HYPERSENSITIVE RESPONSE – A BIOPHYSICAL PHENOMENON OF PRODUCERS

Zoobia Bashir¹, Aqeel Ahmad^{2*}, Sobiya Shafique², Tehmina Anjum², Shazia Shafique² and Waheed Akram²

¹Department of Physics, Bahauddin Zakariya University, Multan, Pakistan

²Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan

Received: March 3, 2013; Accepted: March 5, 2013

Hypersensitive response/reaction is a form of the cellular demise frequently linked alongside plant resistance against pathogen infection. Main transducers for this reaction are the intermediates of reactive oxygen and ion fluxes which are plausibly needed for hypersensitive response (Hpr Sen Rsp). An immediate and enormous energy production and its intra-cellular biochemical conduction are imperative for an Hpr Sen Rsp to be occurred. A number of studies proved that there are such diverse types of factors involved in triggering of Hpr Sen Rsp that morphologies of dead cells have become a vast topic of study. Hpr Sen Rsp could play a frolic role in plants as certain programmed cellular disintegrations in other organisms, to restrict pathogen growth. In fact, Hpr Sen Rsp can be involved in all types of tissues and most of the developmental stages.

Keywords: oxidative burst, ion flux, inducer, effector, defense pathway, pathogenesis-related proteins

Introduction

Plants have adopted a number of urbane and effectual pathways to stop conquest of their own by pathogens and to scarce illness. The most commonly said resistance against pathogens is the quick cell disintegration and/or tissue destruction to inhibit invading pathogen. This cell death quickly encircles the point of infection and makes the tissue nearly impossible for pathogen to penetrate. Thus, due to its quick action, this phenomenon is termed as Hypersensitive Reaction, or Hpr Sen Rsp [1]. Hpr Sen Rsp can be activated by a vast spectrum of pathogenic entities varying both in types and nature. However, in all cases, its success is directly related to the swiftness of the response. If the response would not be quick, then it will tend to be useless or ineffective. It is vital to note that what the plant pathologists conventionally call necrosis is not equivalent or complementary to damage of tissues in other kingdoms (by antagonism to apoptosis). Rather, necrosis factually denoted a naked eye visible phenomenon lacking superficial connotation. Cellular demise is additionally visible in progress of illness symptoms, but it occurs temporally far

afterwards conveying pathogen ingress. In this study, we will denote to Hpr Sen Rsp as progress of cellular demise as a consequence of illness confrontation, and to necrosis as progress of cellular demise across procedure of disease. This use of sentences is not aimed to vitally connote superficial differences. Hpr Sen Rsp is frequently conditioned by attendance in pathogen with a gene of avirulence (avr), which product is understood by a plant owning corresponding confrontation gene (R). A contact managing to illness is denoted compatible, and after confrontation is competent, contact is shouted incompatible. This specific pathogen credit reports for countless, but not all, plant illness defenses [2].

The simplest ideal is that avr gene transcribes a ligand that is understood by product of compatible R gene that next triggers the Hpr Sen Rsp and illness confrontation [3]. In supplement, molecules from pathogen denoted pathogen elicitors are the main reason to trigger Hpr Sen Rsp [4]. Moreover, plant receptors are additionally believed to be encompassed with all types of pathogen elicitors [5]. Consecutive to credit, biochemical and metabolic plant adaptations are well conserved amid disparate plant microbe contact [6].

*Corresponding author: Aqeel Ahmad; E-mail: aqeelahmad1@gmail.com

It is also important that the Hpr Sen Rsp is entirely a process of host transcription. In other words, plant genome is responsible for this type of cell death and there is no involvement of pathogen based losses [7]. Pathogen is detected by the plants, and plant tissues go into self-disintegration process. This phenomenon is genetically controlled and manipulated as well.

