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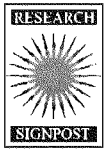

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1 Physiology and defence mechanisms to pathogens in tropical woody plants

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Abstract

Resistance of cotton (Gossypium hirsutum) to Xanthomonas campestris pv. malvacearum (Xcm) and of coffee (Coffea arabica) to the orange rust fungus (Hemileia vastatrix) and root-knot nematodes (Meloidogyne sp.) is characterized by a rapid hypersensitive cell death at the infection sites. To elucidate some of the mechanisms underlying these two plants defence reactions, molecular studies were undertaken by different strategies (candidate gene and global approaches). Two gene families, lipoxygenase

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and peroxidase, were studied for their relationships with cotton defence to Xcm and the role they may have in the resistance strategy was physiologically investigated. A catalogue of EST involved in the coffee/rust interaction was also generated from cDNA subtractive libraries. Expression analyses lead to the identification of genes showing enhanced transcript accumulation in the early stages of coffee resistance to rust and nematodes, providing new insights into tropical woody plants responses to pathogens.

Introduction

Among tropical plants of agronomic interest, coffee (*Coffea* sp.) and cotton (*Gossypium hirsutum* L.) are two of the most valuable world's traded commodities, contributing to several billions US dollars annually. Production is distributed out of more than 70 tropical and subtropical developing countries where these cash crops are considered as an important currency source, but also as a favourable social stability factor [1]. The main cultivated varieties of these two crops are highly susceptible to several diseases and pathogens. For instance, cotton bacterial blight caused by *Xanthomonas campestris* pv. *malvacearum* (*Xcm*) is an important and potentially destructive disease affecting cotton yield losses in excess of 20%. Similarly, the orange rust fungus (*Hemileia vastatrix*) and root-knot nematodes (*Meloidogyne sp.*) are considered as the more important agronomic constraints in the major coffee-growing areas (Brazil, Latin America and Asia), regarding the substantial losses of vigour and yield they can cause in *C. arabica* plantations (70% of the production worldwide) [2].

Limitation of these pests and diseases in woody crops has generally been based on toxic, costly, and poorly effective chemical treatments, now widely restricted due to increasing environmental concerns. As an alternative, resistant varieties have been developed by traditional breeding approaches based on the exploitation of natural resistance sources. Coffee and cotton resistance to *H. vastatrix*, *M. exigua* and *Xcm*, respectively, are conferred by major genes [3, 4, 5, 6] whose phenotypic expression is the so-called hypersensitive response (HR), mediated by the gene-for-gene model [7].

The HR, which is activated early during the infection process, is characterized by the formation of necrotic lesions in the region of pathogen attack resulting from a programmed cell death directly responsible for the pathogen confinement and growth limitation. It is associated with activation of defence mechanisms in the dying area as well as in the surrounding tissues such as changes in protein phosphorylation, generation of reactive oxygen species (the oxidative burst), modification of ion fluxes, cell wall reinforcement by deposition of lignin and callose, lipid peroxidation, synthesis of antimicrobial molecules (phytoalexines), production of signalling hormones, and activation of pathogenesis-related (PR) genes [8, 9, 10]. Understanding of the cellular and

molecular mechanisms involved in plant defence reactions provides others possibilities to develop new management strategies, in addition to the exploitation of resistance genes in field. To this purpose, studies have been initiated to characterize physiological events triggered by the plant/pathogen recognition and identify genes specifically involved in the defence response of cotton to bacterial blight and of coffee to rust and nematodes.

So far, two different strategies have been used: a candidate gene approach for the cotton/*Xcm* interaction and a global strategy concerning the resistance of coffee to rust and nematodes. The candidate gene strategy focus on genes which have already been identified in other plant/pathogen interactions, since defence mechanisms and molecular processes underlying the HR have been extensively investigated in model species (*Arabidopsis thaliana*, rice, tobacco ...), but poorly in tropical woody plants [11, 12, 13]. Thus, the choice of a suitable candidate gene is based on the *a priori* belief that it could play a relevant role in the studied pathosystem and depends on the degree of homology with known-function sequences. The global strategy, (also called without *a priori* approach), consists in the establishment of a catalogue of genes implicated in resistance mechanisms. Construction of cDNA libraries corresponding to diverse interactions, systematic ESTs sequencing, and differential screening of the libraries foster the isolation of clones directly involved in the plant defence responses.

