Systemic acquired resistance: Characterization of genes associated with plant defence response

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Abstract

Plants show their defence mechanism against pathogens and insects by induction of both localized and systemic responses. Of these, systemic acquired resistance (SAR) provides a protection on the uninfected tissues by activating different signaling pathways leading to induction of several pathogen related (PR) genes upon contact with invaders. Details of the molecular mechanisms of SAR, including pathogenesis-related proteins and their expression, and the signals including salicylic acid and jasmonates are still unclear. However, characterization of mutants involved in this response pathway helps to dissect this complex mechanism of SAR. In this paper, it will be discussed genes associated with SAR and the identification of mutants involved in this response pathway.

Key words: Resistance, mutants, salicylic acid, jasmonates, gene
Introduction

Plant diseases and insects are major constraints to plant growth and development. The primary control tactic for managing pathogens has relied solely on chemical control. In recent years, increased awareness of the potential adverse effects that pesticides can have on the environment has underscored the need to develop alternative, nonchemical methods of controlling diseases. The development of plants with enhanced resistance to pathogens is one alternative method for controlling pathogens; however, the incorporation of disease resistance into plants has been restricted by limited genetic variability and the potential for pathogens to evolve resistant biotypes. Molecular techniques, such as recombinant DNA and gene transformation techniques provide useful tools for augment traditional breeding efforts.

One area of research related to diseases resistance that scientists are focusing on is the plant’s defence mechanisms. Plants successfully resist invasion from an array of pathogens by inducing plant responses. A hypersensitive response is one example of a defence response utilized by plant to combat pathogens. The hypersensitive response causes necrosis of plant cells around the site of infection which serves to limit the spread of the infection throughout the plant. Besides localization of infection, hypersensitive response triggers a phenomenon known as systemic acquired resistance (SAR). SAR has recently generated considerable attention; however, details of the signal transduction pathway leading to SAR are still unclear. This paper will strictly discuss SAR related genes and the identification of mutant involved in this response pathway.

Plant defence pathways

Plants respond in a variety of ways to pathogen attack that refers not only microbial pathogens but also nematodes, insects, or herbivores, as well as treatment with certain chemicals, or other types of stresses (Sticher et al., 1997). Plant defence responses to these attacks have been categorized into three primary pathways: the gene for gene resistance pathway, pathways that serve to limit virulent pathogen from spreading throughout the plant, and the SAR pathway (Glazebrook et al., 1997).

In the gene for gene resistance pathway, the host is resistant when the pathogen has a particular avirulence gene that corresponds to a specific resistance (R) gene in the host. Plants containing a specific R gene are able to recognize pathogens that carry a corresponding avirulence (avr) gene that leads to localized cell death called the hyper-sensitive response (HR) and limits the spread of the pathogen (Glazebrook et al., 1997; Rairdan and Delaney, 2002).

The gene for the gene pathway not only stimulates the localized hypersensitive response in plant, but also SAR for future pathogen attacks. In the SAR pathway, infection by a pathogen that causes host cell that triggers a signal to be emitted throughout the plant, this signal triggers the activation of defence genes in uninfected tissues, and results in the plant showing enhanced resistance to subsequent infection by an array of pathogens (Glazebrook et al., 1997).

Systemic acquired resistance (SAR)

Studies to discern the factors responsible for stimulating and controlling SAR proceeded the discovery that salicylic acid produces resistance in plants to an array of pathogens (Gaffney et al., 1993; Delaney et al., 1994). This finding suggested that salicylic acid is an important component for the induction of SAR. Salicylic acid is widely distributed in both monocot and dicot plants (Raskin et al., 1990).

Evidence for the support of salicylic acid to play an important role in SAR come from experiments using plants transformed with bacterial nahG gene (Gaffney et al., 1993). This gene encodes salicylate hydroxylase which is an enzyme that converts salicylic acid to an inactive compound called catechol. These experiments showed that plants expressing salicylate hydroxylase were unable to accumulate salicylic acid following pathogen attack and as a result these plants lacked the ability to activate SAR genes or to develop resistance (Gaffney et al., 1993). Several researches have also demonstrated that the application of salicylic acid or salicylic analogs: 2,6 dichloroisonicotinic acid and benzo-(1,2,3)-thiadiazole-7-carbothioic acid have the ability to stimulate SAR (White, 1979; Metraux et al., 1991; Gaffney et al., 1993; Gorlach et al., 1996); whereas, the elimination of salicylic acid from the plant leads to plants that are incapable of establishing a SAR response (Gaffney et al., 1993; Delaney et al., 1994). These studies further support the notion that salicylic acid plays a role in the SAR pathway. It is still
unclear if salicylic acid serves as systemic signal for plant defence pathway, nonetheless, it is evident that if salicylic acid cannot build-up in the plant, SAR gene expression and disease resistance cannot be induced.

