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Plant nitrogen supply affects the *Botrytis cinerea* infection process and modulates known and novel virulence factors

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Abstract

Plant nitrogen (N) fertilization is known to affect disease; however, the underlying mechanisms remain mostly unknown. We investigated the impact of N supply on the *Arabidopsis thaliana–Botrytis cinerea* interaction. *A. thaliana* plants grown in low nitrate were more tolerant to all wild-type *B. cinerea* strains tested. We determined leaf nitrate concentrations and showed that they had a limited impact on *B. cinerea* growth in vitro. For the first time, we performed a dual RNA-Seq of infected leaves of plants grown with different nitrate concentrations. Transcriptome analysis showed that plant and fungal transcriptomes were marginally affected by plant nitrate supply. Indeed, only a limited set of plant (182) and fungal (22) genes displayed expression profiles altered by nitrate supply. The expression of selected genes was confirmed by quantitative reverse transcription PCR at 6 hr postinfection (hpi) and analysed at a later time point (24 hpi). We selected three of the 22 *B. cinerea* genes identified for further analysis. *B. cinerea* mutants affected in these genes were less aggressive than the wild-type strain. We also showed that plants grown in ammonium were more tolerant to *B. cinerea*. Furthermore, expression of the selected *B. cinerea* genes in planta was altered when plants were grown with ammonium instead of nitrate, demonstrating an impact of the nature of N supplied to plants on the interaction. Identification of *B. cinerea* genes expressed differentially in planta according to plant N supply unveils two novel virulence functions required for full virulence in *A. thaliana*: a secondary metabolite (SM) and an acidic protease (AP).

KEYWORDS

Arabidopsis thaliana, *Botrytis cinerea*, defences, nitrogen, virulence factors

1 | INTRODUCTION

Nitrogen (N) is one of the major plant nutrients, essential for the production of amino acids, proteins, hormones, phenolics, and other cellular compounds (Miller and Cramer, 2005). It is involved in most plant physiological processes for growth and development, and can also impact disease resistance. N is frequently limiting in soils, therefore N commercial fertilizers are applied to cultivated soils to increase

crop yields. These fertilizers can be absorbed by plants as either ammonium or nitrate (Huber and Thompson, 2007; Patterson *et al*., 2010).

Numerous studies have shown that the different forms of N are metabolized differently and may result in differential physiological responses in the plant (Salsac *et al*., 1987; Hirel *et al*., 2011; Lea and Miflin, 2011). For example, Patterson *et al*., (2010) found that ammonium and nitrate triggered different signalling pathways in *Arabidopsis thaliana*. The specificity of the signalling resulted from alterations in extracellular pH associated with ammonium uptake, downstream metabolites in the ammonium assimilation pathway, and the presence or absence of the nitrate ion. Nitrate-specific responses revealed by microarray analysis concern functional categories such as cation transport, cytokinin response, and oxidative pentose phosphate pathway/NAD(P)H generation, which were up-regulated. In contrast, biotic stress/defence and transcriptional regulation categories were induced by ammonium. In a physiological study on long-term ammonium nutrition of *A. thaliana*, Podgorska *et al*. (2013) observed no substantial impairment of photosynthetic capacity but an increase of leaf NAD(P)H/NAD(P)$^+$ ratio, of reactive oxygen species (ROS) content, and an accumulation of biomolecules oxidized by free radicals.

Besides the effects on plant growth and development, the form of N available to plants and pathogens also affects disease severity (Huber and Watson, 1974; Snoeijers *et al*., 2000; Fagard *et al*., 2014). A given form of N may reduce one disease but increase another. For example, ammonium nutrition of tomato plants can either increase or decrease disease caused, respectively, by *Fusarium oxysporum* and *Pseudomonas syringae* (Duffy and Défago, 1999; Fernandes-Crespo *et al*., 2015). High nitrate application resulted in an increased susceptibility of tomato towards *Oidium lycopersicum* and *P. syringae* pv. *tomato* (Hoffland *et al*., 2000) and a decrease in disease severity of rice blast (Long *et al*., 2000). For the interaction with *Botrytis cinerea*, N status of plants influences the infection process differently depending on the plant species. Indeed, high nitrate results in increased disease in grapevine, strawberries, and the model plant *A. thaliana* (Fagard *et al*., 2014), whereas a reduced susceptibility of tomato towards the same pathogen was reported (Lecompte *et al*., 2010; Vega *et al*., 2015).

B. cinerea (perfect stage: *Botryotinia fuckeliana*) is a filamentous ascomycete fungus responsible for grey mould disease, which causes important economic losses (Dean *et al*., 2012). This necrotrophic pathogen is able to infect over 200 dicot hosts, in addition to some monocots. Genome sequences of two strains, B0510 and T4, have been annotated and are publicly available (Amselem *et al*., 2011; Van Kan *et al*., 2017). Currently, *B. cinerea* is controlled through the use of fungicides although serious problems of fungicide resistance have emerged (Leroux *et al*., 2002). It is thus crucial to develop new strategies to limit fungal colonization of plants.

B. cinerea possesses a large arsenal of molecules to kill and degrade host cells, such as nonspecific phytotoxins, cell wall-degrading enzymes and ROS (Van Kan, 2006; Choquer *et al*., 2007). Consistently, the numerous natural strains of *B. cinerea* isolated over the years show extensive variation in their aggressiveness on different plant species (Corwin *et al*., 2016). On the contrary, plants have developed a multitude of defence responses that restrain *B. cinerea* colonization. These defences include production of ROS, pathogenesis-related (PR) proteins, phytoalexins, as well as signalling dependent on the phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (Ferrari *et al*., 2003; Windram *et al*., 2012). Numerous transcriptomic approaches have been undertaken in plants, including *A. thaliana*, infected with *B. cinerea* but only gene profiles of the plant or the fungus alone have been considered (Windram *et al*., 2012; Blanco-Ulate *et al*., 2014; Sham *et al*., 2014; Vega *et al*., 2015). Two recent publications have reported a simultaneous transcriptome analysis of *B. cinerea* and *A. thaliana* in the same infected leaf tissue (Soltis *et al*., 2019; Zhang *et al*., 2019). In parallel, the effects of N supply on *A. thaliana* have been studied at the transcriptional level (Patterson *et al*., 2010; Podgorska *et al*., 2013). However, to our knowledge, no report relates a simultaneous transcriptome analysis of *B. cinerea* and *A. thaliana* in combination with an abiotic stress.

In this work, we characterize the influence of N supply on the tolerance of *A. thaliana* to *B. cinerea*. We also examined the impact of N on pathogen growth in vitro. We observed that the infection was reduced at low nitrate conditions, and thus performed a transcriptomic analysis at an early time point of infection (6 hr postinfection, hpi). A subset of plant and fungal genes affected by N supply was analysed by quantitative reverse transcription PCR (RT-qPCR) 6 and 24 hpi. Our study identified gene candidates susceptible to explain mechanisms underlying the effect of N supply on disease. Analysis of corresponding *B. cinerea* knockout mutants revealed the role of three of these candidates in the infection process, two of which are identified as virulence factors for the first time.

2 | RESULTS

2.1 | Effect of N supply on the susceptibility of *A. thaliana* towards a set of *B. cinerea* wild-type strains

We showed previously (Fagard *et al*., 2014) that *A. thaliana* leaves infected with mycelial plugs of *B. cinerea* wild-type strain B0510 were less susceptible to infection when plants were supplied with 2 mM NO_3^- than with 10 mM NO_3^-. To further understand the impact of N supply on *B. cinerea* infection, we also analysed plants grown at 0.5 mM NO_3^-, which had a mildly limiting effect on biomass (Table S1). Furthermore, we tested the effect of 2 mM NH_4^+, a concentration below the one shown as toxic (Podgorska *et al*., 2013). We did not detect any reduction in biomass (Table S1). Lesion areas measured at day 2 were reduced by about 28% at 0.5 mM compared to high (10 mM) nitrate (Figure 1a). At 2 mM NH_4^+ lesion areas were reduced by about 17%, 35%, and 40% compared to 0.5, 2, and 10 mM nitrate, respectively. These results showed that NH_4^+ nutrition increased *A. thaliana* tolerance to *B. cinerea*. As most infected leaves grown at 10 mM nitrate were almost completely invaded at day 2, we could not follow the study and compare lesion sizes after day 2.

