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The relationship between pathogen-induced systemic resistance (ISR) and multigenic (horizontal) resistance in plants

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Abstract

Plants have developed mechanisms to successfully co-exist in the presence of pathogenic organisms. Some interactions between plants and pathogens are based on recognition of specific elicitor molecules from avirulent pathogen races (*avr* gene products), which is described in the gene-for-gene resistance theory. Another type of resistance, multigenic (horizontal) resistance, is a less well-studied phenomenon that depends upon multiple genes in the plant host. All plants possess resistance mechanisms which can be induced upon pre-treatment of plants with a variety of organisms or compounds. This general phenomenon is known as induced systemic resistance (ISR). At least in some plant species, ISR depends on the timely accumulation of multiple gene products, such as hydrolytic enzymes, peroxidases or other gene products related to plant defences. The pre-treatment of plants with an inducing organism or compound appears to incite the plant to mount an effective defense response upon subsequent encounters with pathogens, converting what would have been a compatible interaction to an incompatible one. Our studies in three plant–pathogen systems clearly document that multigenic-resistant plants constitutively express specific isozymes of hydrolytic enzymes that release cell wall elicitors, which in turn may activate other defense mechanisms. ISR induces constitutive accumulation of these and other gene products prior to challenge. ISR is known to function against multiple organisms, and there is no specificity observed in the accumulation patterns of defense-related gene products when ISR is induced. It is therefore hypothesized that the constitutive accumulation of specific isozymes of hydrolytic enzymes, or other defense related gene products, is an integral part of both multigenic resistance and the phenomenon of ISR. Further, plants in which ISR has been activated appear to move from a latent resistance state to one in which a multigenic, non-specific form of resistance is active.

Introduction – plant disease resistance

Plant defense responses involve the activation of multiple, coordinated and apparently complimentary defense responses, such as the production of phytoalexins or other antimicrobial compounds, the formation of physical barriers through increased cross-linking, the elicitation of the hypersensitive response, or the elevated expression of pathogenesis-related proteins. The induction of plant defense responses also seems to involve several signal transduction cascades (Karban and Kuć, 1999).

'Single gene' disease resistance, also known as gene-for-gene resistance, depends on the possession of a single resistance (R) gene by the plant, which must interact with a specific avirulence (*avr*) gene product produced by the pathogen in order for a defense response to be invoked. If a plant lacks the correct R gene to match at least one of the *avr* genes possessed by an invading pathogen, that plant will be unable to use its R genes to detect and stop the pathogen (Buell, 1999).

Multigenic resistance, also known as 'horizontal', 'quantitative' or 'polygenic' resistance, refers to plant

disease resistance generated via interactions between the products of multiple plant genes, not a single R gene (Nelson, 1978; Simmonds, 1991). Multigenic resistance is considered to be non-specific in that the plant and pathogen do not require matching R and *avr* genes for a timely plant defense response to occur. Multigenic resistant plants which have been bred to resist a specific pathogen tend to resist a greater variety of pathogens and pathogen races than those bred or engineered to expresss particular R genes (Simmonds, 1991).

Another category of disease resistance depends upon the induction of defenses following exposure to organisms or compounds. A variety of organisms, including virulent and avirulent pathogens (Tuzun et al., 1986; 1992; Tuzun and Kuć, 1991), mycorrhizal fungi (Borowicz, l997) and non-pathogenic rhizobacteria (Tuzun and Kloepper, 1995; Benhamou et al., 1998) have been observed to activate plant defense responses. Abiotic inducing agents include compounds isolated from plant pathogens (Wei and Beer, 1996; Norman et al., 1999) and a variety of chemicals (Fought and Kuć, 1996; Benhamou and Belanger, 1998). This general phenomenon is known as induced systemic resistance (ISR), and generally results in a non-specific resistance against a broad specturm of pathogens and pests. The extent of protection has sometimes been observed to vary (e.g. Manhandhar et al., 1999; Ton et al., 1999), and may depend upon the genotype and physiological condition of the plant, as well as the nature of the inducing agent used.