Pathogenesis-related proteins (PRs) have also been shown to be associated with the initiation of SAR. PRs accumulate after pathogen attack or related situations (van Loon et al., 1994). Research has identified the expression of PR-1 (Pathogen-Related -1), PR-5, and BGL2 (1, 3-ß-glucanase) to be correlated with the SAR response (Uknes et al., 1992). Because PR genes are associated with the onset of SAR, they can be utilized as target genes to study defence pathways.

White (1979), Ward et al. (1991), and Uknes et al. (1992) demonstrated that an increase in salicylic acid levels stimulates the accumulation of PR proteins. These experiments gave the basis for the proposed hypothesis that the accumulation of salicylic acid triggers the expression of PR proteins that serve to limit infection of the host (Ward et al., 1991). The PR genes: PR-1, BGL2, and PR-5 have been identified as genes regulated by salicylic acid (Uknes et al., 1992). Ward et al. (1991) demonstrated that tobacco salicylic acid stimulates the production of the same PR proteins that infection by the tobacco mosaic virus does.

The isolation of plant mutants with defects in specific defence responses, such as SAR has allowed the roles these responses play in fighting pathogens to be investigated by studying the effects of their absence on plant-pathogen interactions. Method employed to identify and characterize genes associated with SAR include genetic screens of mutants that display altered SAR responses and studying the activity of the BGL2-GUS reported gene (Delaney, 1997; Glazebrook et al., 1997). Research has identified the expression of PR-1 (Pathogen-Related -1), PR-5, and BGL2 (1, 3-ß-glucanase) to be correlated with the SAR response (Uknes et al., 1992). Because PR genes are associated with the onset of SAR, they can be utilized as target genes to study defence pathways.

Arabidopsis thaliana has been the primary plant species, along with tobacco and tomato, for genetic analysis because it possess several characteristics that make it desirable for identifying and cloning genes of interest, including a small genome size, rapid generation time, its map and genome sequence are available, and mutation and transgenic techniques have been developed and optimized for this species (Baker et al., 1997). Several mutants have been isolated that display altered SAR responses. This review will strictly focus on identification of these mutants (cpr, cim, lsd, acd2, nim1, and npr1) and a brief discussion of their role in the SAR defence response. Mutants that have been found to be associated with SAR can be divided into two categories: mutants that are constitutive expressors of PR genes and SAR, and mutants that fail to express PR and develop SAR (Delaney, 1997).

The identification of mutants that display constitutive expression of SAR has been achieved by analyzing RNA from mutagenized seedlings using northern blot analysis. SAR genes were used as probes for the hybridization experiments. Three classes of mutants identified using this technique were the cim (constitutively immune) mutants, the lsd (lesion stimulating disease) mutants, and the acd2 (accelerated cell death) mutant (Lawton et al., 1993; Dietrich et al., 1994; Greenberg et al., 1994). These mutants all have constitutive expression of SAR, however, the lsd and acd2 mutants are different from the cim mutant because they develop lesions as a result of spontaneous cell death. The results of this research have demonstrated that the cim, lsd, and acd2 mutants are associated with increased levels of salicylic acid, expression of defence genes (PR-1, PR-5, and BGL2), and resistance to virulent pathogens.

A second method developed for the identification of mutants that exhibit constitutive expression of SAR is the use of transgenic plants possessing the ß-1, 3-glucanase (PR-2) promoter (BGL2) attached to a reporter gene that encodes GUS. The idea behind this method is to identify mutants with increased expression of PR-2. Using this technique, the cpr1 and cpr6 mutants, a fourth class of mutants having increased expression of defence related proteins (PR-1, PR-5, and BGL2) and enhanced diseases resistance, were identified (Bowling et al., 1994; Clarke et al., 1998) that the cpr6 mutant also expresses the defensin proteins PDF1.2 and Thi2.1. Defensins are small antifungal proteins found in the animal and plant kingdom (Broekaert et al., 1997). In Arabidopsis, systemic expression of the defensin gene PDF1.2 is induced during infection by the fungus Alternaria brassicicola (Penninckx et al., 1996). The PDF1.2 gene is up-regulated by jasmonic acid (JA) but not by salicylic acid (SA) and, importantly, after infection with A. brassicicola, JA accumulates in both infected and non-infected leaves (Kachroo et al., 2001; Shah et al., 2001; Yoshioka et al., 2001).

