Basic insight in plant-pathogen interaction

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REVISIÓN

ABSTRACT

The molecular mechanisms involved in the perception, signaling and response in plant-pathogen interactions are major elements in the study of true resistance or susceptibility. As yet, there is no clear idea on what is really happening during certain molecular events. Nevertheless, this new insight offers us the possible answers to many questions on this topic. In this review, we deal with the basic and new hypotheses on the biochemical and molecular mechanisms that are activated in the plant during its interaction with the pathogen.

Keywords: resistance gene, plant-pathogen interaction, plant defense response

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RESUMEN

Fundamentos de la interacción planta-patógeno. Los mecanismos moleculares involucrados en el reconocimiento, señalización y respuesta durante interacciones planta-patógeno constituyen elementos importantes a tener en cuenta para conocer la real resistencia o susceptibilidad de las plantas hacia los patógenos. Hasta el momento, no existe una idea clara con relación a los eventos moleculares que ocurren. Sin embargo, nuevos conocimientos brindan la posible vía para responder muchas preguntas sobre esta temática. En esta revisión, focalizamos los temas fundamentales y nuevas hipótesis relacionadas con los mecanismos bioquímicos y moleculares activados por la planta durante su interacción con el patógeno.

Palabras claves: genes de resistencia, interacción planta-patógeno, respuesta defensiva en plantas

*I*ntroduction

Understanding the basis of why a certain pathogen causes disease in one host plant and not in another has long intrigued and motivated plant pathologists. Plants, in nature, are generally resistant to most pathogens. The ability of a pathogen to produce a disease in a host plant is usually the exception, not the rule. This is because plants have an innate ability to recognize potential invading pathogens and to set up successful defenses. On the other hand, successful pathogens produce diseases because they are able to evade detection or suppress host defense mechanisms, or both.

Since the beginning of the 20th century, classical breeding for disease resistance in plants has been a major method for controlling plant diseases. However, it was not until 1940 when H. H. Flor published his seminal work on the genetics of the interaction between flax and its obligate rust pathogen, *Malamspora lini*, that we gained a substantial understanding of the genetic interactions controlling disease resistance in plants. Flor's work was novel, insightful, and under - appreciated at the time as he concurrently studied the inheritance of resistance in the host and the virulence in the pathogen. This work resulted in the formulation of the gene-for-gene hypothesis.

In its most simple form, the gene-for-gene hypothesis states that plants contain single dominant resistance R genes that specifically recognize pathogens that contain complementary avirulence genes. Avirulence genes can be defined as genes in the pathogen that encode a protein product that is conditionally recognized directly or indirectly only by those plants that contain the complementary R gene [1, 2].

Specific recognition results in the induction of defense gene expression and the inhibition of pathogen growth. However, if the host plant does not contain the R gene, the pathogen can still produce the disease on that plant although it contains the avirulence gene. It was the work of H. H. Flor that set the stage for the subsequent molecular cloning of pathogen avirulence genes and plant R genes. Moreover, the lack of evidence for the direct avr-R interactions stimulated scientists to propose new models for avr perception by resistant plants. One interesting model is that the R proteins confer recognition of avr factors only when these factors are complexed with their host virulence targets. This molecular mechanism has been recently named "guard model" (Figure1).

R Genes

To survive, plants must defend themselves from numerous pathogens. Some defenses are constitutive, such as various pre-formed anti-microbial compounds, whereas others are activated by pathogen recognition. The recognition process includes the product of a dominant or semi-dominant resistance R gene present in the plant and the corresponding dominant avirulence (Avr) factor encoded by or derived from the pathogen. The recognition of the Avr factor by the host plant starts one or more signal transduction pathways that activate several of the plant's defenses, thus compromisingthe ability of the pathogen to colonize the plant [3, 4].

To date, the direct interaction between an R protein and an Avr factor has been demonstrated only for the tomato Pto and the *Pseudomonas syringae* AvrPto proteins. Based on observations, many AVR proteins appear to have a role in pathogen virulence; the 'guard model' was recently proposed for the R gene function. This model predicts that AVR proteins are effectors interacting with particular target proteins in the plant to manipulate host processes in favour of the pathogen. In this scenario, R proteins are guardians

1. Ali G S, Reddy A S N. Inhibition of fungal and bacterial plant pathogens by synthetic peptides: in vitro growth inhibition, interaction between peptides and inhibition of disease progression. Molecular Plant-Microbe Interaction 2000;13:847-59.