B. cinerea isolates are diverse and can have different pathogenic behaviours (Rowe and Kliebenstein, 2007). Therefore, we tested the

effect of nitrate supply on the pathogenicity of different strains: B0510 whose genome was sequenced (Amselem *et al*., 2011), Bd90 collected in 1986 from grapevine in Bordeaux (Reignault *et al*., 1994), and BC1 and BC21 collected from tomato in commercial greenhouses in Avignon (Ajouz *et al*., 2010). For these four strains, we observed a decrease in plant susceptibility to the pathogen at low nitrate. However, the propagation rates were different, indicating different aggressiveness of these strains on *A. thaliana*. The differences between strains were most important at 0.5 mM nitrate: BC1 was more aggressive than B0510 and BC21, the latter being more aggressive than Bd90 (Figure 1b).

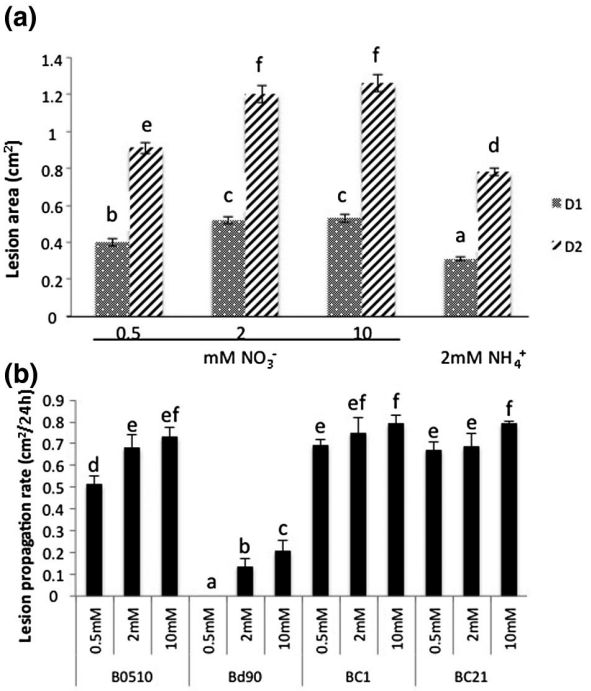


FIGURE 1 Pathogenicity tests on *Arabidopsis thaliana* leaves infected with *Botrytis cinerea* mycelium. Plants were grown with 0.5, 2, or 10 mM NO_3^- (a, b) or 2 mM NH_4^+ (a) and infected with *B. cinerea*. (a) The area of the lesions was measured daily. For each condition, three independent experiments were conducted with 20 leaves for each. Mean values of lesion areas were determined (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .05$). D1, day 1; D2, day 2. (b) Lesion propagation rates obtained after infection of *A. thaliana* leaves with different wild-type-strains (B0510, Bd90, BC1, BC21). Lesion propagation rates were calculated between day 2 and day 1. Values are the mean of three independent experiments (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .1$)

2.2 | Effect of N availability on in vitro growth of *B. cinerea*

We first quantified NO_3^- and NH_4^+ concentrations in *A. thaliana* leaves grown at 0.5, 2, 10 mM NO_3^-, and 2 mM NH_4^+ (Table 1). For NO_3^-, we obtained 94.9, 111, 137.1, and 50 nmol/mg fresh weight (FW), respectively. For NH_4^+, we obtained 1.01, 1.71, 1.31, and 3.25 nmol/mg FW, respectively. To mimic NO_3^- and NH_4^+ leaf concentrations, we then studied *B. cinerea* growth in vitro, using 80 and 140 mM NO_3^- or NH_4^+. We also included two concentrations often used in culture media, 10 and 29 mM. No significant difference was observed in radial growth of B0510 wild-type strain between NO_3^- and NH_4^+ (Figure 2). *B. cinerea* growth reached a maximum at 10 and 29 mM. A slight but significant decrease of about 19% was observed at 140 mM. Similar results were obtained for Bd90, BC1, and BC21 strains (Figure S1). We also tested 3 mM NH_4^+ on *B. cinerea* growth without observing any difference compared to 10 or 29 mM NH_4^+. Although no difference in radial growth was observed, all the strains sporulated better on NO_3^- than NH_4^+, whatever the concentration used, with about 10^6 spores/ml for B0510 in NO_3^- and 7×10^5 spores/ml in NH_4^+. All strains displayed an orange pigmentation on NH_4^+ medium.

Different amino acids were also tested (asparagine, glutamine, proline, histidine, and lysine; Figure S2). We observed no difference in radial growth of B0510 with all amino acids used compared to 29 mM NO_3^- except with lysine, where the network of mycelia was very sparse and thin. No sporulation was observed when proline and lysine were used as the N source.

From these results, it clearly appears that a high NO_3^- concentration (140 mM) reduced slightly B0510 growth in vitro. As an increase in the disease was observed with leaves grown at 10 mM NO_3^- (Figure 1), which corresponds to 140 mM NO_3^- available in leaves, we could conclude that the increased symptoms observed in *A. thaliana* at high nitrate are not simply the consequence of increased nitrate availability for the fungus in leaves.

2.3 | Identification of *A. thaliana* genes differentially modulated by *B. cinerea* infection (6 hpi) under various nitrate regimes

We performed a dual mRNA-Seq analysis to characterize both the transcriptome of *A. thaliana* leaves and that of *B. cinerea* in infected plants (6 hpi) grown at 0.5, 2, or 10 mM NO_3^-.

To determine the effect of NO_3^- supply on leaves in the absence of infection we compared the transcriptome of plants grown in high

TABLE 1 Nitrate and ammonium contents in foliar tissue of *Arabidopsis thaliana* grown with 0.5, 2 or 10 mM NO_3^- or 2 mM NH_4^+

	N supply conditions			
	0.5 mM NO_3^-	2 mM NO_3^-	10 mM NO_3^-	2 mM NH_4^+
Nitrate content in foliar tissue	94.9 ± 8.97	111 ± 15.60	137 ± 9.72	50 ± 3.59
Ammonium content in foliar tissue	1.01 ± 0.15	1.71 ± 0.33	1.31 ± 0.18	3.25 ± 0.47

Note: Values are expressed in nmol/mg fresh weight ± *SD*.

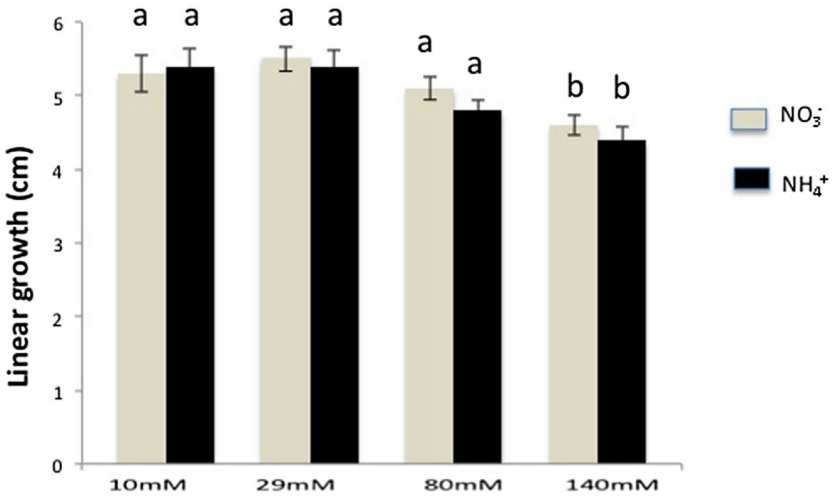


FIGURE 2 Radial growth of B0510 wild-type strain 3 days after inoculation. *Botrytis cinerea* was grown on Czapek agar with different concentrations of NO_3^- or NH_4^+ (10, 29, 80, and 140 mM). Data are the means of three independent experiments (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .05$)

TABLE 2 Modulation of selected *Arabidopsis thaliana* metabolism-related genes

Gene name	Arabidopsis Genome Initiative number	Comparison				
		Infected vs. mock (0.5 mM NO_3^-)	Infected vs. mock (2 mM NO_3^-)	Infected vs. mock (10 mM NO_3^-)	10 vs. 0.5 mM NO_3^- (mock)	10 vs. 0.5 mM NO_3^- (infected)
Nitrate reductase						
NIA1	AT1G77760	0.91*	0.76*	0.04	0.23	−0.66
NIA2	AT1G37130	2.35*	2.48*	2.40*	−0.01	0.02
Asparagine synthase						
ASN1	AT3G47340	−3.61	−1.62	−0.86	0.77*	3.53*
ASN2	AT5G65010	−0.56*	−0.74*	−0.84	−0.16	−0.45
Nitrate transporter						
NRT2.1	AT1G08090	N/A	N/A	N/A	−1.1*	N/A
NRT2.6	AT3G45060	5.31**	6.78**	6.41**	−0.61	0.48
NRT2.7	AT5G14570	−0.92*	−0.61*	−1.14*	0.01	−0.22
Glutamine synthase						
GLN1.1	AT5G37600	0.82*	0.93*	0.89*	−0.12	−0.05
GLN1.2	AT1G66200	−0.74*	−0.73*	−0.75	−0.14	−0.15
GLN1.4	AT5G16570	−0.72*	−0.60	−0.84	−0.08	−0.21
GLN2	AT5G35630	−1.04*	−0.87*	−0.92	0.00	0.12
Ammonium transporter						
AMT1.1	AT4G13510	1.17*	1.08*	1.34*	0.03	0.19
AMT2.1	AT2G38290	2.23**	2.27**	2.44**	0.07	0.27
Glutamate dehydrogenase						
GDH2	AT5G07440	3.12**	3.25**	2.84*	0.95*	0.67

Note: The values represent the $\log_2$ ratios between comparison conditions. Positive and negative values correspond to genes respectively more and less expressed in first condition compared to second condition. N/A: expression too low in one condition to calculate a ratio. Values with asterisks indicate significant differences between the two conditions.

