The IRE1-bZIP60 branch of Unfolded Protein Response is required for *Arabidopsis* immune response to *Botrytis cinerea*

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Abstract

The Unfolded Protein Response (UPR) is a retrograde signalling pathway which is activated when endoplasmic reticulum (ER) proteostasis is disturbed. Here, we have investigated by reverse genetics the contribution of such pathway in *Arabidopsis thaliana* response to two necrotrophic fungi of agricultural importance, *Botrytis cinere*a which is responsible for the development of grey mold disease, and *Alternaria brassicicola* which triggers black spot disease. We found that the branch of UPR dependent on the INOSITOL-REQUIRING ENZYME 1 (IRE1) and the transcription factor (TF) bZIP60 is required to restrict foliar necrotic symptoms induced by both fungi. Accordingly, focussing on *B. cinerea*, we provided evidence for the production of the active bZIP60 form during infection. This activation was accompanied by an increased expression of UPR-responsive genes coding for ER-localized chaperones and co-chaperones that belong to the ER-Quality Control (ER-QC) system. Furthermore, mutants deficient for two ER-QC components were also more susceptible to infection. By contrast, investigating the involvement of CELL DIVISION CYCLE 48 (CDC48) AAA+-ATPAses that assist ER-Associated Degradation (ERAD) pathway for disposal of luminal unfolded proteins, we showed that a series of mutants and transgenics are more resistant to grey mold disease. Seeking for molecular insights into how the ER could shape Arabidopsis immune response to *B. cinerea*, we quantified the expression of defence gene and cell death markers in single *bzip60* and double *ire1* mutants. However, none of those genes were mis-regulated in mutant genetic backgrounds, indicating that IRE1-bZIP60 branch of UPR modulates the Arabidopsis response to *B. cinerea* by a yet-to-be-identified mechanism. Interestingly, we identified the NAC053/NTL4 TF as a potential actor of this unknown mechanism, linking the UPR and proteasome stress regulon.

Author summary

Necrotrophic fungi are one of the most economically significant plant pathogens worldwide, inflicting massive pre- and post-harvest losses on a wide range of fruit and vegetable crops. They adopt a necrotrophic lifestyle, deriving their nutrients predominantly from dead plant tissues to complete their life cycle. *Botrytis cinerea* is the causal agent of grey mold and no plant shows complete resistance towards this pathogen. The use of genetic models such as the plant *Arabidopsis thaliana* has partially enabled the understanding of the immunity mechanisms involved in the plant's response to *B. cinerea*. Our work provides new insights into the cellular mechanisms of how plants cope with this pathogen. In this context, by means of a reverse genetic approach, we explored the role of the Unfolded Protein Response (UPR), a cell signalling pathway regulating protein homeostasis within the endoplasmic reticulum (ER) and thus protecting cells from a harmful over-accumulation of aberrant or misfolded proteins.

Introduction

The maintenance of protein homeostasis is one of the cornerstones of cellular functions. It involves the precise regulation of translation, the folding of newly synthesized proteins, their post-translational modifications, their sorting and trafficking within the cell, and their degradation (1). The endoplasmic reticulum (ER) plays crucial roles in these processes, supporting the synthesis of one third to a quarter of total proteins in eukaryotic cells (2). Environmental factors, such as pathogen infection, intensify the workload on the protein folding machinery. When the endoplasmic reticulum (ER) is unable to meet the cell's demand for protein folding, unfolded or misfolded polypeptides accumulate within the ER lumen, leading to a proteotoxic stress known as ER stress. This situation actuates the Unfolded Protein Response (UPR), a retrograde, ER-to-nucleus, signalling pathway, that is conserved across kingdoms. In Arabidopsis, the canonical UPR invokes three bZIP transcription factors (TF) that are anchored in ER membranes (bZIP17, bZIP28 and bZIP60) and made soluble, therefore active, through two distinct mechanisms when ER stress occurs. One mechanism is the non-conventional splicing of *bZIP60* mRNAs that results in a shift in the open reading frame, eventually causing the translated proteins to be devoid of their transmembrane domains. Such splicing process relies on the ribonuclease activity of two ER-resident transmembrane proteins, the INOSITOL-REQUIRING ENZYME 1A (IRE1A) and B (IRE1B) (3,4). When ER stress is persistent, IRE1 ribonuclease activity can also be employed to dispose of ER-associated mRNAs , a phenomenon known as Regulated IRE1-Dependant Decay (RIDD; (5,6); the latter of which reduces the amount of nascent polypeptides crossing ER membranes, thus preventing the accumulation of unfolded proteins within the ER lumen. The other activation mechanism of UPR involves the translocation of bZIP17 and/or bZIP28 TF to the Golgi apparatus through vesicular trafficking, their intramembrane proteolytic cleavage and subsequent release of their transcription-activating domains that can then reach the nucleus (7,8). Target genes of UPR TF encompasses those coding for chaperones, co-chaperones and additional factors involved in the ER-Quality Control (ER-QC) system, increasing ER folding capacity. This includes calreticulin (CRT) and calnexin (CNX), as well as BINDING-IMMUNOGLOBULIN PROTEINS (BiP) and its interacting proteins STROMAL CELL-DERIVED FACTOR 2

