Host and Nonhost Resistance in *Medicago–Colletotrichum* Interactions

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Medicago truncatula lines resistant (A17) or susceptible (F83005.5) to the alfalfa pathogen *Colletotrichum trifolii* were used to compare defense reactions induced upon inoculation with *C. trifolii* or with the nonadapted pathogens *C. lindemuthianum* and *C. higginsianum*. Nonadapted *Colletotrichum* spp. induced a hypersensitive response (HR)-like reaction similar to the one induced during the host-incompatible interaction. Molecular analyses indicated an induction of *PR10* and chalcone synthase genes in host and nonhost interactions but delayed responses were observed in the F83005.5 line. The *clste12* penetration-deficient *C. lindemuthianum* mutant induced an HR and defense gene expression, showing that perception of nonadapted strains occurs before penetration of epidermal cells. Cytological and transcriptomic analyses performed upon inoculation of near-isogenic *M. truncatula* lines, differing only at the *C. trifolii* resistance locus, *Ct1*, with the nonadapted *Colletotrichum* strain, showed that nonhost responses are similar in the two lines. These included a localized oxidative burst, accumulation of fluorescent compounds, and transient expression of a small number of genes. Host interactions were characterized by a group of defense and signaling-related genes induced at 3 days postinoculation, associated with an accumulation of salicylic acid. Together, these results show that *M. truncatula* displays a rapid and transient response to nonadapted *Colletotrichum* strains and that this response is not linked to the *C. trifolii* resistance locus.

Nonhost resistance (NHR) is defined as the ability of a plant species to resist infection by an entire pathogen species. This form of resistance is less well understood than the one encountered in gene-for-gene systems, in which a specific plant gene confers resistance to a specific pathogen race carrying the cognate avirulence gene. NHR has been linked to multiple constitutive and inducible mechanisms that prevent entry or colonization of the plant (Thordal-Christensen 2003). In particular, NHR may result from the inability of a given pathogen species to overcome, or suppress, basal plant defense reactions triggered by the perception of pattern- or damage-associated molecular

patterns (PAMPs or DAMPs) found in microbes or initiated by their damaging effects (Zipfel 2008; Hein et al. 2009). Pathogens unable to suppress this initial line of defense are then designated as nonadapted, whereas closely related pathogen species that acquired the ability to overcome PAMP-triggered immunity are designated as adapted.

This situation is particularly illustrated in the case of biotrophic and hemibiotrophic fungal pathogens that exhibit a very narrow host range, such as *Blumeria graminis*, the powdery mildew pathogen of cereals, whose formae speciales are pathogenic only on individual plant species; for example, *B. graminis* f. sp. *graminis* on wheat and *B. graminis* f. sp. *hordei* on barley (Lipka et al. 2008). Other powdery mildew species that infect *Arabidopsis thaliana* allowed a direct comparison between defense reactions induced by adapted and nonadapted powdery mildew pathogens on this plant. It was shown that grass and legume powdery mildew pathogens induce a rapid and localized accumulation of cell wall material at the infection site and, in some cases, a hypersensitive response (HR) that restricts the development of the pathogen (Lipka et al. 2008). Mutants impaired in NHR identified PEN-1, a membrane protein implicated in the transport of vesicle required for the delivery of vesicle cargo at the cell periphery, with an essential role in NHR to powdery mildews for rapid deposition of cell-wall materials at the site of pathogen entry (Assaad et al. 2004). However, formation of papillary callose is not required for prepenetration resistance toward three nonadapted *Colletotrichum* spp.: *Colletotrichum truncatum*, *C. lagenarium*, and *C. gleosporioides* (Shimada et al. 2006), suggesting that NHR of *Arabidopsis* to these hemibiotrophic pathogens requires additional mechanisms. Indeed, preinvasion resistance was shown to require actin cytoskeleton function. The above examples show that NHR mechanisms displayed by a plant may differ, depending on the nature of the invader; notably, its taxonomic position and pathogenic strategy.

In the course of our studies on legume–*Colletotrichum* spp. interactions, we have taken advantage of the model plant *Medicago truncatula* to characterize the responses of this plant to adapted and nonadapted *Colletotrichum* spp. The three species used in this work (*C. trifolii*, *C. lindemuthianum*, and *C. higginsianum*) interact with *M. truncatula*, *Phaseolus vulgaris*, and *A. thaliana*, respectively, in a race- or cultivar-specific manner (O'Connell et al. 2004; Geffroy et al. 2008; Yang et al. 2008). Like many *Colletotrichum* spp., these aerial pathogens develop a two-stage hemibiotrophic mode of parasitism on a susceptible host. Infection is initiated by the differentiation of an appressorium, a melanized cell that generates a high internal turgor pressure, allowing mechanical breaching of the plant

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cuticle. The pathogen then penetrates epidermal cells in which it develops intracellularly as a biotroph for 2 to 3 days. It then switches to a necrotrophic, destructive mode of invasion coincident with the differentiation of secondary hyphae. In resistant cultivars, pathogen arrest occurs very soon after initial penetration of host cells as a result of the rapid HR developed at infection sites. Resistance to *C. trifolii* is governed in *M. truncatula* by a single dominant locus, named *CtI*, located at the upper part of chromosome 4 (Ameline-Torregrosa et al. 2008) from which *RCT1*, a gene belonging to the toll interleukin 1 receptor nucleotide-binding site leucine-rich repeat family, recently has been shown to confer resistance to *C. trifolii* when transferred to *M. sativa* (Yang et al. 2008). The resistance of *M. truncatula* to *C. trifolii* is associated with the production of reactive oxygen species, the localized accumulation of fluorescent compounds, and induction of defense gene expression (Torregrosa et al. 2004).

The availability of genetically defined *M. truncatula* lines with contrasting phenotypes (i.e., resistant or susceptible to *C. trifolii*) and of closely related, nonadapted *Colletotrichum* spp. prompted us to use this pathosystem to compare host and NHR. Cytological and large-scale transcriptomic studies were done, together with the use of a mutant strain impaired in the penetration step, in order to assess the effect of host background on NHR and the requirement of epidermal penetration for NHR to occur.

RESULTS

Symptoms and defense responses of *M. truncatula* to nonadapted *Colletotrichum* spp.