Candidate gene strategy: Cotton resistance to *Xanthomonas campestris*

Resistance of cotton plants (*Gossypium hirsutum* L.) challenged by the bacterial pathogen *Xcm* responds hypersensitively [14]. The Réba B50 cultivar carrying the B_2B_3 blight resistance genes which confer immunity to the avirulent race 18 of *Xcm* [15] was used to characterize early events involved in the establishment of cotton HR to the parasite [16]. Two gene families, lipoxygenase and peroxidase, were studied for their relationships with plant defence. The role these proteins may have in the resistance strategy of cotton was physiologically investigated.

1. Lipoxygenases

Dramatic damage undergone by membranes during HR was correlated with production of poly-unsaturated fatty acid (PUFA) hydroperoxides and associated with active oxygen species (AOS) generation. Induction of alteration of membrane structure by lipoxygenases (LOX) during HR was suggested as an alternative hypothesis to the AOS role for lipid peroxidation [17]. Activation of LOX was demonstrated in several plants both during compatible and incompatible interactions.

Accumulation of hydroperoxydes correlated with HR symptoms

Microscopical investigations of infected tissues revealed irreversible membrane disorganization during HR of the cotton cultivar Reba B50 to *Xcm* race 18 [18, 19], suggesting the existence of a causal link between membrane lipid peroxidation and hypersensitive cell death. Based on a detailed biochemical and molecular description [20], investigations on cotton cotyledon tissues undergoing HR discriminated between a free radical-mediated process, and a LOX pathway, i.e. non-specific *vs.* specific peroxidation, respectively. Hypersensitive death induced by the avirulent race 18 from *Xcm* is correlated with a massive LOX-mediated production of 9S-hydroperoxides, tissue dehydration and apparition of HR lesions on infected leaves at 24 hours post-infection (hpi). Regiospecificity and enantioselectivity of LOX activity characterized in cotyledon extracts at 24 hpi was in accordance with its involvement in the production of the 9S-hydroperoxides. Upstream from this LOX-mediated lipid peroxidation, a narrow peak of intense LOX activity was observed at 9 hpi (Figure 1-A). Analysis of the corresponding extracts by electrophoresis on IEF gels revealed the presence of different LOX isoforms. The activity of the acidic isoforms (particularly the pI 4.6 one) was correlated with induction of the HR, although it was slightly induced at 9 hpi also. The increase

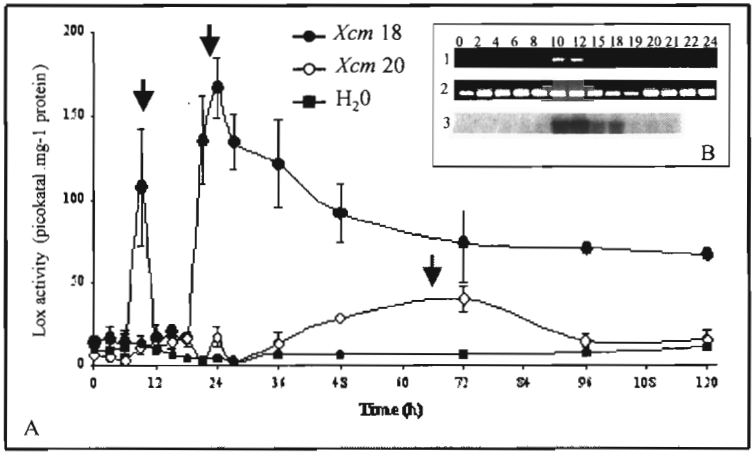

Figure 1. Activation of cotton lipoxygenase during the HR to *Xcm*. A: Time course of lipoxygenase activity in *Xcm*-infected cotton cotyledons showing two peaks in resistant plants (black arrows) and one peak, later and weaker, in susceptible plants (grey arrow); controls were performed on H_2O-infiltrated cotyledons. **B**: Transcriptional activity of cotton *GhLox1* gene during the HR (1: RT-PCR with *GhLox1* specific primers; 2: RT-PCR with actin primers; 3: Northern blot with *GhLox1* as probe).

in pl 4.6 LOX isoform activity and the appearance of a pl 7.4 band paralleled the increase in 9S-LOX activity at 9hpi, suggesting this band to likely have also a 9S-LOX specificity.