2. Beers E P, McDowell J M. Regulation and execution of programmed cell death in response to pathogens, stress and developmental cues. Current Opinion in Plant Biology 2001;4:561-7.

3. Bent A F. Plant mitogen-activated protein kinase cascades: ?negative regulatory roles turn out positive. Proceeding of the National Academy of Sciences 2001;98: 784-6.

4. Blondelle S E, Lohner K. Combinatorial libraries: a tool to design anti-microbial and antifungal peptide analogues having lytic specificities for structure-activity relationship studies. Biopolymers 2000; 55:74-87.

that recognise the complexes formed by the target proteins and the Avr gene-encoded modulators. This recognition consequently initiates the plant defense response. AVR proteins are therefore important tools allowing the identification and characterization of these crucial protein complexes and the ensuing processes [5, 4, 6].

An array of *R* genes that provide protection against viruses, bacteria, fungi, and oomycetes has been cloned from both monocots and dicots. Many contain a nucleotide-binding site (NBS). It is often located closer to the N terminus of the R protein and is either a leucine zipper or a TIR domain, which is similar to the intracellular C-terminal signaling domain of the integral membrane of the *Drosophila* Toll protein and the mammalian interleukin-1 receptor. Both the Toll protein and the interleukin-1 receptor are involved in signaling pathways that lead to the activation of the defense responses to pathogens in *Drosophila* and mammals, respectively. Two R proteins have also been shown to contain a serine/threonine kinase domain [7].

In addition to these motifs, all but two R proteins involved in gene-for-gene interactions have a leucine-rich repeat (LRR) region. This domain consists of imperfect repeats of nine to >40 units, each of which is of about 25 amino acids long. In the central region of each repeat is a β strand/β turn structure, which is hypervariable and has the consensus sequence XX(L)X(L)XXXX, where L corresponds to the conserved leucines (or other aliphatic amino acids) and X denotes the flanking hypervariable aminoacids. This structure in the different repeats is thought to fit together to form a solvent-exposed parallel ß sheet. Such a solvent-exposed, hypervariable surface could facilitate the interaction of the R protein with its cognate Avr factor (ligand) and could provide different recognition specificities for altered Avr factors [8].

Monogenic resistance is not durable in most cases due to the high mutation rate of many plant pathogens. Mutants, which have changed from avirulent to virulent, will have a selective advantage as their host range has been broadened and they will therefore multiply more efficiently. Plants, however, have a wide range of recognitional specificities and susceptibility is the exception, suggesting that the co-evolution between the host and the pathogen frequently occurs in nature. During evolution, new resistance specificities must have been generated to cope with the newly evolved virulent strains of pathogens [9].

A clue to the mechanisms by which sequence diversification in plant resistance genes is promoted, comes from their genomic organization. Some R genes, such as *Hm1* and *RPM1* [10, 11], are only present as a single copy gene, and are absent in susceptible plants. Most *R* genes, however, are organized in complex loci that contain an array of homologous genes. Examples of *R* genes that are present in clusters include *Rp1, Rpp5, Xa21, Pto, Dm3, I2, N, M* and the *Cf* genes. The tandem array organization of homologous sequences probably facilitates inter and intragenic recombination events, unequal crossing-over and gene duplication [12, 13].