A cascade of genes is usually recognized as resistance-related genes. They are inducible in the presence of variance biotic and abiotic factors. To develop resistance in plant tissue transcription of these genes is necessarily important. Compounds which produce due to a microbe-derived signal are called phytoalexins. These are defense-related compounds and enzymes, other structural compounds, and also pathogenesis-related proteins (PR). Induction and modulation of these resistance conferring genes are not strictly associated to plant pathogen interactions. Abiotic agents and physical stresses applied to plants play equal role in the elevation of defense responses. Lee et al. [8] argued upon the induction of similar behavior of non-pathogenic bacteria as the pathogens exhibit, but their potential to boost resistance is encountered by coronatine, which delays cell death started by non-pathogenic bacteria.

Here we will address pursuing key aspects of Hpr Sen Rsp:

- Conflicts in studying Hpr Sen Rsp
- Morphological appearance of response
- Inducers of Hpr Sen Rsp
- Induction pathways.

Conflicts in studying hypersensitive response

“Hypersensitive response is too intermingled/confusing phenomenon”

Hypersensitive response is intrinsically based upon the production of lesions on plant. There are a number of factors other than Hpr Sen Rsp which cause comparable lesions on plant tissues and, hence, cause confusion in the study of Hpr Sen Rsp. Here, these factors have been categorized and discussed briefly.

Plant mutations

There are some mutated organisms which show illness lesions just resembling with the lesions of hypersensitive response [9]. Hypersensitive response cannot be interpreted in illness of plants because it gives rise to enhance defense responses in plants such as the quantity of structural biochemicals. The major and frequently proved examples of these which enhances structural building units are cellulose, callose, and lignin, etc. Enhancement of defenses has been proved up to the genetic level in terms of gene expression levels [10]. Thus, it is assumed that these mutated individuals have abnormal defense pathways due to which

they readily produce dead cell tissues without the presence of any disease causing entity. There are two main types of these mutated organisms: (1) first, initiation mutated organisms and (2) propagation mutated organisms.

Initiation mutated organisms

First category of mutated organism exhibits lesions which are manipulated in size. Major example of such mutation is “*lsd5 Arabidopsis*,” which was studied by Zheng et al. [11]. These organisms have defects in activation of cell disintegration when pathogen comes in contact.

Propagation mutated organisms

In the second class of mutated organisms, there are lesions present that, after commenced, increase in number and range to engulf available tissue area. In fact, these organisms have defective metabolic pathways which negatively contribute in Hpr Sen Rsp [12]. This hypothesis has been strengthened by another study of Hu et al. [13], in which it has been shown that the mutated defenses in maize can raise an unstoppable series of cell death. It does not even need to receive the signals of pathogen presence. These secondary lesions can be termed as pathogen-independent lesions.

Assortment of transgenes

One major factor of increased abilities of plants to start Hpr Sen Rsp is the assortment of transgenes from generation to the next. Researchers and farmers more likely want to produce and cultivate crop plants which have increased resistance against pathogens. For this purpose, it is also essential to stabilize and assort defense-related gene in downstream progenies of crop plants. As these new transgenic varieties possess extra efficient defenses against pathogens, they show an elevated number of lesions on their surfaces [14, 15]. A lot of studies prove such type of results in which activation of more efficient defense gene started self-destruction of plant tissue even on the minor pathogen derived signaling. Same has happened in case of Atkinson and Baker [16] and Mittler et al. [17]. According to these studies, over-expression of plant gene which recognizes bacteria due to its light driven proton became a cause of significant increase in Hpr Sen Rsp lesions on tobacco plants.

Share of abiotic factors

One other fact about Hpr Sen Rsp is that it is not strictly associated with the presence of a biotic entity. The main reason behind is that it is a quick and severe modification of plant metabolic pathways, and there are a number of modifiers reported for such purposes. Elicitors originated

from living organisms are one of them, but there are also a number of agents which have abiotic/synthetic origin and they can start cell disintegration. Anything which is related to the activation plant defense responses can be involved in Hpr Sen Rsp [18]. Sometimes, lesions caused by Hpr Sen Res are too resembling to disease lesions that they are difficult to differentiate [19, 20].

