cells immediately upon penetration by the pathogen (Naton et al., 1996). The processes related to HR, such as reorganisation of cytoplasm and cytoskeleton architecture, the synthesis of cell wall phenolics and callose, or the activation of defence-related genes, are apparently so energy consuming that increased mitochondrial activity is needed to provide sufficient amounts of ATP.

We also monitored the oxidative burst in single cells by cytochemical staining and observed that upon infection the levels of intracellular ROI was tightly correlated with the rate of rapid cell death (Naton et al., 1996). Analysis by transmission electron microscopy further revealed that the cellular membrane system was subjected to irreversible damage during the course of infection and that this process was closely related to ROI production. It is conceivable that peroxidation of fatty acids and lipids is responsible for increased membrane deterioration. Various additional lines of evidence strongly suggest that the intracellular accumulation of ROI is important for HR induction, whereas the extracellular generated ROI are not of major significance, at least in our *in vitro* model system (Naton et al., 1996). Since in cultured parsley cells, the observed metabolite changes were largely confined to individual infected cells within a microcallus and occurred in the same manner in single cells, we consider the HR, including rapid cell death, a cell-autonomous process that does not depend on the presence of adjacent cells.

Local defence gene activation in response to HR

In an incompatible interaction, pathogen recognition and generation of a primary intracellular signal, which activates defense responses, is restricted to the first penetrated cell. However, many of these activated defense responses, in particular the up-regulation of defence-related genes, are not restricted to the directly infected cells suggesting that secondary signals are involved in cell-to-cell communication. The expression patterns for a large number of such genes have been determined in infected potato, parsley, and many other plants, as well as in single infected cells of our parsley model system, by the technique of *in situ* mRNA hybridisation (Schmelzer et al., 1989; Schröder et al., 1992; Gross et al., 1993; Kombrink et al., 1993; Freytag et al., 1994; Kawalleck et al., 1995; Büchter et al., 1997; Ancillo et al., 1999). Two essential questions could be conclusively answered by these studies, namely the role of the directly infected cell and whether or not HR is required for gene activation. In infected tissue, a frequent observation is that the necrotic cells are devoid of signal and only the surrounding tissue shows high mRNA levels of a defence-related gene (Schmelzer et al., 1989; Taylor et al., 1990; Schröder et al., 1992; Kombrink et al., 1993). But this is presumably only a secondary effect of tissue necrosis at relatively advanced infection stages. By contrast, in cultured parsley cells a rapid and strong accumulation of mRNA encoding PR-1 was observed in both single, infected cells, as well as in all cells of a small aggregate or microcallus (Gross et al., 1993). Since the timing of induction was more or less identical in infected and neighbouring cells and occurred prior to any detectable cell death, we conclude that defence gene activation is not dependent on HR or cell death (Gross et al., 1993; Naton et al., 1996).

Is HR cell death required for resistance?

Another interesting question is whether or not the occurrence of HR is a prerequisite for resistance. Although cell death and resistance in plants are apparently tightly connected, it remains to be proved whether the HR has a decisive role in resistance. In other words, is the cell death occurring during HR the actual cause of disease resistance? Or is cell death not important for pathogen restriction and only closely associated with other factors, mechanisms or cellular changes that limit pathogen spread and hence are the basis for resistance? Thus, cell death may only be a consequence of other mechanisms that limit the pathogen. Indeed, there are several reports on plants interacting with viral, bacterial or fungal pathogens that are resistant to various pathogens without employing HR, which throw doubt on its direct involvement in resistance. Examples include *Rx*-dependent resistance of potato against potato virus X (Bendahmane et al., 1999), resistance of bean against *Pseudomonas syringae Hrp*-mutants (Jakobek and Lindgren, 1993), race-specific resistance to powdery mildew of barley mediated by the *mlg* gene (Görg et al., 1993), and resistance of tomato to

Cladosporium fulvum (Hammond-Kosack and Jones, 1994). In addition, *Arabidopsis* mutants have been derived which display effective gene-for-gene bacterial resistance without HR cell death (Bowling et al., 1994; Yu et al., 1998).

In the interaction between potato and *P. infestans*, numerous defence responses are activated to the same extent in both resistant and susceptible plants (Schröder et al., 1992; Freytag et al., 1994). This also included the occurrence of HR cell death that was observed in both incompatible and compatible interactions, although the final phenotype was different, resulting in small necrotic spots and large lesions, respectively. Thus, despite the presence or absence of a functional resistance gene, major defence reactions are apparently executed in both cases and the resistance phenotype is apparently the result of small quantitative differences in cellular and biochemical responses (Freytag et al., 1994; Vleeshouwers et al., 2000). Thus, despite the striking association observed between host HR cell death and the inhibition of pathogen growth, there is no evidence which shows a need for HR to limit pathogen proliferation.

Systemic defence responses and SAR in relation to HR

Many studies have shown that a HR is accompanied not only by biochemical changes at the site of infection but also at distant sites in the plant (Madamanchi and Kuc, 1991; Sticher et al., 1997). In potato, SAR to late blight can be induced by local treatment with various pathogens, including *P. infestans* or *Pseudomonas syringae* (Strömberg and Brishammar, 1991; Kombrink et al., 1996), as well as by treatment with arachidonic acid (Cohen et al., 1991; Coquoz et al., 1995) or pathogen cell wall components (Doke et al., 1987).

