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Interactions between *Xanthomonas* Species and *Arabidopsis thaliana*

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Abbreviations: colony forming units (CFU), Columbia (Col-0), days post inoculation (dpi), hypersensitive response (HR), Landsberg *erecta* (Ler), pathogenesis-related protein 1 (PR-1), phenylalanine ammonia lyase (PAL), *Xanthomonas campestris* pv *campestris* (*Xcc*)

Key Words: *Xanthomonas*, hypersensitive response, tolerance, defense response, black rot

ABSTRACT

***Arabidopsis* has been well studied as a model plant for plant pathogen interactions. While a large portion of the literature has been devoted to interactions between *Arabidopsis* and *Pseudomonas* and *Peronospora* species, a small cadre of researchers have been making inroads on the response of *Arabidopsis* to *Xanthomonas*. Differential responses of *Arabidopsis* accessions to isolates of *Xanthomonas campestris* pv *campestris* include tolerance, a hypersensitive response, resistance without a hypersensitive response and disease which is characterized by chlorosis and necrosis. Loci that govern the recognition of *X. c. campestris* have been identified and are the focus of on-going positional cloning efforts. Signaling and other downstream molecules involved in manifestation of resistance to *Xanthomonas* have been investigated resulting in the identification of many components of the resistance response. Parallel to the characterization of the host response, molecular and genomic efforts focused on the pathogen have the potential to reveal the mechanisms by which this bacterium can invade and colonize host tissues.**

Pathogens of *Arabidopsis*

Like other plant species, *Arabidopsis thaliana* is susceptible to only a limited number of pathogens including viruses, bacteria, fungi, nematodes and insect pests. Diseases resulting from these pathogens have been reported in the wild (Holub *et al*. 1994, 1995; Tsuji and Somerville 1992) suggesting both the pathogen and the host share an ecological niche and when the appropriate environmental conditions are present disease can occur. Diseases have also been observed in a laboratory setting where the host is deliberately exposed to the pathogen. Regardless of the setting, nature or the laboratory, *Arabidopsis* responds in a similar fashion as other higher plants when exposed to viral, prokaryotic, or eukaryotic pathogens.

Perhaps the more facile class of pathogen to work with in a laboratory setting are the bacterial pathogens. Bacteria have several advantages over the other classes of pathogens for pathological studies in that they can be cultured *in vitro* and have relatively rapid generation times (minutes not days). In addition, most bacterial plant pathogens elicit rapid host responses (hours to days) and have pathogenicity and avirulence factors that have been documented in other plant species thereby providing a foundation to begin work in *Arabidopsis*. Only a small number of bacterial species are pathogenic on *Arabidopsis.* The predominant bacterial pathogen utilized in *Arabidopsis* studies is *Pseudomonas syringae* and the reader is referred to other

chapters in this book describing *Arabidopsis* responses to this pathogen. Additional bacterial pathogens utilized in *Arabidopsis* research include *Erwinia* species which are causal agents of soft-rots, *Ralstonia* species which are causal agents of vascular wilts, and *Xanthomonas campestris* pathovars which are causal agents of blights and rots and are the focus of this chapter.

The Genus *Xanthomonas*

The genus *Xanthomonas* has been well described in several definitive publications (Starr 1981; Leyns *et al.,* 1984; Swings *et al.,* 1993) and the reader is referred to these for more details regarding this genus. In brief, *Xanthomonas* species are gram-negative rod-shaped aerobic bacteria with polar flagella and are found primarily in association with plants (diseased lesions, soil, plant debris) (Starr 1981). *Xanthomonas* species produce diagnostic pigments (termed xanthomonadins) and exude large amounts of xanthan gum, an extracellular polysaccharide which is used as a food additive (Williams 1980; Starr 1981; Swings *et al.,* 1993). *Xanthomonas* species, similar to other plant pathogens, can cause symptoms such as chlorosis, necrosis, cankers, and vascular wilts (Leyns *et al.,* 1984). Collectively, *Xanthomonas* isolates have been reported to cause disease on ~400 different monocotyledonous and dicotyledonous species (Leyns *et al.,* 1984), making *Xanthomonas* a significant plant pathogen. As will be discussed in more detail below, current mechanisms for control of *Xanthomonas* diseases rely extensively on breeding for resistance and/or cultural practices such as crop rotation and sanitation. An example of the extreme costs that can arise from lack of control of a *Xanthomonas* species, either through lack of breeding for resistance or ineffective control through cultural practices, is the recent epidemic of citrus canker in the United States (Brown 2001). Attempts to control citrus canker in Florida through an aggressive eradication program have cost over $200 million to date, with no end in sight to control of the disease (Brown 2001).