Phytotoxins

Phytotoxins of pathogens play a major role in production of lesions on plant surface. These lesions may also be confused with the lesions of Hpr Sen Rsp [21]. Hence, there are three factors extremely interfering in the study of Hpr Sen Rsp: (1) plant mutations, (2) assortment of transgenes, and (3) synthetic chemicals and phytotoxins of pathogens. These factors produce the similar symptoms on plant tissues as the Hpr Sen Rsp, even in the absence of Pathogen.

Morphological appearance of response

In most of the explored pathosystems, pathogen infection is nonsynchronous. This renders chronological arrangements of cytological events involved in progressive managing of Hpr Sen Rsp much difficult. A number of efforts have been made, and countless efforts have been made to delineate progress of Hpr Sen Rsp in living plant tissues. These studies have major focus upon single infections, and the most prominent success has been attained in a host pathogen system of *Uromyces vignae*, a biotrophic fungus and cowpea. Chen and Heath [22] recorded the sequential occurrence of a series of events after 15 h of inoculation when the inoculation tended to be incompatible. Series of cellular events was: (1) nucleus migration towards fungal penetration point and intense cytoplasmic streaming, (2) ending of cytoplasmic streaming, organelles of brownian similarity, nucleus condensation, granules accumulation at periphery of cytoplasm, protoplast shrinkage, and (3) cytoplasm downfall and demise of infected cell.

There is not even a single parameter to differentiate between the intermingled symptoms of Hpr Sen Rsp and necrosis. Thus, to make initial proceedings in this direction requires sufficient transfer of knowledge from animal apoptosis. It gives a sound basis in terms of rate of transpiration, shrinkage of cell and cellular organelles, intra-cellular translocation of nucleus, and accumulation of tough materials leading towards the construction of corky materials across point of infestation/infection [23]. The susceptible varieties of plants do not exhibit any of the phenomenon previously described. In some plants, Hpr Sen Rsp resulted into DNA fragmentation without producing any rhythmic pattern or ladder formation. The major example of this kind of response has been noticed by Mittler et al. [24]. He observed DNA fragmentation in tobacco plants resulted due to the attack of TMV12. In the continuation of this aspect, Ryerson and Heath [25] described the similar

type of fragmentation (oligonucleosomal fragments) produced in the cowpea plants when they were treated with an incompatible fungal species, *U. vignae*. All such types of studies give rise to the conclusion that endonucleases are produced in a plant when it undergoes an Hpr Sen Rsp [26].

Wang et al. [27] studied that the feedback of plants produced in response of AAL-toxin and recorded the formation of tough apoptotic objects in susceptible varieties. Most of the plants produce necrotic lesions when HPr Sen Rsp gets started while only few plants produce characteristic disease symptoms other than necrosis [23].

Inducers of Hpr Sen Rsp

There is a dearth of studies present on signaling pathways directly relevant to the HPr Sen Rsp. However, studies on general signaling processes give a comprehensive view of the phenomenon [3, 6]. There are three basic parts of these studies: (1) biochemicals which are detected as pathogen presence and (2) transduction of signals to the effectors (3) the way in which effectors respond.

Elicitors of pathogen

It is not compulsory for a pathogen to activate all of its complex metabolic processes along the plant surface to be detected by plant, but only a single or a combination of few of its characteristic biochemicals is sufficient to get it identified. In case of bacterial pathogens, the outside capsule generally acts as an elicitor. Bacteria need this outer capsule as an adhesive material, which allows bacteria to stick to a damp surface. Bacteria penetrate during this period by disrupting the plant surface. There are a number of structural proteins present in this capsule which give the adhesive nature to the bacteria, and the same proteins are detected by plants as an elicitor of pathogen.

