MOLECULAR PLANT PATHOLOGY (2004) **5**(5), 453–464 DOI: 10.1111/J.1364-3703.2004.00245.X

An *Arabidopsis* mutant with altered hypersensitive response to *Xanthomonas campestris* pv. *campestris*, *hxc1*, displays a complex pathophenotype

MARIE LUMMERZHEIM[1,2], THOMAS KROJ[1], MARCIO FERREIRA[3], MAURICE TRONCHET[1], FRANÇOIS GODARD[1], MARK VAN MONTAGU[2] AND DOMINIQUE ROBY[1]*

[1]*Laboratoire des Interactions Plantes–Microorganismes, UMR CNRS/INRA 2594, 31326 Castanet-Tolosan, France*
[2]*Laboratorium voor Genetika, Universiteit Gent, Ledeganckstraat 35, 9000 Gent, Belgium*
[3]*Laboratório de Genética Molecular Vegetal, Universidade Federal de Rio de Janeiro, Brazil*

SUMMARY

The *hxc1* mutant was identified by screening an EMS (ethylmethane sulphonate) mutagenized population of *Arabidopsis* Col-0 plants for an altered hypersensitive response (HR), after spray inoculation with an HR-inducing isolate of *Xanthomonas campestris* pv. *campestris* (*Xcc*) (strain 147). The *hxc1* mutant shows a susceptible phenotype several days after initiation of the interaction with the avirulent strain. This macroscopically observed phenotype was confirmed by measurement of *in planta* bacterial growth and by microscopical analysis. Interestingly, the *hxc1* mutation acts very specifically. *Hxc1* displays a pathophenotype identical to that observed in the wild-type with several extensively characterized avirulent and virulent bacteria, except in response to *Pseudomonas syringae* pv. *tomato* strain DC3000/*avrRpm1*, for which a partial loss of resistance was observed. Finally, the mutation causes an attenuation of expression of several defence markers regulated through different signalling pathways. Together, these data underline the complexity of this novel defence mutant, and support the hypothesis of a mutation affecting a key component acting during the first steps of the plant defence response leading to resistance to *Xcc147* and *Pseudomonas syringae* pv. *tomato* containing the *avr* gene, *avrRpm1*.

INTRODUCTION

In plants, resistance to pathogenic micro-organisms frequently results from specific recognition of pathogen-derived proteins, called avirulence (avr) proteins. In most cases, this recognition event leads to the activation of host defence responses (Glazebrook, 2001). One common plant resistance response is the hypersensitive response (HR), which involves rapid and localized host cell death at the site of attempted infection (Klement, 1982). Although the HR involves some tissue damage, it effectively protects the plant by depriving the pathogen of living host cells, and limits the spread of infection. Many studies have shown that the HR is accompanied by physiological changes in the plant cells both at the site of infection and at a distance. These changes involve intracellular signalling processes such as ion fluxes across the plant cell membrane (Atkinson *et al*., 1985), generation of active oxygen species (Lamb and Dixon, 1997; Levine *et al*., 1994) and changes in protein phosphorylation (Zhou *et al*., 1995), which are followed by disruption of the cell membrane, decompartmentalization of the cell contents, and finally result in cellular collapse and formation of a limited necrosis (Mansfield and Richardson, 1981). In addition, signalling to the cells in the immediate vicinity of this necrotic lesion is most likely responsible for the establishment of a local defence response. Finally, systemic signalling from the location of infection site to distant tissues initiates transcriptional activation of plant defence genes (including the biosynthesis and accumulation of antimicrobial compounds) (Glazebrook, 2001). Although a definitive causal relationship between HR and resistance remains to be established (Greenberg, 1997), the HR constitutes a phenotype that has been instrumental for the cloning of genes involved in pathogen resistance.

Genetic screens provided a means to isolate mutant lines affected in their recognition of specific pathogens. Several *R* genes were cloned and, based on their sequences, similarities in their structural organization could be predicted, suggesting their role as receptors of *avr* gene-encoded signals and in downstream signalling (Hammond-Kosack and Jones, 1997). However, the signalling cascades activated after recognition are just beginning to be elucidated, emphasizing the necessity to isolate mutants affected in these pathways. Several genetically distinguishable

* *Correspondence*: Tel.: 05 61 28 53 26; Fax: 05 61 28 50 61; E-mail: roby@toulouse.inra.fr

pathogen response pathways have been identified, involving (or not) the NDR1 and EDS1 proteins (Aarts *et al.*, 1998; Glazebrook, 2001; Thomma *et al.*, 2001). Mutations such as *pad4* and *pbs1* and *2* alter resistance; PAD4-mediated resistance is conditioned by the same spectrum of *R* genes as EDS1 (Glazebrook *et al.*, 1997); *pbs1* affects only resistance to *RPS5* (Warren *et al.*, 1999). Interestingly, the recent discovery of two downstream resistance components, RAR1 and SGT1, introduced further complexities in our understanding of HR. These components are required for the function of most known R proteins (Shirasu and Schulze-Lefert, 2003). In addition, a number of *Arabidopsis* mutants exhibiting signs of pathogen-independent HR have been isolated by Dietrich *et al.* (1994), Greenberg *et al.* (1994) and Lorrain *et al.* (2003). These mutations affect loci that are thought to be involved in signal transduction activating a cell death programme.

New screens for isolating mutants that are affected in their quantitative and/or qualitative responses to a given stimulus should further contribute to the unravelling of the signal-transducing steps downstream from the receptor. Thus, *Arabidopsis*-enhanced disease susceptibility or resistance mutants have been identified (Rogers and Ausubel, 1997; Tierens *et al.*, 2002; Vogel and Somerville, 2000), which has allowed (and will still allow) new defence-related functions to be defined.

Adopting this kind of approach, we have screened for *Arabidopsis* mutants displaying a spatial and/or temporal alteration in the HR when challenged with an avirulent bacterial pathogen, *Xcc*, the causal agent of black rot of the Brassicaceae (Parker *et al.*, 1993). Resistance to *Xcc* involves different mechanisms in *Arabidopsis*, depending on the bacterial strain considered. Somerville and collaborators showed that the *Arabidopsis* ecotype Col-0 is able to support a substantial *Xcc* 2D520 population in the intercellular leaf space and that this tolerance is governed by a dominant allele of the single nuclear gene *RXC1* (Tsuji *et al.*, 1991). The same authors reported the existence of three other loci: *RXC2*, which governs resistance to *Xcc* 2D250 by restricting pathogen growth, and *RXC3* and *4*, which suppress symptom formation (Buell and Sommerville, 1997). We have shown the induction of an HR on *Arabidopsis* leaves after spray inoculation with the isolate 147 of *Xcc*, and characterized the observed lesions at a phenotypic, microscopic and molecular level (Lummerzheim *et al.*, 1993). More recently, we reported the identification of three classes of mutations causing loss-of-HR (complete or partial), hyper-responsiveness and susceptibility to the avirulent bacterial strain. One mutant, belonging to the susceptible class, *hxc2* (for hypersensitivity to *Xanthomonas campestris*), has been extensively characterized (Godard *et al.*, 2000). The present study describes another mutant, *hxc1*, belonging to the same mutant class but not to the same complementation group. This mutation confers a complex pathophenotype, which suggests that the *HXC1* locus might be a new component of the resistance signalling pathways in *Arabidopsis*.

RESULTS

Phenotypic characterization of *hxc1*, an *Arabidopsis* mutant with an altered hypersensitive response to *Xcc*

Among the different mutants that we isolated (Godard *et al.*, 2000), we focused our interest on a mutant, *hxc1*, which displayed a complex pathophenotype in response to the *Xcc* strain 147, which is avirulent on plants of the wild-type, Col-0. Twenty-four hours after spray-inoculation, Col-0 exhibits small necrotic lesions, which dry out 2–3 days post-inoculation (dpi), as shown in Fig. 1C,D and no further symptom development can be observed later. Similar necrotic lesions are visible on *hxc1* leaves 1 dpi, but during the second day of infection, a halo of chlorosis surrounds the individual lesions in the mutant, and 3 dpi a generalized spreading chlorosis can be observed (Fig. 1A,B). After inoculation by infiltration, a similar sequence of events could be observed (Fig. 1E). Twenty-four hours post-inoculation, necrotic symptoms limited to the infiltrated area appear in Col-0, and a similar necrotic area can be observed on the mutant *hxc1*. Three days post-inoculation, no chlorotic symptoms are seen in Col-0, whereas in the mutant *hxc1* strong chlorosis progresses far beyond the infiltration zone, gaining the whole leaf and rapidly evolving to necrosis 6 dpi. The disease phenotypes of the susceptible ecotype, Sf-2 infiltrated with the *Xcc147* strain, and of Col-0 infiltrated with the virulent strain 8004, are shown (Fig. 1F) in comparison with this disease-like mutant phenotype. In both cases, a spreading chlorosis, evolving to necrosis, can be observed. However, they only rarely gain the whole infected leaf.