Reports of *Arabidopsis*-infecting Isolates of *Xanthomonas*

With respect to infection of *Arabidopsis*, only isolates of *X. campestris* have been reported to cause disease. The species, *X. campestris,* is a large collection of isolates that share similar biochemical and physiological phenotypes yet exhibit different host specificity and are separated into pathovars to reflect host specificity or host origin (Leyns *et al.,* 1984; Van den Mooter and Swings 1990). Table 1 lists the *X. campestris* pathovars that have been examined on *Arabidopsis* accessions. Both resistance and disease, as demonstrated by chlorosis and necrosis on inoculated leaves as well as systemic infections, have been reported on *Arabidopsis* accessions, suggesting that a full range of host responses occur in *Arabidopsis* (Tsuji and Somerville 1988; Simpson and Johnson 1990; Davis *et al.,* 1991; Tsuji *et al.,* 1991; Lummerzheim *et al.,* 1993; Parker *et al.,* 1993; Aufsatz and Grimm 1994).

Although a number of these pathovars can incite a response on *Arabidopsis*, the most prevalent pathovar utilized in *Xanthomonas-Arabidopsis* studies is the pathovar *campestris*. Various strains of this pathovar have demonstrated typical race-cultivar specificity indicative of the presence of a gene-for-gene relationship between the host and the pathogen. Thus, due to the abundance of literature with *X. campestris* pv *campestris* (*Xcc*), a majority of this chapter will be devoted to the interactions of *Arabidopsis* with various strains of *Xcc*.

Xcc and Black Rot of Crucifers

Xcc is the causal agent of black rot of crucifers (for review see Williams 1980). *Xcc* has a broad host range that includes a majority of members of the Cruciferae family. Numerous agronomically important species of crucifers including broccoli, brussel sprouts, cabbage, cauliflower, radish, and turnip are susceptible to *Xcc*. *Xcc* can also infect weed species within the Cruciferae (Schaad and Dianese 1981) making weed reservoirs of *Xcc* a factor in the control of black rot in crucifer production fields.

Xcc typically infects host plants through natural openings such as hydathodes, stomates, or wounds (for review see Williams 1980). Once the bacterium is in the vascular system, it can become systemic resulting in blackened veins and thus the name “black rot”. Under favorable environmental conditions, the pathogen can become seed-borne (for review see Williams 1980). For commercial crucifers, black rot is a significant issue. The fact that the pathogen can be seed-borne has led to seed certification programs in developed countries. The certification process adds extensive cost and effort for commercial seed production.

Several lines of evidence have firmly established *Arabidopsis* as a natural host of *Xcc*. First, classic black rot

symptoms have been reported following infection of *Arabidopsis* with specific *Xcc* strains (Simpson and Johnson 1990; Tsuji *et al.,* 1991). Second, systemic infection occurs in highly susceptible accessions following challenge with *Xcc* (Figure 1; Buell and Somerville 1997). Third, a preference for hydathode infection, rather than stomatal infection, was observed in *Arabidopsis* leaves which is consistent with the entry route favored by *Xcc* in other cruciferous hosts (Hugouvieux *et al.,* 1998). Fourth, infections of *Arabidopsis* by *Xcc* do occur in nature as reported by Tsuji and Somerville (1992).

Pathogenicity Mechanisms of *Xanthomonas* species

There have been several excellent reviews on pathogenicity and virulence mechanisms in gram-negative plant pathogenic bacteria and the reader is referred to these articles for more in-depth discussions (Collmer 1998; Staskawicz *et al.,* 2001). With respect to *Xcc* pathogenicity on non-*Arabidopsis* crucifers, there are a number of publications by M. J. Daniels and colleagues and the reader is referred to these for a summary of *Xcc* virulence mechanisms (Daniels *et al.,* 1993; Dow and Daniels 1994). In brief, *Xcc* employs a vast array of degradative and regulatory mechanisms to parasitize its host. Extracellular polysaccharide production, cell wall degrading enzymes, proteases, as well as genes that encode for secretion of these products are involved in virulence on cruciferous hosts. In addition to these structural components, regulatory components of pathogenicity and virulence have been identified in *Xanthomonas* (for review see Daniels *et al.,* 1993; Dow and Daniels 1994).