*$p < 0.05$; **$p < 0.01$.

(10 mM) or low (0.5 mM) NO_3^-. We found that 330 and 353 genes were significantly more expressed in plants grown in high (10 mM) or low (0.5 mM) NO_3^-, respectively, but only 24 and 38, respectively, of those genes showed a $\log_2$ ratio higher than 1 (Table S2). This indicated that the modulation of plant gene expression due to NO_3^- availability, in the absence of infection, was mild. The expression of several genes related to nitrate transport and/or metabolism was repressed as expected under low NO_3^- in uninfected leaves (*ASN1*, *NRT2.1*, and *GDH2*: Table 2). On the other hand, defence-associated genes showed an increase in expression in plants grown in low NO_3^-.

For example, several WRKY transcription factors, such as *WRKY54*, *WRKY29*, and *WRKY38*, were more expressed in low NO_3^- (log_2 ratios of −1.01, −1.19, and − 1.86, respectively) but none were known to be involved in *B. cinerea* tolerance (Table S2). However, the *PDF1.2a* gene, a marker of the JA signalling pathway known to be involved in resistance against *B. cinerea*, showed a lower expression in plants grown at 10 mM NO_3^- (log_2 ratio −1.10; Table S2).

We then compared *A. thaliana* gene modulation in response to *B. cinerea* infection in plants grown under low (0.5 mM), medium (2 mM), or high (10 mM) NO_3^-. We found 2,920, 3,005, and 2,915 genes induced and 1,646, 1,322, and 110 genes repressed significantly (more than twofold) following *B. cinerea* infection 6 hpi in plants grown at 0.5, 2, or 10 mM NO_3^-, respectively (Figure S3a,b). Analysis of the functional categories of these genes using the FunCatDB indicated that, as previously described (Moreau *et al*., 2012; Zarattini *et al*., 2017), the functional category containing the highest number of genes modulated by infection is the metabolism-associated category (Figure S3c). This was true for both *B. cinerea*-induced and -repressed genes in all NO_3^- supply conditions. Furthermore, gene ontology enrichment analysis indicated that the functional categories associated with metabolism, defence, cellular transport, and cellular communication were over-represented in the *A. thaliana* genes induced by *B. cinerea* infection compared to the whole genome. The categories associated with metabolism, cellular transport, and cellular biogenesis were over-represented in the *A. thaliana* genes repressed

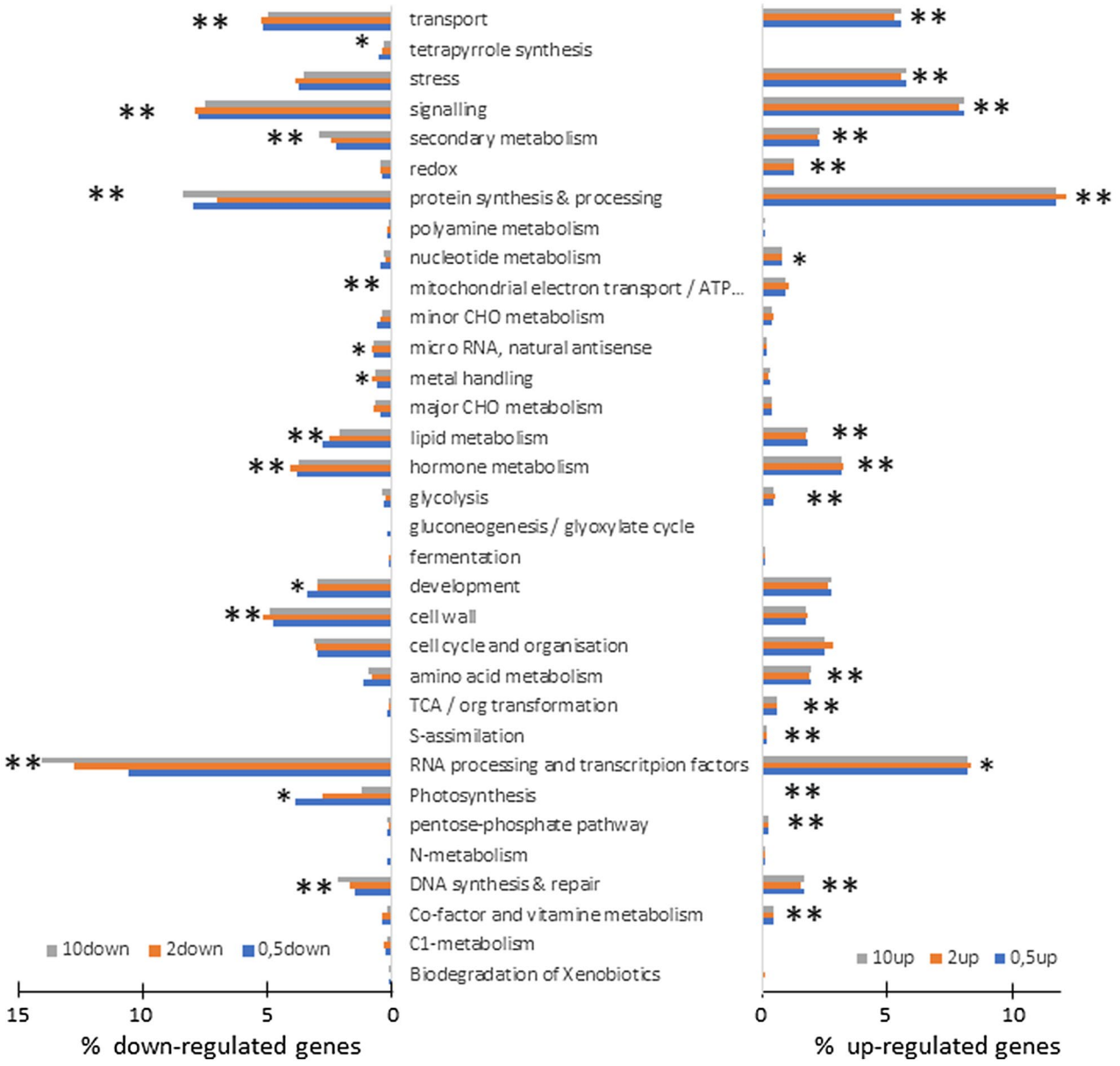


FIGURE 3 Genes down- (left) or up- (right) regulated by *Botrytis cinerea* infection were analysed using the MapMan categories of the "Classification SuperViewer Tool" (http://bar.utoronto.ca). Categories over-represented were compared to the whole genome: $^*p < .05$, $^{**}p < .01$. The indicated categories were over-represented in all NO_3^- conditions except for down-regulation of tetrapyrrole synthesis genes, which was specific to 0.5 and 2 mM NO_3^-

by *B. cinerea* infection (Figure S3c). To characterize in more detail *A. thaliana* genes modulated by infection under different nitrate regimes, we used the MapMan categories, which are more detailed (Thimm *et al*., 2004). We found that most MapMan categories were represented in similar proportions among genes modulated by infection in plants grown under contrasting N regimes (Figure 3). As expected, several categories were significantly over-represented compared to the whole genome, for example the "stress", "signalling", "redox", and "hormone metabolism" categories in the up-regulated genes, and the "photosynthesis" category in the down-regulated genes. Interestingly, we found slight differences between N regimes only in the down-regulated genes. Indeed, relatively fewer transcription factors-encoding and secondary metabolism-associated genes were repressed under lower NO_3^- concentrations, although they were still significantly over-represented compared to the whole genome (Figure 3).