In planta *bacterial growth in* hxc1

One characteristic of plant resistance is the restriction of *in planta* growth of bacteria. In order to quantify the effect of the *hxc1* mutation on bacterial growth, we evaluated the growth of the strain 147 in leaves of the *hxc1* mutant, as compared with the resistant wild-type Col-0 and the susceptible ecotype Sf-2, 2, 3, 5 and 7 dpi (Fig. 2). The population of *Xcc147* in *hxc1* mutant leaves was 2–3 orders of magnitude higher than in the wild-type, Col-0 (144-fold higher, 3 dpi and 2845-fold higher 5 dpi) and was similar, or slightly higher, as compared with Sf-2 leaves, demonstrating that the *hxc1*/*Xcc147* interaction is compatible.

Response of the hxc1 *mutant to other avirulent bacteria*

In order to determine the specificity of the *hxc1* mutation, we analysed the pathophenotype of *hxc1* in response to inoculation by various virulent or avirulent bacterial pathogens. We infiltrated the mutant with several *Xanthomonas* isolates inducing an asymptomatic resistance response: *Xcc* 8004/*avrXca*, the mutant *XchB2*, or the strain 2D520, and the virulent strain 8004. All these

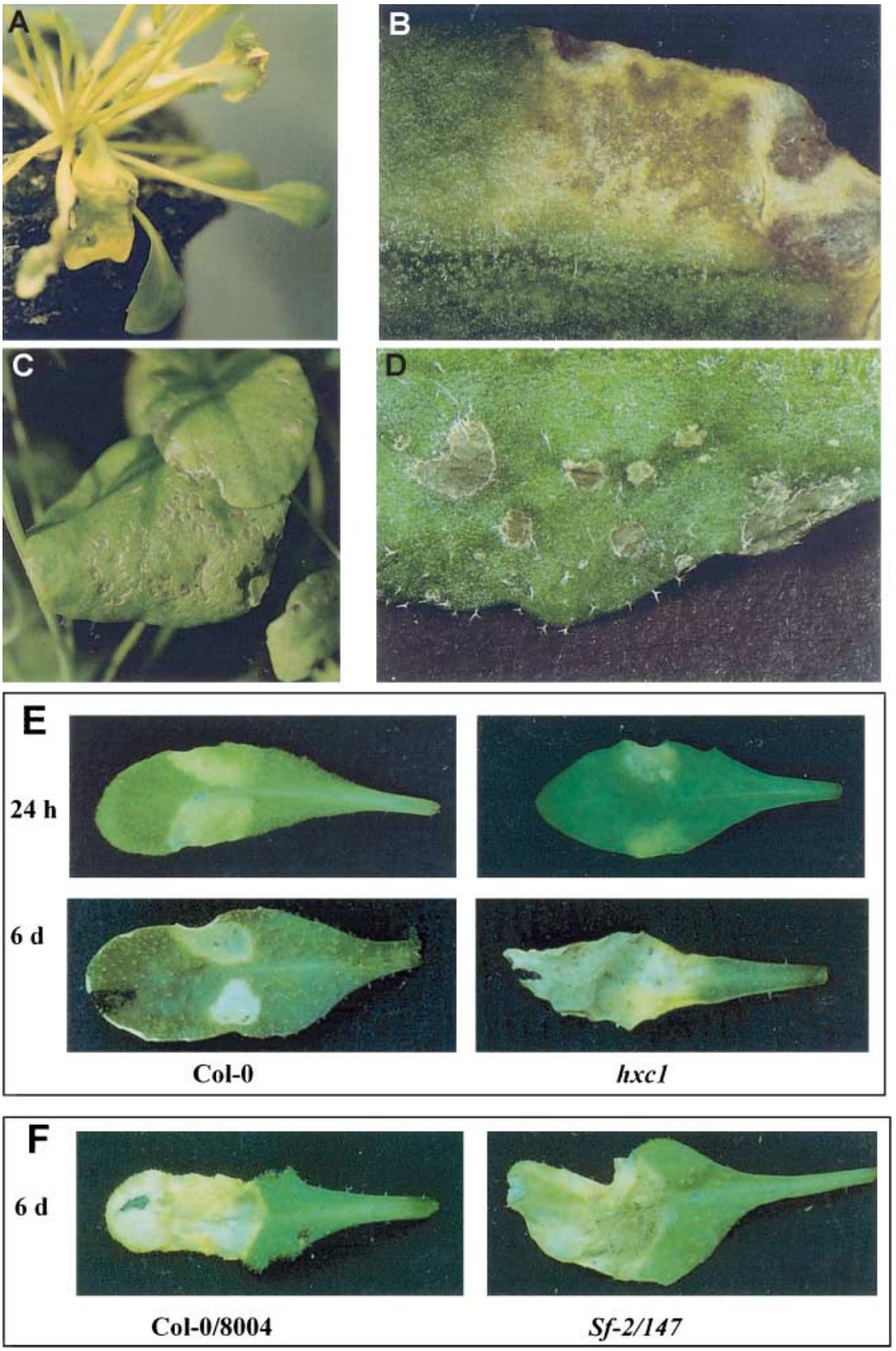


Fig. 1 Macroscopic observation of the *hxc1* mutant phenotype. Col-0 (C and D) and *hxc1* (A and B) after spray-inoculation with the avirulent strain of *Xcc*147. Col-0, Sf-2 and *hxc1* (E and F) after infiltration inoculation with virulent (8004) and avirulent (147) *Xcc* strains.

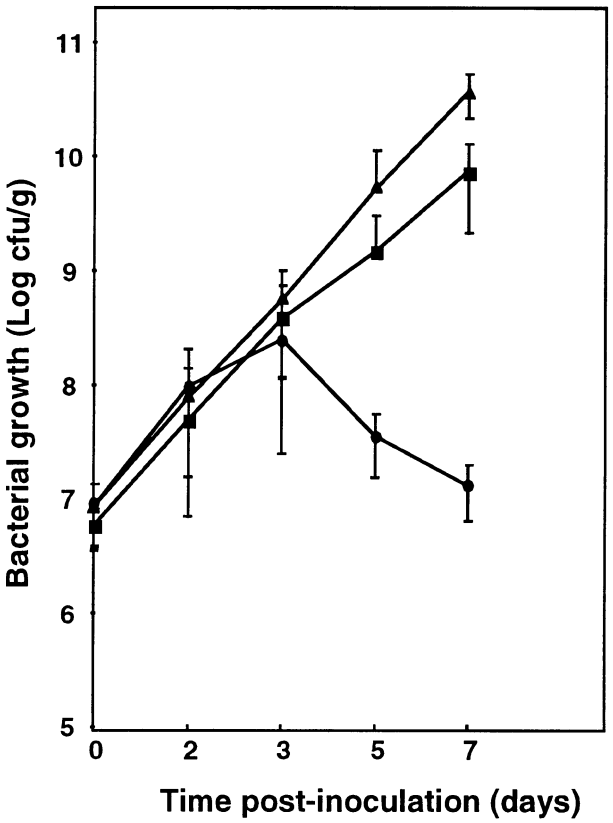


Fig. 2 *In planta* growth of *Xcc147* in the *hxc1* mutant (triangles), as compared with Col-0 (circles) and Sf-2 (squares) ecotypes. Leaves were pressure-infiltrated with *Xcc147* (10^8 cfu/mL) and the bacterial growth was determined as described in Experimental procedures. Each data point represents the mean and standard error of four replicates of each treatment, with five leaves per plant and four plants for each time point. The experiment was performed twice.

