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# Phytoalexins: Defence or just a response to stress?

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**Abstract**

Inducible plant defence responses include antimicrobial compounds called phytoalexins, lytic enzymes, oxidizing agents, cell wall lignification and a number of pathogenesis related proteins. The antimicrobial properties of phytoalexins suggest that they are an important component of plant defence. A role for these compounds in defence has been revealed through several experimental approaches. Data have come, for example, through studies on the rate of phytoalexins in relation to cessation of pathogen development, quantification of phytoalexins at the infection site and relationship of pathogen virulence to the phytoalexin tolerance. Although there are some evidence suggesting a role for phytoalexins accumulation in resistance, there are also examples against it. Progress in the development of our understanding of the role of phytoalexins in resistance is discussed in this review.

***Key words:*** Phytoalexins, plants, resistance, defence, pathogen

## Fitoaleksinler: Savunma mı yoksa sadece strese bir tepki mi?

**Özet**

Uyarılabilir bitki savunma mekanizması, fitoaleksinler, litik enzimler okside olan maddeler, hücre duvarı lignifikasyonları ve patojenite ile ilgili birkaç proteini de kapsayan antimikrobiyal maddeleri içerir. Fitoaleksinlerin antimikrobiyal özellikleri, onların bitkilerin savunmasında önemli olduğunu göstermektedir. Bu maddelerin dayanıklılıktaki rolü birkaç değişik yaklaşımla çalışılmıştır. Biriken fitoaleksin oranının patojen gelişimini durdurması, infeksiyonun oluştuğu yerdeki fitoaleksin miktarı ve patojen virülensliğinin fitoaleksini tolere etme yeteneğiyle ilişkisi şimdiye kadar yapılan çalışmalar arasındadır. Fitoaleksinin birikiminin dayanıklılıkta bir rolü olduğunu kanıtlayan sonuçlar olmasına rağmen, bu düşüncenin tam aksi örnekler de bulunmaktadır. Bu derlemede fitoaleksinlerin dayanıklılıktaki rolünü anlamamızı sağlayacak gelişmeler tartışılmıştır.

***Anahtar sözcükler:*** Fitoaleksinler, bitkiler, dayanıklılık, savunma, patojen

## Introduction

Higher plants are routinely exposed to micro-organisms, both above and below the ground. Fortunately, only a handful of them cause diseases on them. Indeed, as with animals, compatibility of micro-organisms (susceptible) with the host is an exception in the nature, while incompatibility (resistance) is the rule (Panopoulus et al., 1984). In many cases, following an attack, an encounter leaves no obvious trace of its occurrence and the microbe fails to establish itself due to a lack in activation of pathogenicity functions or to highly effective plant defence mechanisms. Others leave evidence of an intense host-pathogen interaction that eventually results in the restriction of the pathogen (Delaney,

1997). In this case, host tissues often display activated defence functions that produce antimicrobial compounds, enzymes and structural reinforcement that may limit pathogen growth (Dixon and Lamb, 1999).

Activated (infection-induced) defence mechanisms operates only once the pathogen attempts to infect the host, thus their products are normally either absent from healthy plant tissues, or present only in lower amounts than that can be detected during incompatible interactions. They therefore provide an excellent model for investigation of events involved in cellular signalling, and to evaluate the role played by specific defence mechanisms in resistance (Smith, 1996).

## Phytoalexin concept

Antimicrobial compounds from plants are broadly classified into two categories: phytoantipicins and phytoalexins (Mansfield, 1999). Phytoanticipins are described as "low molecular weight, antimicrobial compounds that are present in plants before challenge by micro-organisms, or are produced after infection solely, from pre-existing precursors". Phytoalexins are defined as "low molecular weight, anti-microbial compounds that are both synthesized and accumulated in plants after exposure to micro-organisms or abiotic agents" (Paxton, 1980; VanEtten et al., 1994). Phytoalexins represents one component of a battery of induced defence mechanisms used by plants including lytic enzymes such as chitinases and glucanases, oxidizing agents, cell wall lignification and a number of pathogenesis-related (PR) proteins and transcripts of unknown functions (Dixon and Lamb, 1990; Lamb et al., 1989). It is important to recognise that phytoalexin accumulation may be part of a co-ordinated defence strategy, in which any one factor may alone be unable to account for restriction of the potential pathogen (Mansfield, 1999).

## Elicitors of phytoalexin accumulation

The molecules that signal plants to begin the process of phytoalexin synthesis are called elicitors. Elicitors of biotic origin may be involved in the interaction of plants and potential pathogens, whereas abiotic elicitors are not involved in normal host-pathogen interactions (Darvill and Albersheim, 1984).

