
ELICITATION OF PLANTS

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ABSTRACT

Elicitors are compounds stimulating any type of plant defense. This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors and compounds released from plants by the action of the pathogen (endogenous elicitors). Also elicitors could be used as enhancers of plant-secondary-metabolite synthesis and could play an important role in biosynthetic pathways to enhanced production of commercially important compounds. The increased production, through elicitation, of the secondary metabolites from plant cell cultures has opened up a new area of research, which could have important economical benefits for bio industry.

Introduction

Plants exhibit a wide array of defense strategies against pathogen attack. The resistance against pathogens is performed by both preexisting (constitutive) and induced defense systems. Inducible defense responses are triggered following recognition of a range of chemical factors termed 'elicitors' (1). Originally the term elicitor was used for molecules capable of inducing the production of phytoalexins, but it is now commonly used for compounds stimulating any type of plant defense (2, 3, 4). They might be both of biotic or abiotic origin (see below).

The first biotic elicitors were described in the early 1970 (5). Since then, numerous publications have accumulated evidence for pathogen-derived compounds that induce defense responses in intact plants or plant cell cultures. They comprise distinct compounds among either oligosaccharides or lipo- and glycoproteins. Such biotic elicitors often originate from the pathogen (exogenous elicitors) but in some cases are liberated from the attacked plant by the action of enzymes of the pathogen (endogenous elicitors) (6, 2). A prominent early example is the work of Albersheim et

al., (7) who first isolated oligosaccharides that activate a variety of plant defense genes.

Elicitors are usually capable to induce various modes of plant defense including the production of ROS (reactive oxygen species), the hypersensitive response and the production of phytoalexins, i.e. antimicrobial secondary compounds (8, 2, 3, 4). The induction of phytoalexin biosynthesis has gained special importance in biotechnological approaches to improve the production of secondary metabolites. Many of these compounds are of high value as therapeutics or otherwise biologically active agents. An example is the bioproduction of taxol, a diterpenoid found in the bark of *Taxus* trees. This compound is approved by the Food and Drug administration (FDA) for the treatment of ovarian and breast cancer. There is a high demand for taxol, but its synthetic production is extremely costly, so biosynthesis in *Taxus* spp. cell cultures has become the focus of extensive research (9). In general, plant cell cultures are rich sources of valuable pharmaceuticals and other biologically active compounds (10). However, relatively few cultivars and derived cell cultures synthesize secondary metabolites over extended

TABLE 1

Elicitors of plants

Elicitors						Reported effects on		
Physical Elicitors	Injury					P		
	Abiotic		Metal ions (lanthanum, europium, calcium, silver, cadmium), oxalate			Pc		
Chemical Elicitors	Biotic	Complex Composition	Yeast cell wall, Mycelia cell wall, Fungal spores			Pc ,F		
		Defined Composition	Carbohydrates	Polysaccharides	Alginate LBG Pectin Chitosan Guar Gum	Pc, F, B F Pc, F Pc Pc		
					Oligosaccharides	Mannuronate Guluronate Mannan Galacturonides	F F F Pc	
						Peptides	Glutathione	Pc
						Proteics	Proteins	Cellulase, Elic- itins, Oligandrin
			Lipids		Lipopolysaccharides	Pc		
			Glycoproteins		Not characterized	Pc		
			Volatiles		C ₆ -C ₁₀	Pc		

Abbreviations: P, plants; Pc, plant cell culture; B, bacterial cell culture; F, fungal cell culture.

periods and in amounts suitable for commercial exploitation. Elicitation studies have shown promise in increasing yields and cutting production costs (11, 12, 13, 14). Among the various examples are biotechnological approaches for the production of isoflavonoid phytoalexins (15), sesquiterpenoid phytoalexins (16), coumarins (17) and podophyllotoxin (18). Likewise, enzymes of secondary metabolism or detoxification can be obtained from elicited cell cultures, e.g. phenylalanine ammonia lyase (PAL) (19) and glutathione S-transferase (GST) (20). Elicitors have also been used as research tools to understand elements of the complex pathways and signaling interactions in plant secondary metabolism.