To determine whether the effect of NO_3^- supply on the infection process occurred through the modulation of known N metabolism and/or defence gene expression, we first compared $\log_2$ ratios of selected genes in low and high NO_3^- (Tables 2 and 3). As shown previously (Fagard *et al*., 2014), the expression of several genes related to N transport and/or metabolism was affected by infection. In most cases the modulation occurred in all NO_3^- conditions, although there were some variations in the intensity of modulation (Table 2). *NIA1* and *ASN1* on the contrary were modulated by infection only in plants grown in low NO_3^- (Table 2). We then focused on genes involved in defence known to be expressed in response to *B. cinerea* infection and/or involved in resistance against *B. cinerea*, in particular genes involved in the JA and SA signalling pathways (Table 3). As expected, many of these genes were strongly induced in response to infection; however, several of these genes were not induced in leaves grown in high NO_3^-. This was true for several downstream response genes of both the SA and JA pathways and was most strongly marked for *PDF1.2* genes, which are responsive to JA signalling.

As described above, analysis of the functional categories of the RNA-Seq data revealed only slight differences in the enrichment between the different N regimes (Figure 3). Thus, to identify plant genes modulated differently by nitrate, we compared *B. cinerea*-modulated genes in plants grown in the different N regimes. This allowed us to identify the genes most differentially expressed in response to infection in low NO_3^- compared to high NO_3^- (Table S3). This confirmed that several defence genes, including the JA-dependent *PDF1.2* genes, were among the most strongly expressed in plants grown in 0.5 mM NO_3^-, which are more tolerant to *B. cinerea*. Furthermore, in these conditions, plants also expressed several genes involved in signalling, such as a calcium-dependent kinase (*CIPK20*), two transcription factors (*MYB59* and *ANAC90*), and a putative disease-resistance protein (At3g25010; Table S3), all of which could potentially be involved in the increased tolerance of plants grown in low NO_3^- to *B. cinerea*.

Altogether, our data indicate that NO_3^- supply modulates defence activation in response to *B. cinerea* as well as genes of unknown function.

TABLE 3 Modulation of selected *Arabidopsis thaliana* defence-related genes following *Botrytis cinerea* infection (6 hr postinfection))

		Comparison			
Gene name	Arabidopsis Genome Initiative number	Infected vs. mock (0.5 mM NO_3^-)	Infected vs. mock (2 mM NO_3^-)	Infected vs. mock (10 mM NO_3^-)	10 vs 0.5 mM (infected)
Salicylic acid pathway					
ICS1	AT1G74710	2.16**	2.35**	2.00*	−0.09
PR1	AT2G14610	1.66*	0.72*	−0.57	−1.43*
PR5	AT1G75040	1.16*	−0.06	−0.48	−1.41**
PAD4	AT3G52430	1.14*	1.02*	0.79*	−0.59
EDS1	AT3G48090	0.71*	0.06	0.54	−0.35
Jasmonic acid pathway					
AOS	AT5G42650	0.59	0.68*	0.60	0.15
AOC1	AT3G25760	2.89**	3.12**	3.33**	0.55
PDF1.2a	AT5G44420	2.61**	2.51**	0.80	−2.91*
PDF1.2c	AT5G44430	3.66**	3.59**	2.40	−3.18*
VSP2	AT5G24770	−1.69	−0.49	1.23	2.22
LOX2	AT3G45140	0.95*	0.76	0.63	−0.27
Cell wall-degrading enzyme					
PME17	AT2G45220	10.53**	11.24**	11.25**	0.36

Note: The values represent the $\log_2$ ratios between comparison conditions. Positive and negative values correspond to genes respectively more and less expressed in first condition compared to second condition. N/A: expression too low in one condition to calculate a ratio. Values with asterisks indicate significant differences between the two conditions.

*$p < 0.05$; **$p < 0.01$.

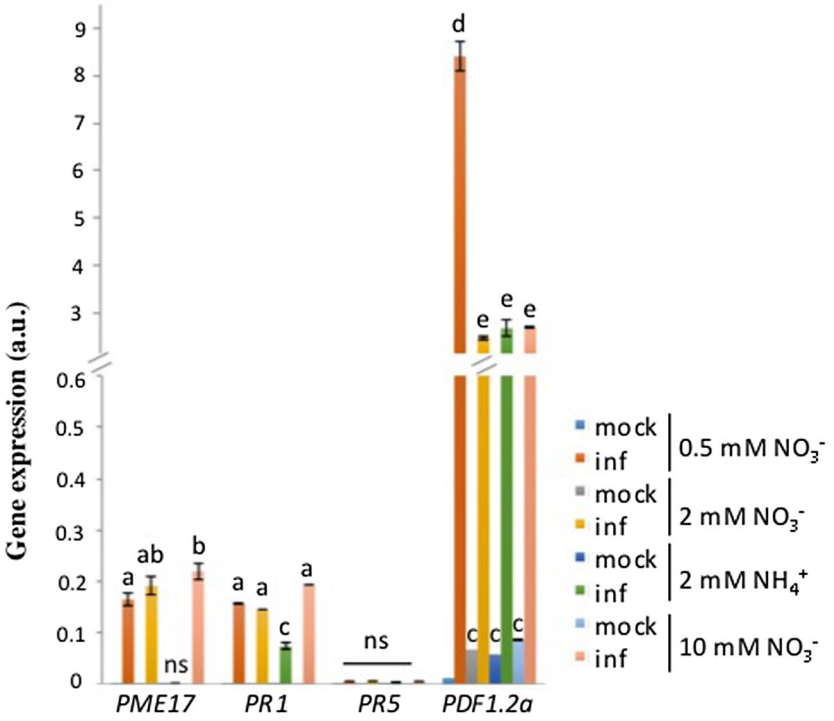


FIGURE 4 Expression of different *Arabidopsis thaliana* genes in leaves grown at 0.5, 2, or 10 mM NO_3^- or 2 mM NH_4^+ and infected (inf) or not (mock) with *Botrytis cinerea* (B0510). Expression levels were quantified 24 hr postinfection. Transcript levels were normalized to the transcript level of *A. thaliana* reference gene *APT*. Data are expressed as mean normalized expression in arbitrary units (a.u.) and are the means of triplicates (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .1$). ns, nonsignificant. The experiment was repeated three times with similar results

2.4 | Expression profile of a subset of *A. thaliana* genes at 24 hpi

We selected four genes for further analysis, *PDF1.2a*, *PR1*, and *PR5*, differentially modulated by infection under different NO_3^- regimes, and *PME17*, a pectin esterase, which was among the most strongly induced genes following infection independently of NO_3^- supply (Table 3).

All four selected genes showed very low or no expression in mock foliar tissue, whatever the NO_3^- concentration (Figure 4). Three of the four selected genes (*PME17*, *PR1*, *PDF1.2a*) showed induced expression in response to *B. cinerea* infection at 24 hpi. The expression profile of *PDF1.2a* was similar to that observed at 6 hpi with a level of expression four times higher at 0.5 mM NO_3^- than in the other N supply conditions. *PR1* did not show differences of expression under different NO_3^- regimes; however, we noticed a weaker induction of this gene following *B. cinerea* infection when *A. thaliana* plants were grown with NH_4^+. *PME17* was not differentially expressed under different NO_3^- regimes, but was not induced by *B. cinerea* in leaves grown in 2 mM NH_4^+.

Altogether these results confirm an increase in the expression level of the *PDF1.2a* gene marker of JA signalling, which could explain the increased tolerance to *B. cinerea* of leaves grown at 0.5 mM NO_3^-.

2.5 | Identification of *B. cinerea* genes differentially expressed in planta under various nitrate regimes at 6 hpi

The genome of *B. cinerea* was first sequenced in 2011 (Amselem *et al*., 2011) and completed in 2017 (Van Kan *et al*., 2017). This 42.9 Mb genome comprises 18 chromosomes and 16,448 genes. In our transcriptomic analysis at 6 hpi, we found no expression for 17% of *B. cinerea* genes. We also arbitrarily eliminated from our study genes for which more than 10 mRNA-Seq reads/million were found in noninfected leaves as this could reflect either a slight contamination or a homology too important to orthologous plant genes. As a result, 3,196 genes were considered as expressed 6 hpi and used for further analysis (19% of the *B. cinerea* genome). Among them, 22 were differentially expressed under different nitrate regimes (Table 4). Several of these genes have putative functions that could be important for pathogenesis, such as ROS detoxification, toxin production, protein degradation, or metabolite biosynthesis. None were related to N metabolism except for a putative glutamine amidotransferase that showed, however, very low expression at 6 hpi. Transcriptomic data were confirmed for selected genes by RT-qPCR (Pearson correlation of 0.74 to 0.95, indicating a high correlation between these two sets of data; correlation is significant at the 0.05 level) (Figure S4).