Although there is a substantial amount of information currently available on pathogenicity mechanisms in *Xanthomonas*, the level of information is about to increase exponentially. The first genome of a plant pathogen completely sequenced was *Xylella fastidosa* (Simpson *et al.,* 2000) which is the causal agent of citrus variegated chlorosis and is closely related to *Xanthomonas*. Not surprisingly, several components of virulence and pathogenicity in *Xcc* have orthologs in *X. fastidosa*, including genes involved in xanthan gum (extracellular polysaccharide) production, regulation of pathogenicity factors, and the type II secretion system that is necessary for export of degradative enzymes (Dow and Daniels 2000; Simpson *et al.,* 2000).

Even more exciting is the new genome sequencing projects focused on *Xanthomonas* species. *X. axononpodis* pv *citri*, the causal agent of citrus canker, is being sequenced by the Organization for Nucleotide Sequencing and Analysis (ONSA), a consortium of laboratories in Brazil (Kamoun and Hogenhout 2001; http://genoma4.iq.usp.br/*Xanthomonas*/). This pathogen is closely related to *Xcc* and major insights into the virulence mechanisms of *Xanthomonas* will be revealed from this project. The other *Xanthomonas* genome project is focused on *Xcc* (Kamoun and Hogenhout 2001; http://genoma.fcav.unesp.br/xc-campestris/home/xc_menu.html). In addition, the genome of *Ralstonia solanacearum* (Kamoun and Hogenhout 2001; http://www.genoscope.cns.fr/externe/English/Projets/Projet_Y/Y.html) and *Pseudomonas syringae* pv *tomato* (http://www.tigr.org; http://ppi.cornell.edu/), both pathogens of *Arabidopsis*, are the focus of genome sequencing efforts and these genomic sequences will provide additional resources for the identification of pathogenicity mechanisms in *Xanthomonas*.

Table 1. Pathovars of *X. campestris* tested on *Arabidopsis*.

Pathovar	Reference
aberrans	Parker *et al.,* 1993
armoraciae	Davis *et al.,* 1991
carotae	Simpson & Johnson 1990
campestris	Aufsatz & Grimm 1994
	Davis *et al.,* 1991
	Lummerzheim *et al.,* 1993
	Parker *et al.,* 1993
	Simpson & Johnson 1990
	Tsuji & Somerville 1988
	Tsuji *et al.,*1991
	Tsuji & Somerville 1992
raphani	Parker *et al.,* 1993
vesicatoria	Lummerzheim *et al.,* 1993
vitans	Lummerzheim *et al.,* 1993

Genetic Basis for Resistance to *Xanthomonas*

Responses in *Arabidopsis* to *Xanthomonas* infections are dependent on genetic factors present in the host and in the pathogen. Several types of resistance responses have been documented in *Arabidopsis-Xanthomonas* interactions. These include tolerance, resistance mediated through a hypersensitive response (HR) and non-HR-mediated resistance. Genes involved in the resistance response can be classified into three classes: (1) those involved in the recognition of the pathogen (also known as R genes), (2) genes involved in signal transduction events, and (3) genes involved in the suppression of pathogen growth and development (defense response genes). Currently, five genes have been identified genetically that are hypothesized to be involved in recognition of the pathogen (Table 2; Tsuji *et al.,* 1991; Buell and Somerville

1997; Godard *et al.,* 2000). These have been termed *RXC* for reaction to *Xanthomonas campestris* and are named *RXC1-5*. Additional genes have been identified in both the signal transduction pathway and in the suppression of pathogen growth/development and are discussed in more detail below (see also Table 3).