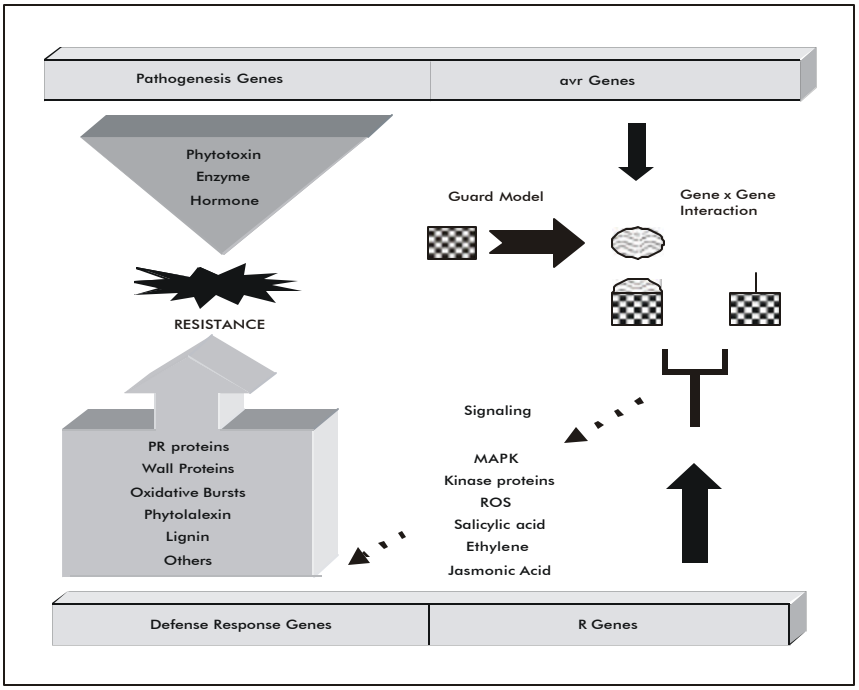

Figure 1. General mechanism during plant-pathogen interaction.

Signaling

Plants have integrated signaling networks that mediate the perception of and responses to the hormones, nutrients, and environmental cues and stresses that govern plant growth and development. The current knowledge of plant signal transduction pathways has come from the identification of the sensors and receptors that perceive the signal, and of the transcription factors and target genes that coordinate the response [14].

Protein kinases play a central role in signaling during pathogen recognition and the subsequent activation of plant defense mechanisms. Members of different kinase subfamilies, such as calcium-dependent protein kinases and MAP kinases, are involved. The future challenge is to understand how these kinases work, which cellular responses they mediate, and how they fit into the bigger picture of defense signaling [15].

Mitogen-activated protein kinase (MAPK) cascades have emerged as a universal signal transduction mechanism that connects diverse receptors/sensors to cellular and nuclear responses in eukaryotes. New findings have revealed the complexity and redundancy of the signaling components, the antagonistic nature of distinct pathways, and the use of both positive and negative regulatory mechanisms, components that link sensors / receptors to target genes and other cellular responses [16, 17].

In recent years, it has become apparent that MAPK cascades play some of the most essential roles in plant signal transduction pathways from cell division to cell death. MAPK cascades are evolutionarily conserved signaling modules with essential regulatory functions in eukaryotes, including yeasts, worms, flies, frogs, mammals and plants. The recent enthusiasm

5. Cardinale F, Jonak C, Ligterink W, Niehaus K, Boller T, Hirt H. Differential activation of four specific MAPK pathways by distinct elicitors. Journal of Biological Chemistry 2000;275:36734-40.

6. Conrath U, Pieterse C M J, Mauch-Mani B. Priming in plant-pathogen interactions. Trends in Plant Science 2002;7:210-6.

7. Dangl J L, Jones J D G. Plant pathogens and integrated defense responses to infection. Nature 2001;411:826-33.

8. De Wit P J G M. On guard. Nature 2002; 416:80-3.

9. Droillard M J, Thibivilliers S, Cazale A C, Barbier-Brygoo H, Lauriere C. Protein kinases induced by osmotic stresses and elicitor molecules in tobacco cell suspensions: two crossroad MAP kinases and one osmoregulation-specific protein kinase. FEBS Letter 2000;474:217-22.

10. Flor HH. Current status of the gene for gene concept. Annu Rev Phytopathol 1971;9:275-96.

11. Erik A van der Biezen. Quest for antimicrobial genes to engineer disease resistant Crops. Trends in Plant Science 2001;6:89-91.

12. Gao AG, Hakimi SM, Mittanck CA, Wu Y, Woerner BM, Stark DM, Shah DM, Liang J, Rommens CM. Fungal pathogen protection in potato by expression of a plant defensin peptide Nature Biotechnology 2000;18:1307-10.

13. Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A, Innes RW, Dangl JL. Structure of the Arabidopsis RPM1 gene enabling dual specificity disease resistance. Science 1995;269:843-6.

14. Hammond-Kosack KE, Jones JDG. Resistance gene dependent plant defense responses. Plant Cell 1996;8:1773-91.

for plant MAPK cascades is backed by numerous studies showing that plant MAPKs are activated by hormones, abiotic stresses, pathogens and pathogen-derived elicitors, and they are also activated at specific stages during the cell cycle [18].

MAPK activation by pathogens, pathogen-derived elicitors and defense related second messengers is complicated. Two tobacco MAPKs, SIPK and WIPK (wound-inducible protein kinase), are activated by various pathogen-related signals through both race-specific and non-race-specific elicitation mechanisms [19, 20]. As both of these MAPKs are also activated by diverse abiotic stresses, pathogen defense signaling is a part of the integrated stress-signaling network in plants. SIPK and WIPK may provide convergence points for many distinct signaling cascades in plant defense and stress responses [21, 22, 23].

Orthologs of SIPK and WIPK in *Arabidopsis* (AtMPK6 and AtMPK3, respectively) and alfalfa (SIMK and SAMK, stress-activated MAPK, respectively) are also activated by both biotic and abiotic stresses, further supporting this idea [24]. The question then is how can these MAPKs mediate the induction of stimulus specific defense responses. Recent studies suggest that different stimuli activate these MAPKs to different levels and with different kinetics. Thus, these MAPKs may participate in distinct signaling complexes [25, 26].

The characterization of the loss of function mutants of MAPK signaling components would undoubtedly foster the understanding of their functions in whole plants; however, it appears to be difficult to obtain such mutants. It is likely that some MAPK signaling components are essential for cell growth and development. It is also possible that many single-knockout mutants lack readily detectable phenotypes as a result of functional redundancy [27]. Because of the transient nature of MAPK activation in many responses, the indirect and long lasting phenotypes of MAPK signaling mutants could be misleading or confusing. Mutant phenotypes may not always represent the primary targets of the mutated signaling pathway [28].

Curiously, all of the MAPK signaling mutants isolated so far *ctr1*, *edr1*, *mpk4* and *mkp1* indicate only a negative regulatory role of MAPK cascades in *Arabidopsis*. Therefore, it is essential to combine various assay techniques to identify the true functions of MAPK signaling cascades in plants. Besides the core MAPK cascade components and scaffold/anchoring proteins, the role of negative regulators such as various protein phosphatases and the identification of upstream signals, receptors/sensors, adaptor proteins, transcription factors, MAPK substrates and target genes will help us piece together the biological functions of a large number of plant gene products that are involved in the essential signaling network of protein phosphorylation [6].

*R*esponses

After an *R* gene-mediated recognition of the pathogen attack, various defense responses are often activated. Localized activation of programmed cell death (PCD) in response to microbial attack is thought to act as a defense mechanism that inhibits the growth of pathogens within infected plant tissues. By killing cells at and around the site of infection this process generates a physical barrier composed of dead plant cells and limits the availability of nutrients to the pathogen because of the rapid dehydration that accompanies tissue death [29, 19].

Also termed the hypersensitive response (HR), this cell death response is accompanied by the induction of numerous anti-microbial defenses. Among these are pathogenesis-related (PR) proteins, such as glucanases and chitinases, and phytoalexins. It is believed that the coordinated activation of PCD and defense mechanisms at the site of pathogen entry provides the plant with an efficient defense response that prevents pathogen proliferation and its possible consequence: systemic infection [30].

PCD that occurs during the HR is accompanied by an increase in the production of reactive oxygen intermediates (ROI). Recent studies indicated that ROI in the form of H_2O_2 and O_2 may be the key mediators of PCD during the HR. ROI were also involved as signal transduction agents that lead to the induction of other defense mechanisms such as PR proteins, salicylic acid (SA), biosynthesis, and systemic acquired resistance [31].

Anti-microbial peptides are ancient mediators of the innate defenses of all species of life. These small lytic peptides are being used to genetically engineer disease-resistant crop plants. It is anticipated that certain (combinations of) potent anti-microbial peptides will provide relevant agronomical levels of disease control and should contribute to more sustainable agricultural practices [32].