Transcription of LOX genes

A cotton LOX gene (*GhLox1*) was cloned from cotyledon tissues. Its expression during HR was studied by semi-quantitative RT-PCR and Northern blot (Figure 1-B). *GhLox1* transcripts were detected only during the incompatible reaction, between 6 and 18 hpi by RT-PCR, with a higher accumulation at 12 hpi, and between 10 and 18 hpi by Northern blot. The molecular determinants of the specificity of *GhLox1* were identified in the sequence and characterized this LOX gene as a 9-LOX. These results showed the correlation between LOX gene transcription and LOX activity.

Taken together, the present work gives evidence for a crucial role of 9S-LOX-mediated lipid peroxidation in the execution of HR cell death in cotton. Through which signalling pathway this LOX-dependent mechanism operates still remains debatable, but salicylic acid, methyl-jasmonate (MeJA), and hydrogen peroxide (H_2O_2) are putative candidates to be involved in LOX induction. In addition, the early and narrow production of MeJA 2 hpi during cotton HR indicated that other LOX genes (i.e. 13-LOX) could be associated with resistance.

2. Peroxidases

Peroxidases (POD; EC 1.11.1.1.7), a group of haem-containing glycosylated proteins, are known to be activated in response to pathogen attacks [21]. Several roles have been attributed to plant POD in host/pathogen interactions [22], including involvement in the HR. These proteins were studied for their potent activities during resistance of cotton to the bacterial blight.

Peroxidases are associated with the oxidative burst in cotton

The oxidative burst in plants infected by incompatible pathogens is a key early event in the expression of resistance [23, 24, 25]. In cotton, the oxidative burst generated AOS during resistance, including anion superoxides ($O_2^{\cdot-}$) and H_2O_2, resulting from dismutation of $O_2^{\cdot-}$ by a MnSOD [59]. Several lines of evidences strongly suggested that a wall-bound peroxidase was involved in the production of $O_2^{\cdot-}$, 3 hpi (Figure 2-A) [26]. Increase in activity of cationic POD isoforms (pI 9-9.4), positive effects of POD inhibitors on $O_2^{\cdot-}$ generation, immunolocalization of POD, and analysis of POD genes transcriptional activity reinforced the idea of a strong role of POD in the cotton burst to *Xcm* [27].

Changes in POD activity during infection was assessed spectrophotometrically according to time course of infection. It increased significantly during the

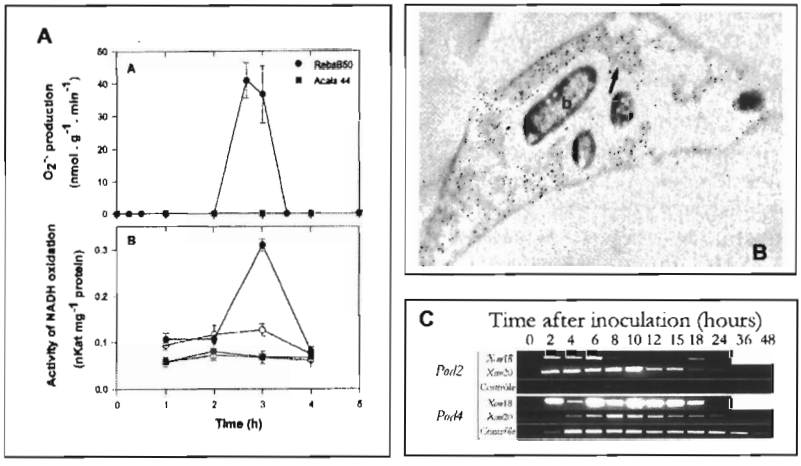

Figure 2. The oxidative burst in *Xcm* race 18-infected cotton cotyledons. A: Production of superoxide anions 3 hpi associated with NAD-oxidation; **B**: Immunolocalization of peroxidases 3 hpi, close to the bacteria, in the intercellular areas of exhibiting-HR cells; **C**: expression of POD genes assessed by RT-PCR: *pod2* was transcripted from 2 to 8 hours during HR, while the expression of *pod4* is higher in HR cells than in susceptible infected cells.