At the present, only a single corresponding gene for avirulence has been reported from a crucifer-infecting *Xanthomonas* isolate. This avirulence gene, *avrXca*, was isolated from *X. c. raphani* and confers incompatibility in a large number of *Arabidopsis* accessions (Parker *et al.,* 1993). *avrXca* encodes a large protein (~67 kDa) that is possibly secreted from the bacterium and the upstream region of *avrXca* contains a putative *hrp* box suggesting *avrXca* is controlled by the *hrp* regulatory system. The *hrp* pathway is an essential component of pathogenicity and

Figure 1. Systemic infection of *Xcc*2D520 in differential *Arabidopsis* accessions. Select basal leaves (3-5) of 3-4 week old plants were infiltrated with *Xcc*2D520 (~10^7 CFU ml^{-1}). Photographs were taken 23 d.p.i. The accessions from left to right are Col-0, Pr-0, and Ler.

virulence in bacterial plant pathogens and the reader is referred to these reviews for more information (Alfano and Collmer 1997; Cornelis and Van Gijsegem 2000). The lack of identification of corresponding avirulence gene(s) in *Xcc* isolates may reflect either technical difficulties in screening for such a gene or a lack of the gene in this pathogen. Genome sequencing efforts (see above) should provide valuable insight into this unresolved issue.

Tolerance to *Xcc*

Using the 2D520 strain of *Xcc*, Tsuji *et al.,* (1991) reported tolerance to the pathogen in the Columbia accession (Col-0). While the Pr-0 accession developed chlorosis and necrosis following infiltration with *Xcc*2D520, Col-0 remained asymptomatic. As the *in planta* bacterial levels were indistinguishable between these two accessions, the response in Col-0 was termed tolerance as limited bacterial growth was supported in Col-0 without the development of symptoms. Thus, an uncoupling of symptom formation and pathogen growth is seen in the interaction of Col-0 and Pr-0 with *Xcc* 2D520. Genetic mapping efforts indicated that a single dominant gene, termed *RXC1* (Tsuji *et al.,* 1991), confers tolerance. *RXC1* was been mapped to the lower arm of chromosome 2 (Buell and Somerville 1997).

The biochemical and physiological mechanism by which Col-0 is able to tolerate 2D520 is not well understood. This tolerance does not involve the synthesis of the phytoalexin, camalexin (Tsuji *et al.,* 1992). At the molecular level, examination of the mRNA accumulation levels of several genes involved in defense responses did not reveal an accumulation of mRNA unique to Col-0 and thus no genes could be correlated specifically with tolerance (Buell and Somerville 1995). Although an accumulation of pathogenesis-related protein 1 (PR-1) mRNA was observed, it was expressed at high levels in both Col-0 and Pr-0.

Non-HR Resistance to *Xcc*

Previous work with *Xcc*2D520 utilized the differential accessions Col-0 and Pr-0. A further survey of *Arabidopsis* accessions revealed additional susceptible accessions. Specifically, the accession, Landsberg *erecta* (Ler), is highly susceptible to *Xcc*2D520 (Tsuji and Somerville 1988; Buell and Somerville 1997). Inoculation of Ler results in severe chlorosis and necrosis which can spread systemically resulting in necrosis of the entire plant (Figure 1; Buell and Somerville 1997). A qualitative difference in the susceptible response is apparent between Pr-0 and Ler. Whereas Pr-0 lesions are primarily chlorotic in nature, Ler lesions are more necrotic with darkening of the vascular tissue that is absent from Pr-0 lesions (Figure 2). *In planta* bacterial levels in Ler are 10-100- fold higher than in Col-0 and in Pr-0, revealing a suppression of bacterial growth in Col-0 and Pr-0 in comparison to Ler (Buell and Somerville 1995, 1997). Thus, Col-0 exhibits two responses to *Xcc*2D520: tolerance to limited bacterial growth (as compared to the susceptible accession Pr-0) and resistance without a HR (when compared to the highly susceptible accession Ler).

Examination of the genetic basis for resistance to the 2D520 isolate indicated three loci, *RXC2*, *RXC3*, and *RXC4*, were involved in resistance (Buell and Somerville 1997). The major locus for resistance, *RXC2*, behaved as a single dominant gene whereas the other two loci, *RXC3* and *RXC4*, functioned in a digenic manner. These loci have been placed on the genetic map and map to chromosome 5 (*RXC2*, *RXC3*) and chromosome 2 (*RXC4*) (Buell and Somerville 1997). Although *RXC2* and *RXC3* map to distinct locations on chromosome 5, *RXC4* maps to the same region of chromosome 2 as *RXC1*. Due to limited recombination events, it is not clear whether *RXC1* and *RXC4* are the same gene or simply map to similar regions of the chromosome.

Table 2. Major loci involved in the recognition of *X. campestris*.