In the remainder of this article, evidence concerning the nature of multigenic and induced plant defense responses will be reviewed. The induction patterns and possible functions of specific genes (encoding hydrolase isozymes in particular) related to these forms of resistance in several plant-pathogen systems will be discussed.

Enzymes associated with disease resistance

A wide variety of enzymes have been associated with disease resistance, only a few of which will be discussed in this article. For a more comprehensive review, see van Loon and van Strien (1999).

Chitinases and β-1,3-glucanases

The production of hydrolytic enzymes alone may not be sufficient for the protection of all plants from disease (e.g. Dalisay and Kuć, 1995a,b). However, this does not mean that hydrolase isozymes are not involved in disease resistance, or that they do not play an important role in resistance to some pathogens. Hydrolytic enzymes may have a dual function in disease resistance. Some isozymes will have direct antimicrobial effects against an invading pathogen. These isozymes, and/or others, may also accelerate and amplify the disease resistance process by generating hypersensitive response elicitors upon encountering a pathogen.

Unfortunately, a great deal of the work regarding the role of specific enzymes in disease resistance fails to distinguish between the different isozymes that are present. Significant changes in the expression of a particular isozyme may go undetected.

Chitinases catalyze the hydrolysis of chitin, a linear polymer of β-1,4-linked N-acetylglucosamine residues that is the predominant constituent of fungal cell walls, nematode eggs, and mid gut layers of insects. Some plant chitinases also exhibit lysozymal activity (Boiler, 1985; Dodson et al., 1993). Three classes of plant chitinases have been proposed based upon protein primary structure (Shinshi et al., 1990). The highly variable nature of chitinases, and the multiplicity of chitinase isozymes in plants, suggest that plant chitinase isozymes may carry out specific and differing roles. Some chitinase isozymes, for example, have antifungal activity while others do not, and the activity of antifungal chitinase isozymes isolated from tobacco (Sela-Buurlage et al., 1993) and tomato (Lawrence et al., 1996) have been found to be specific for certain pathogens.

Many plant pathogenic fungi contain β-1,3-glucans in their cell walls in addition to chitin. Chitinases and β-1,3-glucanases purified from tomato (Lawrence et al., 1996), tobacco (Sela-Buurlage et al., 1993), pea (Mauch et al., 1988) and the tropical forage plant *Stylosanthes guianensis* (Brown and Davis, 1992) have been found to have synergistic antifungal effects *in vitro*. The *in planta* antifungal effects of tomato and tobacco chitinases and β-1,3-glucanases have also been recorded (Benhamou et al., 1990; Benhamou, 1992). It has been suggested that the synergistic effects of these enzymes, and the specificity of their effects, may be attributed to the structure of a particular fungal cell wall. For example, the chitin layers of some fungal cell walls appear to be buried in β-glucans, rendering the chitin inaccessible to chitinases unless there is prior hydrolysis with β-1,3-glucanases (Benhamou et al., 1990).

Oligosaccharide elicitors of plant defense responses can also be generated by chitinases and β-1,3-glucanases. Soybean β-1,3-glucanases (Keen and Yoshikawa, 1993; Ham et al., 1991) and specific isozymes of tomato chitinase and β-1,3-glucanase (Lawrence et al., 1996; 2000) generated elicitors from fungal pathogens. Tomato chitinases generated elicitors from germinating spores of *Alternaria solani*, but not the mature cell walls of this pathogen (Lawrence et al., 2000).

Antioxdant enzymes

Plant cells are protected against damage from active oxygen species generated during the hypersensitive response by a complex antioxidant system, including enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase, and catalase (Zhang and Kirkham, 1994).

Several species of active oxygen (O_2^-, H_2O_2, and OH^-) result from the reduction of molecular oxygen, and there are numerous possible reactions which allow these species to interconvert (Elstner, 1987; Mader et al., 1980). Hydrogen peroxide, which has the longest half-life, provides a good estimate of the relative active oxygen level in the system. There is an opinion that elicitor or pathogen-stimulated accumulation of H_2O_2 comes only from SOD-catalysed dismutation of superoxide radicals (Auh and Murphy, 1995). SOD and catalase are critical to the immediate level of H_2O_2 since they are involved in production and utilization of the molecule. The existence of multiple molecular forms of SOD, peroxidase, catalase and other related enzymes and the variation in the activity of these during plant development suggests that each isozyme may have a separate role (Scandalios, 1993).