incompatible interaction between 8 and 10 hpi, as compared to the activity in the compatible interaction (Réba B50/*Xcm* race 20) and the control. A subsequent systemic increase in POD activity was also recorded one day after bacterial treatment, both locally in non-infected areas of cotyledons, and in the whole plant [28]. The highly localized accumulation of POD proteins revealed by immunocytochemistry in cotton cells 3 hpi (Figure 2-B) [27] is consistent with diamino-benzidine cytochemistry observations showing that POD activity was confined in the apoplasm and close to the bacteria [26]. POD identified in the apoplastic washing fluid were shown to be (1) cationic, (2) responsible for the production of superoxide anions, subsequently dismutated into wall-bound H_2O_2, (3) pre-formed, since they were detected in healthy cotyledons, and (4) inducible for their $O_2^{\cdot-}$ -generating NADH-oxidase activity in non-infected tissues [26]. POD accumulation in *Xcm*-encapsulating material is a response to HR development and suggests that cotton cells challenged by the pathogen create a localized, highly toxic environment, in line with AOS production, that results in limiting bacterial growth. This confined apoplastic localization of active POD may explain why the change in activity 3 hpi was not observed spectrophotometrically, but only on IEF gels with reference to apoplastic cationic isoforms [26].

Differential expression of peroxidase genes during HR

POD genes were cloned from two cDNA libraries made at early stages (2.5 and 5.5 hpi) of the cotton incompatible reaction [27]. Seven cotton POD genes were cloned (named *pod1*, *pod2*, *pod3*, *pod4*, *pod5*, *pod6* and *pod10*), with similarities to plant class III POD [29]. Putative signal peptide cleavage sites were identified in the coding sequence of all clones and no clear peroxisomal targeting signal sequences were detected, thus suggesting that these POD are located extracellularly.

The expression profile of each cDNA clone was determined by RT-PCR using specific primers, at different pi times for incompatible, compatible reactions and water infiltration. Analysis of gene expression showed variation in transcript accumulation during both compatible (race 20) and incompatible interactions for four of these genes. *pod2* was induced by pathogen infection and weakly stimulated in the control (Figure 2-C); *pod3* was specifically down-regulated during the HR after the oxidative burst; *pod4* and *pod6* were more intensely up-regulated during disease and in the control. All these data suggest that cotton peroxidases may have various functions in the defence response to *Xcm* infections.

Recently, an extensive study on the expression of *A. thaliana* class III POD [30] suggested that POD do not fullfill similar biological roles, even sequences are similar (>70% identical). Hence, it is difficult to predict the role of a particular POD on the basis of its similarity with another known POD. One response of cotton to *Xcm* was found to be a drastic accumulation of flavonoids in walls and cytoplasm of cells undergoing HR [19], suggesting a possible relation between increased POD activity and phenol oxidation. POD were shown to catalyze H_2O_2-dependent oxidation of flavonols [31] suggesting that the flavonoid-POD reaction can function as an H_2O_2 scavenging mechanism. Consistent with observations that H_2O_2 is synthesized apoplastically and flavonoids produced abundantly in cells at the edge of lesions during cotton HR to *Xcm*, the role of POD as an oxidative damage protectant must be examined in light of the fact that flavonoids are electron donors.

Global strategy: Coffee resistance to the orange rust fungus and root-knot nematodes

1. Coffee leaf rust resistance

Resistance of *C. arabica* varieties to leaf rust caused by *H. vastatrix* is conditioned by gene-for-gene interactions [32, 6, 33]. The resistance is expressed by rapid hypersensitive cell death at the infection sites (stomata) as early as 24 hpi [6, 34]. Macroscopically, HR lesions appear as chlorotic flecks 12 days post-inoculation (dpi) [6]. Biochemical and cytological analyses of coffee leaves showed that growth of the fungus in resistant coffee plants

usually ceased in the early stages of infection process, after the formation of the first haustorium. Host cell death was associated with precocious haustoria encasement with callose and ß-1,4-glucans. In addition, a peak of phenylalanine ammonia-lyase (PAL) activity was detected 2 dpi and coincided with the early accumulation of phenolic compounds [34].

Isolation of coffee genes involved in rust resistance: Subtractive EST libraries construction

The suppression subtractive hybridization (SSH) method [35] was used to generate cDNA libraries enriched in sequences expressed in coffee leaves during the early stages of HR [36]. Several studies indicated that a high number of plant genes are transcriptionally regulated upon challenge by a pathogen [37, 38, 39] but that most of them may be common to both compatible and incompatible interactions [40]. To focus on genes strictly involved in the HR, cDNA from plants infected with an avirulent *H. vastatrix* race (incompatible interaction) were subtracted with cDNA from plants infected with a virulent *H. vastatrix* race (compatible interaction).