Classification of elicitors

According Radman et al., (21) elicitors are classified as physical or chemical, biotic or

abiotic and complex or defined depending on their origin and molecular structure (Table 1).

• Biotic elicitors

Biotic elicitors are molecules of either pathogen or host origin that can induce defense responses (such as phytoalexin accumulation or hypersensitive response) in plant tissue.

Often complex biological preparations have been used as elicitors, where the molecular structure of the active ingredients is unknown. Examples of such elicitors are yeast extract and microbial cell-wall preparations.

In recent years, the exact molecular structure of an increasing number of elicitors has been elucidated, including various polysaccharides, oligosaccharides, proteins, glycoproteins, and fatty acids (22, 23).

• Proteins and glycoproteins as elicitors

Proteins and enzymes are another class of

elicitors that trigger defense reactions e.g. in plant cell cultures. Cellulase causes rapid accumulation of phytoalexins in *N. tabacum* cell cultures, an increase in the production of capsidol and debneyol and production of two previously unknown phytoalexins (16).

The pathogenic fungus *Phytophthora drechleri* secretes elicitors (protein elicitors) that induce necrosis in tobacco leaves: holoproteins (proteins involved in the phototropic signal perception) causes concentration-dependent leaf necrosis. At least three isoforms of this elicitor are produced by *P. drechleri* (24). Huet and Pernollet, (25) described eight different elicitors from fungi *P. cryptogea*, *P. cinnamomi*, *P. capsici*, *P. megasperma* and *P. drechleri*. Within this group two classes of elicitors were identified, acidic molecule (α) and basic molecule (β). Differences were observed in the elicitation of necrosis between the different elicitors, their basic and acid forms and the optimum concentration required for the necrosis.

Protein elicitors have been used to elucidate the role of ion channels in plant cell membranes in the signal transfer triggered by external stimuli.

Pectolyase, a cell-wall-degrading enzyme, is a potent inducer of membrane depolarization (chloride effluxes) in cell membrane of *N. tabacum* (26). Another protein, cryptogein, secreted by *Phytophthora cryptogea*, elicits depolarization of the membrane. Both proteins generate similar depolarizations of the plant cell membrane, but the induced chloride effluxes are of different intensities.

A recently added protein to the family of elicitors is the elicitor-like molecule 'oligandrin'. Oligandrin is a low-molecular-mass peptide secreted by the fungus *Pythium oligandrum* and induces resistance against *Phytophthora parasitica* in tomato (*Lycopersicon esculentum*) plants (27). The remarkable characteristic of this protein is that it does not induce hypersensitive reac-

tion and thus it can be considered as a potential tool in biocontrol. Oligandrin elicits systematic resistance to *Fusarium* crown and root rot in tomato plants (28).

Glycoproteins have also been shown to elicit phytoalexins in plant cell cultures. The native conformation of a glycoprotein extracted from cell suspensions of the fungus *Ceratocystis fimbriata* causes an increase in coumarin concentration in *Plantanus acerifolia* cell cultures (29). Glycoprotein preparations from bakers' yeast elicit the formation of the benzophenanthridine alkaloids in cultured cells of *Eschscholzia californica* (30, 31).

• Oligosaccharides as elicitors

From early studies carbohydrates have been implicated in the overproduction of secondary metabolites in plant cell cultures. Albersheim et al., (7) first isolated oligosaccharides that activate a variety of plant defense genes. Sharp et al., (32) investigated the role of specific carbohydrate elicitors on phytoalexin production in soybean cell cultures where they identified eight distinct oligosaccharides after partial acid hydrolysis of mycelial walls of *Phytophthora gasperma*. Characterization of these oligosaccharides revealed that all but one elicitor-inactive oligosaccharide had 3,6-linked glucopyranose residues with the eight having β -linked glucopyranose residues. The fact that the active and the inactive carbohydrate elicitors show only small structural differences suggests a highly specific recognition of the carbohydrate structure by the elicitor receptor(s). The authors point out that the active elicitor could be one of over 150 elicitor-inactive oligosaccharides found in the mycelial extract.