Fungal pathogens are detected by their mycelial nature and from the proteinaceous excretions of their bodies. There are some investigations available that conclude upon non-proteinaceous materials of fungal origin acting as elicitors. Sometimes the cell wall proteins of fungi play a major role in detection of fungal pathogens. In the same way, nematodes are detected due to biochemicals present in their saliva and physical injuries caused by them. All of these genes for which transcription is being detected are actually the pathogenesis-related genes of microbes and contribute into disease development.

Induction pathways

The most common hypothesis in case of Hpr Sen Rsp is that it is the correspondence phenomenon between pathogen avr-gene and plant resistant conferring gene. Product of the avr-gene initiates defense pathway in plants in result of which resistance gene of plant is transcribed. A very

sound proof in this direction was given by Scofield et al. [28] and Tang et al. [29], which discovered the relation between tomato resistance gene *Pto* and *avr-Pto* of *Pseudomonas syringae* pv tomato. Another researcher Mackey et al. [30] explained the interaction between RIN4 with *P. syringae* Type III effectors and also described RPM1-mediated resistance in *Arabidopsis*.

Further studies proved that the surface contact of the host-pathogen is not sufficient for induction of such a severe type of response in plants, but a nucleic or cytoplasmic invasion is necessary [31–33]. The biochemistry of this induction is the extreme oxidative activities of pathogen on/within plant tissue. It gives rise to higher quantities of reactive oxygen intermediates [34], which ultimately needs a bulk of ion fluxes across cell membrane [35].

Now, there are different opinions of researchers upon reactive oxygen intermediates. One opinion is that H_2O_2 is the key substance, which successfully gives rise to cell or tissue disintegration [23]. Jabs et al. (1996) also reported a severe necrosis in *Arabidopsis* (*lsl1* mutated) plant raised due to superoxide generated by H_2O_2 . Because the superoxide has very weak diffusion properties, so the necrotic lesion is not quickly progressive in Hpr Sen Rsp. If the next cell or tissue is needed to be disintegrated, then its transfer would be in isoform of H_2O .

Second opinion of reactive oxygen is inclined towards NADPH oxidase of cell membrane [37]. This phenomenon is also strengthened from the animal apoptotic studies. According to Heath [38] reactive oxygen alone cannot lead a cell towards death, but the presence of salicylic acid is essential for this purpose. Thus, to support disease and infectious pathogen, it is necessary to reduce the metabolic pathway of salicylic acid. This goal is successfully achieved by using coronatine, which increase bacterial infection [11].

Third opinion is about lipid peroxides formation [20], which is due to the Ca^{2+} uptake [23]. During this process, Cl and K^+ are also directed out by H-ATPases, when cytoplasm gets alkalization [16]. Another in its support is of Levine et al. [23], in which it was concluded that an immediate influx of Ca^+ is due to the eruption of oxidative burst.

There are also some studies which negatively argue upon the oxidative burst and Hpr Sen Rsp. According to Glazener et al. [39], it is not necessary for an Hpr Sen Rsp to have a strong oxidative burst because *P. syringae* pv *syringae* cause same response on tobacco plant without adequate involvement of oxidative burst.

There are few genes which have been reported as *avr*-genes in plants. One of them is *Prf*. It is necessarily important for the proper functioning of *Pto* gene [40]. This gene brings about the transcription of protein along with leucine-zipper, which has been assertively proved in many researches [3]. It has been well known that *Pto* transcribes a serine-threonine kinase in cell cytoplasm of plants [41] and it leads towards the occurrence of Hpr Sen Rsp [42, 43]. Another investigator Zhou et al. [44] also reported a new gene of *Pti* in plants, which is responsible for Hpe Sen Rsp. Brueggeman et al. [45] discovered RPG1 gene in

maize, which is responsible for resistance against pathogens and has similarity with receptor kinase.