Since we are interested in the natural mechanisms of induced resistance in potato, we set up conditions for the biological induction of SAR that would allow us to determine the molecular mechanisms leading to the state of induced resistance. Different potato cultivars with and without specific resistance genes (Bintje, R0; Datura, R1; Isola, R4), were chosen and these were inoculated with virulent or avirulent races of *P. infestans* at two lower leaves. At various times thereafter the whole plants were inoculated with a virulent race of the pathogen. In all plant/pathogen combinations tested, this local preinoculation resulted in a significant reduction in disease symptoms, scored as number and size of lesions. Thus, the induction of SAR in potato is independent of the genetic background of the plant and the pathogen, as reported for other systems (Madamanchi and Kuc, 1991; Hammerschmidt, 1999). However, the quantitative evaluation of the SAR status resulted in highly variable data, which was mainly due to varying effectiveness of the primary infection as a result of varying fitness of the *P. infestans* zoospores. Such variation was not observed when the primary infection was carried out with the bacterial *Brassica* pathogen, *Pseudomonas syringae* pv. *maculicola*, for which potato is a non-host. Injection of a bacterial suspension into potato leaves resulted in the rapid development of necrotic lesions and upon challenge inoculation with a virulent race of *P. infestans* 2–4 days later, a significant and reproducible reduction in disease symptoms was observed in comparison to control plants (Kombrink et al., 1996). In addition, a considerable delay in pathogen colonisation of induced plants and thus reduction in *P. infestans* biomass could also be demonstrated (Kombrink et al., 1996).

When analysing the expression patterns of various PR proteins in potato plants showing the SAR phenotype, a strong local and systemic accumulation of mRNAs encoding acidic (class II) and basic (class I) chitinases and 1,3-β-glucanases was observed in response to the primary infection (Kombrink et al., 1996). The time course of systemic mRNA and protein accumulation in upper uninfected potato leaves correlated well with the induction of the SAR phenotype which is manifested at 2–4 days after the primary inoculation. As in many other plants, chitinases and 1,3-β-glucanases seem to be suitable SAR marker genes also in potato. However, in contrast to other plants not only the acidic isoforms but also the basic ones are systemically upregulated.

Since *P. infestans* is an oomycete lacking chitin in its cell wall, it is unlikely that the induced chitinases are directly responsible for restricting the growth of this pathogen, unless they displayed additional enzymatic functions. Therefore, other proteins and mechanisms are presumably responsible for retarding *P. infestans* during SAR. In search for additional systemically activated defence genes with the potential to participate in the establishment of the SAR phenotype, various screening approaches utilising 'differential mRNA display' and 'differential cDNA hybridisation' techniques were employed. As a result, several cDNAs encoding proteins with defence potential were isolated, including

the basic isoform of the PR-1 protein (Kombrink and Somssich, 1997), the tomato homologue of which has inhibitory activity against *P. infestans* (Niderman et al., 1995), a novel extracellular matrix protein which in tomato has been associated with lignified secondary walls (Domingo et al., 1994), and a glutathion *S*-transferase, which has been demonstrated to be selectively induced by various types of pathogen (Taylor et al., 1990; Martini et al., 1993; Strittmatter et al., 1996). The systemic activation of these genes during SAR is virtually identical to that of acidic chitinase (ChtA2) marker gene (Büchter et al., 1997), but their functional significance for establishment of the SAR phenotype needs to be demonstrated.

In addition to proteins with potential defence function or novel proteins with unknown functions (Kombrink et al., 1996), a number of cDNAs encoding enzymes of primary metabolism or proteins engaged in photosynthesis resulted from the screens described above. Systemic upregulation of aldolase, glyceraldehyde-3-phosphate dehydrogenase, ribulose-1,5-bisphosphate carboxylase, chlorophyll-a/b-binding protein, ferredoxin, and others, although quite unexpected, may reflect the increased energy requirements related to the activation of defence responses, as well as the demand for precursors of different biosynthetic reactions leading to phytoalexins, phenylpropanoids, etc. The activation of primary metabolism or housekeeping genes during infection or SAR induction is not commonly found, but a precedent has been reported for elicitor-treated and infected parsley and alfalfa cells, in which the transcriptional activation of genes encoding enzymes of the oxidative pentose phosphate pathway has been observed (Kombrink and Hahlbrock, 1986; Fahrendorf et al., 1995; Batz et al., 1998).

Conclusions

Despite species-specific differences, plants have apparently evolved common basic defence strategies comprising very rapid, localised and delayed, systemic defence mechanisms to resist pathogens. Significant advances have been made in understanding the genes involved in regulating the resistant state as well as the chemical signals modulating the responses. However, the actual mechanism(s) stopping pathogen development has not conclusively been revealed. This also applies to cell death as a common phenotype of the HR. Its activation may be involved in the resistance response, but the functional role of cell death is not clear. The use of transgenic plants and of mutants defective in expression of HR cell death or specific defence responses should be ideally suited to uncover the mechanisms that are causally related to locally and systemically induced disease resistance. Although a generally accepted benefit of the HR cell death is its proposed ability to restrict and eventually kill the pathogen, results obtained with the potato/*Phytophthora* system indicate that host cell death may only contribute to a limitation in pathogen growth, not its total cessation. In fact, a fine balance between induction of defence responses and growth of the pathogen seems to determine resistance or susceptibility, illustrating the quantitative nature of resistance in this system. From the extensive research performed in recent years, it is apparent that different forms of cell death and mechanisms leading to HR are existing and executed in plant/pathogen interactions. But still, many intriguing questions related to HR cell death, its initiation and execution, and its relation to local and systemic disease resistance are not yet answered. Further investigations by genetic, biochemical and cytological techniques are required to dissect the mechanisms controlling the HR, and to elucidate the signal transduction events leading to local and systemic gene activation and eventually to the disease resistance phenotype.

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