The signal transfer triggered by carbohydrate elicitors has been studied with regard to calcium influx, pH shifts and production of H_2O_2 in tobacco cell cultures (33).

Oligogalacturonides derived from plant cell walls were used to induce defense responses in tobacco plants. Oligogalacturoni-

TABLE 2

Carbohydrate elicitors and metabolites in plant cell cultures (after Radman et al., (21))

Elicitor	Culture	Metabolites/products
β -Linked glucopyranosyl	<i>Glycine max</i>	Phytoalexins
α -1,4-Oligogalacturonide	<i>Glycine max</i>	Phytoalexins
Chitosan	<i>N. tabacum</i> , <i>E. californica</i>	Phytoalexins
Hepta- β -glucoside	<i>Glycine max</i>	Phytoalexins
Pectic oligomers	<i>Citrus limon</i>	Phytoalexins
β -1,6-1,3-Glucans	<i>Glycine max</i>	Isoflavonoids
β -Glucan	<i>Glycine max</i>	H ₂ O ₂
β -D-Glucans	<i>N. tabacum</i>	Disease resistance
Chitin	<i>Papaver somniferum</i>	Sanguinarine
Chitosan	<i>Polygonum tinctorium</i>	Iridubin
Chitosan	<i>Lupinus albus</i>	Isoflavonoids
Chito-oligosaccharides	<i>Juniperus chinensis</i>	Podophyllotoxin
Oligogalacturonides	<i>N. tabacum</i>	H ₂ O ₂
Chitosan	<i>Lupinus albus</i>	Genistein
Chitin and chitosan oligosaccharides	<i>Taxus canadensis</i>	Taxol
Chitin, alginate, pectin, guar gum		
rhamsan, xanthan	<i>Morinda citrifolia</i>	Anthraquinones
Laminarin	<i>N. tabacum</i>	H ₂ O ₂
Mannan	<i>Hypericum perforatum</i>	Hypercins
<i>N</i> -Acetylchitohexaose	<i>Taxus canadensis</i>	Taxol
Chitosan	<i>Rheum palmatum</i>	Anthracene
<i>N</i> -Acetylchito-oligosaccharide	<i>Avena sativa</i>	Anthranilate

des triggered an increase in Ca²⁺ uptake. Ca²⁺ influx is important in maintaining H₂O₂ levels and a significant increase in the extracellular pH compared with the control.

Chitosan is a mostly acetylated β -1,4-linked D-glucosamine polymer, which acts as a structural component of the cell wall of several plant fungal pathogens, such as *Fusarium sp.* The effect of chitosan on the membrane permeability and secondary-metabolite production was investigated in *Nicotiana tabacum* and *Eschscholzia californica* (34). Chitosan did not affect membrane permeability, but the plant cells were shown to elicit phytoalexins. Chitosan-derived oligosaccharides were used in over-production (six-fold increase) of taxol in cultures of *Taxus canadensis* (35) **Table 2**, shows a list of carbohydrate elicitors of metabolites in plants.

• Plant hormones as elicitors

Salicylic acid (SA) and jasmonic acid (JA) are seen as the key signals for defense gene

expression (36). It was generally thought that SA regulates resistance to fungal, bacterial, and viral pathogens (37, 38), whereas JA induces the production of various proteins via the octadecanoid pathway that provides plants with resistance against insects (39, 40). However, this distinction between the two pathways is not that clear and pathogens and arthropods may sometimes trigger both (41, 36, 42). SA and JA, as well as synthetic mimics, can be applied exogenously to plants to induce the same metabolic changes that lead to resistance as induced by pathogens and insects (43, 44). The common elicitors like JA and SA and knowledge about the biochemical pathways that they induce makes them useful in the studying of plant elicitation processes, for example various genes that are induced by JA and related compounds have been identified (36, 45).