Twelve, 24 and 48 hours were chosen as the appropriate time-points for isolation of the RNAs used to construct the subtractive cDNA libraries. Indeed, microscopic observations of fungal development indicated that the *H. vastatrix* isolates have germinated and developed appressoria 12 hpi, then penetrated through stomata 24 hpi and finally developed haustorial mother cells 48 hpi. At this time, in the incompatible interaction, the fungus ceased its growth and death of host cells is initiated [34].

In order to obtain a catalogue of expressed genes in rust infected-coffee plants, hundreds of ESTs were generated from the subtracted libraries (GenBank accession numbers: CF588584 to CF589197). ESTs showing similarities to plant protein database entries were classified into functional categories (Figure 3). They were mainly distributed in cell signalling/communication, cell/organism defence, gene/protein expression, and metabolism classes.

The EST from the cell defence category presented homologies with proteins known to be involved in apoptosis regulation in animal cells (Beclin and macrophage migration inhibitory factor), in several oxidative pathways (cytochrome P450 and oxidoreductases), in metal homeostasis and detoxification (metallothioneins) and in response to several stresses (heat-shock proteins). ESTs with homologies to components of *A. thaliana* resistance signalisation pathways such as NDR1 (non race-specific disease resistance) [41] and DND1 (defence no death) [42] proteins were also isolated. Other clones matched proteins involved in defence reactions, such as the pathogenesis-related (PR) proteins (chitinases, ß1,3-glucanases, PR-10), the chalcone synthase and the lipoxygenase enzymes. Finally, ESTs presented similarities to proteins involved in specific resistance to pathogens (tomato Asc-1

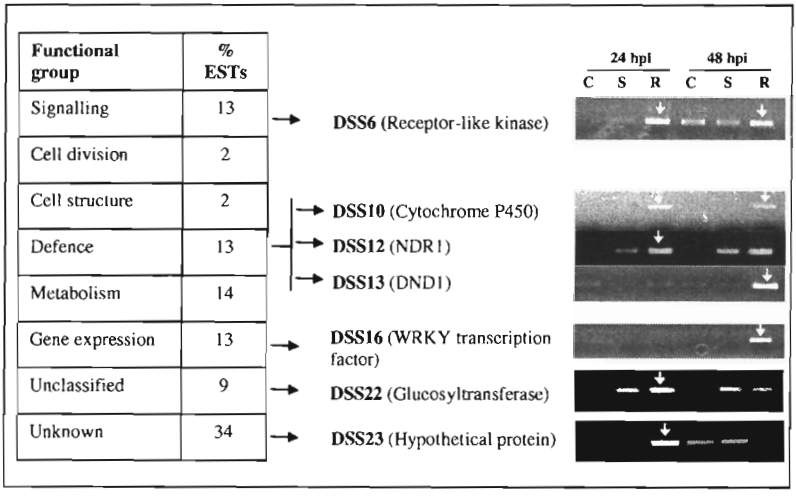

Figure 3. ESTs classification and examples of DSSs expression in *C. arabica* leaves inoculated with *H. vastatrix*. ESTs were classified into functional categories following the Expressed Gene Anatomy Database cellular role classification scheme [43]. DSSs expression analyses were performed by RT-PCR, 24 and 48 hpi. Putative function of DSSs is indicated into brackets. C: non-inoculated plants sprayed with water; S: compatible interaction; R: incompatible interaction. Arrows indicate gene differential expression in incompatible *vs.* compatible interactions. For experimental procedure details see [36].

protein, pepper importin alpha2) and disease resistance (R) proteins (CC-NBS-LRR class, tomato *Cf*-family). These data indicate the conservation in coffee plant of *R*-gene mediated resistance signalling pathways described in model plants.

In addition, a high proportion of the ESTs (34 %) matched genes with unknown function and 17 % had no or low similarity to protein database entries. These cDNA clones may represent an additional source of coffee genes potentially involved in the resistance response.

Gene expression during HR

Selection of cDNA clones specifically expressed in the resistant samples was performed by differential screening of the subtractive libraries. Clones showing a strong hybridization signal with the probes originating from the resistant samples and weak or no hybridization with those from the susceptible samples were selected and called DSS for Differentially Screened Sequences. Differential expression of DSSs during the coffee HR was confirmed by RT-PCR analyses [36]. Tested DSSs clearly showed an enhanced transcript accumulation

in inoculated plants over the time-course experiment as compared with the control plants. In addition, several of them showed induction during the incompatible interaction when comparing with the compatible interaction (Figure 3). Up-regulation of most of the genes occurred around 24 hpi. These results correlate with cytological observations of the coffee rust resistance reaction [34, 44] and suggest that induction of defence responses in cell leaves occurs early after penetration of fungal hyphae into the substomatal chamber.