Two *M. truncatula* lines showing a differential phenotype in response to the alfalfa anthracnose pathogen *C. trifolii* were inoculated with *C. lindemuthianum*, a bean pathogen, and *C. higginsianum*, a pathogen of the family *Brassicaceae* (O'Connell et al. 2004). The *C. trifolii*-resistant A17 line displayed localized lesions which began to appear 2 to 3 days postinoculation (dpi) on detached leaves and were still visible 7 dpi, typical of the HR previously described (Torregrosa et al. 2004) (Fig. 1A). In contrast, the susceptible-F83005.5 line showed severe anthracnose lesions at the inoculation sites, as observed at 7 dpi (Fig. 1D). Inoculation of the two lines with *C. lindemuthianum* or *C. higginsianum* induced HR-like lesions (Fig. 1B, C, E, and F), confirming that these two fungal species are nonadapted on *M. truncatula*.

A kinetic study of the expression of two defense genes, a chalcone synthase (*CHS1*; GenBank accession no. AJ277211) (Bonanomi et al. 2001) and a pathogenesis-related class 10 protein (*PR10-1*; GenBank accession no. Y08641) (Gamas et al. 1998), previously shown to be induced in response to *C. trifolii* inoculation (Torregrosa et al. 2004), was performed after

inoculation of detached leaves with adapted or nonadapted *Colletotrichum* spp. Plant tissues were sampled at times corresponding to different stages of pathogenesis: i) conidia germination and appressoria differentiation, (compatible and incompatible interactions) at 16 h postinoculation (hpi); ii) penetration and establishment of the biotrophic phase (compatible interactions) or beginning of the hypersensitive cell death (incompatible interactions) at 40 hpi; and iii) switch to the necrotrophic phase (compatible interaction) or completion of the HR (incompatible interaction) at 72 hpi. Transcript accumulation of the two marker genes was observed at 16 hpi in the resistant line inoculated by either the adapted or the nonadapted *Colletotrichum* strains but transcripts were not detected in the susceptible line at this time point (Fig. 2). At 40 hpi, transcript accumulation of marker genes was observed in the two lines in compatible and incompatible interactions. At 72 hpi, transcript accumulation of *PR10-1* and *CHS1* was essentially found in the compatible interaction (F83005.5-*C. trifolii*).

Together, these analyses showed that nonadapted *Colletotrichum* spp. triggered similar HR-like lesions in the two lines A17 and F83005.5. However, gene expression studies revealed a faster defense response in A17 than in F83005.5 to the nonadapted and adapted *Colletotrichum* strain on A17.

Perception of a nonadapted *C. lindemuthianum* mutant strain impaired in the appressoria-mediated penetration step.

The finding that defense gene expression was induced on A17 plants at the onset of fungal penetration prompted us to examine whether inductive signals were released at an earlier stage of pathogenesis. For these experiments, we used a previously characterized nonpathogenic mutant of *C. lindemuthianum* disrupted in the *CLSTE12* transcription factor (Wong Sak Hoi et al. 2007). The *clste12* mutant formed melanized appressoria with basal penetration pores but was impaired in the subsequent steps required for pathogen entry and invasive growth in its host, a defect that has also been shown in other pathogens (Park et al. 2002; Deng et al. 2007; Yang and Chung 2010). In addition, the mutant is defective in the production of cell surface adhesion components and hydrolytic enzymes (Wong Sak Hoi et al. 2007). The *clste12* mutant formed appressoria on *M. truncatula* leaves within 16 hpi (data not shown), and induced the accumulation of fluorescent compounds at 3 dpi and the formation of HR-like lesions at 7 dpi (Fig. 3), similar to the wild-type *C. lindemuthianum* strain. This indicates that perception of *C. lindemuthianum* is independent of epidermal cell penetration and invasive growth.

At 16 hpi, the *clste12* mutant induced an expression pattern of *PR10-1* and *CHS* similar to the wild-type strain (Fig. 4). However, expression of *PR10-1* and *CHS1* in *clste12*-inoculated A17 leaves at 40 hpi was significantly lower than in the

Fig. 1. Macroscopic symptoms of *Medicago truncatula* leaves inoculated with *Colletotrichum* spp. Macroscopic symptoms at 7 days postinoculation. **A**, A17 inoculated with *Colletotrichum trifolii* (*C.t*). **B**, A17 inoculated with *C. lindemuthianum* (*C.l*). **C**, A17 inoculated with *C. higginsianum* (*C.h*). **D**, F83005.5 inoculated with *C. trifolii*. **E**, F83005.5 inoculated with *C. lindemuthianum*. **F**, F83005.5 inoculated with *C. higginsianum*.

case of the wild-type strain, indicating that development of a penetration peg, or production of CLSTE12-regulated effectors, is required for the duration of the defense response.

Transcriptomic signatures associated with host and NHR.

A major quantitative trait locus, named *Ct1*, involved in resistance to *C. trifolii* races 1 and 2, was previously detected near the top of chromosome 4, using the LR5 (A17-Jemalong × F83005.5) F7 recombinant inbred line (RIL) population (Ameline-Torregrosa et al. 2008). This region also contains the *RCT1* gene, which confers resistance to *C. trifolii* when transferred to susceptible alfalfa plants (Yang et al. 2008). Two near-isogenic lines (NIL), named 210A and 210F, were selected after selfing of an F7 line (line 210) showing residual heterozygosity inside the *Ct1* region. 210A is resistant to *C. trifolii* whereas 210F is susceptible (Supplementary Fig. S1) (M. Cazaux and C. Jacquet, unpublished work). To determine the role in the nonhost response of this *C. trifolii* resistance locus, these two isogenic lines were inoculated with *C. trifolii* or *C. lindemuthianum* co-

nidia. Macroscopic symptoms identical to those obtained with the parental lines were obtained (Fig. 5A). In the case of host and nonhost incompatible interactions, a localized accumulation of hydrogen peroxide was detected at 2 dpi upon staining the tissues with diaminobenzidine (DAB) (Fig. 5B). Hypersensitive lesions were surrounded by cells that accumulated fluorescent compounds (Fig. 5C). No clear differences were noticed at the cellular levels between host and nonhost defense reactions except that, in the case of the host incompatible interactions, DAB staining heavily labeled conidia and appressoria as well as the germ tube, reflecting a strong stress response in fungal cells. The expression of the two defense marker genes, *PR10-1* and *CHS1* (Fig. 5D), was studied in the two lines inoculated by spraying conidial suspensions on whole plants. As previously observed in the case of the parental lines, no specific expression pattern distinguishing host and nonhost response emerged from these analyses, except that the stronger gene induction was observed in the incompatible interaction with the adapted strain (i.e., the 210A line in response to *C. trifolii* at 1 dpi).