• Abiotic elicitors

The use of abiotic elicitors in plant cell

cultures has received less attention compared with the biotic elicitors (21). Some heavy metal salts are often found to trigger phytoalexin production. For example, AgNO₃ or CdCl₂ elicited overproduction of two tropane alkaloids, scopolamine and hyoscyamine, by hairy root cultures of *Brugmansia candida* (angel's trumpet) (46). Wu et al. (47) investigated the effects of the rare-earth metal lanthanum on production of taxol in cell culture of *Taxus sp.* Significant enhancement (280%) of taxol yield was detected in the supplemented cultures without notable changes in the biomass. Some synthetic chemicals, though not themselves active as elicitors, proved to influence the signal transfer triggered by pathogens. For example, Siegrist et al. (48) found that benzol [1,2,3]-thiadiazole-7-carbothioic acid S-methyl ester (BTH) acted as a 'conditioner' in the elicitation of systemic acquired resistance in parsley (*Petroselinum crispum*) cells treated with a crude elicitor from the cell wall of *Phytophthora sojae*. Under an optimal concentration of BTH, parsley cells produced enhanced levels of coumarin.

Plant pathogen resistance and elicitor recognition in plants

Plants are built of immobile cells embedded in rigid cell walls. Migration of specialized defensive cells to the sites of microbial invasion, as known for animals, is therefore impossible. Instead, plant defense systems have evolved in such a way that each cell has acquired the capability to respond to attempted infection and to build up a defense response. As a consequence, plants are resistant to most potential pathogens in their environment.

In the early infection stage of plant disease, recognition of the pathogen is an important event for the resistant plant. Elicitor recognition by the plant is assumed to be mediated by specific receptors in the plant cell, been localized either on the cell surface for a number of fungal elicitors

(49) or within the cell for certain bacterial elicitors (50, 51, 52), which initiate signaling processes that activate plant defenses.

The specificity of plant responses to pathogens can be classified into two broad categories. Non-host resistance (general, non-specific or basic resistance) is a response to all races of a particular pathogen, and occurs in all cultivars of a host plant species. In contrast, host-specific resistance (race-specific resistance) is dependent upon the presence of a particular pathogen race, a particular host plant cultivar, or both.

Plant pathogen resistance which occurs at the cultivar or species level is believed to be maintained by recognition of race-specific or race-nonspecific (general) elicitors, respectively (49). While general elicitors are able to trigger defense in host and non-host plants, race-specific elicitors induce defense responses leading to disease resistance only in specific cultivars (8). **Table 3**, depicts some general and race-specific elicitors which belong to a wide range of different classes of compounds.

Fungal elicitors of the general type appear to be constitutively present in the cell wall, for example as structural components. In contrast, harpins, a class of bacterial elicitors of the hypersensitive response in non-host plants as well as in resistant genotypes of some host plants, are produced and secreted only upon contact or under experimental conditions mimicking the apoplastic space of plants (70, 71). Race-specific elicitors are very often synthesized and secreted only upon infection of the host plant.

The underlying genetic basis of each type of plant disease resistance differs according to the genetic makeup of both plant and pathogen. Non-host disease resistance is multi-component, relying upon a foundation of passive plant defense, and usually also involving the activation of active defenses by non-specific elicitors of biotic origin. Generally, the genetic determination of non-host resistance is poorly understood (4).