The majority of the DSSs belonged to the defence, signalling or gene expression categories. DSS12 and DSS13 best matched the *A. thaliana dnd1* and *ndr1* genes. The DND1 protein is a cyclic nucleotide-gated ion channel (AtCNGC2) involved in the HR signalling pathway to *P. syringae* [42]. The NDR1 protein is a key component of the signalling pathway of many CC-NBS-LRR resistance proteins [41]. DSS16 and DSS17 putatively encoded an AP2-type and a WRKY transcription factor. A number of studies have shown the implication of several transcription factors in potentiating the plant responses to pathogen infection [45]. Particularly involved are several WRKY proteins which genes may be rapidly induced by pathogens or treatment with salicylic acid [46, 47, 48].

Although far to be exhaustive, the ESTs reported here may provide a significant set of data for improving our knowledge of coffee resistance to rust. With the availability of high-density cDNA filters technology, the expression profiles of hundreds ESTs will be monitored simultaneously in several coffee/rust interactions to help determine the mechanisms of these biological processes.

2. Coffee root-knot nematode resistance

In the main coffee-growing areas (Latin and Central America), root-knot nematodes (*Meloidogyne* sp.) are the most serious and damaging pest facing coffee production. More than 17 *Meloidogyne* species have been described on coffee but only a limited number is commonly observed (i.e. *M. exigua*, *M. incognita*, *M. arabicida* and *M. paranaensis*) [49]. Some specific nematode-resistant coffee cultivars have recently been engineered by conventional plant breeding based on the introgression of major resistance genes from wild coffee germplasms (*C. canephora*) into the cultivated varieties genomes (*C. arabica*) [50]. Resistance to *M. exigua* is controlled by a single dominant gene called *Mex1* [41] whose phenotypic expression is a HR [51]. When challenged with *M. exigua*, resistant coffee root tips exhibit the characteristic features of localized cell death, similar to those already described in other plant/nematode interactions [52, 53].

Choice of candidates from the coffee/rust EST libraries

Compared to other plant/pathogen interactions (fungal or bacterial), knowledge of mechanisms involve in resistance of woody plants to nematode

remains sketchy. The rare data available in the literature were obtained on *Mi*-resistant tomato since *Mi* is the only resistance gene to root-knot nematodes cloned up to now [54]. They result from the analysis of mutants affected in resistance [55] or from cDNA libraries differential screening [56].

As a consequence, the EST and specific genes (DSS) identified for the coffee/rust interaction represent a pool of potentially interesting candidates to be tested in the nematode interaction. In addition, they offer the opportunity to compare the implication of some genes in the defence response against two different pathogens on a same plant species. Some clones were then chosen based on their homologies with known defence genes and their expression was investigated by semi-quantitative RT-PCR.

Differential expression of selected genes upon nematode infection time-course

The RT-PCR experiments were performed on cDNAs obtained from inoculated and non-inoculated coffee root tips (0.5 cm section) of both susceptible and resistant varieties upon a *M. exigua* infection time-course (2 to 7 dpi). The four time points were conditioned by the results of cytological studies pinpointing the main stages of the HR [51].

The susceptible and resistant cultivars used in this study are both *C. arabica* varieties. Although they display a very low genetic diversity [58], they are not isogenic for the resistance gene *Mex1*. Therefore, differences in the plant genetic backgrounds (in addition to *Mex1*) may explain variations in the basal expression levels of the tested genes in the non-inoculated plants. The expression patterns of the chosen genes are showed in Figure 4. Only the genes displaying a differential expression are presented.

Expression profiles of the selected genes clusterized into 2 groups.

The EST clones, encoding respectively a chitinase, a LTP and a miraculin (LeMir), displayed a somewhat different profile between the susceptible and the resistant hosts. Their RT-PCR patterns in the inoculated susceptible plants showed a constant and linear decrease of the transcript accumulation from 2 to 5 dpi compared to the control, with an assumed return to the basal level by 7 dpi. On the opposite, the infected resistant roots expression pattern transiently peaked at the 3 dpi time point and dropped back to the non-inoculated level by 5 dpi.