Among these 22 genes, we selected the most highly expressed for further analysis: an acidic protease I (*AP*), an oxidoreductase (*OR*), and a secondary metabolite biosynthesis gene (*SM*). We also selected four genes not differentially expressed under different nitrate regimes but known as pathogenicity factors such as the phytotoxins botcinic acid (*BoA6*) and botrydial (*Bot2*), and cell wall-degrading enzymes such as polygalacturonase I (*PG1*) and pectin methylesterase 3 (*PME3*).

2.6 | Expression profiles of selected *B. cinerea* genes at 24 hpi

Expression of the selected genes was first analysed in B0510 mycelium grown in vitro on Czapek medium supplemented with NO_3^- or NH_4^+ at

TABLE 4 Expression profile of *Botrytis cinerea* genes in infected *Arabidopsis thaliana* leaves grown under different nitrate conditions

Gene number	Gene description	Fold change (log_2)		
		2 mM vs. 0.5 mM	10 mM vs. 2 mM	10 mM vs. 0.5 mM
BC1G_05082	Alcohol dehydrogenase superfamily, zinc-type	1.72*	1.39*	3.10*
BC1G_10664	Alcohol dehydrogenase superfamily, zinc-type	−0.48	1.67*	1.18
BC1G_01745	NADP-dependent leukotriene b4 12-hydroxydehydrogenase	0.08	0.90*	0.97
BC1G_12880	Isoflavone reductase	0.87	1.91*	2.76*
BC1G_01112	NAD(P)-binding domain	0.65	2.33	2.97*
BC1G_02778	Class I glutamine amidotransferase-like	0.95	1.24*	2.19*
BC1G_14715	Protein of unknown function DUF3632	0.27	1.54	1.81*
BC1G_08890	β-Lactamase-like	0.62	1.32	1.93*
BC1G_14153	Acidic protease 1	0.34	0.60	0.94*
BC1G_15304	Quinone oxidoreductase	0.95	0.95	1.88*
BC1G_12671	Quinone oxidoreductase	0.38	1.06	1.43*
BC1G_08847	Oxidoreductase	0.29	0.90*	1.19
BC1G_10231	β-Glucosidase	−0.19	0.81*	0.62
BC1G_03253	Pyridoxal reductase	−0.05	0.83*	0.78
BC1G_13293	Unknown	−0.03	1.15*	1.11
BC1G_02021	Glucose-methanol-choline oxidoreductase	0.81*	0.01	0.81
BC1G_14185	Signalling protein	−0.65	−0.52	−1.18*
BC1G_07052	Pectate lyase	−0.80	−0.73	−1.54*
BC1G_07391	Glutathione S-transferase (BcGST1)	−0.08	1.45*	1.37
BC1G_12605	Glutathione S-transferase (BcGST14)	−0.27	1.15*	0.87
BC1G_03433	Glutathione S-transferase (BcGST13)	0.48	1.18*	1.66*
BC1G_09645	Glutathione S-transferase (BcGST3)	0.80	0.67	1.46*

Note: Gene description corresponds to putative function as described in BcPortal (http://botbioger.versailles.inra.fr/botportal/index.html) except for BC1G_14153, which is known to encode an acidic protease (Rolland *et al*., 2009). The leaves were sampled 6 hr postinfection. Values are log_2 signal ratios of infected leaves between 2 and 0.5 mM, 10 and 2 mM or 10 and 0.5 mM of nitrate. Positive and negative values correspond to genes up- and down-regulated. Asteriks (*) indicate that gene expression was significantly changed.

29, 80, or 140 mM (Figure 5). Three of the selected genes displayed strong expression in vitro (*AP*, *OR*, and *PG1*). However, only the *AP* gene was differentially expressed on different nitrate concentrations and reached a maximum at 140 mM. The four other genes (*SM*, *BoA6*, *Bot2*, and *PME3*) showed very low expression. For NH_4^+ treatment, we established two types of profile compared to NO_3^- treatment: no difference in expression profile for *OR* and *PG1*, or a drastic repression of expression for *AP*, *SM*, *PME3*, and *Bot2*.

In infected plants grown at different concentrations of NO_3^- or with 2 mM NH_4^+, all the selected *B. cinerea* genes were more expressed 24 hpi than during in vitro growth, the *PG1*-encoding gene displaying a maximal expression level (Figure 6). The *AP* gene was the only one differentially expressed in contrasting nitrate conditions while the expression of the *OR* gene was practically nonexistent. The *BOA6* gene showed a slight repression in higher nitrate conditions when lesion sizes on leaves reached a maximum. Expression of the *SM* and *Bot2* genes was not affected by the nitrate conditions at 24 hpi. With NH_4^+ treatment, we found two types of profiles compared to NO_3^- treatment: a repression for the *AP*, *PME3*, and *BOA6* genes, or an increase in expression for the *SM*, *PG1*, and *Bot2* genes. The increased expression of these genes under NH_4^+ conditions was observed although foliar lesions were reduced (Figure 1). This result suggests that plants grown in NH_4^+ create a different environment for *B. cinerea* compared to plants grown in NO_3^-.

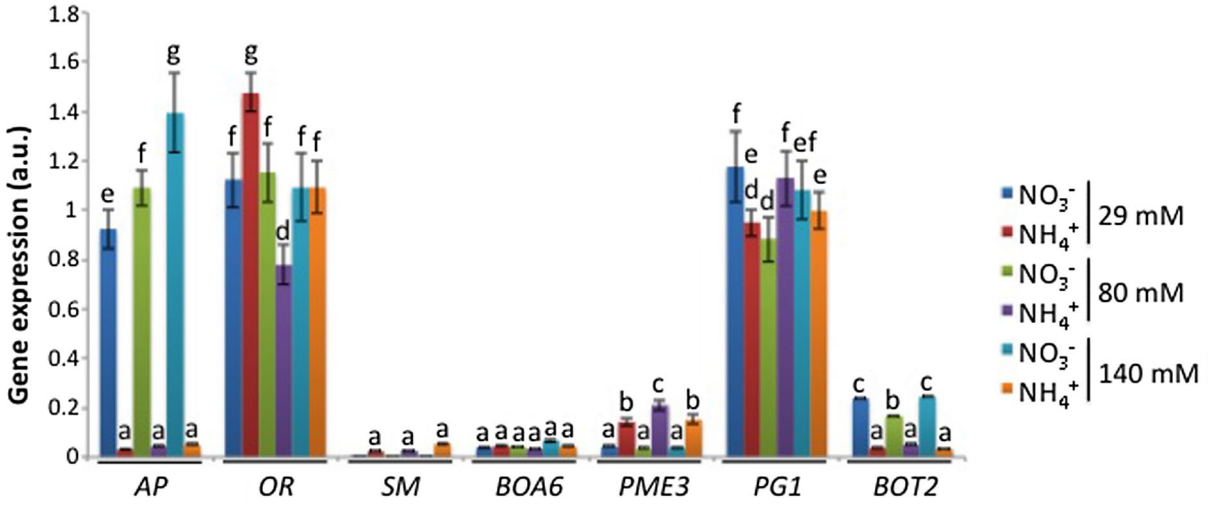
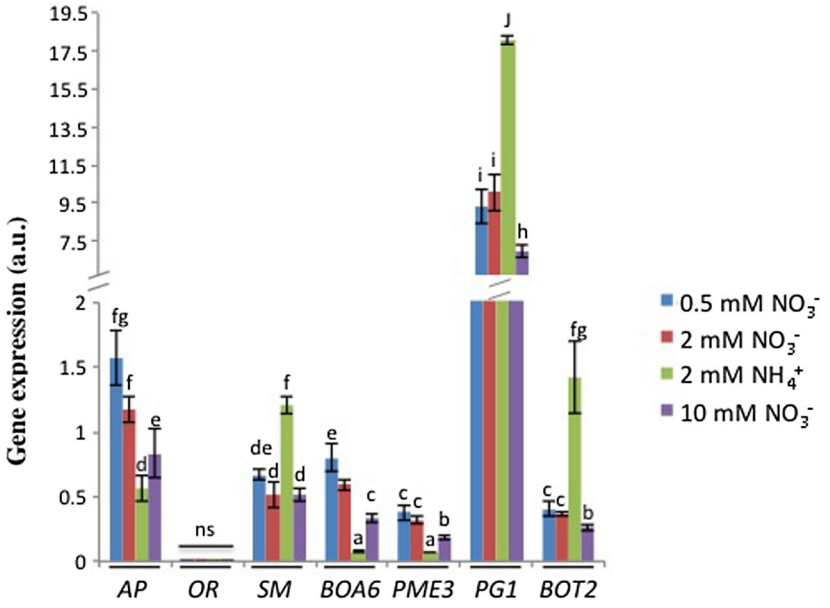