Locus	Phenotype	Map position	Reference
RXC1	Tolerance to *Xcc*2D520	Chromosome 2	Tsuji *et al.,*1991 Buell & Somerville 1997
RXC2	Non-HR resistance (monogenic) to *Xcc*2D520	Chromosome 5	Buell & Somerville 1997
RXC3	Non-HR resistance (digenic) to *Xcc*2D520	Chromosome 5	Buell & Somerville 1997
RXC4	Non-HR resistance (digenic) to *Xcc*2D520	Chromosome 2	Buell & Somerville 1997
RXC5	HR to *Xcc*147	Unknown	Godard *et al.,* 2000

Hypersensitive Response: Interactions with *Xcc*750

The *Xcc*750 isolate has been shown to induce a HR-like response on resistant accessions of *Arabidopsis* such as Col-0 while causing chlorosis on susceptible accessions such as Oy-0 (Aufsatz and Grimm 1994). The HR observed on Col-0 is coupled with a lack of significant bacterial growth, consistent with the HR observed in *Arabidopsis* with other pathogens such as *Pseudomonas*. Resistance in *Arabidopsis* is associated with high basal expression and an induction of expression of a low molecular weight protein termed ECS1 (formerly named CXc750; Aufsatz and Grimm 1994). Subsequent work using transgenic plants and polyclonal antibodies generated to ECS1 revealed it is associated with the cell wall (Aufsatz *et al.,* 1998). Although *ECS1* had been tightly correlated with the resistance phenotype, definitive genetic and transgenic experiments have revealed *ECS1* is not a resistance gene for *Xcc*750 but instead is linked to a locus involved in resistance to *Xcc*750.

Hypersensitive Response: Interactions with Xcc147

The response of *Arabidopsis* to the HR-inducing strain 147 of *Xcc* has been described in a series of papers by the Roby laboratory and other researchers. *Xcc*147 induces a HR on resistant accessions of *Arabidopsis* such as Col-0 (Lummerzheim *et al.,* 1993). At the molecular level, a correlation between mRNA accumulation of two defense genes, phenylalanine ammonia lyase (PAL) and β-1,3-glucanase, and the HR was observed (Lummerzheim *et al.,* 1993). Lummerzheim *et al.* (1993) also examined transcript accumulation of a basic class I chitinase and an ascorbate peroxidase and although they were up-regulated upon infection with *Xcc147*, they were also expressed in the compatible interaction with *Xcc*8004 revealing a lack of specificity with the HR. There are four classes of chitinase (I to IV) and Gerhardt *et al.* (1997) were able to demonstrate that an extracellular class IV chitinase was expressed in *Arabidopsis* leaves following challenge with *Xcc147*. Unfortunately, class IV chitinase expression was not examined in leaves challenged with a compatible *Xcc* isolate and no conclusion can be made regarding the specificity of class IV chitinase expression in the HR. In healthy plants, the class IV chitinase was only expressed in siliques, suggesting a specific developmental pattern for this gene.

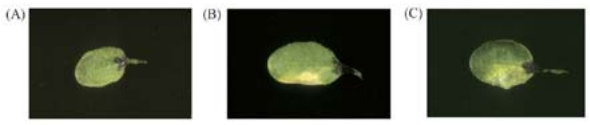

Figure 2. Comparison of symptom formation in differential *Arabidopsis* accessions following *X. c. campestris* infection. Leaves of 3-4 week old plants were infiltrated with a suspension of *Xcc*2D520 (~10^7 CFU ml^{-1}). Photographs were taken at 6 d.p.i. (A) Col-0, (B) Pr-0, and (C) Ler.

While the above studies with *Xcc*147 were performed using whole plant studies, a number of new genes involved in the HR to *Xcc*147 have been identified using cell suspension cultures. A cDNA clone encoding a putative sulfotransferase was identified from a library constructed from pathogen-challenged cell suspension cultures. The sulfotransferase, *RaR047*, is similar to flavonol sulfotransferases from *Flaveria* species (Lacomme and Roby 1996). *RaR047* is developmentally regulated as mRNA could only be detected in cell cultures and in developing aerial tissues (Lacomme and Roby 1996). Expression of *RaR047* was strongly induced in young seedlings by treatment with several elicitors of defense responses, including salicylic acid and jasmonic acid. The induction of *RaR047* expression by elicitors is consistent with the induction of expression observed with pathogen treatment of *Arabidopsis* leaves (Lacomme and Roby 1996). Inoculation with either *Xcc*147 or an incompatible strain of *Pseudomonas syringae* pv *maculicola* (M2) resulted in accumulation of *RaR047* mRNA. Compatible strains of *Xcc* (*Xcc*8004) and *P. s. maculicola* (M4) were also able to induce accumulation of *RaR047* although the extent of accumulation was substantially less than that in incompatible interactions. Although there is a correlation of *RaR407* expression with resistance (Lacomme and Roby 1996), the function of the encoded protein is unknown. It is speculated that RaR047 may function in synthesis of a molecule that is involved in signal transduction or that RaR047 functions directly in the suppression of pathogen growth (Lacomme and Roby 1996). Definitive biochemical studies will establish an enzymatic function for RaR047 and allow placement of this sulfotransferase in the defense response pathway.