Effectors of cellular disintegration

It has been previously described that in plants many of the Hpr Sen Rsp-related phenomenon have been studied indirectly. Sometimes, animal systems give a more comprehensive view regarding this behavior of plants. The main cell disintegrating factor in plants is the reactive oxygen species. It can readily react with lipids, proteins and other cellular structures and can destroy their identity leading to complete destruction of cells and cellular structures [46]. Cysteine and serine proteinases are thought to be the second effectors of this category, which ensure cell disintegration [23, 26]. Cysteine proteases are also involved in inter cellular signaling. Thus, it can be concluded that Hpr Sen Rsp is not only the phenomenon of plant defense but it also serves for the alert signal to other plant cells and tissues. In this way plant activates its defense system (systemic acquired resistance) and gets protected [38].

In *Arabidopsis*, a gene (i.e., *NDR1*) encodes a membrane associated protein, but if it is mutated, then it affects another class of resistance genes viz. LZ-NB-LRR. Mutation in *NDR1* does not affect TIR-NB-LRR class of genes which also confer resistance in plants. To cause reduction in function of TIR-NB-LRR mutation is required in *EDS1* (putative lipase of L-family) gene of *Arabidopsis* [47]. In this way, *NDR1* and *EDS1* both are the control centers for plant genes encoding effectors of Hpr Sen Rsp. Del Pozo and Lam [48] concluded that expression of a protein (p35) enhances viral movement in plant cell and promote the infection caused by baculovirus.

Pandey et al. [49] enlisted the possible effectors which individually or in group cause cell death in plants. These effectors are included: *Athsr3*, *Athsr4*, and ion leakage. All these effectors are induced under the influence of pathogenic entity. The basic gene controlling the quantity of these effectors is “*hrp*.” Its constitutive expression multiplies the normal amounts of effectors. VPE has been reported as a vacuolar protein which initiates Hpr Sen Rsp in plants after the infection of virus [50, 51].

Concluding words

Intrinsically, Hpr Sen Rsp is an highly controlled and well programmed phenomenon. Due to a number of triggering factors and countless disintegration elements, there are many pathways through which a cell can die. There is a need to find some ways through which cell death due to environmental factors and cell death due to internal Hpa Sen Rsp can be efficiently differentiated. Genetic program for Hpr Sen Rsp is under progress, and still, there are some evidences for the assortment of gesture pathways existing. In the future, genetics of Hpr Snen Rsp would be a more detailed and well understood phenomenon than it is now.