Peroxidases represent another component of an early response system in plants to pathogen attack (Mader and Fussi, 1982; Mader et al., 1980). The products of these enzymes, in the presence of a suitable hydrogen donor and hydrogen peroxide, can have direct antimicrobial and antiviral effects (van Loon and Callow, 1983). The extracellular location of peroxidase isozymes stimulated during pathogen attack (Birecka et al., 1975), and their affinity for substrates involved in lignification, as well as the capacity of peroxidases to form hydrogen peroxide (Ride, 1975), suggest that peroxidase isozymes may also be involved in the formation of barrier substances which limit the extent of pathogen spread. The release of superoxide and free radical intermediates during lignin polymerization (Grisebach, 1981) may be involved in restricting the growth of both fungal and bacterial pathogens (Klement, 1982; Ride, 1975; Tiburzy and Reisner, 1990). For example, antibacterial components active against *Xanthomonas oryzae* pv. *oryzae* were isolated from rice leaves and found to be lignin precursors (Reimers and Leach, 1991).

Patterns of expression of enzymes in multigenic resistant and induced resistant plants

In this section, the manner in which hydrolytic and antioxidant enzymes are expressed in plants which express multigenic resistance, and plants in which systemic resistance has been induced, will be compared. Three plant systems (tobacco, tomato and cabbage) will be discussed in some detail, while work in other plant systems will be mentioned briefly.

Tobacco

Resistance to *Peronospora tabacina* (blue mold) in tobacco is considered to be due to a few genes acting in an additive fashion (Rufty, 1989). Several breeding lines have been developed by the use of intraspecific hybridization of wild *Nicotania* species *N. tabacum*. Results from SDS-PAGE and Western blot analyses consistently revealed the presence of chitinase and β-1,3-glucanase isozymes prior to pathogen attack, as well as an earlier induction of isozyme accumulation following attack, in the resistant lines (Tuzun et al., 1997). Enzyme activity assays closely correlated with the Western blot analysis (Robertson, 1995).

Induced systemic resistance to *Peronospora tabacina* (blue mold) occurs naturally under field conditions (i.e. in plants not inoculated by human beings) (Tuzun et al., 1992). Inoculation of tobacco with *Peronospora tabacina* spores or tobacco mosaic virus (TMV) resulted in the induction of systemic resistance against a variety of pathogens (McIntyre et al., 1981) and the accumulation of β-1,3-glucanase and chitinase isozymes prior to foliar inoculation (Tuzun et al., 1989; Ye et al., 1990; Pan et al., 1991; 1992). Similar results were observed for tobacco inoculated with viruses, PGPR or various chemical inducers (Maurhofer et al., 1994, Schneider and Ullrich, 1994; Lusso and Kuć, 1995). Increases in lysozyme, peroxidase, polyphenol

oxidase and phenylalanine ammonium lyase activity, correlated with the induction of ISR, have also been reported (Ye et al., 1990; Schneider and Ullrich, 1994). Inhibition of fungal pathogen growth was found to precede host cell necrosis in induced tobacco, and it is thought that this might be due to the production of defense response elicitors by hydrolytic enzymes (Ye et al., 1992).

Elevated constitutive expression of an endochitinase gene from *Trichoderma viride* in tobacco and potato resulted in significant protection against multiple fungal pathogens (Lorito et al., 1998). Reduced levels of anionic peroxidase, however, did not result in reduced lignification in transgenic tobacco (Lagrimini et al., 1997).