In natural conditions, the stimulus is provided by the presence of the micro-organism and its perception by the host initiates the chain of events leading to phytoalexin synthesis. Biotic elicitors may originate in the invading organism, in which case they are referred to as "exogenous", whereas "endogenous" elicitors are of plant origin and are generated by the interaction between micro-organism and plant. Molecules with elicitor activity have been identified across a wide range of structural types including polysaccharides, glycoproteins, lipids, lipopolysaccharides, oligosaccharides and even enzymes, though their activity can be attributed to their effect in releasing elicitor-active components from the cell walls of the pathogen or host (Anderson, 1989; Blein et al., 1991; Hahn et al., 1992; Ricker and Bostock, 1992; Alghisi and Favaron, 1995).

Abiotic elicitors form a diverse collection of molecules that are not derived from natural sources, such as the tissues of the pathogen or host. Under normal circumstances, they would not be encountered by the plant. The group includes compounds such as fungicides; salts of heavy metals, for example $Cu^{2+}$ and $Hg^{2+}$; the detergents, basic molecules such as polylysine and histone; reagents that are intercalated DNA (Dixon et al., 1983; Darvill and Albersheim, 1984). Treatment of plant tissues with factors that cause stress, for example repeated freezing and thawing, wounding or exposure to UV light (Kodama et al., 1988; Kodama et al., 1992; Mert-Türk et al., 1998) can also induce phytoalexin synthesis.

## Phytoalexins in disease resistance

Phytoalexins accumulate at infection sites and they inhibit the growth of fungi and bacteria *in vitro* therefore, it is logical to consider them as possible plant-defence compounds against diseases caused by fungi and bacteria. Depending upon the phytoalexin, fungus and bioassay, the $EC_{50}$ for fungi is generally $10^{-3}$ to $10^{-5}$ M (reviewed by Kuc, 1995). Thus they are comparatively weak as antifungal agents. Although there is no evidence that are phytoalexins are translocated, the speed of their accumulation and

localization at the infection site may permit the pathogen to encounter concentrations far in excess of the $EC_{50}$ at early stages in the infection process. There is evidence for this presumption. Concerning the accumulation of pisatin in pea and phaseollin in green bean, it was apparent that the phytoalexins accumulated to fungitoxic concentrations not only in inoculum droplets placed on opened pea or bean pods but also in the tissues immediately below the inoculum droplets (Cruickshank and Perrin, 1968). These data supported a role for phytoalexins in plant disease resistance, but there were and still are exceptions.

There are also examples that phytoalexins accumulated during compatible plant-pathogen interactions. These include the induction of pisatin by the virulent Oomycete *Aphanomyces eutiches* (Pueppke and VanEtten, 1976) and by the pathogenic strains of the fungus *Nectria hematococca* and induction of spirobrassinin by virulent races of *Leptosphaeria maculans* (Pedras and Seguin-Swartz, 1992). Similarly Glazebrook and Ausubel (1994) reported that the virulent pathogen *Pseudomonas syringae* pv. *maculicola* elicits the synthesis of high levels of camalexin in *Arabidopsis thaliana*. Mert-Türk et al. (1998) also showed that camalexin accumulated during both compatible and incompatible interaction in *A. thaliana* when challenged with an Oomycete, *Peronospora parasitica*.

If the results exemplified above are interpreted, in incompatible interactions, phytoalexin accumulation limits or stops pathogen growth, thereby conferring resistance to the plant (Figure 1). In compatible interactions, the pathogen apparently, either tolerates the accumulated phytoalexins, detoxifies them, suppresses phytoalexin accumulation, and/or avoids eliciting phytoalexin production (Mansfield, 1982).

## Gene manipulation for resistance

Phytoalexin definition described above does not include any criteria that would allow discrimination between a role for phytoalexins in defence versus just a response to stress. In order to evaluate the importance of phytoalexins in defence the following criteria can be used: 1. The restriction of the pathogen development must be associated with phytoalexin production, 2. Phytoalexins must accumulate to antimicrobial levels at the infection site in resistant plants or cultivars that could result the cessation of the pathogen growth, 3. There must be strong evidence that the phytoalexins have vital importance in resistance, and absence of these compounds would result enhanced susceptibility (Hammerschmidt, 1999).

The first two criteria are easy to satisfy through direct observation of pathogen development in relation to phytoalexin accumulation. The third criterion is the most difficult and complicated one that requires detailed analysis as exemplified below.