Table 1 Phenotypes of the mutant *hxc1* and the wild-type ecotype Col-0 after inoculation with different *Xanthomonas* strains (R, resistant; S, susceptible; 0, null response).

Bacterial strain	Isolate	Reference	Col-0	*hxc1*
Xanthomonas	147	Lummerzheim *et al.* (1993)	R	S
campetris pv.	8004	Daniels *et al.* (1984)	S	S
campestris	8004/*avrXca*	Parker *et al.* (1993)	R	R
	XchB2	Arlat *et al.* (1993)	0	0
	Xcc2D520	Buell and Sommerville (1997)	0	0

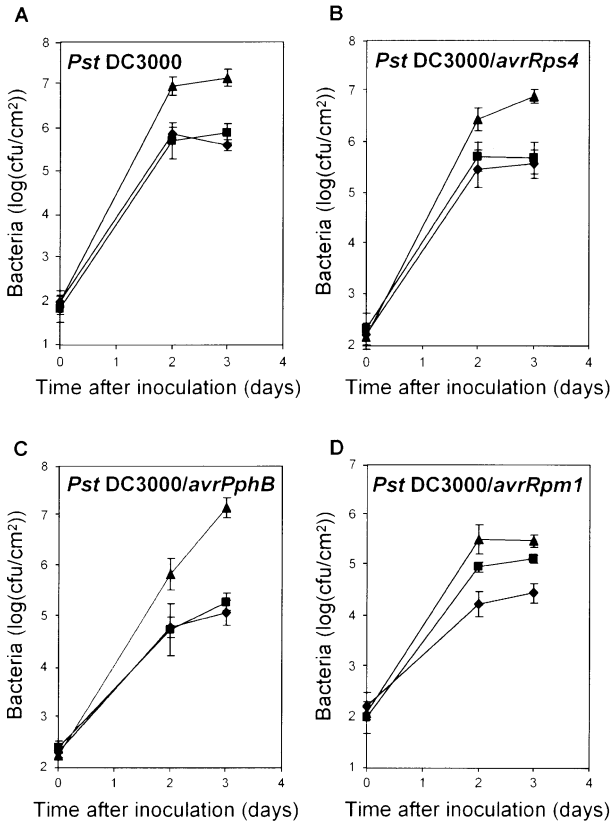


Fig. 3 *In planta* growth of different strains of *Pseudomonas syringae* pv. *tomato* in the *hxc1* mutant (squares), as compared with the wild-type Col-0 (diamonds) and a mutant used as a control (triangles): *pad4-1* (for DC3000 and DC3000/*avrRps4*) and *ndr1* (for DC3000/*avrPphB* and DC3000/*avrRpm1*). Leaves were infiltrated with the strains DC3000 (A), DC3000/*avrRps4* (B), DC3000/*avrPphB* (C) and DC3000/*avrRpm1* (D) (10^5 cfu/mL) and the bacterial growth was determined as described in Experimental procedures. Each data point represents the mean and standard error of four replicates of each treatment, with three leaves per plant and four plants for each time point. The experiment was performed three times.

bacteria induced in *hxc1* a response identical to that observed in the wild-type, as judged by analysis of symptom development (Table 1). We also used different isolates of *Pseudomonas syringae* pv. *tomato* (*P. s. tomato*), with each being diagnostic for a different *R* gene, to determine whether *hxc1* mutation affected resistance/HR to *P. s. tomato.* Thus, *P. s. tomato* strain DC3000 either expressing or not expressing an avirulence gene, was investigated by evaluating growth of the bacteria at different times after inoculation in *hxc1*, the wild-type Col-0 and a mutant with reduced resistance (*pad4-1* for *avrRps4* and *ndr1-1* for *avrRpm1*, *avrRpt2* and *avrPphB*) or enhanced susceptibility (*pad4-1*) (Fig. 3, and data not shown). In all cases but one, *hxc1* behaved identically to the wild-type, and neither reduced resistance nor enhanced susceptibility could be observed in *hxc1*. Only in the case of DC3000/*avrRpm1* did we observe a partial but significant loss of resistance that was similar or slightly weaker than that found in *ndr1*. These results were consistent in three independent experiments and indicate that disease resistance conferred by different *R* loci is not affected by the *hxc1* mutation, except in the case of *RPM1*.

Table 2 Genetic analysis of the mutant *hxc1*.

Cross	Generation	Number of plants* 'Susceptible'	HR	Total	χ^2
Col-0 × *hxc1*	F1	—	13	13	—
	F2	94	268	362	0.18
hxc1 × Col-0	F1	—	10	10	—
	F2	37	105	142	0.084
hxc2 × Sf-2†	F1	—	32	32	—
	F2	54	66	110	0.076
hxc1 × hxc2	F1	—	8	8	—
hxc2 × hxc1	F1	—	12	12	—

*Leaves (five) of each plant were infiltrated with *Xcc147* (5×10^7 cfu/mL) and scored for symptoms at 6 days post-inoculation.

†The numbers presented here result from crosses *hxc2* × Sf-2 and Sf-2 × *hxc2*.

Genetic analysis of the mutant *hxc1*

Genetic analysis of *hxc1* is shown in Table 2. The mutant was back-crossed to Col-0 (Lummerzheim *et al.*, 1993) and F1 and F2 progenies were inoculated with *Xcc147* and scored 6 dpi for appearance of HR lesions and lack of spreading chlorosis. All the F1 plants developed a typical HR, indicating that the mutation was recessive. The segregation of resistance and susceptibility in the F2 progenies was consistent with a 3 : 1 ratio, and the symptoms observed in the resistant progeny were indistinguishable from those observed in the wild-type, Col-0. We can thus conclude that the susceptible phenotype in the *hxc1* mutant plants is caused by a single recessive mutation.

In order to determine whether *HXC1* is allelic to the resistance gene *RXC5*, governing resistance to *Xcc* strain 147 in Col-0, a cross between *hxc1* and the susceptible ecotype Sf-2 was performed. All the F1 plants developed a typical HR (Table 2), and in the F2 generation resistance and susceptibility segregated with a 9 : 7 ratio, indicating that *hxc1* is not allelic with the susceptibility locus present in Sf-2.

Finally, the *hxc1* mutant was crossed to the previously identified *hxc2* mutant (Godard *et al.*, 2000). All the F1 plants tested exhibited a clear, unambiguous HR, demonstrating that the two mutations are not allelic.

Microscopic characterization of *hxc1*

Figure 4 shows transverse leaf sections of Col-0 and *hxc1*, 12, 36 and 72 h after infiltration-inoculation with *Xcc147*. No apparent differences between Col-0 and *hxc1* could be observed at 12 h post-inoculation (hpi) (Fig. 4A,B), suggesting that the mutant and the wild-type do not differ qualitatively at this time point. In both leaf sections, cells can be found with their plasma membrane pulled away from the cell wall, indicating the onset of plasmolysis. Thirty-six hours post-inoculation, significant differences can be seen in the leaf section of the wild-type compared with the mutant (Fig. 4C,D). In Col-0, most cells are undergoing a general decompartmentation preceding a cytoplasmic collapse. The chloroplasts are moving away from the plasma membrane into a more densely staining cytoplasm. They are swelling, assuming a more rounded shape, and their boundary membranes are disintegrating (Fig. 4C). In *hxc1* the symptoms are far more attenuated: the plasmolysis observed 12 hpi is now generalized and chloroplast breakdown is initiated (Fig. 4D). The Col-0 leaf mesophyll 72 hpi (for sections made in the infiltrated area) is completely necrotic and cell structures are no longer recognizable, with the exception of the rigid lignin structure of the sieve tubes (Fig. 4E). Leaf mesophyll of Col-0 outside the necrotic zone appears healthy (data not shown), in contrast to *hxc1*, where different degenerative stages can be observed: cells with an advanced plasmolysis, cells with disrupted membrane systems and organelles, and collapsed cells with amorphous necrotic cytoplasm (Fig. 4F). As shown in Fig. 4G,H, *hxc1* and Col-0, infiltrated with *Xcc147* and *Xcc8004*, respectively, show both similar and typical features of a compatible interaction 72 hpi (Brown *et al.*, 1993; Jones and Fett, 1985; Lummerzheim *et al.*, 1993).