References

- Mur, LAJ: Hypersensitive response in plants. In: eLS. John Wiley & Sons Ltd., Chichester (2007). <http://www.els.net> [doi: 10.1002/9780470015902.a0020103].
- Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG: Molecular genetics of plant disease resistance. *Science* 268, 661–667 (1995)
- Bent AF: Plant disease resistance genes: function meets structure. *Plant Cell* 8, 1757–1771 (1996)
- Ebel J, Cosio EG: Elicitors of plant defense responses. *Int Rev Cytol* 148, 1–36 (1994)
- Boller T, Felix G: A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* 60, 379–406 (2009)
- Hammond-Kosack KE, Jones JDG: Resistance gene-dependent plant defense responses. *Plant Cell* 8, 1773–1791 (1996)
- Dangl JL, Jones JTG: Plant pathogens and integrated defense responses to infection. *Nature* 411, 826–833 (2001)
- Lee S, Ishiga Y, Clermont K, Mysore KS: Coronatine inhibits stomatal closure and delays hypersensitive response cell death induced by nonhost bacterial pathogens. *PeerJ* 1:e34 (2013). <http://dx.doi.org/10.7717/peerj.34>
- Cui J, Bahrami AK, Pringle EG, Hernandez-Guzman G, Bender CL, Pierce NE, Ausubel FM: *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores. *Proc Natl Acad Sci USA* 102(5), 1791–1796 (2005)
- Greenberg JT, Guo A, Klessig DF, Ausubel FM: Programmed cell death in plants – a pathogen-triggered response activated coordinately with multiple defense functions. *Cell* 77, 551–563 (1994)
- Zheng XY, Spivey NW, Zeng W, Liu PP, Fu ZQ, Klessig DF, He SY, Dong X: Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. *Cell Host Microbe* 11(6), 587–596 (2012)
- Zheng G, Bachinsky DR, Stamenkovic I, Strickland DK, Brown D, Andres G, McCluskey RT: Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/a2MR, and the receptor-associated protein, (RAP). *J Histochem Cytochem* 42, 531–542 (1994)
- Hu G, Richter TE, Hulbert SH, Pryor T: Disease lesion mimicry caused by mutations in the rust *rpl* resistance gene. *Plant Cell* 8, 1367–1376 (1996)
- Dangl JL, Dietrich RA, Richberg MH: Death don't have no mercy: cell death programs in plant-microbe interactions. *Plant Cell* 8, 1793–1807 (1996)
- Mittler R, Lam E: Sacrifice in the face of foes: pathogen-induced programmed cell death in plants. *Trends Microbiol* 4, 10–15 (1996)
- Atkinson MM, Baker CJ: Role of the plasmalemma H-AT-Pase in *Pseudomonas syringae*-induced K⁺/H⁺ exchange in suspension-cultured tobacco cells. *Plant Physiol* 91, 298–303 (1989)
- Mittler R, Shulaev V, Lam E: Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell* 7, 29–42 (1995)
- Nürnberg T, Nennsteil D, Jabs T, Sacks WR, Hahlbrock K, Schell D: High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. *Cell* 78, 449–460 (1994)
- He SY, Huang HC, Collmer A: *Pseudomonas syringae* pv *syringae* Harpin: a protein that is secreted via the hrp pathway and elicits the hypersensitive response in plants. *Cell* 73, 1255–1266 (1993)
- May MJ, Hammond-Kosack KE, Jones JDG: Involvement of reactive oxygen species, glutathione metabolism, and lipid peroxidation in the Cf-gene dependent defense response of tomato cotyledons induced by race-specific elicitors of *Cladosporium fulvum*. *Plant Physiol* 110, 1367–1379 (1996)
- Zhao Y, Thilmony R, Bender CL, Schaller A, He SY, Howe GA: Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. *The Plant Journal* 36(4), 485–499 (2003)
- Chen CY, Heath MC: Cytological studies of the hypersensitive death of cowpea epidermal cells induced by basidiospores-derived infection by the cowpea rust fungus. *Can J Bot* 69, 1199–1206 (1991)
- Levine A, Pennell RI, Alvarez ME, Palmer R, Lamb CJ: Calcium-mediated apoptosis in plant hypersensitive disease resistance response. *Curr Biol* 6, 427–437 (1996)
- Mittler R, Shulaev V, Seskar M, Lam E: Inhibition of programmed cell death in tobacco plants during a pathogen-induced hypersensitive response at low oxygen pressure. *Plant Cell* 8, 1991–2001 (1996)
- Ryerson DE, Heath MC: Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. *Plant Cell* 8, 393–402 (1996)
- Mittler R, Lam E: Identification, characterization, and purification of a tobacco endonuclease activity induced upon hypersensitive response cell death. *Plant Cell* 7, 1951–1962 (1995)
- Wang H, Li J, Bostock RM, Gilchrist DG: Apoptosis: a functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. *Plant Cell* 8, 375–391 (1996)
- Scofield SR, Tobias CM, Rathjen JP, Chang JH, Lavelle DT, Micheltore RW, Staskawicz BJ: Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science* 274, 2063–2065 (1996)
- Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB: Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science* 274, 2060–2063 (1996)
- Mackey D, Holt B, Wiig A, Dangl J: RIN4 Interacts with *Pseudomonas syringae* Type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell* 108, 743–754 (2002)
- Leister RT, Ausubel FM, Katagiri F: Molecular recognition of pathogen attack occurs inside of plant cells in plant disease resistance specified by the *Arabidopsis* genes RPS2 and RPM1. *Proc Natl Acad Sci USA* 93, 15497–15502 (1996)
- Van den Ackerveken G, Marois E, Bonas U: Recognition of the bacterial protein AvrBs3 occurs inside the host plant cell. *Cell* 87, 1307–1316 (1996)
- Jones JDG: A kinase with keen eyes. *Nature* 385, 397–398 (1997)
- Yoshioka H, Asai S, Yoshioka M, Kobayashi M: Molecular mechanisms of generation for nitric oxide and reactive oxy-

