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Staying alive – is cell death dispensable for plant disease resistance during the hypersensitive response?

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ABSTRACT

Probably the most known and best studied type of plant resistance to pathogenic infections is the hypersensitive response (HR), a form of localized programmed cell death associated with restriction or killing of pathogens that often leads to macroscopically visible localized tissue necrosis. It is generally assumed that cell death and resistance within the HR are physiologically and genetically linked. However, there has been considerable speculation about whether cell death is an absolute requirement for resistance conditioned by the HR. This review discusses the relation of cell death and resistance in the HR, in particular, the importance of cell death in this process. We intend to focus on the increasing amount of research evidence showing that in several plant-pathogen interactions, the two main components of the HR – resistance and cell death – can be physiologically, genetically and temporally uncoupled. In other words, HR should be considered as a combination of resistance and cell death responses, where cell death may be dispensable for plant disease resistance. The varying contribution of these two components (i.e. cell death and resistance) generates an array of defense strategies differing in efficiency. Thus, a very early and rapid defense response seems to contribute to the development of macroscopically symptomless (extreme) resistance, while a moderately early defense response results in resistance with the concomitant development of controlled and limited cell and tissue death (HR). Accordingly, a delayed and failed attempt by the host to elicit resistance responses would result in massively stressed plant tissues (e.g. "systemic HR") and a partial or almost complete loss of control over pathogen invasion. The dynamic nature of resistance responses in plants implies that resistance can be effective with or without cell death but its outcome and efficiency may depend primarily on the timing and speed of the host response.

A hallmark of eukaryotic immune systems is the ability to selectively recognize invading pathogens and to mount appropriate defense responses. Although plants lack the adaptive immunity found in mammals, they are able to defend themselves against a wide range of pathogens using sophisticated mechanisms of recognition and response (see e.g. Ref. [111]). During plant-pathogen interactions, the first line of plant defense consists primarily of the pathogen or microbe-associated molecular pattern (PAMP or MAMP) recognition system (sensitive also to non-pathogenic microbes) that has been shown to confer a so-called basal resistance (pattern-triggered immunity, PTI) [15,73]. If the MAMP-responsive system fails to recognize the (pathogenic) microbe as an invader, a second line of plant defense is induced. This is a cultivar/pathogen race specific resistance that can be regularly broken down by newly emerging pathogen races [33,43,73]. This type of plant disease resistance (effector-triggered immunity, ETI) is activated when a plant resistance gene (*R*) product recognizes - directly or indirectly – a specific pathogen gene product (effector) encoded by an avirulence (*Avr*) gene (also called gene-for-gene resistance, see e.g. in Refs. [43,47,63,96]). In case of both lines of plant defense (PTI and ETI) the final result of pathogen recognition may be the hypersensitive response (HR), a form of localized programmed cell death associated with restriction of pathogens to the infection site that often leads to the

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appearance of macroscopically visible localized tissue necrosis (see e.g. Refs. [51,52,57,83]). However, ETI is much more often associated with an HR than PTI [73,111,118]. In fact, PTI is typically a symptomless plant response, unless its development is partially arrested by e.g. inhibition of plant protein synthesis or pretreatment with a compatible bacterium or bacterial effectors, in these cases PTI can be accompanied by an HR [16,84,29].

In general it is assumed that, within the HR, cell death and resistance are physiologically and genetically linked. This would mean that cell death contributes to or is essential for resistance which makes sense when the invader is a biotrophic pathogen (i.e. it prefers live host tissues) but seems difficult to interpret in the case of necrotrophic pathogens (i.e. pathogens that kill invaded host tissues). It is likely therefore that HR-type cell death occurs as a consequence of overactive defense responses during resistance [26] as first suggested several decades ago [17,81]. In fact, through the years there has been considerable speculation about whether cell death is an absolute requirement for resistance conditioned by the HR.

The purpose of this review is to discuss the relation of cell death and resistance in the HR, in particular, the importance of cell death in the process of disease resistance. We intend to focus on the increasing amount of research evidence showing that, at least in certain plant–pathogen interactions, the two main components of the HR – resistance and cell death – can be uncoupled in a physiological, genetic or temporal sense.