Defence responses in the *hxc1* mutant

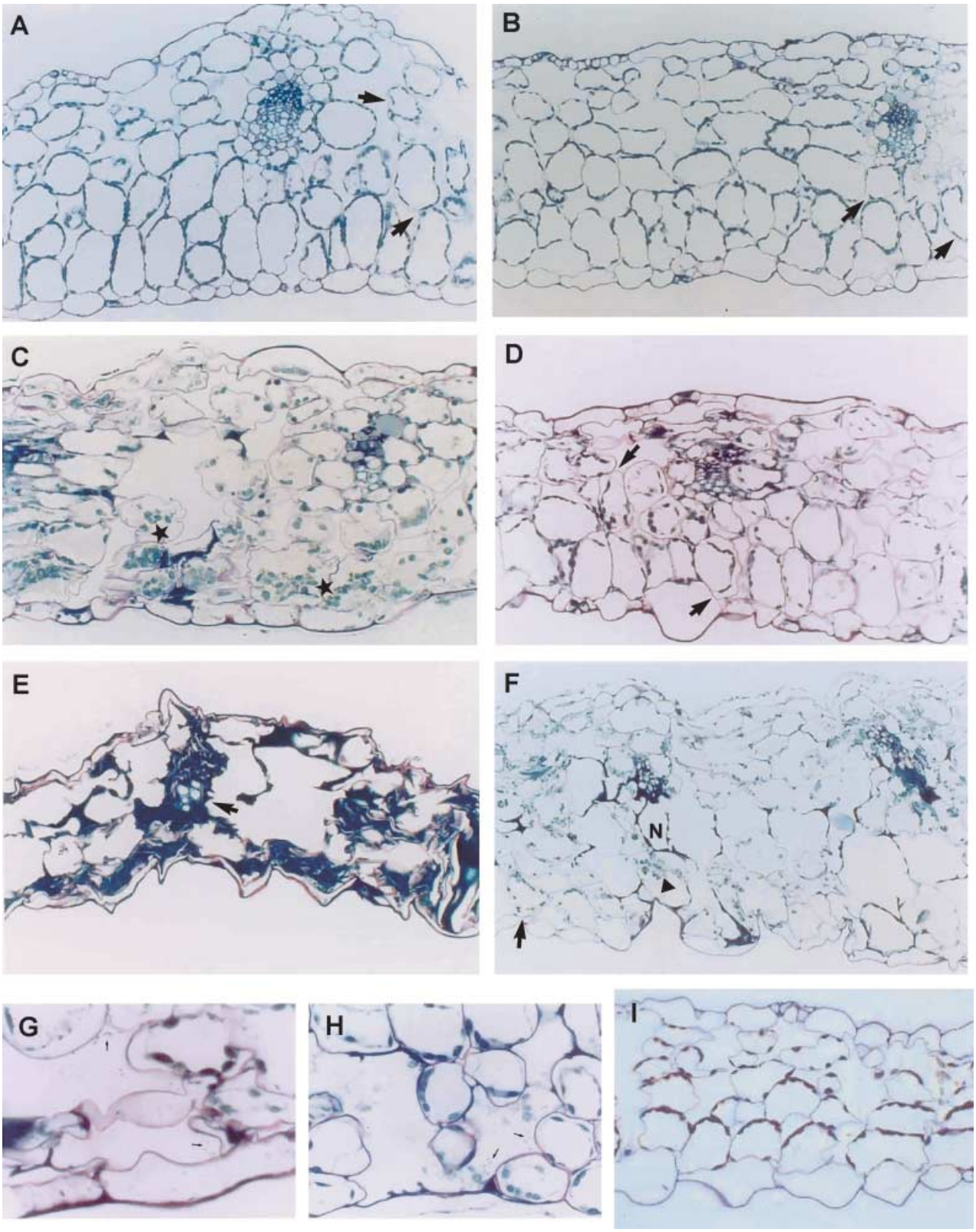
Rapid and sustained O_2^- generation is induced in hxc1

Early production of O_2^- or derivatives of superoxide anion is a hallmark of the plant defence response and has been more particularly shown to be involved in HR to attempted infection by avirulent pathogens (for reviews see Doke *et al.*, 1994; Lamb and Dixon, 1997).

In order to evaluate the oxygen radical release, reduction of extracellular cytochrome *c* was measured spectrophotometrically (Doke, 1983) in leaves of Col-0 and *hxc1* infiltrated with the strains 147 and 8004 (Fig. 5). In control leaves (infiltrated with water), production of O_2^- could be measured 1 h after mock inoculation in Col-0 and *hxc1* and did not increase over the experiment (data not shown). In Col-0 leaf discs infiltrated with strain 147, a transient burst of O_2^- release was measured 3–5 hpi (Fig. 5). At these time points a slightly delayed but more substantial O_2^- release could be detected in *hxc1* leaf discs. This O_2^- release increased until 10 hpi, whereas in Col-0, no significant O_2^- release could be detected any longer. When infiltrating Col-0 and *hxc1* leaves with the virulent strain 8004, a 2-h delay was observed before being able to measure O_2^- release values equivalent to the highest values obtained in Col-0 during the incompatible interaction. The O_2^- production increased further until 10 hpi, reaching rates comparable with those monitored in *hxc1* leaf discs infiltrated with *Xcc147*.

Defence gene induction in the hxc1 *mutant*

Infection of *Arabidopsis* with *Xcc147* results in the activation of different defence-related genes (Lummerzheim *et al.*, 1993),


A
B
C
D
E
F
N
G
H
I

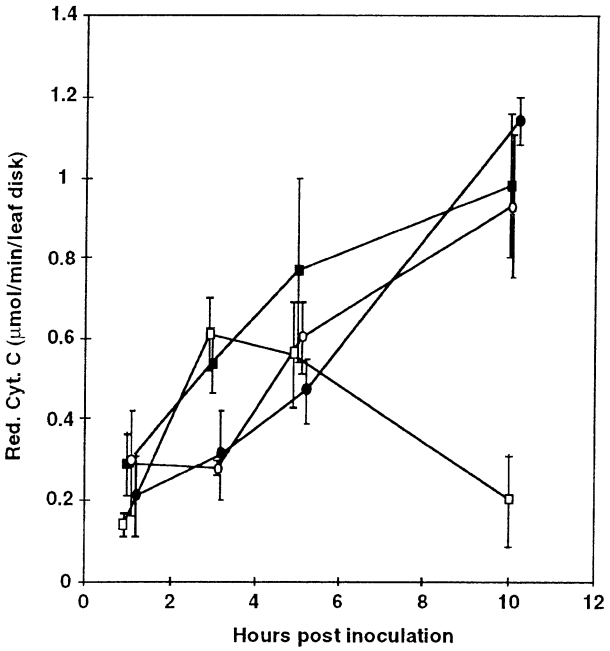


Fig. 5 O_2^- generation in *hxc1* and the wild-type after inoculation with different strains of *Xcc*. Leaf discs of Col-0 (open symbols) and *hxc1* (closed symbols) were infiltrated with cytochrome *c* solution, at different times after inoculation with *Xcc147* (squares) and 8004 (circles), and the OD was measured at 550 nm, as described in Experimental procedures. The results presented are the average of three independent experiments. Water controls were performed in parallel, and reduction of cytochrome C could be detected in Col-0 and *hxc1* 1 hpi (0.20 mmol/min/leaf disc in Col-0; 0.33 mmol/min/leaf disc in *hxc-1*), but did not increase over the experiment (0.21 mmol/min/leaf disc in Col-0; 0.12 mmol/min/leaf disc in *hxc-1*, 10 hpi).