TABLE 3

General and race specific elicitors with diverse chemical structure and their effects in plants

	Elicitor*	Source	Function in producing organism	Type	Examples for effects in plants
I	Branched (1,3-1,6) β -glucans; active with DP ≥ 7 or 5, depending on host	<i>Phytophthora</i> , <i>Pythium</i>	Component of the fungal cell wall	General	Phytoalexin in soybean, rice
	Chitin oligomers; active with DP $\geq 4-6$, depending on host	Higher fungi	Chitin (linear β -1,4-linked polymer of N-acetyl-glucosamine) of the fungal cell wall	General	Phytoalexin in rice; lignification in wheat leaves
I+II	Pectolytic enzymes degrading plant cell walls and releasing endogenous elicitors, e.g. oligogalacturonides	Various fungi and bacteria	Enzymes provide nutrients for the pathogen	General	Protein inhibitors and defence genes in <i>Arabidopsis</i> , tomato
II	Elicitor activity independent from enzyme activity, e.g. endoxylanase	<i>Trichoderma viride</i>	Enzyme of fungal metabolism	Race specific	HR and defence genes in tobacco
	Elicitins (10 kDa)	<i>Phytophthora</i> , <i>Pythium</i>	Sterol scavengers?	Narrow	HR in tobacco
	PaNie 25 kDa	<i>Pythium aphanidermatum</i>	?	General	PCD and callose formation in tobacco, carrot, <i>Arabidopsis</i>
	avr gene products, e.g. AVR4, AVR9	<i>Cladosporium fulvum</i> ; avr products also from other fungi and bacteria	Role in virulence?	Race specific	HR in tomato
	Viral proteins, e.g. viral coat protein	TMV	Structural component	Race specific	HR in tomato, tobacco
	Harpins (kDa)	Several Gram-negative bacteria	Involved in Type III secretion. Exact function?	General	HR and defence genes in tobacco and <i>Arabidopsis</i>
	Flagellin (33 kDa); flg15 is sufficient for activity	Gram-negative bacteria	Part of bacterial flagellum	General	Ca Callose deposition, defence gg genes, ROS in <i>Arabidopsis</i>
	Protein or peptide toxins, e.g. victorin	<i>Helminthosporium victoriae</i> (rust)	Toxin for host plants	Race specific	PCD in oat
III	Glycoproteins, e.g. a 42 kDa protein where a Pep-13 fragment without glycosylation site is sufficient for elicitor function	<i>Phytophthora sojae</i>	?	General	Phytoalexin and defence genes in parsley
	Glycopeptide fragments of invertase	Yeast	Enzyme in yeast metabolism	General	Defence genes and ethylene in tomato
IV	Syringolids (acyl glycosides)	<i>Pseudomonas syringae</i> pv.	Signal compound for the bacterium?	Race specific	HR in soybean carrying Rpg 4 resistance gene
	Nod factors (lipo-chito-oligo-saccharides)	<i>Rhizobium</i> and other rhizobia	Signal in symbiosis communication	General	Nod formation in legumes but also alkalization in tomato cell cultures

V	FACs (fatty acid amino acid conjugates)	Various <i>Lepidoptera</i>	Emulsification of lipids during digestion?	General	Monoterpenes in tobacco- 'indirect defence'
	Ergosterol	Various fungi	Main sterol of higher fungi	General	Alkalinization in tomato cell cultures
	Bacterial toxins, e.g. coronatine	<i>Pseudomonas syringae</i>	Toxin in compatible interactions, disturbs 'proper' salicylic acid response, mimics jasmonate action	General	Defence genes and defence compounds in <i>Arabidopsis</i> , <i>Brassica</i>
	Sphinganine analogue mycotoxins, e.g. fumonisin B1	<i>Fusarium moniliforme</i>	Toxin in necrotrophic interaction; disturbs sphingolipid metabolism	General	PCD and defence genes in tomato, <i>Arabidopsis</i>

* DP=degree of polymerization; I=oligosaccharides; II=peptides and proteins; III=glycopeptides and -proteins; IV=glycolipids; V=lipophilic elicitors;

Data from reviews (6, 53, 2, 3, 54, 55, 4, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68 and 69).