FIGURE 5 Expression of different genes of wild-type *Botrytis cinerea* (B0510) grown in vitro on Czapek medium supplemented with 29, 80, or 140 mM NO_3^- or NH_4^+. Expression levels were quantified after 3 days of mycelium growth. Transcript levels were normalized to the transcript level of *B. cinerea* reference gene *ACTIN*. Data are expressed as mean normalized expression in arbitrary units (a.u) and are the means of triplicates (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .1$). The experiment was repeated twice with similar results

FIGURE 6 Expression of different genes of wild-type *Botrytis cinerea* (B0510) in infected leaves grown at 0.5, 2, or 10 mM NO_3^- or 2 mM NH_4^+. Expression levels were quantified 24 hr postinfection. Transcript levels were normalized to the transcript level of *B. cinerea* reference gene *ACTIN*. Data are expressed as mean normalized expression in arbitrary units (a.u.) and are the means of triplicates (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .1$). ns, nonsignificant. The experiment was repeated three times with similar results



2.7 | Pathogenicity of *B. cinerea* mutants deficient in *AP*, *SM*, or *Bot2*

We selected the *AP* and *SM B. cinerea* genes for further analysis as they displayed strong expression in planta and were affected by N supply at both 6 and 24 hpi. We also included the *BOT2* gene in our analysis as it was highly expressed under NH_4^+ in planta but not in vitro. To determine whether these genes were involved in pathogenicity, we analysed the behaviour of corresponding mutants on leaves from *A. thaliana* plants grown in 2 mM NO_3^-. Because all three genes showed a very different expression profile in plants grown in 2 mM NH_4^+ (Figures 5 and 6), we also tested the mutants in this condition. For each gene, two mutants were tested. Detached leaves were infected with the parental strain B0510 and the mutants, and lesion areas were measured daily (Figure 7). As shown above (Figure 1) the wild-type strain was less aggressive under NH_4^+ than NO_3^-. Compared to the wild-type strain, the mutants displayed reduced aggressiveness. The reduction was particularly drastic with the *ap* and *bot2* mutants. Similar results were obtained with the second mutant of each gene (data not shown). Globally, these results reveal a crucial role for the acidic protease and Botrydial in the *A. thaliana*–*B. cinerea* interaction.

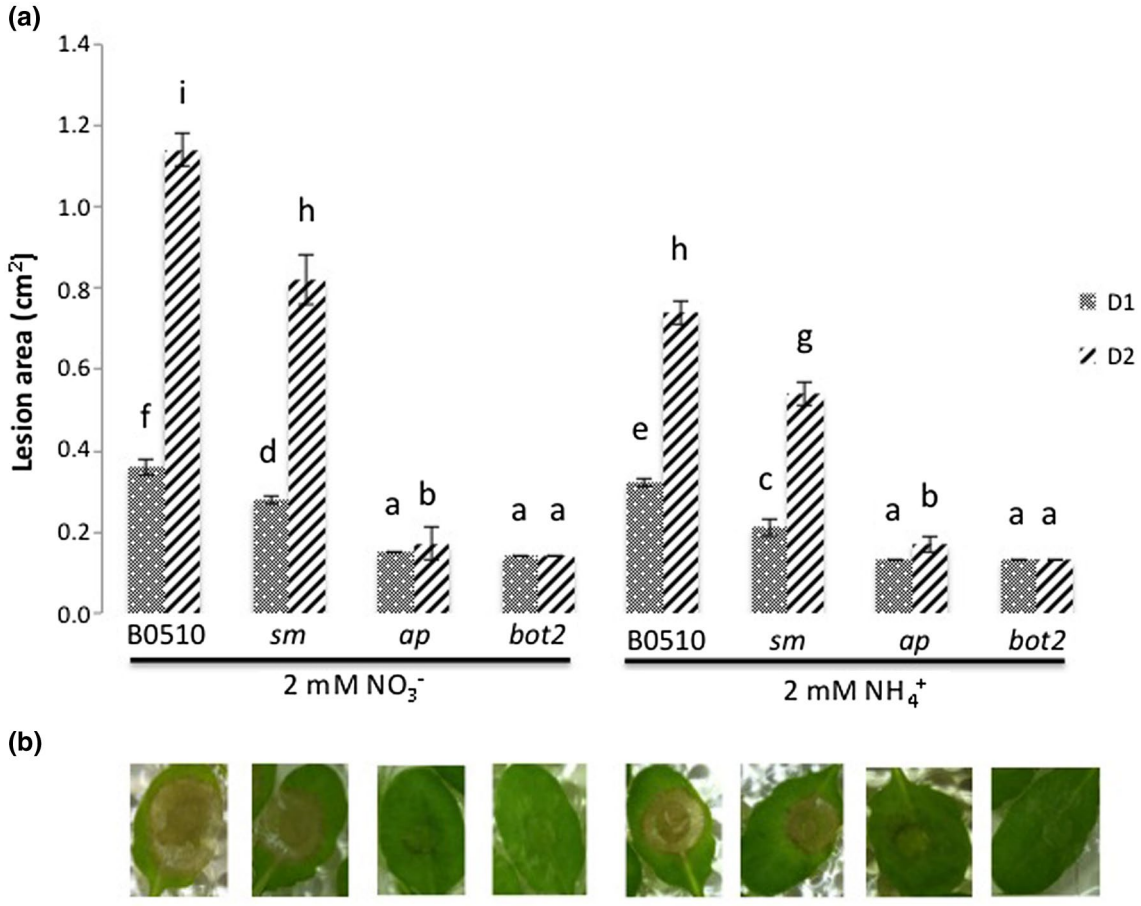


FIGURE 7 Pathogenicity tests on *Arabidopsis thaliana* leaves infected with different *Botrytis cinerea* strains. Plants were grown on 2 mM NO_3^- or NH_4^+ before infection with either the B0510 wild-type strain or mutants altered in secondary metabolism (*sm*), acidic protease (*ap*), or botrydial toxin (*bot2*). (a) Lesion areas were measured daily. (b) A representative picture of disease symptoms (2 days postinfection) is shown for each condition tested. For lesion areas, three independent experiments were conducted with 30 leaves each. Mean values of lesion areas were determined (±*SD*). Different letters indicate significantly unequal values (Mann–Whitney test, $p = .05$)

3 | DISCUSSION

Despite numerous studies about the influence of N supply on plant disease, the underlying mechanisms have not been investigated in detail (Walters and Bingham, 2007; Fagard *et al*., 2014; Vega *et al*., 2015). Several hypotheses have been proposed, such as an increase in nutrient availability for the pathogen (Gupta *et al*., 2013), an increase in plant immunity partly associated with ammonium nutrition (Patterson *et al*., 2010), or a direct effect on regulation of pathogenicity factors by N availability (Zhou *et al*., 2017). In this study, we investigated the impact of N supply on both sides of the *A thaliana*–*B. cinerea* interaction.

Indeed, low nitrate supply was shown to increase tolerance of *A. thaliana* towards *B. cinerea* strain B0510 (Fagard *et al*., 2014). With the use of a collection of *B. cinerea* isolates differing in their aggressiveness, we ensured that the tolerance conferred by low nitrate supply was not strictly related to the fungal strain. These results were similar to those obtained with other plant hosts such as vine (Delas *et al*., 1991), strawberry, lettuce (Lecompte *et al*., 2013), and legumes (Davidson and Krysinska-Kaczmarek, 2007). On the contrary, an increase in severity of the disease caused by *B. cinerea* was observed on tomato (Hoffland *et al*., 1999; Lecompte *et al*., 2010). We also found that ammonium conferred more tolerance of *A. thaliana* to *B. cinerea* than nitrate at the same concentration. Therefore, we assumed that the influence of N on the interaction between *B. cinerea* and host plants could affect any important factor governing the interaction, namely fungal growth, plant defence, and fungal virulence.