In addition to sulfotransferase, cinnamoyl-CoA reductase, an enzyme involved in lignification, is associated with the development of the HR to *Xcc*147. Two cinnamoyl-CoA reductase genes, *AtCCR1* and *AtCCR2*, exhibit different substrate specificities and do not exhibit coordinate regulation during development and pathogen challenge (Lauvergeat *et al.,* 2001). *AtCCR1* is expressed throughout normal development and is expressed more in highly lignified tissues such as stems in comparison to leaf tissue. In contrast, *AtCCR2* is weakly expressed in developing tissue. Following challenge with *Xcc*147, *AtCCR2* and not

AtCCR1, was highly upregulated. *AtCCR2* expression was correlated with the HR as *AtCCR2* expression was not detectable in compatible tissues. *AtCCR2* was also inducible by treatment with salicylic acid, further supporting its role in defense responses. Although these two genes share substantial similarity (81.6 % identity at the amino acid level) they clearly have distinct expression profiles *in planta*. With the completion of the *Arabidopsis* Genome Initiative (2000), it would be interesting to examine the regulatory regions of these genes for promoter sequences that may reflect the differential expression patterns.

An additional set of genes involved in the HR to *Xcc*147 was described by Lacomme and Roby in 1999. A total of 27 cDNA clones (*Athsr*) was identified by differential screening of a cDNA library that was constructed from cell suspension cells challenged with *Xcc*147. The 27 clones were then grouped into "cDNA clone families" based on cross-hybridization and sequencing results. The *Athsr2* cDNA family was the most heavily represented family with 16 clones identified. The *Athsr2* cDNA family encodes voltage-dependent anion channel proteins (Lacomme and Roby 1999) that are localized in the mitochondrion and function to transport small molecules across the mitochondrial membrane. Membrane integrity and control over solute movement across the membrane are central components in apoptotic cell death and voltage-dependent anion channel proteins have been well studied in mammalian apoptosis (for review see Green and Reed 1998; Boya *et al.,* 2001). The *Athsr3* cDNA family, represented by 6 clones from the screening, encodes an alterative oxidase (Lacomme and Roby 1999). In tobacco, Chivasa *et al.* (1997) demonstrated that alternative oxidase was a component of the signal transduction pathway that leads to the HR following challenge with tobacco mosaic virus. While *Athsr2* and *Athsr3* were associated with the mitochondrion, *Athsr4* (represented by a single clone) encodes a protein with similarity to a Rab GDP-dissociation inhibitor protein. Rab proteins are involved in membrane/vesicle trafficking and Rab GDP-dissociation inhibitor proteins are intimately involved in the regulation of Rab proteins and ultimately in the regulation of membrane/vesicle trafficking (for a review on Rab proteins and their regulation see Stenmark and Olkkonen, 2001). The other 3 cDNA families (*Athsr5, 6, 7*), also represented by a single clone, did not have significant similarity to any entries in the database (Lacomme and Roby 1999).

The expression patterns of the *Athsr2-7* cDNAs were assessed in cell suspension cultures challenged with differential isolates of *Xanthomonas*: *Xcc*147 (avirulent), *Xcc*8004 (virulent), and *Xcc* 8B2 (control). All of the cDNAs were exclusively or preferentially expressed in cells challenged with *Xcc*147, confirming the success of the differential screening and suggesting a role for these genes in the HR (Lacomme and Roby 1999). Further research using a biochemical and a functional genomics approach will be essential for defining the roles of these genes in the HR.