The majority of cases of race-specific resistance appear to result from the generation by a pathogen of race-specific elicitors of active plant defense, and the recognition of these by the plant host. Specific elicitors are encoded by avirulence (*avr*) genes (72, 73), and these peptides are believed to bind to receptor peptides, encoded by host resistance (*R*) genes. The resistance relationship between the *avr* gene in the pathogen and *R* gene in the host plant has been studied extensively in plant pathology. Flor (74) firstly demonstrated this relationship with the "gene-for gene-concept". According to this hypothesis, the host plant contains an *R* gene corresponding to a specific elicitor (ligand) encoded by a pathogen *avr* gene wherein the interaction produces an "incompatible" or resistant reaction to the pathogen. Since Flor's research was first reported, numerous *R* gene mediated plant-pathogen interactions have been described (75). Recognition of the *avr* gene products by the host triggers signal transduction pathways that cause a massive shift in gene transcription and plant cell metabolism, and local and systemic signals are released that prime the rest of the plant against further infection. The presence of general elicitors, such as the release of host and pathogen wall fragments, during this process may amplify the defense response.

Most *R*-gene mediated resistance shows

high specificity to the elicitors, which is supported by the fact that most *R* genes can recognize only one specific elicitor and a few *R* genes, can recognize two elicitors (76). Five classes *R* proteins are now recognized: intracellular protein kinases; receptorlike protein kinases with an extracellular leucine-rich repeat (LRR) domain; intracellular LRR proteins with a nucleotide binding site (NBS) and a leucine zipper (LZ) motif; intracellular NBS-LRR proteins with a region with similarity to the Toll and interleukin-1 receptor (TIR) proteins from *Drosophila* and mammals; and LRR proteins that encode membrane bound extracellular proteins (77). The majority of *R* proteins have common features that include variable-length leucine rich repeats (LRR) domains, whose functions are assumed to mediate protein-protein interactions (78). However, the direct interaction of an *R* protein with a receptor for an *avr* protein has been reported in a few cases. For example, rice plants that are resistant to the rice blast disease contain the *Pi-ta* gene corresponding to the *avr Pi-ta* gene of the fungus. Using the yeast two-hybrid system, Jia et al. (79) showed that *avr Pi-ta* protein did bind to the *Pi-ta* protein of the resistant rice plant.

The spectrum of reactions elicited in plants undergoing either type of resistance is complex, but nevertheless strikingly

similar (4). However, *R*-gene mediated recognition of the elicitor triggers a highly effective resistant defense system leading to prevention of pathogen growth and spread to other adjacent cells, which is termed as an incompatible interaction. On the other hand, the absence of an *R* gene corresponding to the specific elicitor of the pathogen allows pathogen growth and spread, which is termed as a compatible interaction. However, Maleck et al. (80) reported that expression patterns of many defense related genes were similarly changed in incompatible and compatible interactions between *A. thaliana* and the fungus *Peronospora parasitica*. Thus, they suggested that the resistance in the incompatible interaction triggered by recognition of the elicitor may result from more rapid and higher amounts of defense gene expressions than in the compatible interaction in which the susceptible plant failed to stop pathogen growth due to late and/or low levels of defense gene expression.

Mechanism of elicitation in plant cells

Intensive research has been devoted to establishing the mechanism of elicitation in plants. Research was focused mainly on the biotic and particularly carbohydrate elicitors, and the effects of abiotic elicitors on the overproduction of secondary metabolites in plants is poorly understood; elicitation is hypothesized to involve the key messenger Ca^{2+} , factors affecting cell membrane integrity, inhibition/ activation of intracellular pathways and changes in osmotic pressure acting as stress agents (46, 47)

Initial studies into the elicitation phenomenon by biotic elicitors in plant systems were based on the defense mechanism in animal cell systems (81). In animal cells, plasma-membrane-localized receptors activate ion channels and protein kinases. The evidence in support of the existence of plasma-membrane receptors in plants has

been gathered, particularly over the last decade (82).