Recently, two groups have published papers on the ectopic expression of anti-microbial genes that confer resistance to bacterial and fungal phytopathogens in transgenic potato. Whereas Caius Rommens' group directly used the natural alfalfa defensin gene alfAFP [33], Santosh Misra and co-workers designed the synthetic gene MsrA1 [34]. The Misra group constructed this chimera by fusing the cecropin and melittin genes, derived from a giant silk-moth and a bee, respectively. Defensins, cecropins and melittins are a part of many (>500) small anti-microbial peptides (26-50 amino acid residues) that are ancient mediators of the innate defenses of all life forms [35]. The antifungal peptide alfAFP [33] and other plant, mammalian and insect defensins belong to the class of anti-microbial peptides characterized by β-sheet structures [35]. These complex folded molecules contain four, six or eight invariant cysteine residues that form several intramolecular disulfide bonds. The 5.6 kDa alfAFP peptide was, like most plant defensins, isolated from seeds where it contributes to the protection of germlings against harmful microorganisms [33] (analogous to the common fungicide coating of crop seeds). Defensins display lytic activity through binding and disruption of microbial plasma membranes. The plant defensin DmAMP1, for example, specifically binds to fungal microsomal fractions, and yeast mutants resistant to DmAMP1 show reduced binding affinity [36].

In addition, most promising anti-microbial peptides exhibit agronomic relevant activities against a broad range of pathogenic microorganisms, or alternatively, target specific pathogens that are difficult

15. Hammond-Kosack KE, Jones JDG. Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1997;48: 575-607.

16. Hancock REW, Diamond D. The role of cationic anti-microbial peptides in innate host defenses. Trends in Microbiology 2000;8:402-10.

17. Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K. Various ?abiotic stresses rapidly activates Arabidopsis MAP kinases AtMPK4 and AtMPK6. Plant Journal 2000;24:655-65.

18. Johal GS, Briggs SP. Reductase activity encoded by the HM1 disease resistance gene in maize. Science 1992;258:985-7.

19. Jones JDG. Putting knowledge of plant disease resistance genes to work. Current Opinion in Plant Biology 2001;4:281-7.

20.Kovtun Y, Chiu WL, Tena G, Sheen J. Functional analysis of oxidative stress activated mitogen-activated protein kinase cascade in plants. Proceeding of the National Academy of Sciences 2000;97: 2940-5.

21.Ligterink W, Hirt H. Mitogen-activated protein (MAP) kinase pathways in plants: versatile signaling tools. Int Rev Cytol 2001;201:209-75.

22.López-García B, Gonzalez-CandelasL, Pérez-Paya E, Marcos JF. Identification and characterization of a hexapeptide with activity against phytopathogenic fungi that cause postharvest decay in fruits. Molecular Plant-Microbe Interaction 2000;13:837-46.

23.MAPK Group (Kazuya Ichimura et al.) Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends in Plant Science 2002; 7: 301-308.

24.Michelmore RW, Meyers BC. Clusters of resistance genes in plants evolve by divergent selection and a birth-and death process. Genome Research 1998;8:1113-30.

25.Mikolajczyk M, Awotunde O, Muszynska G, Klessig D, Dobrowolska G. Osmotic stress induces rapid activation of a salicylic acid-induced protein kinase and a homolog of protein kinase ASK1 in tobacco cells. Plant Cell 2000;12:165-78.

26.Mitsuhara I, Matsufuru H, Ohshima M, Kaku H, Nakajima Y, Murai N, Natori S, Ohashi Y. Induced expression of sarcotoxin IA enhanced host resistance against both bacterial and fungal pathogens in transgenic tobacco. Molecular Plant-Microbe Interaction 2000;13:860-8.

27.Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 2002;7:405-10.

28. Muskett PR, Kahn K, Austin MJ, Moisan LJ, Sadanandom A, Shirasu K, Jones JDG, Parker JE. Arabidopsis RAR1 Exerts Rate-Limiting Control of R Gene-Mediated Defenses against Multiple Pathogens. The Plant Cell 2002;14:979-992.