The acquisition of nutrients is a key component in the life of any microbial pathogen and is crucial for maintaining growth in the host. N assimilation and regulation in mycelial fungi is well documented, especially for *Aspergillus nidulans*, *Neurospora crassa*, and *Saccharomyces cerevisiae* (Caddick, 2004). We know that NH_4^+, which is directly metabolized to glutamate and glutamine, is an excellent and often preferential N source for fungi. Nitrate, the major form of inorganic N in the environment also used by fungi must be metabolized to NH_4^+. When these primary N sources are not available, many other sources can be used, such as nitrite, amides, most amino acids, and proteins (Marzluf, 1997). To mimic the N environment that *B. cinerea* may encounter during growth in planta, we determined NO_3^- and NH_4^+ concentrations in healthy leaves grown at 0.5, 2, and 10 mM NO_3^- and 2 mM NH_4^+ and tested these concentrations on *B. cinerea* growth in vitro. Apart from

a slight growth reduction at high concentration of the two forms of N, no difference in the growth rate of *B. cinerea* was observed between NO_3^- and NH_4^+. Lecompte *et al*., (2010) also observed a decrease in radial growth of *B. cinerea* at high concentration of ammonitrate. Amino acids like asparagine, glutamine, proline, histidine, and lysine were also tested in a previous study, the first three having been found to accumulate in *A. thaliana* plants grown at low nitrate (Fagard *et al*., 2014). There was no difference in radial growth of *B. cinerea* compared to nitrate except with lysine for which the network of mycelia was very sparse and thin. Consequently, reduced fungal growth observed at high nitrate in vitro is not consistent with the increased size of foliar lesions found at the same concentration. These results indicate that a simple nutritional effect of nitrate on *B. cinerea* growth cannot explain the in planta observations. Interestingly, Zhou *et al*. (2017) found that in the interaction between cucumber and *Fusarium oxysporum*, the effect of nitrate on disease development was different under in vitro and in vivo conditions. These authors concluded that N could affect not only pathogen growth and virulence, but also plant metabolism. Therefore, we performed a transcriptomic analysis of *A. thaliana* leaves infected by *B. cinerea* (6 hpi) to analyse both *A. thaliana* and *B. cinerea* genes differentially expressed, under low and high nitrate. As early as 6 hpi, 83% of *B. cinerea* genes were already expressed in leaves regardless of the nitrate condition. We found that 3,196 *B. cinerea* genes (19% of *B. cinerea* genome) were significantly expressed in planta 6 hpi with 22 of them differentially expressed under different nitrate regimes. The potential functions of these latter concern the production of secondary metabolites, ROS, and enzymes involved in detoxification processes. There were only two enzymes probably involved in nitrogen metabolism, an acidic protease and a glutamine amidotransferase-like. These genes are mostly expressed in high nitrate, suggesting the existence of a better detoxification by *B. cinerea* of defence molecules produced by the plant host. These results could explain why more severe symptoms are observed at high nitrate.

Concerning the major pathogenicity factors such as cell wall-degrading enzymes, our transcriptomic analysis revealed that at 6 hpi, the corresponding genes were highly expressed in planta, in particular *PG1*, regardless of nitrate supply. As it was reported that fungal pathogenicity factors were induced similarly during growth in planta as in vitro under N-limiting conditions, Donofrio *et al*. (2006) suggested the occurrence for the fungus of N-starvation conditions in planta. We thus analysed by RT-qPCR *PG1* expression in *B. cinerea* grown in vitro with 29, 80, and 140 mM nitrate. These nitrate concentrations correspond to those determined in *A. thaliana* leaves grown at 0.5, 2, and 10 mM nitrate. For the three nitrate concentrations, the expression level of *PG1* was 10 times lower in vitro than in planta, which indicates that there is not a direct relationship between N starvation and effector gene expression during fungal growth and colonization in planta.

Concerning plant genes, our transcriptomic analysis showed that under low nitrate, several N metabolism marker genes were slightly repressed, indicating as shown previously (Peng *et al*., 2008) that plants grown in low nitrate adapt their metabolism. We also found that a number of known or putative sugar transporters were repressed under low nitrate conditions, consistent with a conservation of the C/N balance under N limitation. Under low nitrate, several defence-associated genes were slightly up-regulated in the absence of infection (Table S1); however, the differences in expression levels were often low compared to expression levels observed following infection. Furthermore, most of these genes did not correspond to major known defences against *B. cinerea*, and thus the biological significance of these observations remains to be clarified. Analysis of functional categories of modulated genes showed that transcription factors and secondary metabolism-associated genes were over-represented in induced genes. Interestingly, under low nitrate, the relative number of genes in these two categories was higher than under high nitrate, suggesting that activation of these genes could contribute to the lower susceptibility observed under low nitrate.

Concerning the plant response to *B. cinerea* infection, our data show that N limitation does not strongly affect the plant's qualitative response. Indeed, the vast majority of *A. thaliana* genes were modulated in their expression with similar patterns in response to *B. cinerea* in all nitrate conditions, sometimes with slight differences in the intensity of the response. A subset of N metabolism and/or defence-related genes showed strong differences in their pattern of expression in response to *B. cinerea* in plants grown in different nitrate conditions. Among those genes, we found the asparagine synthetase-encoding *ASN1* gene (Table 1), involved in N metabolism as well as in defence against microbial pathogens in pepper (Hwang *et al*., 2011). However, contrary to previous observations with bacterial pathogens, *ASN1* is repressed following *B. cinerea* infection and its expression level is lower in tolerant conditions (low nitrate). Thus, *ASN1* expression levels are unlikely to explain differences in tolerance levels observed in this study.

On the other hand, we found that JA-dependent gene expression was strongly modulated by nitrate availability with expression levels higher in low NO_3^-, as shown previously in tomato (Vega *et al*., 2015). Consistently the JA-signalling deficient mutant, *jar1*, was less tolerant in low nitrate, indicating that at least part of the increased tolerance in low nitrate could result from higher JA-dependent defence (Figure S4a,b). However, higher JA-dependent defence does not seem to explain higher tolerance in plants grown in ammonium (Figure 4). This is consistent with a previous study (Fernandez-Crespo *et al*., 2015) in which the authors showed that plants grown in NH_4^+ were more resistant to *P. syringae*. Interestingly, this increased resistance of plants grown in NH_4^+ was correlated with enhanced H_2O_2, abscisic acid, and putrescine accumulation.

Interestingly, the reduced pathogenicity of *B. cinerea* mutants deficient in a secondary metabolite (*sm*), an acidic protease (*ap*), or a toxin (*bot2*) on *A. thaliana* was visible under both N conditions (2 mM NO_3^- or NH_4^+) in the plant culture medium. The *sm* mutant is interesting because it was found as aggressive as the wild-type on French bean and tomato leaves (Aguileta *et al*., 2012). The loss of pathogenicity of AP- and Bot2-deficient mutants is striking. Furthermore,

Bot2 remained as pathogenic as the wild-type on bean (Pinedo *et al*., 2008). Thus, our study reveals that the genes responsible for production of AP and Bot2 play a crucial role in the pathogenicity of *B. cinerea* on *A. thaliana* and that AP and Bot2 may be considered as potential virulence factors involved in host specificity for *B. cinerea*. AP has been shown to be secreted during infection and to have protease activity (Rolland *et al*., 2009). Thus, a possible role for AP in planta would be to degrade plant proteins during the first phases of the infection. Possible targets of AP are plant defence proteins, to repress defence activation, cell wall proteins, to help dismantle the cells, or less specifically plant proteins to provide amino acids for fungal growth.

Altogether, our results show that N supply could be used as part of disease control strategies because N availability affects the pattern of defence activation, particularly JA-dependent defence. Further investigations are required to identify the other sources of tolerance affected by N supply as well as the plant signals that affect fungal in planta virulence expression depending on N supply.

4 | EXPERIMENTAL PROCEDURES

4.1 | Plant growth

A. thaliana ecotype Col-0 was obtained from the INRA-Versailles collection. The *jar1-1* mutant (Staswick *et al*., 1992) was provided by the Nottingham Arabidopsis Stock Center. Plants were grown on nonsterile sand in a growth chamber (65% relative humidity, 8 hr photoperiod, 21°C). Plants were supplied for 6 weeks with a nutrient solution containing 0.5, 2, or 10 mM NO_3^- or 2 mM NH_4^+.