Large-scale transcriptomic analyses were performed in order to compare NHR and host resistance in these genetically defined lines differing at the *Ct1* locus. Inoculations were done on 2-week-old intact plants by spraying a conidial suspension of *C. trifolii* or *C. lindemuthianum*. Due to their taxonomic proximity, *C. trifolii* and *C. lindemuthianum* were used in this experiment. RNA was extracted from leaves collected at 1 and 3 dpi from three independent experiments and transcript profiling was performed using Mt16kOLI1Plus chips (Thompson et al. 2005), allowing us to monitor the expression of approximately 16,000 genes. Data from inoculated and corresponding control (noninoculated) plants were used to assess the expression ratio of each gene. After analysis of variance (ANOVA), a gene was considered responsive to the pathogen if it was associated with a *P* value ≤ 0.05 and regarded as differentially expressed if the absolute value of the log₂ ratio was ≥ 0.584 (>1.5 fold induction or <0.67 fold repression). Examining each experimental data set individually, a low number of genes were differentially expressed at 1 dpi in 210A and 210F inoculated with nonadapted *C. lindemuthianum* and adapted *C. trifolii*, respectively. This result is probably due to the inoculation procedure which produced dispersed and localized micro-HR

Fig. 2. Gene expression after inoculation by *Colletotrichum* spp. A17 and F83005.5 were inoculated with *Colletotrichum trifolii* (*C.t*), *C. lindemuthianum* (*C.l*), or *C. higginsianum* (*C.h*). Relative transcript levels ratios were calculated between gene expression measured in inoculated and noninoculated leaves and was expressed in log₂. Gene expression was measured at 16, 40, and 72 h after inoculation. **A**, Expression of the *PR10-1* gene (Y08641). **B**, Expression of the chalcone synthase 1 gene (*CHS1*; AJ277211.1).

Fig. 3. Macroscopic symptoms and epifluorescence microscopy of *Medicago truncatula* leaves inoculated with *Colletotrichum lindemuthianum*. Macroscopic symptoms at 7 days postinoculation (dpi) (upper panel) and epifluorescence microscopy at 2 dpi (lower panel). **A** and **E**, A17 inoculated with the wild-type strain of *C. lindemuthianum* (*C.l*). **B** and **F**, A17 inoculated with the *clste12* mutant of *C. lindemuthianum*. **C** and **G**, F83005.5 inoculated with the wild-type strain of *C. lindemuthianum*. **D** and **H**, F83005.5 inoculated with the *clste12* mutant of *C. lindemuthianum*.

lesions, leading to a strong dilution of induced cells to unaffected tissues. More than half of these genes were differentially regulated by adapted and nonadapted *Colletotrichum* strains, suggesting that host and nonhost incompatible reactions largely overlap (Fig. 6A).

At 3 dpi, differential expression of many more genes was observed from 210A and 210F inoculated with *C. trifolii* than with *C. lindemuthianum* (Table 1). This was obviously related to the continuing development of the pathogen during the compatible host interaction but also during the incompatible interaction, suggesting that, in this case, the pathogen was arrested at a later stage of development than during nonhost interactions.

Hierarchical clustering was performed for all genes differentially expressed in at least one interaction. Four major groups

Fig. 4. Defense gene expression in *Medicago truncatula* leaves after inoculation by a penetration mutant of *Colletotrichum lindemuthianum*. The A17 and F83005.5 lines of *M. truncatula* were inoculated with the wild-type or *clste12* mutant strains of *C. lindemuthianum*. Relative transcript level corresponding to **A**, the *PR10-1* (Y08641) gene and **B**, the chalcone synthase 1 gene (*CHS1*; AJ277211.1) gene was measured at 16, 40, and 72 h after inoculation. Data are expressed as the $\log_2$ ratio between gene expression measured in inoculated and noninoculated leaves.

Fig. 5. Nonhost and host defense reactions induced by *Colletotrichum* spp. in near-isogenic *Medicago* lines. The 210A (*Colletotrichum trifolii* resistant) and 210F lines (*C. trifolii* susceptible) were inoculated with conidial suspensions of the *C. lindemuthianum* (*C. l.*) or the *C. trifolii* (*C. t.*) strains. **A**, Macroscopic symptoms were observed at 6 days postinoculation (dpi). **A** and **E**, 210A inoculated with *C. trifolii* (*C. t.*). **B**, Diaminobenzidine (DAB) staining of inoculated leaves at 2 dpi revealing single-cell oxidative burst. Note the detection of DAB-positive cells only in the case of nonhost and host-incompatible interactions. Bar = 40 μ m. Ap, Appressorium; PH, primary hyphae. **C**, Epifluorescence microscopy at 3 dpi revealing a fluorescent ring of cells around the hypersensitive response cell. Bar = 40 μ m. **D**, *PR10-1* and *CHS1* expression at 1 and 3 dpi in 210A or 210F leaves inoculated with conidial suspension of *C. lindemuthianum* (gray bars) or *C. trifolii* (black bars).

could be distinguished (Fig. 6B). Group I represented genes induced at 1 dpi, mainly during NHR of the two lines to *C. lindemuthianum* spp. and during host resistance of 210A to *C. trifolii* (Table 2). Group I was enriched in genes with a signaling and defense function (Fig. 6B). Among them, genes associated with cell wall structure and modification, such as glycine- or hydroxyproline-rich (glyco) proteins and peroxidase, were only induced in incompatible interactions. In contrast, induction was very low in the susceptible line 210F inoculated with *C. trifolii* (compatible interaction), suggesting the ability of the pathogen to partially suppress early defense induction by this line. Some defense genes from group I, including isoflavone

Fig. 6. Clustering of microarray data and functional classification of co-regulated genes. **A**, Venn diagram to compare the set of induced genes at 1 day postinoculation (dpi) during host and nonhost incompatible interactions. **B**, Hierarchical clustering of microarray data issued from each of the *Colletotrichum* spp.–*Medicago truncatula* interactions using the HCE3.5 software with the complete linkage method and the Manhattan distance measure. Three classes of genes were defined according to their expression profiles. Class I corresponds to genes upregulated at 1 dpi, class II to genes upregulated at 3 dpi, and class 3 to genes downregulated at 3 dpi. Genes corresponding to the three classes were further grouped according to their predicted function.

reductase and chalcone synthase, were induced during the compatible situation but at a later stage of infection. Group II included genes specifically induced in the host-incompatible interaction at 3 dpi (Table 3), while group III comprised repressed genes at 3 dpi, mainly in the host-incompatible and, to a lesser extent, the compatible interactions (Table 4). Most group II genes fell into the signaling functional class, whereas group III genes belonged to signaling and primary metabolism classes. Interestingly, among repressed genes, two of them were similar to the *Arabidopsis* negative regulators of defense responses, NPR3 and SAC9 (Williams et al. 2005; Zhang et al. 2006), and were only repressed by 210A in response to nonadapted and adapted *Colletotrichum* spp.