Genetic studies have determined the cpr1 mutation to be recessive which suggests that CPR1 gene may
serve to suppress salicylic acid build-up in the plant (Bowling et al., 1994). The cpr6 and cim mutations, on the other hand, are dominant and therefore these mutants may be responsible for constitutive expression of factors that are positive regulators of salicylic acid accumulation. It is believed that these mutations act upstream of salicylic acid in the SAR pathway because depletion of salicylic acid prevents SAR gene expression (Glazebrook et al., 1997; Clarke et al., 1998).

In addition to mutants that have constitutive expression of SAR, several mutants have been identified that fail to develop SAR. Screening BGL2-GUS transgenic plant treated with salicylic acid or 2,6-dichloroisonicotinic acid and identifying mutants that fail to exhibit increased expression of GUS has led to identification of the npr mutant (Cao et al., 1994). This mutant fails to exhibit expression of PR proteins and induction of SAR genes despite treatment with salicylic acid or 2,6-dichloroisonicotinic acid (Cao et al., 1994).

Screening plants treated with chemicals that induce SAR for resistance to certain pathogens has been another technique for isolating mutants that fail to exhibit SAR. A nim1 mutant has been isolated that does not express PR proteins and fails to develop resistance to *Pseudomonas parasitica* after treatment with 2,6-dichloroisonicotinic acid and identifying mutants that fail to exhibit increased expression of GUS has led to identification of the npr mutant (Cao et al., 1994). This mutant fails to exhibit expression of PR proteins and induction of SAR genes despite treatment with salicylic acid or 2,6-dichloroisonicotinic acid (Cao et al., 1994).

Experiments conducted by Delaney (1997) and Shah et al. (1997) showed that the nim1 and npr1 mutants are allelic. Because both of these mutants fail to induce expression of SAR despite treatment with salicylic acid and 2,6-dichloroisonicotinic acid, it is proposed that *NPR1* and *NIM1* genes act in the SAR signal transduction pathway; however, at a location downstream from salicylic acid (Delaney et al., 1995).

A tomato mutant, def1, deficient in jasmonate biosynthesis, fails to accumulate PinII in response to wounding and exhibits susceptibility to feeding by tobacco hornworm, *Manduca sexta* (Howe et al., 1996). However, methyl jasmonate when applied to the surface of tomato leaves, induces the synthesis of defensive proteinase inhibitor proteins in the treated plants and in nearby plants incubated in the same chamber with MeJA sprayed plants (Farmer and Ryan, 1990). It was also shown that when tomato and 5 g of leafy branches of *Artemisia tridentata*, a plant shown to possess methyl jasmonate in leave surface structure, were incubated in the same chamber with no physical contact, proteinase inhibitor accumulation is induced in tomato leaves (Farmer and Ryan, 1990).

Clearly, further studies are needed to assess the relationship among these components discussed to determine their exact regulatory effect and/or role in the SAR response. These types of studies will ultimately help us to understand the SAR signal transduction pathway. For instance, Clarke et al. (1998) have investigated the cpr6 mutant and as a result of their work they have proposed that CPR6 may express PR genes by the two distinct mechanisms: (1) direct interaction by which CPR6 expresses PR genes will provide useful insight into the plant’s defence response to pathogen attack. In addition, microarray analysis of gene expression related to SAR started to give a broader perspective on this manner (Glazebrook et al., 2003).

**Conclusion**

Genetic analysis of mutants has been demonstrated to be a useful and powerful tool for investigating defence pathways. The understanding of the mechanisms associated with SAR pathway has advanced significantly since the initial discovery of this pathway. However, much work remains to be done in elucidating the signal transduction pathway responsible for the induction of this defence response and components that regulate this response.

The study of SAR has provided valuable insight into plant disease resistance and will undoubtedly aid in the development of novel strategies for achieving disease control. Delaney (1997) proposed three potential ways the information generated from studies on the SAR pathway can be used for developing novel disease suppression methods: (1) the development of agrochemicals that elicit the SAR; (2) the development of plants with enhanced disease resistance, i.e. the isolation and incorporation of traits with increased inducible defence systems or constitutive expression of SAR; and (3) the isolation and incorporation of genes that regulate the SAR pathway in an attempt to manipulate plants to express SAR at optimal times and conditions.

It is evident after preparing this review that significant progress has been made in identifying the SAR signal transduction pathway and components that
serve to regulate this defence response. Nonetheless, additional research is needed in order to fully understand how this pathway functions and the potential for manipulation of this defence for the development of novel disease control strategies.

References