which can at least in some cases be correlated with the action of plant hormones such as salicylic acid (SA), jasmonate (JA) and ethylene (ET). To analyse further the phenotype of the *hxc1* mutant, the expression of the following defence genes was analysed in plants inoculated with *Xcc147* or water, by quantitative RT-PCR (Fig. 6): *ICS1* (isochorismate synthase 1), a gene involved in SA biosynthesis (Wildermuth *et al.*, 2001); *PR1*, the expression of which is dependent on SA and the SA signalling pathway; *ACS2* (ACC synthase 2), a gene involved in the biosynthesis of ethylene (Wang *et al.*, 2002); and *PDF1-2* and *PR3*, the expression of which depends on the ET/JA signalling pathway (Thomma *et al.*, 1998, 1999). In wild-type and mutant plants infiltrated with water, the expression of these defence genes remains at a basal level; in contrast, in the wild-type plants inoculated with *Xcc147*, expression of these markers was strongly induced, with different kinetics (Fig. 6). In the mutant, the defence genes were still induced but their expression profile was altered. The *ICS1* gene was induced in the mutant with the same timing and the same amplitude as in the wild-type, but its transcript levels declined far more rapidly in the mutant than in the wild-type. The *PR1* gene, located downstream of SA production, appeared to be expressed at lower levels in the *hxc1* mutant, although the overall kinetics remained similar. The same results were obtained for another SA-inducible *PR* gene, *PR5* (data not shown). This indicates that the *hxc1* mutation affects components of the SA signalling pathways located upstream of SA production. Interestingly, whereas *PDF1-2* induction, which occurs late in the interaction, was reduced in the mutant, expression of *PR3* (Fig. 6) and *PR4* (data not shown), which have been shown to be co-regulated with *PDF1-2* by jasmonate and ethylene (Thomma *et al.*, 1999), is not affected in the mutant. Finally, expression of *ACS2*, which is involved in the biosynthesis of ethylene, is significantly up-regulated in the mutant. In summary, *hxc1* mutation clearly affects not only SA signalling, but also jasmonate/ethylene signalling in a complex way.

DISCUSSION

This paper describes a novel *Arabidopsis* mutant, *hxc1*, that was identified by screening for altered HR to an avirulent strain of *Xanthomonas campestris* pv. *campestris*. The *hxc1* mutant appears morphologically normal in the absence of pathogens and no spontaneous cell death could be observed macroscopically (data not shown). In response to inoculation by *Xcc147*, *hxc1* displays a complex pathophenotype, because it shows sensitivity to the bacterial pathogen at a very early stage, which rapidly evolves into a severe disease. Genetic analysis of the mutant *hxc1* indicates that the mutation is recessive, affects a single nuclear locus and is not allelic to the previously identified mutation *hxc2* and to the susceptibility locus of the ecotype Sf-2. *In planta* growth measurements of *Xcc147* show that symptoms in *hxc1* are accompanied by increased multiplication of the pathogen. These data indicate that the mutant does not just mimic the disease response, such as the lesion mimic mutants belonging to the propagation class (Dietrich *et al.*, 1994), but has lost resistance to *Xcc147*. Interestingly, the *hxc1* mutation acts very specifically. Mutant plants display a pathophenotype identical to that observed in the wild-type with several extensively characterized avirulent and virulent bacteria, the only exception

Fig. 4 Microscopic analysis of leaf sections of *hxc1* after inoculation with *Xcc147*. Semithin cuts of Col-0 (A, C, E, G) and *hxc1* (B, D, F, H) leaves, 12 (A, B), 36 (C, D) and 72 h (E–H) after infiltration inoculation with *Xcc147* (A–F, and H) and *Xcc* 8004 (G). A semi-thin cut of a Col-0 healthy leaf is shown as a control (I). Arrows indicate cells whose plasmamembrane peels off from the cell wall in A, B, D and F; at bacterial clusters in G and H; in E the arrow indicates a vascular bundle. Asteriks indicate the chloroplasts, moving away from the plasma membrane into a more densely staining cytoplasm. Triangles indicate cells undergoing a cytoplasmic collapse. N, necrotic cells.

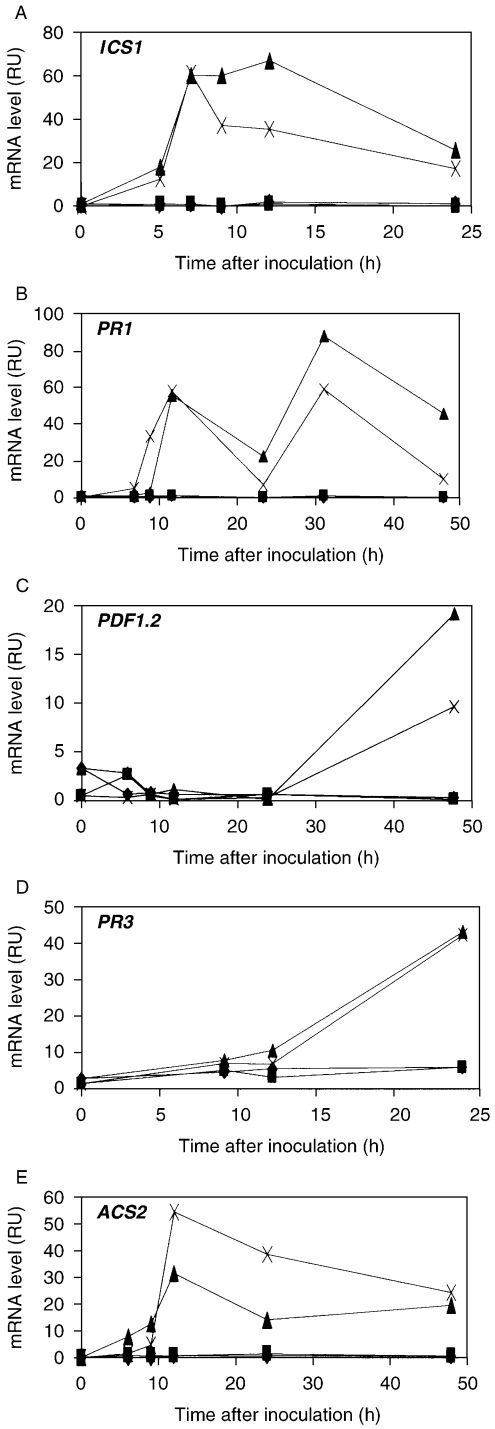


Fig. 6 Defence gene expression in wild-type and *hxc1* mutant plants after inoculation with *Xcc147*. Transcript levels of *ICS-1*, *PR-1*, *PDF1-2*, *PR3* and *ACS2* were determined by quantitative PCR, in plants inoculated with *Xcc147* (5×10^7 cfu/mL) or water (symbols: diamonds—Col-0 treated with water, squares—*hxc1* treated with water, triangles—Col-0 inoculated with *Xcc147*, crosses—*hxc1* inoculated with *Xcc147*). See Experimental procedures for further details.

being the response to *P. s. tomato/avrRpm1*. Finally, the mutation causes an attenuation of expression of several defence markers regulated through different signalling pathways.

All these data allow us to gain an insight into the position of the *hxc1* mutation in the disease resistance signalling pathways so far identified in *Arabidopsis*. *HXC1* is required for recognition of the *Xcc* isolate 147 but it is not identical to the *R* gene governing resistance to this bacterium. It is rather an early component of the signalling pathway leading to resistance, and resembles in this the previously described, non-allelic *HXC2* gene. Our results also suggest that *HXC1* is different from the genes *RXC-2*, *3* and *4*, which condition resistance to *Xcc* strain 2D520 (Buell and Sommerville, 1997) because (i) different mechanisms of resistance are involved in response to *Xcc*2D520 and 147 (absence of symptom and hypersensitive response, respectively), and (ii) the *hxc1* mutant is not affected in its response to *Xcc*2D520. The *hxc1* mutation was found to act very specifically and does not resemble any of the known loci required for *R*-gene-dependent responses. Tests with virulent *P. s. tomato* DC3000 and *Xcc*8004 indicate that it is not a component of the basal resistance response, and experiments with *P. s. tomato* strain DC3000 carrying various *avr* genes demonstrated that the function of any of the corresponding *R* genes was not impaired (Fig. 3), with the only exception being *RPM1*. In response to DC3000/*avrRpm1*, we observed repeatedly a partial but significant loss of resistance that was similar or slightly weaker than the reduction of resistance observed in *ndr1-1*. *ndr1-1* is also known to impair RPM1 function only partially, but whereas *ndr1* plants showed strong and rapid development of chlorosis in the interaction with DC3000/*avrRpm1*, *hxc1* at a similar bacterial titre showed only moderate disease symptoms. We suggest that the *hxc1* mutation affects a common component of the signalling pathways leading to resistance to *P. s. tomato/avrRpm1* and *Xcc147.* Recently, mutations (*lra2*) in a single *Arabidopsis* cytosolic HSP90 isoform (HSP90.2) have been shown to compromise the function of RPM1, and not that of related disease resistance proteins (Hubert *et al.*, 2003). *lra2*, similarly to *hxc1*, presents only a partial loss of resistance to DC3000/*avrRpm1*, and is not affected in basal resistance against virulent pathogens, suggesting that *hxc1* and *lra2* might affect the same signalling component. However, the *RPM1*-mediated HR is severely attenuated in *lra2*, but does not seem to be compromised in *hxc1* as in *ndr1*. Experiments to investigate these hypotheses are under way.