To validate microarray data, expression of five differentially expressed genes was analyzed by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) (Supplementary Fig. S2). The results were similar to the microarray data except for some experimental points for which contrasting results were obtained. Importantly, similar expression patterns were observed for genes showing a differential expression in a specific interaction such as the adenylate-kinase gene and the HRGP gene which were induced at 1 dpi in the resistant line upon inoculation of the nonadapted strain.

NHR may involve different hormone signaling pathways depending on the nature of the pathogen (Nurnberger and Lipka, 2005). The transcriptomic analyses of nonhost and host interactions did not reveal clear effects of inoculation by *Colletotrichum* spp. on plant hormone signaling. However, it was previously shown that the salicylic acid (SA) pathway is involved in anthracnose basal resistance, as suggested by the increased susceptibility to *C. higginsianum* of an *Arabidopsis* line expressing the *NahG* gene (O'Connell et al. 2004). Thus, accumulation of SA was investigated in 210A and 210F leaves inoculated with *C. trifolii* or *C. lindemuthianum*. A significant increase of SA concentration was detected only in *C. trifolii*-inoculated plants at 3 dpi (Fig. 7). However, SA accumulation was higher in the susceptible line compared with the resistant one, suggesting that SA accumulation is induced by the development of anthracnose symptoms, which results in a greater number of cells inducing defense reactions.

DISCUSSION

Although NHR has been recognized for many years, the molecular mechanisms that underlie its various steps are still largely unknown. The knowledge gained on PAMP-triggered basal defense immunity and on gene-for-gene resistance, together with the use of model plant and pathogen systems, will increase understanding of this important aspect of durable resistance (Boller and Felix 2009). This was recently illustrated by comparative analyses of the NHR of *A. thaliana* to nonadapted biotrophic pathogens (powdery mildews) and hemibiotrophic pathogens (*Colletotrichum* spp.) (Zimmerli et al. 2004; Shimada et al. 2006; Lipka et al. 2008). It was notable that NHR can share common cellular and molecular traits with host basal defense. However, the effect of the genetic background

Table 1. Number of up- and downregulated genes in *Medicago truncatula* near-isogenic lines (NIL) at 1 and 3 days postinoculation (dpi) with *Colletotrichum lindemuthianum* and *C. trifolii*

NIL	Species	Upregulated		Downregulated	
		1 dpi	3 dpi	1 dpi	3 dpi
210A	<i>C. lindemuthianum</i>	22	4	15	12
	<i>C. trifolii</i>	25	47	22	33
210F	<i>C. lindemuthianum</i>	15	10	3	2
	<i>C. trifolii</i>	11	49	7	23

of the plant was not controlled in these studies. Our purpose was to use the interaction of the model plant *M. truncatula* with *C. trifolii* to directly compare the effect of the plant genetic background on host resistance and NHR to *Colletotrichum* spp. that display an identical mode of parasitism.

In this study, we showed that many aspects of the resistance of *M. truncatula* to nonadapted *Colletotrichum* strains are quite similar to the incompatible reaction induced by the adapted strain on the resistant line. Most notably, all incompatible interactions were characterized by the rapid formation of a localized HR-like lesion at inoculation sites, at times when the compatible interaction was symptomless. Recently, Mysore and Ryu (2004) distinguished two types of NHR. Type I relates to pathogens that are not able to overcome a first layer of preformed and general-elicitor induced defense, whereas type II corresponds to pathogens that induce an HR and are recognized through the perception of more specific motifs. The present study shows that the response of *M. truncatula* to nonadapted *Colletotrichum* spp. falls into type II NHR. This is in contrast with nonhost interactions involving *A. thaliana* and the two nonadapted *C. lagenarium* and *C. gleosporioides* pathogens, in which papillary callose formation was observed but not HR cell death (Shimada et al. 2006). Absence of an HR also characterized the NHR of *Arabidopsis* to the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* (Tao et al. 2003). This does not reflect a general failure of *Arabidopsis* to mount an HR upon NHR because cell death had been detected in this plant in response to nonadapted powdery mildew strains (Zimmerli et al. 2004).

The present work also highlighted additional features of the HR response. Thus, it appeared that the HR symptoms of NHR can occur in the absence of epidermal cell penetration, as ob-

served in response to the *clste12* mutant of *C. lindemuthianum*. Although impaired in cell surface properties and invasive growth, as previously reported (Wong Sak Hoi et al. 2007), we found that this mutant had retained the ability to induce HR and associated defense gene expression in *M. truncatula*. This is reminiscent of another *C. lindemuthianum* mutant affected in appressorial-mediated penetration which was still able to induce plant responses such as superoxide production and induction of PR proteins when inoculated onto bean leaves (Veneault-Fourrey et al. 2005). These results indicate that fungal signals are produced before establishment of the biotrophic phase during both host and nonhost interactions.

These signals can be either general elicitors, such as polysaccharide fragments or cell-wall-degrading enzymes, or more specific microbial effectors. *C. lindemuthianum* is a potential source of general elicitors, notably derived from chitin; it also secretes an endopolygalacturonase that releases oligogalacturonide elicitors from plant cell walls (Herbert et al. 2002; Boudart et al. 2003). However, the resistance of *M. truncatula* to *C. trifolii* is mediated by the *Ct1* locus, which comprised several resistance-like genes, notably *RCT1* (Ameline-Torregrosa et al. 2008; Yang et al. 2008) and a close ortholog of *RCT1*, present in the susceptible line. This gene is constitutively expressed and alternatively spliced, giving rise to two transcripts in the resistant line; however, only the longest one in the susceptible line (Yang et al. 2008). One may hypothesize that the type II NHR to *C. lindemuthianum* involves functional homologues of the *resistance-avirulence* gene pairs that govern host resistance in this system. In such a context, the remnant expression of *RCT1* might significantly contribute to the NHR of 210F but is clearly insufficient to confer resistance of *C. trifolii*.