The last gene identified from *Xcc*147-challenged cell suspension cells is the *AtMYB30* gene which encodes an orthologue of the myb oncogene (Daniel *et al.,* 1999; Lacomme and Roby 1999). MYB proteins are transcription factors and are involved in many cellular functions including plant defense responses (Yang and Klessig 1996). *AtMYB30* encodes a 323 amino acid protein and consistent with other MYB proteins contains MYB repeats in its

Table 3. Components of the signal transduction and defense response pathway in incompatible interactions between *Arabidopsis* and *X. c.* pv *campestris*

Component	Putative Identification	Function	Type of Incompatible Interaction	Reference
AtCCR2	Cinnamoyl-CoA reductase	Lignification	HR	Lauvergeat *et al.,* 2001
AtMYB30	MYB domain containing protein	Transcription Factor	HR	Daniel *et al.*, 1999
Ap3.3a	Centrin	Cytoskeleton	HR	Cordeiro *et al.,* 1998
Athsr2 family	Mitochondrial voltage-dependent anion channel proteins	Membrane potential and transport	HR	Lacomme & Roby 1999
Athsr3 family	Alternative oxidase	Cyanide insensitive respiration	HR	Lacomme & Roby 1999
Athsr4	Rab-GDP dissociation inhibitor protein	Regulation of membrane/vesicle trafficking	HR	Lacomme & Roby 1999
Athsr5	Unknown	Unknown	HR	Lacomme & Roby 1999
Athsr6	Unknown	Unknown	HR	Lacomme & Roby 1999
Athsr7	Unknown	Unknown	HR	Lacomme & Roby 1999
BG2	β-1,3-glucanase	Pathogen cell wall degradation	HR	Lummerzheim *et al.*, 1993
COP2	Unknown	Apical hook formation; light response	HR	Buell, unpublished
ECS1	Cell wall protein	Unknown	HR	Aufsatz & Grimm 1994; Aufsatz *et al.*, 1998
HLS1	Acetyltransferase	Apical hook formation	Non-HR	Buell 1998
HXC2	Unknown	Unknown	HR	Godard *et al.,* 2000
PAL	Phenylalanine ammonia lyase	Key enzyme in synthesis of secondary metabolites	HR	Lummerzheim *et al.,* 1993
PR1	Pathogenesis-related protein 1	Unknown; defense-associated	HR; Non-HR resistance	Buell & Somerville 1995
RaR047	Sulfotransferase	Unknown	HR	Lacomme & Roby 1996

N-terminus (Daniel *et al.,* 1999). At the developmental level, *AtMYB30* is expressed at extremely low levels and is detectable only in developing seedlings. Expression of *AtMYB30* was induced by challenge with avirulent *Xcc*147 cells and avirulent *P. syringae* strains. Expression was correlated with phenotypic expression of the HR, as *AtMYB30* expression was not detectable in the susceptible accession Sf-2 or with virulent strains of *Xcc* or *P. syringae*. Expression of *AtMYB30* was also examined in various lesion-mimic mutants (*lsd*) which develop HR-like lesions in the absence of pathogens. In three *lsd* mutants (*lsd3*, *lsd4*, *lsd5*), a positive correlation between *AtMYB30* expression and lesion presence was found (Daniel *et al.,* 1999), further supporting the hypothesis that AtMYB30 is a positive regulator of cell death.

Using differential display, Cordeiro *et al.,* (1998) were able to identify two novel genes involved in the early stages of the HR of *Arabidopsis* to *Xcc*147. One gene, *ap3.3a*, encodes a protein with similarity to centrin, a cytoskeletal protein. Similar to the other centrins, the *Arabidopsis ap3.3a* protein contains four regions that are hypothesized to be involved in Ca^{2+}-binding (Cordeiro *et al.,* 1998). Expression of *ap3.3a* was measured in compatible and incompatible interactions with *Xcc*. A rapid (1 hr) up-regulation of *ap3.3a* was observed in the incompatible interaction with *Xcc*147 whereas accumulation of *ap3.3a* was not observed in the compatible interaction until 1-2 dpi. A second gene identified through differential display is the *ap4.3a* gene which encodes a protein with multiple kinase domains. *ap4.3a* is rapidly induced (15 min) upon challenge with *Xcc*, although specificity of the induction was not apparent between compatible and incompatible *Xcc* strains.