- gen species, and role of the radical burst in plant immunity. *Mol Cells* 28, 321–329 (2009)
35. Watanabe N, Lam E: The hypersensitive response in plant disease resistance. In: *Multigenic and induced systemic resistance in plants*. Springer, Berlin, 83–111 (2006)
 36. Jabs T, Dietrich RA, Dangl JL: Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. *Science* 273, 1853–1856 (1996)
 37. Zurbriggen MD, Carrillo N, Hajirezaei MR: ROS signaling in the hypersensitive response. *Plant Signal Behav* 5(4), 393–396 (2010)
 38. Heath MC: Hypersensitive response-related death. *Plant Molecular Biology* 44, 321–334 (2000)
 39. Glazener JA, Orlandi EW, Baker CJ: The active oxygen response of cell suspensions to incompatible bacteria is not sufficient to cause hypersensitive cell death. *Plant Physiol* 110, 759–763 (1996)
 40. Salmerson JM, Oldroyd GED, Rommens CM, Scofield SR, Kim H-C, Lavelle SR, Dahlbeck D, Staskawicz BJ: Tomato Prf is a member of the leucine-rich repeat class of plant disease resistance and lies embedded within the Pto kinase gene cluster. *Cell* 86, 123–133 (1996)
 41. Michelmore RW, Meyers BC: Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res* 8, 1113–1130 (1998)
 42. Ren D, Yang KY, Li GJ, Liu Y, Zhang S: Activation of Ntf4, a tobacco mitogen-activated protein kinase, during plant defense response and its involvement in hypersensitive response-like cell death. *Plant Physiol* 141, 1482–1493 (2006)
 43. Liu Y, Ren D, Pike S, Pallardy S, Gassmann W, Zhang S: Chloroplast-generated reactive oxygen species are involved in hypersensitive response-like cell death mediated by a mitogen-activated protein kinase cascade. *Plant J* 51, 941–954 (2007)
 44. Zhou J, Loh YT, Bressan RA, Martin GB: The tomato gene Pti1 encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. *Cell* 83, 925–935 (1995)
 45. Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhof A: The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. *Proc Natl Acad Sci USA* 99, 9328–9333 (2002)
 46. Baker CJ, Orlandi EW: Active oxygen in plant pathogenesis. *Annu Rev Phytopathol* 33, 299–321 (1995)
 47. Falk A, Feys BJ, Frost LN, Jones JDG, Daniels MJ, Parker JE: *EDS1*, an essential component of *R* gene mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proc Natl Acad Sci USA* 96, 3292–3297 (1999)
 48. del Pozo O, Lam E: Expression of the baculovirus p35 protein in tobacco delays cell death progression and enhanced systemic movement of tobacco mosaic virus during the hypersensitive responses. *Mol Plant Microbe Interact* 16, 485–494 (2003)
 49. Pandey AK, Ger MJ, Huang HE, Yip MK, Zeng J, Feng TY: Expression of the hypersensitive response-assisting protein in *Arabidopsis* results in harpin-dependent hypersensitive cell death in response to *Erwinia carotovora*. *Plant Mol Biol* 59(5), 771–780 (2005)
 50. Hatsugai N, Kuroyanagi M, Yamada K et al.: A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science* 305, 855–858 (2004)
 51. Melotto M, Underwood W, He SY: Role of stomata in plant innate immunity and foliar bacterial diseases. *Annu Rev Phytopathol* 46, 101–122 (2008)