Tomato

Tomato breeding lines and several plant introductions of *Lycopersicon* spp. have already been identified in studies with heritable foliar resistance to the early blight pathogen *Alternaria solani*, conferred by the presence of multiple genes (Barksdale and Stoner, 1973; Gardner, 1988; Maiero et al., 1990; Maiero and Ng, 1989; Nash and Gardner, 1988). These studies also suggest that expression of a resistant phenotype in a given individual relies on various genetic interactions of an additive and/or epistatic nature. All tomato breeding lines resistant to *A. solani* expressed significantly higher constitutive levels of chitinase and β-1,3-glucanase isozymes than susceptible plants (Lawrence et al., 1996; 2000). The same 30 kDa chitinase isozyme, expressed to a high level in resistant lines, also accumulated more rapidly, and to significantly higher levels, in the resistant lines than in the susceptible ones during pathogenesis (Lawrence et al., 1996). The resistant tomato lines expressing elevated levels of chitinase and β-1,3-glucanase isozymes were also able to produce a greater number of, or more effective, elicitors of the hypersensitive response from *A. solani* cell walls than susceptible tomato lines (Lawrence et al., 2000). It is thought that the higher constitutive expression of hydrolytic enzymes might therefore contribute to disease resistance to *A. solani* via the more rapid and greater production of oligosaccharide elicitors upon contact with the pathogen, that in turn activate other defense mechanisms. More rapid accumulation of chitinases in resistant plants during incompatible tomato–pathogen interactions have also been observed *in planta* by other researchers (Benhamou et al., 1990). Two genes encoding basic chitinases which accumulate during pathogenesis in tomato have been sequenced, and the promoter region of one of these genes has been cloned (Baykal and Tuzun, unpublished data). The manner in which the gene is regulated is currently being determined.

Tomato plants treated with β-amino butyric acid (BABA) accumulated β-1,3-glucanase and chitinase (Cohen et al., 1994), while tomato plants treated with 4-hydroxybenzoic hydrazide, salicylic hydrazide or 2-furoic acid accumulated an acidic peroxidase (Miyazawa et al., 1998). Interestingly, this peroxidase was not produced as a result of pathogenesis or wounding, suggesting that different kinds of inducing agents may have different effects on plant physiology. Enkerli et al. (1993) reported correlations between increased tomato chitinase activity, but not β-1,3-glucanase activity, with induction of resistance. Similarly, correlations between induced resistance in tomato and increased production of various antifungal proteins or activity of peroxidases, but not β-1,3-glucanases, have been reported (Anfoka and Buchenauer, 1997). Treatment of tomato roots with the mycoparasite *Pythium oligandrum* (Benhamou et al., 1997), chitosan and *Bacillus pumilis* (Benhamou et al., 1998) or with benzothiadiazole (Behnamou and Belanger, 1998) triggered and amplified defense responses, including the deposition of newly formed barriers containing callose and phenolic compounds.

Cabbage

A high level of resistance to the black rot pathogen, *Xanthomonas campestris* pv. *campestris* (XCC), was observed decades ago in the cabbage cultivars. Early Fuji and Hugenot (Bain, 1952), and the heritable nature of this resistance was found to involve one major and several modifying genes (Bain, 1955). Cabbage varieties demonstrated to be resistant to a virulent strain of XCC constitutively expressed higher levels of the chitinase-lysozyme isozyme CH2 than susceptible cabbage varieties (Dodson et al., 1993). The level of CH2 expression was correlated with the extent of black rot disease resistance. Acidic protein extraction and denaturing electrophoresis identified at least twelve acid-extractable proteins which accumulated in both black-rot resistant and susceptible varieties following XCC infection. However, accumulation was earlier and more pronounced in the resistant varieties (Tuzun et al., 1997). The chitinase-lysozyme CH2, as

well as peroxidase and superoxide dismutase isozymes, accumulated more rapidly and to a greater extent following inoculation with XCC than susceptible varieties (Dodson et al., 1993; Gay and Tuzun, 2000b). Increases in chitinase, lysozyme, peroxidase and superoxide dismutase activities have also been correlated with increased expression of these isozymes. Higher peroxidase activity in the hydathodal fluids of black rot-resistant cabbage varieties than in susceptible ones was related to increased supression of XCC growth in the hydathodal fluids (Gay and Tuzun, 2000a). Localized accumulations of peroxidase may function to protect plants against XCC infection, since this pathogen initially invades cabbage via the hydathodes (Staub and Williams, 1972).