Table 2. Genes belonging to group I, mainly upregulated in at least one interaction between *Medicago truncatula* and *Colletotrichum* spp. at 1 dpi^a

Mt16kOLIplus	Annotation	TIGR v5	210A		210F	
			Nonhost	Host	Nonhost	Host
Signaling/transcription						
MT003492	Adenylate kinase	TC47188	0.64	−0.10	−0.02	−0.10
MT009512	Steroid-binding protein	TC54196	0.65	−0.44	−0.03	−0.21
MT016327	Transcription factor B3-type	BG646843	0.93	1.03	1.07	0.67
MT009107	Transcription factor CCCH-type	TC53754	0.85	0.58	1.01	0.66
MT012881	Frigida like	TC57871	0.62	0.58	0.68	0.58
MT016420	NAC transcription factor	CA917398	0.64	0.61	0.50	0.37
MT004642	RING-finger protein	TC48442	1.29	1.17	1.62	1.21
MT007046	SNF2 related H box helicase	TC51155	0.69	0.61	0.41	−0.01
MT002007	Polyadenylate-binding protein	TC45554	0.46	0.27	0.59	0.29
Defense						
MT014134	Hydroxyproline-rich protein	TC43063	0.59	0.45	0.39	0.00
MT014142	Hydroxyproline-rich protein	TC43019	0.73	0.56	0.43	0.02
MT014143	Hydroxyproline-rich protein	TC43082	0.68	0.52	0.38	0.02
MT009425	Glycine-rich protein	TC54102	0.56	0.64	0.73	0.51
MT014250	Glycine-rich protein	TC43292	0.68	0.66	0.61	0.41
MT015604	Peroxidase	TC53280	0.63	0.50	0.24	0.21
MT014168	PR10	TC43135	0.18	0.47	0.60	0.39
MT015101	PR10	TC51050	0.22	0.59	0.63	0.29
MT015362	PR10	TC51765	0.64	0.91	0.57	0.39
MT014167	PR10	TC43134	1.05	1.51	1.05	1.06
MT007530	Iron-stress-related protein	TC51883	0.62	0.30	0.23	0.08
Secondary metabolism						
MT014241	Isoflavone reductase	TC43281	0.81	1.00	0.61	0.47
MT014270	Chalcone reductase	TC43329	0.57	1.04	0.82	0.73
Primary metabolism						
MT001267	Carbonic anhydrase	TC44723	0.82	0.13	0.10	0.15
MT013904	Phosphoglucosmutase	TC58970	0.59	0.64	0.48	0.33
Unclassed/unknown						
MT013021	Unknown	TC58019	1.09	0.60	0.91	0.59
MT004228	Unknown	TC47998	0.67	0.32	−0.38	−0.35
MT011615	Unknown	TC56486	0.62	0.26	0.71	0.39
MT015286	Unknown	TC51492	0.43	0.48	0.61	0.52
MT005919	Unknown	TC49804	0.61	0.57	0.53	0.21

^a Expression ratio is expressed in log₂. Values greater than 0.58 (log₂ 1.5) are in bold. dpi = days postinoculation.

For a more precise insight into the effect of the genetic background on NHR, we then concentrated on two *M. truncatula* NIL and performed cytological analyses and large-scale transcriptomic analyses after inoculation with the two closely related species, *C. lindemuthianum* and *C. trifolii*. Nonhost responses observed in 210A and 210F were highly similar, with the development of necrotic spots and, at an earlier stage, the detection of DAB-positive cells suggesting that nonhost responses are independent of the *C. trifolii* resistance locus. As mentioned above, these cytological reactions are typical of the type II NHR detected in various plant–pathogen combinations such as interactions between rice genotypes and nonadapted *Magnaporthe* isolates (Faivre-Rampant et al. 2008).

Results from the transcriptomic analyses revealed that most of the genes induced at 1 dpi by *C. lindemuthianum* in the 210F line are also induced in the 210A line, confirming that the nonhost response is similar in these two lines, differing only in the *CtI* region. Those similar patterns were strictly correlated with the appearance of an HR, in contrast to what was reported for *Arabidopsis*–*P. syringae* interactions, in which similar molecular responses to host resistance and NHR were observed but without an HR (Tao et al. 2003).

At 3 dpi, a small number of genes were differentially expressed in the nonhost combinations, whereas the host interac-

tions were characterized by the sustained expression of additional sets of defense genes and an increase in free SA. This indicates that restriction of fungal development mediated by the *CtI* locus occurred later in the host-incompatible interaction than in the nonhost interactions and that host and NHR involve different recognition mechanisms.

Our study shows that the susceptible line is able to mount a rapid and efficient HR toward nonhost strains but not toward the adapted *Colletotrichum* spp. This could be due to the inability to recognize a *C. trifolii*-specific cell-death elicitor but could also involve fungal effectors involved in cell-death suppression. Specific cell death or basal immunity suppressors have been identified in bacteria (Abramovitch et al. 2003), oomycetes (Dou et al. 2008; Bos et al. 2009), and fungi (Uppalapati et al. 2004) but still await their identification in *Colletotrichum* spp. The *Colletotrichum* genus, which comprises highly specialized species, is particularly suited to investigate the basis of host–pathogen compatibility.

MATERIALS AND METHODS

Plant materials and growth conditions.

M. truncatula Gaertn. A17 and F83005.5 lines were provided by J. M. Prospéri (INRA SGAP Laboratory, Mauguio, France).

Table 3. Genes belonging to group II, mainly upregulated during interaction between the *Medicago truncatula* resistant line (210A) and *Colletotrichum* spp. at 3 dpi^a