1. Resistance and cell death in the HR – uncoupling in a physiological sense

As a result of recognition of pathogens by plant hosts as non-self, the HR is preceded by a series of biochemical and cellular signals, defense responses that include ion fluxes, the induction of kinase cascades, a burst of reactive oxygen species (ROS), NO, increased salicylic acid content, and a specific set of so-called pathogenesis related- and other defense-associated genes (see e.g. Refs. [14,32,34,39,63,96,108,116]). However, it is difficult to judge which subsets of these processes are responsible for resistance and cell death, respectively, during HR. Although dead cells of a plant host cannot provide nutrients and other growth substrates for biotrophic pathogens, these dying cells might release some of the above mentioned signals that are themselves antibiotics or disinfectants [87]. This is supported by previous studies on the *N* gene-mediated resistance of tobacco species to *Tobacco mosaic virus* (TMV). TMV particles could be found in live cells close to the necrotic HR-lesion, even when lesion expansion had stopped [31,122]. These results, however, also imply that pathogen localization (i.e. resistance) and cell death during a viral HR can be separated physiologically, temporally and spatially. Similar conclusions can be drawn from the early work of Farkas et al. [42] which showed that treatment of leaves with antioxidant compounds (ascorbic acid, glutathione, etc.) markedly decreased the number of TMV-elicited HR-lesions, while virus titers did not change significantly. This is in line with our finding that pretreatment of tobacco leaves with low concentrations (5–10 mM) of the ROS hydrogen peroxide (H_2O_2) suppress HR-type cell death associated with resistance to TMV or the bacterium *Pseudomonas syringae* pv. *phaseolicola* by up-regulating antioxidant enzymes, while pathogen levels do not change [59]. Furthermore, during a compatible TMV infection virus titers remain high regardless of the absence or presence of HR-type cell death generated by ROS-treatments [79].

However, it seems that virus accumulation and necrotic lesion formation may or may not correlate during *N* gene-mediated resistance to TMV, depending on many factors. For example, substantially elevated levels of glutathione following pretreatment by L-2-oxo-4-thiazolidine-carboxylic acid (OTC) significantly decreased both the number of HR-type lesions and virus content (TMV coat protein levels), while moderate increases in glutathione induced by the monoterpene (S)-carvone strongly reduced the number and size of necrotic lesions but TMV concentrations remained unchanged [58]. In addition, Hatsugai et al. [68,69] have shown that the tobacco vacuolar processing enzyme (VPE) is a protease essential for programmed cell death (PCD) initiated by vacuolar collapse (i.e. disintegration of vacuolar membranes) during TMV-induced, *N* gene-mediated HR. VPE deficiency induced by gene silencing markedly suppressed HR-type necrotization and increased TMV accumulation suggesting that certain forms of PCD (e.g. initiated by vacuolar collapse) could play a role in virus limitation [68,69]. Also, we have shown that *N* gene-containing cultivated tobacco (*Nicotiana tabacum*) grown with sufficient sulfate (+S plants) developed significantly less necrotic lesions during an HR when compared to plants grown without sulfate (–S plants) [80]. Interestingly, in +S plants TMV particle numbers did not change in comparison to -S plants, however, a reduced accumulation of TMV was evident on the level of viral mRNA that encodes the TMV coat protein, since + S plants showed a more than 50% lower accumulation of TMV CP mRNA as compared to -S plants. This implies that during incompatible TMV infections a reduction in HR-type cell/tissue death might affect certain stages of the viral replication cycle but does not necessarily influence overall pathogen levels.

Pathogen localization (i.e. resistance) and cell death during a viral HR can be physiologically separated not only in case of TMV but other viruses as well. Allopurinol [4-hydroxypyrazolo (3,4-*d*) pyrimidine], a hypoxanthine isomer applied to tobacco plant roots has been shown to strongly interfere with the HR induced by *Tobacco necrosis virus* (TNV) [95]. Remarkably, allopurinol exerts this effect via two independent metabolic pathways. A short allopurinol treatment (4–6 days) before TNV inoculation caused a delayed appearance of smaller than normal HR-type lesions, possibly due to inhibition of xanthine oxidase-mediated production of the ROS superoxide ($O_2^{\bullet -}$), On the other hand, a prolonged allopurinol pretreatment (6–8 days) resulted in a significant reduction of HR lesion numbers coupled to a several fold drop in levels of TNV, likely caused by synthesis of allopurinol and oxypurinol ribunucleosides which could interfere with TNV replication [95]. In a different study, Pogány et al. [100] demonstrated that cytokinin overproduction in transgenic tobaccos significantly suppress TNV-induced HR-type cell and tissue death, with only a partial reduction of virus titers.

It seems apparent from the studies mentioned above that in many cases of virus-elicited HR cell death is not necessarily required for resistance. For plant–fungus interactions, this was first suggested by Brown et al. [17] while studying the correlation of HR-type tissue necrosis and resistance to wheat stem rust (*Puccinia graminis* f.sp. *tritici*) in several resistant and susceptible wheat cultivars. The authors found no apparent relationship between the area of rust colonies at any given time after inoculation and the amount of hypersensitive necrotic tissue per unit area of colonized leaves suggesting that HR-type cell death of host tissues is a consequence and not the cause of resistance of wheat to infection by this rust pathogen. This finding was later functionally tested and confirmed for several other plant–pathogen interactions by Király et al. [81]. When pathogen growth in host tissues was selectively inhibited by chemical or heat treatments HR-type necrotic lesions were induced in potato tubers and wheat or bean leaf tissues infected with compatible fungal races (*Phytophthora infestans*, *P. graminis* and *Uromyces phaseoli*, respectively). It was concluded that HR-type cell- and tissue death is a result of inhibition of pathogen growth. In other words, cell- and tissue death indeed seemed to be