The *hxc1* mutation affects resistance to *Xcc* in a pleiotropic way, suggesting that it plays a role in the regulation of pathogen-induced defence responses, rather than acting directly by pathogen containment. This hypothesis is supported by our observations at microscopic, biochemical and molecular levels. Microscopic observation of leaf sections at different stages of the infectious process clearly demonstrates that the mutant is no longer able to mount an HR and shows a susceptible phenotype

in response to *Xcc147*. Measurement of early production of reactive oxygen intermediates (ROI) in the mutant vs. the wild-type is in line with these data. During plant–pathogen interactions, ROI may fulfil multiple physiological functions. They can act directly as toxic defence agents against pathogens (Levine *et al*., 1994), play a role in peroxidase-catalysed cross-linking of cell wall polymers (Brisson *et al*., 1994), or serve as inducers of various defence reactions (Lamb and Dixon, 1997). ROI also could play an important signalling role in the initiation of the HR (Torres *et al*., 2002). It has indeed been widely observed that HR is preceded by a rapid outburst of O_2^-, H_2O_2 and OH (Doke, 1983; Keppler *et al*., 1989; Levine *et al*., 1994). In our experiments, a significant increase in O_2^- production was not only observed in the incompatible interaction between 3 and 5 hpi, but also during the compatible interaction, starting at 6 hpi. Surprisingly, almost no data are available about AOS (active oxygen species) generation during a compatible interaction, probably owing to the experimental setup using very time-limited experiments (latest time point 4–6 hpi). The delayed but substantial O_2^- production measured can be explained by the fact that O_2^- release could be a consequence and not a cause of plant cell membrane disruption. At early time points (3–5 hpi) *hxc1* produced an O_2^- release almost as large as the transient O_2^- burst observed in the resistant wild-type Col-0. However, unlike Col-0, O_2^- production in *hxc1* increased over time reaching, at 10 hpi, the values that were otherwise only obtained during a compatible interaction at that time point. This O_2^- production profile of *hxc1* can be interpreted either (1) as the addition of an early defence response (the HR) with the establishment of a compatible interaction, or (2) as the consequence of an enhanced susceptibility to *Xcc147*, as compared with the wild-type compatible interaction Col-0/*Xcc* 8004. The first hypothesis is supported by the observation that a reduced SA-response has been found in the *hxc1* mutant, which could lead to a less active hypersensitive/defence response, and consequently to enhanced susceptibility. However, the up-regulated expression of *ACS2*, encoding an enzyme involved in ethylene biosynthesis, without (or with little) alteration of expression of defence markers for this pathway, is in favour of the second hypothesis.

The signalling pathways that lead to disease resistance are complex. Previous work had established that some but not all *R* genes require salicylic acid accumulation and NPR1 function (Glazebrook, 2001). ET- and JA-dependent signals also can influence *R* gene function (Clarke *et al*., 2000). The data reported in here are in favour of an alteration of SA biosynthesis and related defence by *hxc1*.

Surprisingly, alteration of the SA pathway in *hxc1* results in the decrease not only in *PR1* expression, but also in *PDF1-2* gene expression, suggesting that the well-described antagonism between SA- and JA/ET defence pathways (Clarke *et al*., 2000; Gupta *et al*., 2000; Jirage *et al*., 2001) is no longer operating in *hxc1*. In addition, *PR3* and *PR4*, two genes regulated through ET/JA pathways, are not affected in *hxc1*, demonstrating the complexity of regulatory crosstalk between these signalling pathways (Kunkel and Brooks, 2002). Transcriptome analyses suggest that SA and JA/ET pathways can act together (Glazebrook *et al*., 2003; Schenk *et al*., 2000), some genes being induced by both pathways. However, *PDF1-2* do not belong to this class of genes, suggesting that in *hxc1*, this negative crosstalk between the two pathways is no longer functional, or that *hxc1* acts upstream in the regulatory pathway. It also suggests that *PR3* and *PR4* would belong to a set of genes differentially regulated in this context.

An even more surprising result was the observation that *ACS2* is up-regulated in the mutant. Ethylene production as a response to stress has been observed many times, and more particularly in response to infection by pathogens (Pegg, 1976). This hormone is thought to be a modulator of cell death processes, such as senescence, or those observed during interactions with pathogens (Greenberg, 1997). In this latter case, its role was not only demonstrated in the activation of disease resistance mechanisms, but also in disease lesion development (Lund *et al*., 1998). The rate-limiting step in ethylene production is ACC synthase (*ACS*), which is encoded by a multigene family in *Arabidopsis* (Liang *et al*., 1992), and transcription of *ACS* genes is precisely regulated (Wang *et al*., 2002). We chose to analyse the expression pattern of *ACS2*, a member of the multigene family which is known to be expressed in response to diverse stresses (Liang *et al*., 1992) and also in response to elicitor treatment (Oetiker *et al*., 1997). Taking in account that the expression of *ACS2* is expressed at higher levels during compatible interactions with *Xcc* strain 8004, than in response to *Xcc147* (data not shown), this enhanced expression can be interpreted as an amplification of the expression of the symptoms.

Taken together, these results suggest that the ET biosynthesis and signalling pathways are affected in the mutant, in addition to the SA pathway. Measurement of SA, JA and ET in the mutant and the wild-type during the interaction with *Xcc147* would certainly help the interpretation of this complex defence phenotype.

The biochemical and molecular markers used to characterize *hxc1* underline the complexity of this novel defence mutant and support the hypothesis of a mutation affecting a key component activated during the first steps of the plant defence response leading to resistance. This unique phenotype is not entirely unexpected in light of the recent data unveiling the complexity of the *R*-gene-mediated signal transduction pathways.

EXPERIMENTAL PROCEDURES

Plant growth conditions

Arabidopsis thaliana plants were maintained as described previously by Lummerzheim *et al*. (1993). *pad4-1* and *ndr1-1*

plants were obtained from the NASC stock centre. Four- to 5-week-old plants were used for all experiments unless otherwise stated.

Bacterial strains

Bacterial strains have been described elsewhere: *Xcc* strain 147 by Lummerzheim *et al.* (1993), *Xcc* strain 8004 by Daniels *et al.* (1984), *Pseudomonas syringae* pv. *maculicola* strain m2, m4, and m4/*avrRpm1* by Debener *et al.* (1991), *Pseudomonas syringae* pv. *tomato* strain DC3000 and DC3000/*avrB* by Staskawicz *et al.* (1984), DC3000/*avrRpt2* by Whalen *et al.* (1991), DC3000/*avrPphB* by Simonich and Innes (1995) and DC3000/*avrRps4* by Hinsch and Staskawicz (1996).

Inoculation of plants and *in planta* bacterial growth measurements

Arabidopsis leaves were infiltrated with bacteria as described by Lummerzheim *et al.* (1993). The *Xanthomonas* strains were prepared as described by Lummerzheim *et al.* (1993), except that the overnight cultures were washed and adjusted to the desired OD_{600} of 0.1, which was equivalent to 10^8 cfu/mL. Plants grown in a growth chamber were hand-inoculated with a spontaneous rifampicin-resistant mutant of *Xcc147* with an inoculum concentration of 10^8 cfu/mL. The bacterial growth *in planta* was measured 0, 2, 3, 5 and 7 dpi. The leaves were washed once in 0.1% SDS and rinsed three times in distilled water. Bacterial growth was assayed by plating serial dilutions of the extracts on to bacterial growth medium. Five leaves from four plants were harvested per assay, and four replicates of each assay were performed. Each experiment was repeated twice. For the determination of *in planta* bacterial growth of *Pseudomonas* strains, four leaves of 4–6 plants per genotype were infiltrated with a bacterial suspension of 2×10^5 cfu/mL using a blunt syringae. With a cork borer, three leaf discs per plant were harvested at 0, 2 and 3 dpi and ground in 1 mL 10 mM $MgCl_2$. A series of 10-fold dilutions of this extract was prepared in 10 mM $MgCl_2$, plated on King's B medium and incubated at 28 °C for 2 days.