29. Nuhse TS, Peck SC, Hirt H, Boller T. Microbial elicitors induces activation and dual phosphorylation of the Arabidopsis thaliana MAPK 6. Journal of Biological Chemistry 2000;275:7521-6.

30. Nürnberger T, Scheel D. Signal transmission in the plant immune response. Trends in Plant Science 2001;6:372-9.

to control by conventional means. Ongoing clinical trials indicate that anti-microbial peptides can be used as an alternative source for human therapeutic antibiotics [35]. For exploitation in agriculture, the future challenge is to find (combinations of) potent anti-microbial peptides that target relevant pathogens [37, 38]. The efficacy in plants of a new class of synthetic anti-microbial peptides is already under intense scrutiny [39, 40], and synthetic combinatorial libraries are being developed to design novel biologically active peptides [41]. The small genes (<200 base pairs) encoding anti-microbial peptides facilitate the stacking of multiple activities on single transgenes. Transgenically produced anti-microbial peptides should be directed to the relevant plant tissues and cell types, and peptide stability and proper folding have to be considered. The further discovery of anti-microbial peptides with relevant agronomic performance is keenly anticipated and should contribute to more sustainable agricultural practices.

Approaches and perspective

Today's plants are products of eons of evolution from primal living organisms in response to abiotic and biotic environmental changes.

The interactions between plants and pathogens are specific, complex and dynamic. The identification of resistant genes in the germplasm of wild species of field crops and their subsequent introgression into commercial cultivars has been the main approach of many plant breeders.

Several strategies for the identification, characterization and functional analysis of plant genes involved in the triggering, signaling and response to biotic factors have been recently envisaged. Suppression subtractive hybridization and cDNA-AFLP are used to generate new data in plant-pathogen interaction. On the other hand, the use of vectors for virus induced gene silencing (VIGS) and microarray technology to seek gene functions, are strong tools in plant science.

Finally, the novel genes involved in the recognition, signaling and response to pathogen invasion will provide information for the search of novel strategies to develop durable resistance in plants, either through marker assisted selection or biotechnology approaches within the genetic breeding program. Also, results should contribute to fundamental knowledge in the wider field of plant pathology by providing a deeper understanding of mechanisms involved in the interaction.

31.Osusky M, Zhou G, Osuska L, Hancock RE, Kay WW, Misra S. Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. Nature Biotechnology 2000;18:1162-6.

32. Romeis T, Piedras P, Zhang S, Klessig DF, Hirt H, Jones JDG. Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. Plant Cell 1999;11:273-87.

33. Romeis T. Protein kinases in the plant defense response. Current Opinion in Plant Biology 2001;4:407-14.

34. Samuel MA, Miles GP, Ellis BE. Ozone treatment rapidly activates MAP kinase signaling in plants. Plant Journal 2000;22:367-76.

35. Schmelzer E. Cell polarization, a crucial process in fungal defense. Trends in Plant Science 2002;7:411-5.

36. Sharma A, Sharma R, Imamura M, Yamakawa M, Machii H. Transgenic expression of cecropin B, an antibacterial peptide from Bombyx mori, confers enhanced resistance to bacterial leaf blight in rice. FEBS Letter 2000;484:7-11.

37. Takken FLW, Joosten MHAJ. Plant resistance genes: their structure, function and evolution. European Journal of Plant Pathology 2000;106:699-713.

38. Tena G, Asai T, Chiu WL, Sheen J. Plant mitogen-activated protein kinase signaling cascades. Current Opinion in Plant Biology 2001;4:392-400.

39. Thevissen K, Osborn RW, Acland DP, Broekaert WF. Specific binding sites for an antifungal plant defensin from Dahlia (Dahlia merckii) on fungal cells are required for antifungal activity. Molecular Plant-Microbe Interaction 2000;13:54-61.

40. Van der Hoorn RA, De Wit PJ, Joosten MH. Balancing selection favors guarding resistance proteins. Trends Plant Scien 2002; 7:67-71.

41. Zhang S, Klessig DF. Resistance gene N-mediated de novo synthesis and activation of a tobacco mitogen-activated protein kinase by tobacco mosaic virus infection. Proceeding of the National Academy of Sciences 1998;95:7433-8.

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