4.2 | Nitrate and ammonium quantification

Leaves were frozen and finely ground in liquid nitrogen. Nitrate quantification was performed with 20 mg of ground leaf tissue (Miranda *et al*., 2001). One hundred milligrams of ground leaf tissue was used for ammonium quantification with the phenol hypochlorite assay.

4.3 | Culture of *B. cinerea* and infection method

The wild-type strains of *B. cinerea* used in this study were B0510 collected from *Vitis* in Germany (Quidde *et al*., 1998), Bd90 collected in 1986 from grapevine in Bordeaux (Reignault *et al*., 1994) and two strains, BC1 and BC21, collected from tomatoes in commercial greenhouses (Lecompte *et al*., 2010). Six mutants of *B. cinerea* have also been tested in this study, two strains deleted for each of the following genes: *AP* (Rolland *et al*., 2009), *SM* (Aguileta *et al*., 2012), *Bot2* (Pinedo *et al*., 2008). *B. cinerea* strains were grown on Czapek agar medium containing 0.5 g/L KCl, 0.5 g/L $MgSO_4.7H_2O$, 10 mg/L $FeSO_4.7H_2O$, 1 g/L K_2HPO_4, 10 mg/L $Na_2MoO_4.2H_2O$, 30 g/L glucose supplied with 10, 29, 80, or 140 mM NO_3^- or NH_4^+. For the fungal growth rate, mycelial plugs (6 mm diameter) from 3-day-old culture were inoculated in the centre of Czapek medium for each NO_3^- and NH_4^+ concentration cited above. Cultures were incubated at 21-°C with continuous light and colony diameter was measured daily.

For RNA extraction, B0510 wild-type was inoculated on Czapek agar medium added with the different NO_3^- or NH_4^+ concentrations and covered with a cellophane sheet. After 3 days of incubation, mycelium was scraped and RNA extracted.

For pathogenicity assays, each strain was grown on 1.2% cristomalt agar (MA). One mycelium plug (3 mm diameter), taken from the actively growing edge, was inverted onto the upper surface of one excised *A. thaliana* leaf. Infected leaves were kept in a Petri dish under high humidity and daylight, and incubated at 21°C. For each strain, 20 leaves were inoculated and mean lesion area was determined daily using Optilab/Pro-F2.6.3.

4.4 | RNA extraction and cDNA synthesis for RNA-Seq experiments

Three independent biological replicates were produced. For each biological repetition and each point, RNA samples were obtained by pooling RNAs from more than 24 plants. Leaves were collected on plants at the 3.90 developmental growth stage (Boyes *et al*., 2001), grown in 0.5, 2, or 10 mM nitrate conditions. Total RNA was extracted using RNeasy (Qiagen) according to the supplier's instructions. Sequencing technology used was an Illumina Hiseq2000 (thanks to IG-CNS for giving us privileged access to perform sequencing). RNA-Seq libraries were performed by TruSeq Stranded mRNA protocol (Illumina). The RNA-Seq samples were sequenced in paired-end (PE) with a 260 bp sizing and a 100 bases read length. Four samples by lane of NextSeq500 using individual bar-coded adapters and giving approximately 37 million of PE reads by sample were generated. All steps of the experiment were managed in CATdb database (Gagnot *et al*., 2007, http://tools.ips2.u-psud.fr/CATdb/) ProjectID AAP_BAP_NitroPath_2014 according to the international standard MINSEQE "minimum information about a high-throughput sequencing experiment".

4.5 | RNA-Seq bioinformatic treatment and analysis

To facilitate comparisons, each sample followed the same steps from trimming to count. RNA-Seq preprocessing included trimming library adapters and performing quality controls. The raw data (fastq) were trimmed with the Trimmomatic (Bolger *et al*., 2014) tool for Phred quality score (Q-score) >20, read length >30 bases, and ribosome sequences were removed with tool sortMeRNA (Kopylova *et al*., 2012).

The mapper Bowtie v. 2 (Langmead *et al*., 2009) was used to align reads against *A. thaliana* and *B. cinerea* genomes (with – local

option and other default parameters). The 33,602 genes were extracted from the TAIR v. 10 database with one isoform per gene (corresponding to the longest coding sequence) and 16,389 genes for *B. cinerea*. The abundance of each gene was calculated by a local script that parses SAM files and counts only PE reads for which both reads map unambiguously to one gene, removing multihits. According to these rules, for *A. thaliana* around 94% of PE reads were associated with a gene. In the 6% around 2.5% of PE reads were associated to a *B. cinerea* gene.

Differential analysis followed the procedure described in Rigaill *et al.* (2018). Briefly, genes with less than 1 read after a count per million (CPM) normalization in at least one half of the samples were discarded. Library size was normalized using the trimmed mean of M-value (TMM) method and count distribution was modelled with a negative binomial generalized linear model where the environment factor ("stressed" or "unstressed" plants) and the block number were taken into account. Dispersion was estimated by the edgeR method (v. 1.12.0; McCarthy *et al.*, 2012) in the statistical software R v. 2.15.0 (R Development Core Team, 2005). Expression differences were compared between stressed and unstressed plants using the likelihood ratio test and *p* values were adjusted by the Benjamini–Hochberg procedure to control false discovery rate (FDR). A gene was declared differentially expressed if its adjusted *p* value < .05. Fragments per kilobase of transcript per million mapped (FPKMs) were calculated for visual analysis only, and were obtained by dividing normalized counts by gene length. Likelihood ratio test and *p* values were adjusted by the Benjamini–Hochberg procedure to control FDR. Gene ontology was analysed using MapMan (Thimm *et al.*, 2004) and FunCatDB (Ruepp *et al.*, 2004). Enrichment of FunCatDB categories (Figure S3c) was assessed by employing hypergeometric distribution with a *p* value cut-off of 5×10^{-3}. Enrichment in the MapMan categories compared to the whole genome was analysed using the "Classification SuperViewer Tool" (http://bar.utoronto.ca) with a *p* value cut-off of 10^{-3} or 5×10^{-3}.

4.6 | RNA extraction and cDNA synthesis for real-time PCR

For RT-qPCR, total RNA was isolated from 100 mg FW of infected leaves, control leaves, and of mycelium grown in vitro and extracted using TRIzol reagent (Invitrogen). This experiment was performed on three biological replicates. Reverse transcription was performed using an oligo-dT_{20} for a primer and Superscript II RNaseH-reverse transcriptase (Invitrogen). Real-time quantitative PCR analysis was performed on a CFX96 C1000 thermal cycler (Bio-Rad). A 1:5 dilution of cDNA (2.5 µl) was amplified in 7.5 µl of reaction mix containing SYBR Green PCR MasterMix (Eurogentec) and 0.15 µl of each primer (Table S4). Gene expression values were normalized to expression of the *A. thaliana EF1* and *APT* or *B. cinerea actin* and *UBQ*. We obtained similar results with both genes, therefore results with only one reference gene are shown.

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DATA AVAILABILITY STATEMENT

The RNA-Seq data that support the findings of this study are openly available in the Gene Expression Omnibus (GEO) at http://www.ncbi.nlm.nih.gov/geo, project ID GSE116135.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 *In vitro* radial growth of Bd90, BC1 and BC21 wild-type strains on synthetic medium with different concentrations of NO_3^- or NH_4^+ (10, 29, 80 and 140 mM)

FIGURE S2 Radial growth of *Botrytis cinerea* B0510 strain on synthetic medium supplemented with $NaNO_3$ or different amino acids

FIGURE S3 *Arabidopsis thaliana* genes differentially modulated by *Botrytis cinerea* infection (6 hr postinoculation) under various N regimes

FIGURE S4 Expression levels of a subset of *Botrytis cinerea* and *Arabidopsis thaliana* genes in infected leaves (6 hr postinoculation) grown at 0.5, 2 or 10 mM NO_3^-

FIGURE S5 Pathogenicity test on *Arabidopsis thaliana* leaves of Col-0 ecotype and *jar1-1* mutant infected with wild-type *Botrytis cinerea* B0510

TABLE S1 Fresh weight (g.FW) of *A. thaliana* rosettes grown during 6 weeks on 0.5, 2 or 10 mM of NO_3^- or on 2 mM of NH_4^+. Values are the mean ± *SD* of three independent experiments

TABLE S2 *Arabidopsis thaliana* genes overexpressed more than two-fold in uninfected leaves of plants grown in 10 (top) or 0.5 (bottom) mM NO_3^-

TABLE S3 *Arabidopsis thaliana* genes showing more than a two-fold higher expression in plants more resistant to *Botrytis cinerea* (grown in low NO_3^-)

TABLE S4 List of primers used in this study

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