Genetic analysis

The mutant *hxc1*, carrying the *gl1* mutation, was back-crossed to wild-type Col-0. All F_1 plants had normal trichomes, and the F_2 progeny segregated in a ratio of three wild-type to one glabrous; segregation of *hxc1* and *gl1* was independent.

Allelism tests were performed by crossing the *hxc1* mutant with the *hxc2* mutant and the ecotype Sf-2. Individuals from F_1 progeny were scored for their mutant phenotype by infiltration inoculation with *Xcc147*.

Microscopy

Bright-field light microscopy

The leaves were infiltrated with a bacterial suspension adjusted to 5×10^7 cfu/mL. Sections of control and infected leaves were prepared and prefixed under vacuum for 30 min in 2.5% glutaraldehyde in 0.15 M sodium cacodylate buffer (pH 7.4). Further impregnation was continued at room temperature for 3 h, under constant agitation. After rinsing in cacodylate buffer (0.2 M, pH 7.4), fixation was carried out in 2% OsO_4, 0.8% $K_3Fe(CN)_6$ in cacodylate buffer for 2 h at room temperature. After washing with buffer, the samples were step-dehydrated in ethanol and embedded in Epon epoxy resin.

Transverse sections of control and infected leaves were prepared using a Micron Stemi SV 6 microtome. The 1-μm-thick sections were stained with toluidine blue O (1% in 1% borax), examined and photographed through a Carl Zeiss Jenalumar microscope.

Assay of O_2^- generation

Determination of O_2^- generated by inoculated leaf discs was performed by assaying the reduction of extracellular cytochrome *c* spectrophotometrically (adapted from Chai and Doke, 1987). Five leaves per plant were infiltrated with bacteria as described by Lummerzheim *et al.* (1993). Leaf discs (10 mm in diameter) were cut from the middle of the infiltrated area using a cork borer at 0, 1, 3, 5, 10 and 20 hpi. Ten leaf discs per assay were immediately immersed in 3 mL 0.05 M K-phosphate buffer (pH 7.8) containing 0.1 mM deferoxamine (Desferal, Ciba), and 0.1 mM ortho-phenanthroline (Sigma Chemical Co.). Cytochrome *c* at 0.02 mM (Type VI, from horse heart, Sigma Chemical Co.) was added just before vacuum infiltration. In each assay, 1 mL of the reaction solution was pipetted out, and the optical density (OD) at 550 nm was measured by a double-beam spectrophotometer (Kontron, Uvikon 810). We verified the almost linear increase in OD_{550} in our system for 10 min after initiation of the reaction (Chai and Doke, 1987). Cytochrome *c* reducing activity of the discs was expressed as micromoles reduced cytochrome *c*/disk/min by using an extinction coefficient of 21.1.

RT PCR

Total RNA was isolated from infected and control *Arabidopsis* leaves using Nucleospin RNA plant kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. cDNAs were synthesized from 5 μg of total DNA-free RNA, using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA), following the manufacturer's instructions. In order to verify homogeneous efficiency of reverse transcription, we introduced as a spike 5 fg of *in vitro* produced human nebuline RNA before reverse transcription. Quantitative PCR was performed with the

light cycler technology (Roche Diagnostics, Meylan, France) according to the manufacturer's recommendations. The primers used had the following sequences: 5′-GTCCCGAAGCTTACACATGA-3′ and 5′-GCCATACATCCAGCCTTCATCA-3′ (*nebulin*), 5′-GGAGCTACGCAGAACAACTAAGA-3′ and 5′-CCCACGAGGATCATAGTTCAACTGA-3′ (*PR1*), 5′-TCATGGCTAAGTTTGCTTCC-3′ and 5′-AATACACACGATTTAGCACC-3′ (*PDF1.2*), 5′-GCCGTCTCTGAACTCAAATCTCAA-3′ and 5′-GTTACGAGCAAGAACAACCTTGTT-3′ (*ICS1*), 5′-CGCTTGTCCTGCTAGAGGTT-3′ and 5′-GCTCGGTTCACAGTAGTCTGA-3′ (*PR3*).

ACKNOWLEDGEMENTS

We gratefully acknowledge J. L. Montillet for his suggestions and assistance in the O_2^- generation experiments, M. Nicole for his help in microscopy data interpretation, S. Camut for her technical assistance, and C. Balagué and N. Grimsley for critical reading of the manuscript. This research was supported by the Institut National de la Recherche Agronomique (INRA) (A.I.P. INRA Gènes de resistance). M.L. was supported by fellowships from the EC and INRA.

REFERENCES

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J. and Parker, J.E. (1998) Different requirements for EDS1 and NDR1 by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*. *Proc. Natl Acad. Sci. USA*, **95**, 10306–10311.

Arlat, M., Gough, C.L., Barber, C.E., Boucher, C. and Daniels, M.J. (1991) *Xanthomonas campestris* contains a cluster of *hrp* genes related to the larger *hrp* cluster of *Pseudomonas solanacearum*. *Mol. Plant–Microbe Interact.* **4**, 593–601.

Atkinson, M.M., Huang, J.S. and Knopp, J.A. (1985) The hypersensitive reaction of tobacco to *Pseudomonas syringae* pv. *pisi*. Activation of plasmalemma K^+/H^+ exchange mechanism. *Plant Physiol.* **79**, 843–847.

Brisson, L.F., Tenhaken, R. and Lamb, C. (1994) Function of oxidative cross-linking of cell-wall structural proteins in plant disease resistance. *Plant Cell*, **6**, 1703–1712.

Brown, I., Mansfield, J., Irlam, I., Conrads-Srauch, J. and Bonas, U. (1993) Ultrastructure of interactions between *Xanthomonas campestris* pv. *vesicatoria* and pepper, including immuno-cytochemical localization of extracellular polyssacharides and the *avrBS3* protein. *Mol. Plant–Microbe Interact.* **6**, 376–386.

Buell, C.R. and Sommerville, S.C. (1997) Use of *Arabidopsis* recombinant inbred lines reveals a monogenic and a novel digenic resistance mechanism to *Xanthomonas campestris* pv. *campestris*. *Plant J.* **12**, 21–29.

Chai, H.B. and Doke, N. (1987) Superoxide anion generation: a response of potato leaves to infection with *Phytophthora infestans*. *Phytopathology*, **77**, 645–649.

Clarke, J.D., Volko, S.M., Ledford, H., Ausubel, F.M. and Dong, X. (2000) Role of salicilic acid, jasmonic acid, and ethylene in *cpr*-induced resistance in *Arabidopsis*. *Plant Cell*, **12**, 2175–2190.

Daniels, M.J., Barber, C.E., Turner, P.C., Sawcyc, M.K., Byrde, R.J.W. and Fielding, A.H. (1984) Cloning of genes involved in pathogenicity of *Xanthomonas campestris* pv. *campestris* using the broad host range cosmid pLAFR1. *EMBO J.* **3**, 3323–3328.

Debener, T., Lehnakers, H., Arnold, M. and Dangl, J.L. (1991) Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance of a phytopathogenic *Pseudomonas syringae* isolate. *Plant J.* **1**, 289–302.

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A. and Dangl, J.L. (1994) *Arabidopsis* mutants simulating disease resistance response. *Cell*, **77**, 565–577.

Doke, N. (1983) Generation of superoxide anion by potato tuber protoplasts during the hypersensitive response to hyphal wall components of *Phytophthora infestans* and specific inhibition of the reaction by suppressors of hypersensitivity. *Physiol. Plant Pathol.* **23**, 359–367.

Doke, N., Miura, Y., Sanchez, L.M. and Kawakita, K. (1994) Involvement of superoxide in signal transduction: responses to attack by pathogens, physical and chemical shocks, and UV radiation. In: *Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants* (Foyer, C.H. and M.P.M., eds), pp. 177–197. London: CRC Press.

Glazebrook, J. (2001) Genes controlling expression of defense responses in *Arabidopsis*-2001 status. *Curr. Opin. Plant Biol.* **4**, 301–308.