Mutational Approaches to Characterize Signal Transduction and Defense Pathway Components in *Arabidopsis-Xanthomonas* Interactions

In a complementary approach to differential screening of cDNA libraries, Roby and colleagues identified a mutant of *Arabidopsis* that is deficient in manifestation of the HR following challenge with *Xcc*147. In a screen of 20,700 M_2 plants, a single recessive mutant (*hxc-2*) was identified that is unable to mount a full HR (Godard *et al.,* 2000). Inoculation of *hxc-2* plants results initially in necrosis (48 hr) at the infection site which is followed by a chlorotic halo and ultimately, spreading chlorosis. In the *hxc-2* plants, bacterial growth is unrestricted and is indistinguishable from levels in the susceptible accession Sf-2. The *hxc-2* mutant is able to mount an effective HR against avirulent strains of *P. syringae*, suggesting that the wild-type *HXC2* locus is specific for *Xcc*-mediated HR. At the biochemical level, the *hxc-2* mutants retain full responsiveness to salicylic acid which is an inducer of systemic acquired resistance (Godard *et al.,* 2000). However, accumulation of salicylic acid is impaired following challenge with *Xcc*147. At the molecular level, expression levels of several defense genes (*PAL-1*, *PR-1*, *PDF-1.2*) are altered in *hxc-2*, suggesting a role for *hxc-2* in the signal transduction pathway leading to resistance. Genetic mapping experiments place *HXC2* on chromosome 3 near the Major Recognition Gene Complex MRC-F (Godard *et al.,* 2000). Through allelism tests, it was demonstrated that *HXC2* is not the determinant of specificity to *Xcc*147 and that a second gene, *RXC5*, governs recognition of the pathogen.

Ethylene has been implicated in numerous plant responses, including the contrasting responses of defense in incompatible interactions and symptom formation in compatible interactions (Abeles *et al.,* 1992). The availability of mutants in ethylene-associated developmental processes has provided valuable reagents to dissect the role of ethylene in plant-pathogen interactions (for a recent review on the ethylene response pathway see Bleecker and Kende 2000). Inoculation of the *ethylene insensitive2* (*ein2*) perception mutant with a virulent isolate of *Xcc* resulted in plants with reduced symptoms (Bent *et al.,* 1992), suggesting EIN2 is involved in symptom formation. *EIN2* has been cloned and encodes a novel protein (Alonso *et al.,* 1999). However, although EIN2 has been placed in the ethylene response pathway through epistasis tests, its function at the biochemical and molecular level is still unknown (Bleecker and Kende 2000). Another ethylene-related mutant, *hookless1* (*hls1-1*), is unable to form an apical hook during the triple response in germinating seedlings (Guzman and Ecker 1990). Infection of *hls1-1* with *Xcc*2D520 results in disease symptoms and an increase in bacterial growth in comparison to the asymptomatic parental accession (Col-0), suggesting HLS1 functions in resistance, not tolerance, to *Xcc*2D520 (Buell 1998; Buell, unpublished). The *HLS1* gene encodes an acetyltransferase-like protein yet a specific enzymatic function for the protein has not been determined either in apical hook formation or disease resistance (Lehman *et al.,* 1988). A second mutant in apical hook formation, *constitutive photomorphogenic 2* (*cop2*; Hou *et al.,* 1993), is also suppressed in resistance to *Xcc*2D520 (Buell, C.R., unpublished) consistent with the phenotype observed in the *hls1-1* apical hook mutant. *COP2* has not been cloned but with the recent completion of the *Arabidopsis* genome (Arabidopsis Genome Initiative, 2000), this can be accomplished in the near future. Continued work focused on resolving the biochemical function of these proteins will be

valuable to dissecting their role in pathogen responses.

SUMMARY

In the dozen or so years since *Xanthomonas* was first reported as a pathogen of *Arabidopsis*, significant inroads into how this bacterium is able to parasitize this weed species have been made. A survey of *Xanthomonas* isolates has revealed that *Arabidopsis* is not limited to a single resistance mechanism to prevent pathogen growth and development. Instead, a range of responses is present in *Arabidopsis* to deal with *Xanthomonas* which may be reflective of the natural co-evolution of the host and pathogen. With the complete sequence of *Arabidopsis,* along with the ongoing functional genomic projects, we are now poised to identify all of the components involved in the response to this pathogen. Coupled with the pending complete genome sequence of several *Xanthomonas* species, especially *Xcc*, we will have a comprehensive resource to dissect the pathogen component of the interaction.

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