Clones 1.1/4B and 16/10 encode a lipid transfer protein (LTP) and a chitinase, respectively. LTP are small, basic cystein-rich proteins proposed to have antimicrobial activities and to be involved in plant defence mechanisms. For instance, they were shown to be implicated in pepper (*Capsicum* sp.) resistance to tobacco mosaic virus [58] and grape (*Vitis* sp.) response to fungal elicitor treatments [59]. Chitinases are part of the PR proteins. They are induced by stress factors (mainly upon infections) and some isoforms show antifungal properties in *in vitro* assays. They play a role in the early stages of

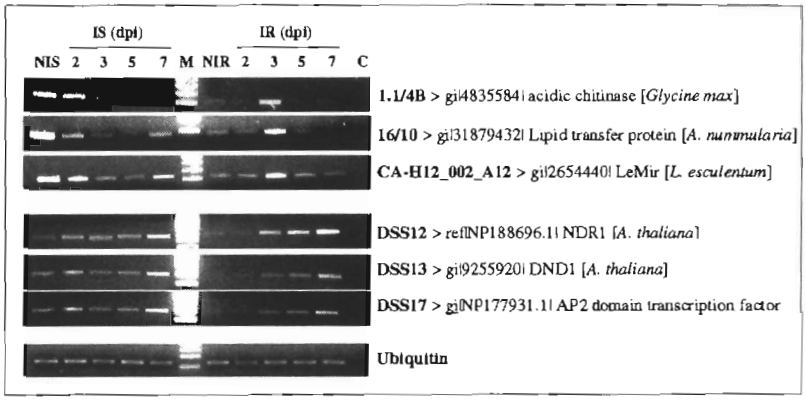

Figure 4. RT-PCR expression patterns of selected genes in *C. arabica* resistant and susceptible roots during an infection time-course with *M. exigua* (from 2 to 7 dpi). NIS: non-inoculated susceptible roots; IS: inoculated susceptible; NIR: non-inoculated resistant; IR: inoculated resistant; C: PCR negative control, M: molecular weight marker. Clone designation and best Blast homology are indicated at the right of each panel. Ubiquitin cDNA was used as internal control.

pathogenesis by releasing elicitor molecules, involved in the transfer of information about the infection [60].

CA-H12_002_A12 encodes the homologue of the tomato protein LeMir (for *L. esculentum* miraculin) shown to be induced specifically on tomato root tips tissues early after infection by *M. incognita* [61]. This protein is secreted within root exudates but its function in resistance to nematode still remains unclear. Nevertheless, its specific transient induction between 2 and 3 dpi may be linked to the penetration/migration stages of the nematodes in the intercellular spaces of the cortical parenchyma and triggering of the early events in HR cell death.

The RT-PCR profiles obtained for the DSS 12, 13 and 17 are less drastically contrasted than the EST clones ones and showed an enhanced expression in the inoculated plants compared to controls, at least at the latest time points of the infection process. The main difference between nematode resistant and susceptible plants lied in the timing and extent of the induction. Transcript accumulation in the susceptible coffee plant, challenged or not with *M. exigua*, remained constant or slightly enhanced from the control to the 5 dpi time point. A faint induction was visible around 7 dpi, whereas under similar conditions, the induction appeared after the 2 dpi time in the resistant plant and continued through the end of the experimental period.

These results are in line with data obtained with the coffee/rust interaction suggesting that DSS12, 13 and 17 encoding NDR1- and DND1-homologs

respectively, and putative AP2 transcription factor, may be key components of the HR regulation in *C. arabica*. To a larger extend, they are consistent with those reported in the literature concerning the response to fungal, bacterial or viral pathogens [62, 63, 64] reinforcing the idea of the occurrence of common points driving the HR process within plant species.

Conclusions

Cotton and coffee defence responses elicited by *Xcm*, the rust fungus and, nematodes respectively were explored by two different strategies (i.e. candidate gene and global approaches) and genes preferentially induced during HR were identified. Their characterization supplies useful data to a better understanding of resistance mechanisms in tropical plants and provides new insights into woody plants responses to biotic stresses.

Some of the identified genes show homologies with well-known components of *R*-mediated resistance in *A. thaliana* and required as regulators of basal defence in several model plants. Although their ubiquitous existence in different plant species suggests the maintenance of common signalling pathways, the role of these effectors in cotton and coffee resistance remains to be validated. So far, current investigations focus on functional analyses performed by *Agrobacterium*-mediated assays.

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