Incompatible interactions with *X. campestris* pv. *vesicatoria* and a less pathogenic strain of XCC were sufficient to induce systemic resistance in cabbage against pathogenic isolates of XCC under both greenhouse and field conditions (Jetiyanon, 1994). Immunized plants produced chitinase/lysozyme, β-1,3-glucanase, osmotin and other pathogenesis-related proteins earlier and in greater quantities than did non-immunized plants (Tuzun et al., 1997).

Other systems

Higher constitutive expression of chitinases and/or glucanases in disease-resistant plants relative to susceptible ones has been noted in barley (Ignatius et al., 1994), grape (Busam et al., 1997) and potato (Wegener et al., 1996). Increases in the expression or activity of chitinase and/or β-1,3-glucanase isozymes in disease resistant plants after pathogen challenge have been reported in barley (Ignatius et al., 1994), pea (Vad et al., 1991) and wheat (Liao et al., 1994; Siefert et al., 1996; Kemp et al., 1999). Chitinase expression increased in wilt-resistant cotton plants following infection by *Verticillium dahliae*, but β-1,3-glucanase expression did not (Cui et al., 2000).

Increases in the expression and activity of chitinases, β-1,3-glucanases and/or peroxidases after the induction of ISR has also been reported in cotton (Dubery and Slater, 1997), wheat (Liao et al., 1994; Siefert et al., 1996), rice (Manandhar et al., 1999), coffee (Guzzo and Martins, 1996), grape (Busam et al., 1997), cucumber (Schneider and Ullrich, 1994; Ju and Kuć, 1995; Dalisay and Kuć, 1995a,b), bean (Dann et al., 1996), pepper (Hwang et al., 1997), chestnut (Schafleitner and Wilhelm, 1997), *Cotoneaster watereri* (Mosch and Zeller, 1996) and *Stylosanthes guianensis* (Brown and Davis, 1992). Kogel et al. (1994) reported that ISR in barley is associated with increases in PR-l, peroxidase and chitinase proteins, but not β-1,3-glucanase. ISR induced in radish by *Pseudomonas fluorescens* has yet to be explained, since no pathogenesis-related proteins accumulate and no changes in cell wall composition occur (Steijl et al., 1999).

Chitosanases, chitinases and β-1,3-glucanases accumulated in infected spruce seedlings (Sharma et al., 1993), and in the vicinity of the pathogenic fungus in infected spruce and pine (Asiegbu et al., 1999). These observations indicate that the defense responses of gymnosperms are similar to those of angiosperms. Induced resistance to pathogenic fungi in mature Norway spruce trees was localized to the immunized bough rather than systemic throughout the plant, but this was attributed to the size of the plant rather than to a fundamental difference in induced resistance mechanisms (Krokene et al., 1999).

Future directions

Plants expressing multigenic resistance, the products of targeted breeding programs, display resistance to a variety of pathogen races. The same is true for plants in which systemic resistance has been induced. In plants bred to express multigenic resistance, higher consititutive levels of specific isozymes of hydrolytic and/or antioxidant enzymes have been observed. In plants in which defense responses have been induced by the application of some agent, higher levels of these isozymes are also observed after induction. Are the genes involved in multigenic resistance also involved in the ISR phenomenon? Recent studies involving a gene locus in *Arabidopsis* suggest that this may be so (Ton et al., 1999), and future studies in plants with larger genomes should elucidate this question.

Another, more intriguing mailer is the regulation of the genes involved in multigenic resistance and ISR. A putative translation initiation factor, NPS45, was recently identified in cabbage via subtractive hybridization and sequenced (Abdullah, 2000). NPS45 expression was observed to vary between black rot-resistant and susceptible plants in response to inoculation with XCC, treatment with jasmonic acid, and thermal stress. Further studies will elucidate the role of this gene in the regulation of plant defense responses, and may also

give clues as to how plant defense responses can be affected by environmental stresses.

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