A central part of evaluating the role of phytoalexins in resistance has been to manipulate phytoalexin accumulation synthesis with inhibitors and enhancers of phytoalexin synthesis and then to determine whether the degree of resistance is altered thereby. If phytoalexin accumulation makes a significant contribution to resistance, cultivars that are normally susceptible to a virulent race of pathogen would be expected to become more resistant, and cultivars that are resistant would become more susceptible to infection, according to whether the phytoalexin content is increased or decreased respectively.

In order to find out the role of phytoalexins in resistance, a few elegant models have been studied. The gene for biosynthesis of stillbene phytoalexins has been transferred from *Vitis vinifera* (grapevine) to tobacco. In response to inoculation with *Botrytis cinerea*, the transformed plants accumulate mRNA for stilbene synthase, the enzyme specifically required for synthesis of the stilbene phytoalexin resveratrol, around the infection site. They also demonstrate an enhanced resistance to the pathogen that corresponds with accumulation of resveratrol to fungitoxic concentrations (Hain et al., 1993). This affords powerful evidence of the role of phytoalexin accumulation in disease resistance.

Glazebrook and Ausubel (1994) and Glazebrook et al. (1997) isolated three phytoalexin-deficient *(pad)* mutants of *A. thaliana* accession Col-0 to help elucidate the role(s) of phytoalexins in plant-pathogen interactions. Infection by *P. syringae* induced the *A. thaliana* phytoalexin, camalexin, in the *pad1*, *pad2* and *pad3* mutants to 30, 10 and <1% (undetectable) of the

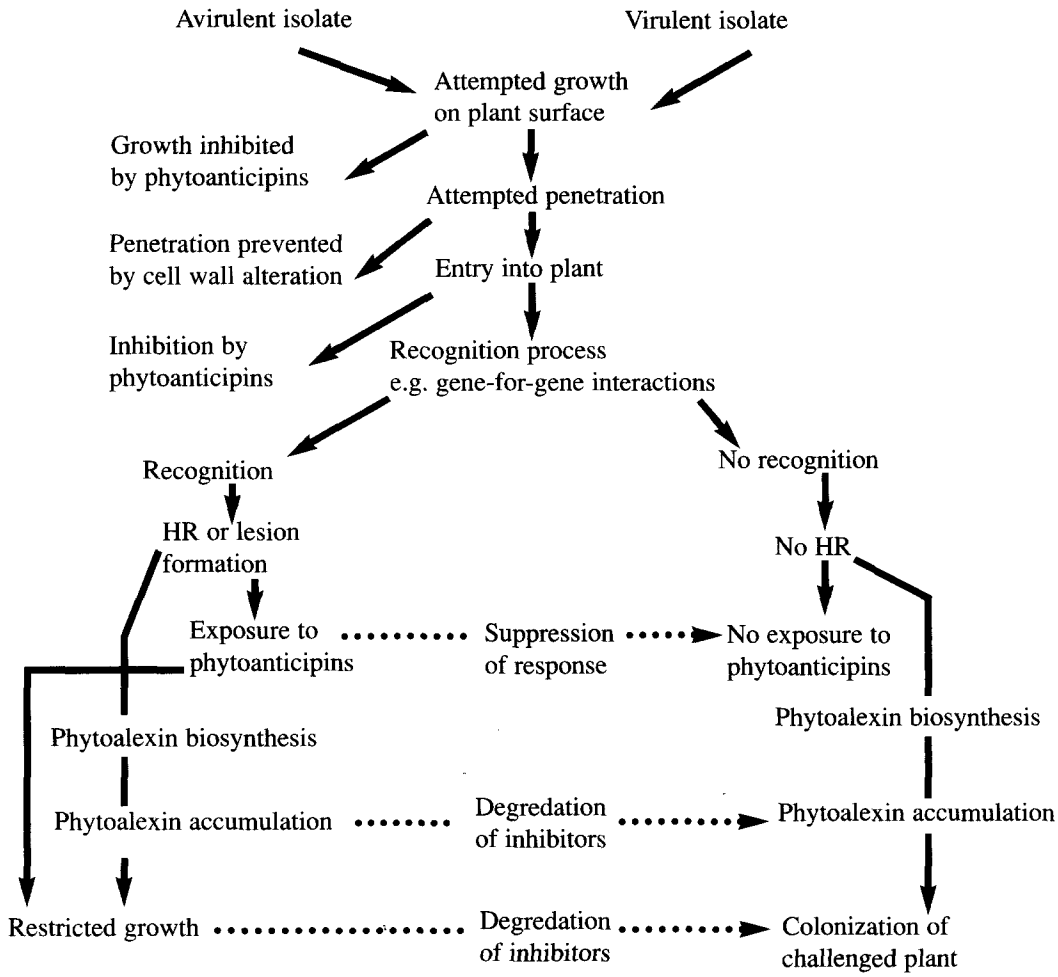


**Figure 1:** Activity of antimicrobial compounds at different stages of the plant-pathogen interaction. The dashed lines refer to the routes taken by virulent pathogens to avoid plant defence (adopted from Mansfield, 1999).