Mt16kOLiplus	Annotation	TIGR v5	210A		210F	
			Nonhost	Host	Nonhost	Host
Signaling/transcription						
MT016329	AP2/EREBP transcription factor	AW689511	−0.01	0.72	−0.12	−0.18
MT009781	Adenylate-cytidine kinase	TC54494	0.22	0.60	0.02	0.16
MT014479	Early flowering transcription factor	TC44345	−0.08	0.72	−0.04	0.07
MT012308	Serine Threonine kinase	TC57250	0.26	0.66	0.38	0.30
MT002241	Serine Threonine kinase	TC45821	0.20	0.59	0.18	0.20
MT001984	Calmodulin-binding protein	TC45530	0.35	0.71	−0.01	0.05
MT007720	CDP-DG:inositol transferase	TC52127	0.38	0.61	0.01	−0.21
MT010770	Phospholipase	TC55571	−0.05	1.06	−0.05	0.02
MT015913	Lipoxygenase	TC56844	0.13	0.60	−0.11	0.46
MT002447	Auxin response factor	TC46043	0.39	0.60	0.19	0.32
Defense						
MT002340	NADP-thioredoxin reductase A	TC45928	0.24	0.74	0.17	0.15
MT013730	Amine oxidase	TC58780	0.27	0.64	0.22	0.23
MT012243	Heat shock protein	TC57180	0.24	0.61	0.13	0.02
MT009896	Dehydrated response protein	TC54626	0.30	0.60	0.15	0.17
MT012742	Extensin	TC57726	−0.11	0.78	0.01	−0.05
Secondary metabolism						
MT008064	Dirigent-like protein	TC52540	0.15	0.62	−0.03	0.41
MT004268	Squalene monooxygenase	TC48042	0.19	0.59	0.26	−0.01
Primary metabolism						
MT005526	Adenylsulfate kinase	TC49389	0.24	0.69	0.14	0.24
MT011342	Glucan phosphorylase	TC56194	0.38	0.64	0.21	0.23
MT003867	Aspartic-type endopeptidase	TC47605	0.07	1.11	0.14	0.37
MT007992	Fribrillin	TC52459	0.39	0.65	0.01	−0.10
Transport/secretion						
MT001650	Small Rab GTPase	TC45153	0.18	0.63	0.05	0.02
MT003691	Prefolding protein	TC47408	0.23	0.62	0.20	0.17
MT008913	Nicastrin-related protein	TC53537	0.11	0.69	0.35	−0.22
MT009415	ABC transporter	TC54092	0.16	0.74	0.09	0.02
MT011180	Na/H exchanger	TC56021	0.35	0.69	0.25	0.06
MT011179	Transportin	TC56020	0.14	0.62	0.11	−0.03
MT002528	Boron transporter	TC46129	0.26	0.61	0.14	0.22
Unclassed/unknown						
MT011051	Unknown	TC55880	0.43	0.75	0.15	0.12
MT003030	Unknown	TC46683	0.20	0.68	0.23	0.15
MT003278	Unknown	TC46952	0.36	0.67	0.10	0.09
MT011318	Unknown	TC56170	0.39	0.66	0.18	0.24
MT003890	Unknown	TC47631	0.09	0.61	0.32	0.23
MT004527	Unknown	TC48313	0.26	0.60	0.22	0.18
MT001354	Unknown	TC44828	0.22	0.75	−0.16	−0.01

^a Expression ratio is expressed in log₂. Values greater than 0.58 (log₂ 1.5) are in bold. dpi = days postinoculation.

The two NIL (210A and 210F) were selected among heterogeneous inbred families, generated following self-fertilization of the no. 210 F7 LR5 (A17 × F83005.5) RIL that is heterozygous for the entire *CtI* region. Five simple-sequence repeat markers

(mtic331, h2_8d13, mtCt12, mtCt14, and mtic510) designed from BAC sequences covering the CtI region (Ameline-Torregrosa et al. 2008) were used to select 210A and 210F that differed by the A17 or F83005.5 alleles throughout the *CtI* locus.

Table 4. Genes belonging to group III, mainly downregulated in the *Medicago truncatula* lines inoculated with *Colletotrichum* spp. at 3 dpi^a