Glazebrook, J., Chen, W., Estes, B., Chang, H.S., Nawrath, C., Metrauk, J.P., Zhu, T. and Katagiri, F. (2003) Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. *Plant J.* **34**, 217–228.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E.E., Crute, I.R., Holub, E.B., Hammerschmidt, R. and Ausubel, F.M. (1997) Phytoalexin-deficient mutants of *Arabidopsis* reveal that *PAD4* encodes a regulatory factor and that four PAD genes contribute to downie mildew resistance. *Genetics*, **146**, 381–392.

Godard, F., Lummerzheim, M., Saindrenan, P., Balagué, C. and Roby, D. (2000) *hxc2*, an *Arabidopsis* mutant with altered hypersensitive response to *Xanthomonas campestris* pv. *campestris*. *Plant J.* **24**, 749–761.

Greenberg, J.T. (1997) Programmed cell death in plant pathogen interactions. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 525–545.

Greenberg, J.T., Guo, A., Klessig, D.F. and Ausubel, F.M. (1994) Programmed cell death in plants: a pathogen-triggered response activated coordinately with multiple defence functions. *Cell*, **77**, 551–563.

Gupta, V., Willits, M.G. and Glazebrook, J. (2000) *Arabidopsis thaliana EDS4* contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jamonic acid signaling by SA. *Mol. Plant–Microbe Interact.* **13**, 503–511.

Hammond-Kosack, K.E. and Jones, J.D.G. (1997) Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 575–607.

Hinsch, M. and Staskawicz, B.J. (1996) Identification of a new *Arabidopsis* disease resistance locus *RPS4*, and cloning of the corresponding avirulence gene, *avrRps4*, from *Pseudomonas syringae* pv. *pisi*. *Mol. Plant–Microbe Interact.* **9**, 55–61.

Hubert, D.A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K. and Dangl, J.L. (2003) Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. *EMBO J.* **22**, 5679–5689.

Jirage, D., Zhou, N., Cooper, B., Clarke, J.D., Dong, X. and Glazebrook, J. (2001) Constitutive salicylic acid-dependent signaling in cpr1 and cpr6 mutants requires PAD4. *Plant J.* **26**, 395–407.

Jones, S.B. and Fett, W.F. (1985) Fate of *Xanthomonas campestris* infiltrated into soybean leaves: an ultrastructural study. *Phytopathology*, **75**, 733–741.

Keppler, L.D., Baker, C.J. and Atkinson, M.M. (1989) Active oxygen production during bacteria induced hypersensitive reaction in tobacco suspension cells. *Phytopathology*, **79**, 974–978.

Klement, Z. (1982) Hypersensitivity. In: *Phytopathogenic Procaryotes* (Mount, M.S. and Lacy, G.H., eds), pp. 149–177. New York: Academic Press.

Kunkel, B.N. and Brooks, D.M. (2002) Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant. Biol*. **5**, 325–331.

Lamb, C. and Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 251–275.

Levine, A., Tenhaken, R., Dixon, R. and Lamb, C. (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*, **79**, 583–593.

Liang, X., Abel, S., Keller, J.A., Shen, N.F. and Theologis, A. (1992) The 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA*, **89**, 11046–11050.

Lorrain, S., Vailleau, F., Balagué, C. and Roby, D. (2003) Lesion mimic mutants: keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci.* **8**, 263–271.

Lummerzheim, M., de Olivera, D., Castresana, C., Miguens, F.C., Louzada, E., Roby, D., Van Montagu, M. and Timmerman, B. (1993) Identification of compatible and incompatible interactions between *Arabidopsis thaliana* and *Xanthomonas campestris* pv. *campestris* and characterization of the hypersensitive response. *Mol. Plant–Microbe Interact.* **6**, 532–544.

Lund, S.T., Stall, R.E. and Klee, H.J. (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. *Plant Cell*, **10**, 371–382.

Mansfield, J.W. and Richardson, A. (1981) The ultrasructure of interactions between *Botrytis* species and broad bean leaves. *Physiol. Plant Pathol.* **19**, 41–48.

Oetiker, J.H., Olson, D.C., Shiu, O.Y. and Yang, S.F. (1997) Differential induction of seven 1-aminocyclopropane-carboxylate synthase genes by elicitor in suspension cultures of tomato (*Lycopersicon esculentum*). *Plant. Mol. Biol.* **34**, 275–286.

Parker, J.E., Barber, C.E., Fan, M.J. and D.M. (1993) Interaction of *Arabidopsis thaliana* with *Xanthomonas campestris*. In: *Arabidopsis Thaliana as a Model for Plant–Pathogen Interactions* (Davis, K.R. and Hammerschmidt, R., eds), pp. 61–72. St Paul: APS Press.

Pegg, G.F. (1976) *The Involvement of Ethylene in Plant Pathogenesis*. Heidelberg: Springer Verlag.

Rogers, E.E. and Ausubel, F.M. (1997) *Arabidopsis* enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in *PR-1* gene expression. *Plant Cell*, **9**, 305–316.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. and Manners, J.M. (2000) Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. *Proc. Natl Acad. Sci. USA*. **97**, 11655–11660.

Shirasu, K. and Schulze-Lefert, P. (2003) Complex formation, promiscuity and multi-functionality: protein interactions in disease-resistance pathways. *Trends Plant Sci.* **8**, 252–258.

Simonich, M.T. and Innes, R.W. (1995) A disease resistance gene in *Arabidopsis* with specificity for the *avrPph3* gene of *Pseudomonas syringae* pv. *phaseolicola*. *Mol. Plant–Microbe Interact.* **8**, 637–640.

Staskawicz, B.J., Dahlbeck, D. and Keen, N.T. (1984) Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. *Proc. Natl Acad. Sci. USA*, **81**, 6024–6028.

Thomma, B.P.H.J., Eggermont, K., Penninckx, I.A.M.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A. and Broekaert, W.F. (1998) Separate jasmonate-dependent and salicylic-dependent defense response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl Acad. Sci. USA*, **95**, 15107–15111.

Thomma, B.P., Eggermont, K., Tierens, K.F. and Broekaert, W.F. (1999) Requirement of functional ethylene-insensitive 2 gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea*. *Plant Physiol.* **121**, 1093–1102.

Thomma, B.P., Penninckx, I.A., Broekaert, W.F. and Cammue, B.P. (2001) The complexity of disease signaling in Arabidopsis. *Curr. Opin. Immunol.* **13**, 63–68.

Tierens, K.F.M.J., Thomma, B.P.H.J., Bari, R.P., Garmier, M., Eggermont, K., Brouwer, M., Penninckx, I.A.M.A., Broeckaert, W.F. and Cammue, B.P.A. (2002) *Esa1*, an *Arabidopsis* mutant with enhanced susceptibility to a range of necrotrophic fungal pathogens, shows a distorted induction of defence responses by reactive oxygen generating compounds. *Plant J.* **29**, 131–140.

Torres, M.A., Dangl, J.L. and Jones, J.D.G. (2002) *Arabidopsis* $gp91^{phox}$ homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc. Natl Acad. Sci. USA*, **99**, 517–522.

Tsuji, J., Sommerville, S.C. and Hammershmidt, R. (1991) Identification of a gene in *Arabidopsis thaliana* that controls resistance in *Xanthomonas campestris* pv. *campestris*. *Physiol. Mol. Plant Pathol.* **3**, 57–65.

Vogel, J. and Somerville, S. (2000) Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. *Proc. Natl Acad. Sci. USA*, **97**, 1897–1902.

Wang, K.L.C., Li, H. and Ecker, J.R. (2002) Ethylene biosynthesis and signaling networks. *Plant Cell*, Suppl., S131–S151.

Warren, R.F., Merritt, P.M., Holub, E. and Innes, R.W. (1999) Identification of three putative signal transduction genes involved in *R* gene-specified resistance in *Arabidopsis*. *Genetics*, **152**, 401–412.

Whalen, M.C., Innes, R.W., Bent, A.F. and Staskawicz, B.J. (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *Plant Cell*, **3**, 49–59.

Wildermuth, M.C., Dewdney, J., Wu, G. and Ausubel, F.M. (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*, **414**, 562–565.

Zhou, J., Loh, Y.-T., Bressan, R.A. and Martin, G.B. (1995) The tomato gene *Pti1* encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. *Cell*, **83**, 925–935.