A general mechanism for biotic elicitation in plants may be summarized on the basis of elicitor-receptor interaction. When a plant or plant cell culture is challenged by the elicitor an array of biochemical activities occur. These include:

- Binding of the elicitor to a plasma membrane receptor (83, 82, 84, 85)
- Altered ion fluxes across the plant cell membrane i.e. Cl^- and K^+ efflux, Ca^{2+} influx, (86, 87, 88, 89, 90, 91, 92, 93).

In plants, Ca^{2+} transients have been found to act as second messengers in a variety of responses to environmental signals, including pathogens. For instance, in parsley cells, an elicitor- responsive calcium channel has been identified and characterized and a transient influx of calcium has been found to occur within minutes after fungal elicitor addition (94).

Another cytoplasmic acidification (95, 96, 97); plasma membrane depolarization (86, 98); extracellular increase of pH in elicitor treated plant tissues (99) and rapid alkalization of the apoplast and the outer medium in cultured plant cells (100, 97) indicate an influx of protons from the apoplast into the cytoplasm. In contrast, elicitor-induced cytoplasmic acidification that is fed by transient efflux of vacuolar protons was first described in cell cultures of *Eschscholzia californica* (30).

- Increased activity of the plant phospholipases was found in some plant tissues and cultured cells after elicitor contact (101, 102, 103, 104, 105, 106, 107); synthesis of secondary messengers Ins (1,4,5) P_3 and diacylglycerol (DAG) (108) mediating intracellular Ca^{2+} release, nitric oxide (109, 110) and octadecanoid signaling pathway (111).
- Rapid changes in protein phosphorylation patterns have been observed upon elicitor treatment of a variety of cell cultures (6, 49, 112, 113, 114, 115). Recent investigations indicate that reversible phospho-

rylations play role in the plant signal transfer during pathogen or stress defence (116, 117); activation of protein kinases, from which MAP-kinases and calcium-dependant kinases are best characterized and catalyze mostly the Thr-Ser phosphorylation in the target proteins (118, 119, 120). The MAP kinase cascade involves MAP kinase kinase kinase (MAPKKK) proteins phosphorylating MAP kinase kinases that in turn phosphorylate MAP kinases. Upon activation, MAPKs are transported to the nucleus where they phosphorylate specific transcription factors. A complete plant MAP kinase cascade in early defence involving MEKKK1, MKK4/MKK5 and MPK3/MPK6 and WRKY22/WRKY29 transcription factors that function downstream of the flagellin receptor FLS2 has been identified (59). A MAP kinase cascade has been speculated to be acting downstream of elicitor responsive ion channels and upstream or independent of the oxidative burst.

- G-protein activation (122, 123, 108, 103) which are also involved in the early responses to elicitors (124).
- Activation of NADPH oxidase responsible for AOS and cytosol acidification (84).
- Cytoskeleton reorganization (125).
- Generation of active oxygen species (126, 127, 128, 129).
- Accumulation of pathogenesis-related proteins such as chitinases and glucanases, endopolygalacturonases, hydroxypyrrolone-rich glycoproteins, protease inhibitors (130, 131).
- Cell death at the infection site (hypersensitive response), (122, 123, 108).
- Structural changes in the cell wall (ligification of the cell wall, callus deposition), (132).
- Transcriptional activation of the corresponding defence response genes (110, 133, 134).
- Plant defence molecules such as tannins

and phytoalexins are detected 2-4 h after stimulation with the elicitor (126, 135, 136, 137).

- Synthesis of jasmonic and salicylic acids as secondary messengers (133, 138).
- Systemic acquired resistance (84).

The study of the chronological order of these events and the interconnection and orchestration between them is complex and is still under investigation. However, not all elicitors follow this sequence of events. Although some peptide elicitors act through plasma-membrane receptors (85), certain peptides of bacterial origin have been reported to enter the cell and get transported to distal tissues acting themselves messengers of the invasion signal. Labeling studies have indicated that the systemic necrosis caused by these proteins is not induced by rapid secondary signaling, but by transport of the elicitor within the tissue (139).

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