Mt16kOLiplus	Annotation	TIGR v5	210A		210F	
			Nonhost	Host	Nonhost	Host
Signaling/transcription						
MT010852	Mitogen-activated protein kinase	TC55662	-1.03	-0.79	0.16	-0.11
MT004670	Serine/threonine protein kinase	TC48472	-0.98	-0.96	-0.06	-0.04
MT009563	Tyrosine protein kinase	TC54253	-0.10	-0.62	-0.22	-0.12
MT015899	Protein kinase	TC56690	-0.15	-0.32	-0.25	-0.59
MT012179	Protein kinase	TC57109	-0.07	-0.07	-0.18	-0.87
MT006189	Phosphatase 2C HAB1	TC50097	-0.58	-0.59	-0.14	0.01
MT006956	Phosphatidylinositol phosphatase, SAC9	TC50939	-1.47	-1.55	0.00	-0.04
MT001262	Regulatory protein NPR3-like	TC44718	-0.88	-0.86	-0.16	0.00
MT016089	SAR DNA-binding protein		-0.30	-0.61	-0.18	-0.04
MT011639	RNA-binding protein	TC56510	-0.17	-0.75	-0.16	-0.19
MT000562	CID4 protein binding	TC43872	-0.76	-0.74	-0.06	-0.19
MT015771	Nucleic-binding protein	TC55006	-0.67	-0.27	0.23	0.00
MT016282	MYB transcription factor	BI266990	-0.18	-0.59	0.02	-0.14
MT011522	Zinc finger protein (CCCH type)	TC56387	-0.09	-0.41	-0.11	-0.62
MT011335	GRAS family transcription factor	TC56187	0.254	0.941	-0.19	-0.59
MT003059	Protein transport factor	TC46716	0.07	0.02	-0.54	-0.75
MT001108	F-box family protein	TC44539	-0.34	-0.60	-0.32	-0.27
MT002615	Ubiquitin, DNA-damage inducible	TC46226	-0.17	-0.61	-0.14	-0.21
MT013464	Ubiquitin-associated protein	TC58492	-0.53	-0.65	0.00	-0.09
MT001795	Endonuclease	TC45314	-0.16	-0.24	-0.29	-0.62
MT014949	Tetratricopeptide-like helical	TC49771	-0.20	-0.69	0.29	0.04
MT007217	Ribosomal protein L5	TC51442	-0.11	-0.63	0.01	0.13
MT011937	60S ribosomal protein L5	TC56837	-0.67	-0.93	-0.11	0.00
MT012504	Translation elongation factor	TC57461	-0.64	-0.42	0.21	-0.02
Defense						
MT007395	Pectate lyase	TC51696	-0.07	-0.59	0.13	-0.02
MT000827	Trypsin/chymotrypsin inhibitor	TC44197	-0.13	0.211	-0.64	-0.62
Secondary metabolism						
MT014003	3-Hydroxy-3-methylglutaryl coA reductase	TC43337	-0.17	-0.43	-0.15	-0.60
Primary metabolism						
MT014115	Chlorophyll a-b binding protein	TC42999	-0.31	-0.58	-0.28	-0.82
MT015098	Chlorophyll a-b binding protein	TC51045	-0.30	-0.55	-0.22	-0.70
MT015099	Chlorophyll a-b binding protein	TC51048	-0.28	-0.58	-0.21	-0.69
MT007170	Chlorophyll a-b binding protein	TC51376	-0.30	-0.53	-0.18	-0.64
MT014000	Chlorophyll a-b binding protein	TC43260	-0.24	-0.37	-0.16	-0.61
MT007180	Chlorophyll a-b binding protein	TC51387	-0.25	-0.22	-0.12	-0.60
MT014110	Chlorophyll a-b binding protein	TC43015	-0.21	-0.57	-0.21	-0.59
MT000102	Light harvesting chlorophyll binding protein	TC43184	-0.16	-0.58	-0.24	-0.80
MT007374	Photosystem reaction center subunit III	TC51661	-0.33	-0.52	-0.17	-0.64
MT003736	Photosystem I apoprotein A2	TC47460	-0.36	-0.85	-0.35	-0.43
MT003797	Photosystem I apoprotein A2	TC47528	-0.29	-0.97	-0.34	-0.57
MT015090	Cytochrome b	TC51032	-0.36	-0.64	-0.17	-0.29
MT007914	Phosphoenolpyruvate carboxylase	TC52360	-0.14	-0.64	-0.20	-0.45
MT000004	RuBisco activator	TC42987	-0.26	-0.69	-0.15	-0.37
MT014109	RuBisco small chain	TC43011	-0.20	-0.53	-0.25	-0.66
MT014122	RuBisco small chain	TC43011	-0.17	-0.50	-0.23	-0.61
MT014158	RuBisco small chain	TC43108	-0.25	-0.53	-0.27	-0.61
MT014123	RuBisco small chain	TC43044	-0.24	-0.47	-0.26	-0.60
MT015214	Glyceraldehyde 3 phosphate dehydrogenase	TC51277	-0.14	-0.55	-0.17	-0.63
MT011509	Glycosyl hydrolase	TC56373	-0.18	-0.66	-0.14	-0.29
MT000153	β-Glucosidase	TC43298	-0.27	-0.46	-0.27	-0.59
MT008500	Nudix hydrolase	TC53066	-1.11	-0.93	0.03	0.00
Unclassed/unknown						
MT000479	Unknown	TC43763	-0.15	-0.64	-0.04	-0.12
MT001113	Unknown	TC44544	-0.22	-0.59	-0.24	-0.21
MT012125	Unknown	TC57053	-0.21	-0.67	0.15	0.03
MT013579	Unknown	TC58616	-0.29	-0.62	-0.16	-0.23
MT014544	Unknown	TC44796	-0.33	-0.63	-0.07	-0.10
MT015334	Unknown	TC51669	-0.34	-0.69	-0.39	-0.37
MT015335	Unknown	TC51670	-0.32	-0.73	-0.24	-0.24
MT012317	Unknown	TC57260	-1.35	-1.41	0.12	-0.01
MT005834	Unknown	TC49714	-0.85	-0.95	-0.02	0.24
MT004344	Unknown	TC48120	-0.62	-0.72	-0.20	0.00
MT000372	Unknown	TC43634	-0.62	-0.64	-0.02	0.08
MT008209	Unknown	TC52709	0.02	-0.07	-0.84	-0.78

^a Values lower than -0.58 (log₂ 0.67) are in bold. dpi = days postinoculation.

Plants seed were surface sterilized for 5 min in sulfuric acid, rinsed several times with sterile water, and germinated on 1% agar plates for 2 days at 20°C in the dark after 1 night at 4°C. Seedlings were transferred to soil and cultivated in a growth chamber with a regime of 16 h of light at 25°C and 8 h of darkness at 22°C.

Inoculation procedures.

C. trifolii Bain race 2 (Ameline-Torregrosa et al. 2008), *C. lindemuthianum* (Sacc. et Magn.) Briosi and Cav. race β (Wong Sak Hoi et al. 2007), *C. higginsianum* IMI349063 (O'Connell et al. 2004), and a *C. lindemuthianum* CLSTE12 mutant (Wong Sak Hoi et al. 2007) were used. Conidia were produced after 7 days at 23°C on the medium described by (Bannerot 1965). Spores were collected in sterile water and the concentration was adjusted to 10^6 cells/ml in 0.01% Tween 20 (Sigma-Aldrich, St. Louis). Inoculations were done on detached leaves or on 2-week-old intact plant as described (Torregrosa et al. 2004). The experiments were repeated at least three times independently. Symptoms were observed at 3 or 7 dpi.

Microscopic analyses.

Accumulation of autofluorescent compounds in the inoculated leaves was followed by epifluorescence microscopy, using an inverted microscope (Leitz DM IRBE, Leica, Germany) with excitation filter BP (340 to 380 nm) and barrier filter LP (425 nm). Images were recorded with the same settings using a CCD color digital camera (Photonic Science, Robertsbridge, East Sussex, U.K.).

qRT-PCR.

Total RNA was extracted from 100 mg of shock-frozen leaves using the Qiagen RNeasy plant mini kit RNA cleanup protocol. RNA quality was checked using the Agilent 2100 Bioanalyzer. cDNAs were synthesized from 2 μ g of total RNA using the SuperScript III kit (Invitrogen, Carlsbad, CA, U.S.A.) with OligodT primers. Real-time qRT-PCR was performed on an ABI Prism SDS 7900HT (Applied Biosystems, Foster City, CA, U.S.A.). Each 10- μ l PCR reaction contained 2 μ l of a 1:50 dilution of the synthesized cDNA, 5 μ l of SYBR Green 2 $\times$ (Invitrogen), and primers (0.15 μ M each). The PCR program consisted of 5 min of incubation at 95°C followed by 40 cycles of 15 s at 95°C and 30 s at 60°C. qPCR reactions were performed in triplicate for each of three biological replications. Data analysis and calculations for comparing expression data were performed using the $\Delta\Delta$ cycle threshold method with the elongation factor EF1 α as the constitutively expressed gene. PCR efficiency was evaluated by performing qPCR on serial dilutions of a mixture of all cDNAs. Amplification specificity for each gene was confirmed by a single dissociation curve peak generated at the end of the PCR cycle. The following primer sequences were used: TC111470 (EF1 α), forward, 5'-GACAAGCGTGTGATCGAGAGATT-3', reverse, 5'-TTTCCGCTCAGCCTTAAGCT-3'; Y08641 (*PR10-1*), forward, 5'-TGGAAAAGCTAGGGGTGATG-3', reverse, 5'-CCGTCTATAAAGCAAGCAAGC-3'; AJ277211.1 (*CHS1*), forward, 5'-TTGCGCAGTGTGCTATATG-3', reverse, 5'-TGAGGGTACATGTTGAACACTAGA-3'; TC53754 (transcription factor, CCCH-type), forward, 5'-AGGCTCCTCCAACAGTAGCA-3', reverse, 5'-GCAGCCTGAATCTTGGACTC-3'; TC48442, (RING-finger protein) forward, 5'-GAAGGGACTGGTGC AAATGT-3', reverse, 5'-TGGGACGATAAAGGAGATGC-3'; TC47188 (adenylate kinase), forward, 5'-TGTCCAGAAACGTGCAGAAG-3', reverse, 5'-ATAATGCCGAGGAGTGATC G-3'; TC43082 (hydroxyproline-rich glycoprotein), forward, 5'-TCTTACCTTGCGATCCCAA-3', reverse, 5'-GTGGTGGGGAAGTGTAAATGG-3'; and TC43281, (isoflavone reductase),