level in wild-type plants, respectively. None of *pad* mutants was compromised in their ability to resist infection by *P. syringae* strains carrying avirulence (avr) genes. However *pad1* and *pad2* exhibited enhanced susceptibility to virulent strains. In contrast, growth of these strains in the *pad3* mutant was not significantly different from that in wild-type accession providing evidence that camalexin does not have an important role in protecting the plants from avirulent pathogens but might have a role on restricting the virulent pathogens. Similar results obtained using an Oomycete *P. parasitica* in a few wild-type accessions and the same mutants (Mert-Türk et al., 1998; Mert-Türk, 2001).

Genetic manipulation of secondary metabolites is an area of molecular biology that remains in its infancy. More detailed understanding of the enzymology of phytoalexin synthesis is needed (Mansfield, 1999). Dixon et al. (1996) pointed out that attempts to engineer metabolic pathways may give unexpected results, revealing fundamentally redundant enzyme systems, metabolic channelling, or unexpected translocational control mechanisms. A continued research focus on phytoalexins is therefore, likely to yield data on fundamental aspects of plant metabolism as well as being of indirect benefit to the development of disease control strategies.

## Conclusions

Phytoalexins are only one components of the complex mechanisms for disease resistance in plants. Studies on phytoalexins alone have contributed a great deal to plant biochemistry and molecular biology. As they accumulate both in susceptible and resistant plants, the real question is that whether they are contributors to defence or just the end product of pathogen- or stress-induced metabolism. More conclusive approaches could be used to answer the question. One of them could be to generate phytoalexin biosynthetic mutants that no longer synthesize phytoalexins, then to assess them whether phytoalexin deficiency causes enhanced susceptibility. There are two extremely critical points here that should be kept in mind. This approach should include genetical analyses using a system in which the biochemical and physiological evidence argues strongly in favour of a key role for phytoalexins in resistance. Second point is that the plants must be evaluated for changes in other defences that may compensate for the loss of phytoalexin production.

As a result of advances in molecular biology, much better view of the role of phytoalexins in defence has established, though we do still not know for certain whether phytoalexin are contributors for defence. Clearly future studies on these compounds will allow us to understand and evaluate plant-pathogen interactions as well as provide new approaches to disease control. All affords in molecular biology and biotechnology is to introduce new approaches into disease control for more friendly environment.

## References


Alghisi P and Favaron F. Pectin-degrading enzymes and plant parasite interactions. *Euro J Plant Pathol.* 101: 365-375, 1995.

Anderson AJ. The biology of glycoproteins as elicitors. In: *Plant-Microbe Interactions.* Kosege T and Nester EW (Ed). McGraw-Hill, New York. Vol.3, 1989.

Blein JP, Mitat ML and Ricci P. Responses of cultured tobacco cells to cryptogein, a proteinaceaus elicitor from *Phytophthora cryptogea. Plant Physiol.* 95: 486-491, 1991.

Cruickshank IA and Perrin DR. The isolation and partial characterisation of Monilicolin A, a polypeptide with phaseollin inducing activity from *Monilinia fructicola. Life Sciences.* 7: 449-458, 1968.

Darvill AG and Albersheim P. Phytoalexins and their elicitors-A defence against microbial infection in plant. *Ann Rev Plant Physiol.* 35: 243-275, 1984.

Delaney TP. Genetic dissection of acquired resistance to disease. *Plant Physiol.* 113:5-12, 1997.

Dixon RA, Dey PM and Lamb CJ. Phytoalexins: enzymology and molecular biology. *Adv in Enzy and Rel A Mol Biol.* 53: 1-36, 1983.

Dixon RA, Lamb CJ. Molecular communication in interaction between plants and microbial pathogens. *Ann Rev Plant Phys and Plant Mol Biol.* 41: 339-367, 1990.