forward, 5'-GAATTTGGGCTAGACGTGGA-3', reverse, 5'-TATCCCCGAGGAGGATCAGTG-3'.

Microarrays.

Total RNA from ground leaves was extracted using TRIzol reagent (Invitrogen) and purified using Microcon-30 columns (Millipore, Billerica, MA, U.S.A.). Total RNA (5 μ g of each sample) was used to synthesize Cy3-labeled cDNA targets using a mixture of 2 μ g of double anchored oligo(dT)15 VN primers and 3 μ g of random primers with the CyScribe first-strand cDNA labeling Kit (GE Healthcare Europe, Orsay, France). Total RNA of each sample was similarly labeled with the Cy5 dye. Labeled cDNA was purified using the ChipShot Membrane Clean Up System (Promega Corp, Madison, WI, U.S.A.), and labeling efficiency was checked by measuring absorbance at 260, 550, and 650 nm with a Nanodrop (Thermo Scientific, Waltham, MA, U.S.A.). Only labeled cDNA having a frequency of incorporation of at least 20 labeled nucleotides per thousand was used for hybridization. Microarray analyses were performed using Mt16kOLI1Plus chips representing all 16,086 tentative consensus sequences of the TIGR *M. truncatula* Gene Index 5, plus a set of 384 oligonucleotides representing transcription factors and regulators. Automatic hybridization of a mix containing the same quantity of Cy3- and Cy5-labeled cDNA was performed using a Ventana's Discovery System (Ventana, Illkirch, France) following the standard procedure. After 16 h of hybridization at 42°C, microarrays slides were washed twice in RiboWash reagent (1 min, 20°C) and once in 0.1 $\times$ SSC (1 min, 20°C) (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Slides were dried by centrifugation (185 $\times$ g, 5 min, 20°C) and scanned using the Genepix 4000 scanner (Axon Instruments, Sunnyvale, CA, U.S.A.). Image processing was performed using the Mapix software (Innopsys, Carbonne, France). Data analysis was done using MAnGO on the R software (Marisa et al. 2007).

Fig. 7. Salicylic acid (SA) quantification in *Medicago truncatula* lines inoculated with *Colletotrichum* spp. Aerial parts of 2-week-old *M. truncatula* plants (210A or 210F) were inoculated by spraying a conidial suspension of *Colletotrichum lindemuthianum* or *C. trifolii*. Free SA was quantified at 1 or 3 days postinoculation according to a protocol adapted from Forcat and associates (2008).

An intraslide normalization was performed using Loess on each array without background correction, followed by a Rquantile interslide normalization. All data were deposited in MIAME compliant format into the ArrayExpress database under accession no. E-MEXP-2349. After ANOVA analysis, a gene was considered responsive to the treatments if it associated with a P value ≤ 0.05 and differentially expressed if the absolute value of $\log_2$ ratio was ≥ 0.584 (>1.5 fold induction or <0.67 repression). Hierarchical clustering of all genes marked as differentially expressed in one condition at least was performed using the HCE3.5 software with the complete linkage method and the Manhattan distance measure.

SA measurements.

SA was quantified in leaf tissues of entire plants inoculated with the different fungi in three biological replicates. Free SA was extracted and measured in *M. truncatula* leaf tissues according to a protocol adapted from Forcat and associates (2008). Briefly, the methanolic supernatant containing free SA and its internal standard, $^2\text{H}_4$ -SA, was analyzed by ultra-performance liquid chromatographic (UPLC)-electrospray tandem mass spectrometry using an Acquity UPLC coupled to a Micro-mass Q-ToF Premier (Waters, Milford, MA, U.S.A.) orthogonal acceleration time-of-flight mass spectrometer. Chromatographic separations were performed on an Acquity UPLC BEH C18 analytical column (100 mm by 2.1 mm by 1.7 μm) (Waters). The binary solvent system included A = water + 0.1% HCOOH and B = acetonitrile (liquid chromatography-mass spectrometry grade). The gradient profile consisted of 0 to 1 min, 5% B; 1 to 8 min, 5 to 68% B; 8 to 9 min, 95% B; 9 to 10 min, 95% B; 10 to 11 min, 5% B; and 11 to 12 min, 5% B with a flow rate of 300 $\mu\text{L}/\text{min}$. The mass spectrometry was operated in the negative mode. Quantification was based on pseudo-multiple reaction monitoring after collision-induced dissociation at 15 eV using the following mass transition: $137.0 > 93.0$ for SA and $141.0 > 97.0$ for $^2\text{H}_4$ -SA. Concentration of free SA in the sample was determined in relation to the $^2\text{H}_4$ -SA internal standard using QuanLynx software (Waters).

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AUTHOR-RECOMMENDED INTERNET RESOURCES

- Colletotrichum.org, a website for the *Colletotrichum* research community: www.colletotrichum.org
- INRA and CNRS Legoo database, a bioinformatics gateway towards integrative legume biology: www.legoo.org/
- The *Medicago truncatula* sequencing project: www.medicago.org/genome
- University of Maryland's Human-Computer Interaction Lab Hierarchical Clustering Explorer vs. 3.5 software: www.cs.umd.edu/hcil/hce