Dixon RA, Lamb CJ, Sewalt VJH and Paiva NL. Metabolic engineering: Prospects for crop improvement through the genetic manipulation of phenylpropanoid biosynthesis and defence responses-a review. *Gene.* 179: 61-71, 1996.

Dixon RA and Lamb CJ. Molecular communication in interactions between plants and microbial pathogens. *Ann Rev Plant Physiol Plant Mol Biol.* 41:229-367, 1999.

Glazebrook J and Ausubel F. Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterisation of their interactions with bacterial pathogens. *Proc Nat Acad Sci, USA.* 91, 8955-8959, 1994.

Glazebrook J, Zook M, Mert F, Kagan I, Rogers EE, Crute IR, Holub EH, Hammerschmidt R and Ausubel FM. Phytoalexin-deficient mutants of *Arabidopsis* reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. *Genetics.* 146:381-392, 1997.

Hahn MG, Darvill A, Albersheim P, Bergmann C, Cheong J-J, Koller A, Lo V-M. Preparation and characterisation of oligosaccharide elicitors of phytoalexin accumulation. In: *Molecular Plant Pathology. A Practhical Approach.* Gurr SJ, McPherson MJ and Bowles DJ (Ed). IRL Press, Oxford, New York, Tokyo. Vol. 2, 1992.

Hain R, Reif HJ, Krause E, Langebartels R and Kndle H. Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature.* 361: 153-156, 1993.

Hammerschmidt R. Phytoalexins: What have we learned after 60 years? *Ann Rev Phytopathol.* 37: 285-306, 1999.

Kodama O, Suzuki T, Miyokawa J, Akatsuka T. Ultraviolet-induced accumulation of phytoalexines in rice leaves. *Agr and Biol Chem.* 52: 2469-2473, 1988.

Kodama O, Li WX, Tamogami S and Akatsuka T. Oryzalexin S, a novel stemarane-type diterpene rice

phytoalexin. *Biosci Biotech Biochem.* 56: 1002-1003, 1992.

Kuc J. Phytoalexins, stress metabolism, and disease resistance in plants. *Ann Rev Phytopathol.* 33: 275-297, 1995.

Lamb CJ, Lawton MA, Dron M and Dixon RA. Signals and transduction mechanisms for activation of plant defences against microbial attack. *Cell.* 56: 215-224, 1989.

Mansfield JW. Role of phytoalexins in disease resistance. In: *Phytoalexins.* Mansfield JW and Bailey J (Ed). Blackie, Glasgow, 1982.

Mansfield JW. Antimicrobial compounds and resistance: the role of phytoalexins and antianticipins. In: *Mechanisms of Resistance to Plant Diseases.* Slusarenko AJ, Fraser RSS and VanLoon LC (Ed). Kluwer, Amsterdam, 1999.

Mert-Türk F, Bennett MH, Glazebrook J, Mansfield J and Holub E. Biotic and abiotic elicitation of camalexin in *Arabidopsis thaliana.* 7th International Congress of Plant Pathology. Edinburgh, Scotland, UK, 1998.

Mert-Türk F. Quantification of downy mildew susceptibility and camalexin accumulation in mutants of *Arabidopsis thaliana.* Ph.D. thesis. University of London, UK, 2001.

Panopoulus NJ, Walton JD and Willis DK. Genetic and biochemical basis of virulence in plant pathogens. In: *Genes Involved in Microbe-Plant Interaction.* Verma DPS and Hohn TH (Ed). Springer-Verlag, Wein. 339-374, 1984.

Paxton JD. Phytoalexins a working redefinition. *Phytopathol.* 132: 1-45, 1980.

Pedras MSC and Seguin-Swartz G. The black-leg fungus: phytotoxins and phytoalexins. *Can J Plant Pathol.* 14: 67-75, 1992.

Pueppke SG and VanEtten HD. The relation between pisatin and the development of *Aphanomyces eluteiches* in diseased *Pisum sativum. Phytopathol.* 66: 1174-1185, 1976.

Ricker KE and Bostock RM. Evidence for release of the elicitor arachidonic acid and its metabolites from sporangia of *Phytophthora infestans* during infection of potato. *Physiol Mol Plant Pathol.* 41: 61-72, 1992.

Smith CJ. Accumulation of phytoalexins: defence mechanism and stimulus response system. *New Phytol.* 132: 1-45, 1996.

VanEtten HD, Mansfield JW, Bailey J and Farmer EE. Letter to the editor: two classes of plant antibiotics: phytoalexins versus phytoanticipins. *Plant Cell.* 1191-1192, 1994.