88 (SDF2) and ENDOPLASMIC RETICULUM DNAJ 3 (ERdj3) (9). In support of the transcriptional
89 reprogramming mediated by the UPR TF, mis-folded and aberrant proteins can also be retro-
90 translocated from ER lumen to cytoplasm where they are ubiquitinated for proteasomal degradation.
91 This process, so-called ER-associated degradation (ERAD), involves the HRD1 (3-Hydroxy-3-
92 methylglutaryl Reductase Degradation 1) complex and the AAA+-ATPase family of proteins CDC48
93 (Cell Division Control protein 48, (9). It aims at restoring ER proteostasis. When adaptive UPR
94 pathway fails to resolve ER stress, a genetic program that ends with the death of malfunctioning cells
95 is engaged. In this context, it has been demonstrated that ER stress-induced cell death (ERSID) is
96 under the control of NAC089 TF (10) and requires several cathepsin B proteases to be executed (11).
97 A plethora of environmental situations can activate the UPR in plants, including heat, salt and
98 drought stress, as well as pathogen infection (12,13). However, only a few data report on UPR
99 involvement in the host plant response to necrotrophic pathogens. For instance, it was
100 demonstrated that inoculating *Nicotiana attenuata* with *Alternaria alternata* resulted in the
101 activation of UPR, and silencing *NaIRE1* or *NabZIP60* genes rendered plants more susceptible to the
102 fungus (14). It was also showed that impairment of the IRE1/bZIP60 branch enhanced Arabidopsis
103 susceptibility to *Drechslera gigantea*, another necrotrophic fungus (15). Although diseases caused by
104 the necrotrophic fungi *Botrytis cinerea* and *Alternaria brassicicola* rank among the most devastating
105 plant diseases worldwide, there are no data documenting the role of UPR in response to these two
106 necrotrophic pathogens. *Botrytis cinerea* causes grey mold disease in a wide range of crops, and
107 *Alternaria brassicicola* is responsible for black spot disease in numerous Brassica species. Both
108 pathogens have the ability to infect the plant model *Arabidopsis thaliana*, making it a valuable tool
109 for unravelling the intricate signalling pathways involved in plant immune response to these
110 pathogens. Innate immunity in plants is orchestrated through signalling cascades involving key
111 hormones like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), inducing significant
112 modifications in gene expression that underlie enhanced resistance (16). The JA signalling pathway is
113 commonly linked to the establishment of plant defence against necrotrophic fungus (17). Arabidopsis
114 mutants with impaired JA perception, production or signalling are notably vulnerable to *B. cinerea* or
115 *A. brassisicola* (18,19). SA is recognized as a pivotal hormone that initiates immune responses against
116 biotrophic pathogens (16), but SA-mediated signalling has also been shown to be involved in
117 resistance against *B. cinerea* in Arabidopsis (19) or against *Alternaria solani* in potato (20). For a
118 comprehensive probing of hormonal signalling cascades, studying the expression profile of marker
119 genes is a widely established method. Activation of JA-dependent signalling pathway is frequently
120 assessed by investigating the expression of genes such as *PLANT DEFENSIN 1.2* (*PDF1.2*) or
121 *OCTADECANOID-RESPONSIVE ARABIDOPSIS 59* (*ORA59*), whereas *PATHOGENESIS-RELATED-1 (PR1)*
122 serves as a marker gene for SA (21). Another typical response of plants facing necrotrophic
123 pathogens is the production in Arabidopsis of the phytoalexin camalexin through SA or JA signalling
124 pathways, depending on the invading pathogen (22). The last step of camalexin synthesis is catalysed
125 by the cytochrome P450 CYP71B15 (PHYTOALEXIN DEFICIENT 3, PAD3) which mRNA accumulation
126 correlates with camalexin production (23). Camalexin has been shown to be toxic for *B. cinerea* and
127 *A. brassicicola* (24,25), and *pad3* mutant shows enhanced susceptibility to both pathogens (19,26).
128 During infection process, necrotrophic pathogens secrete compounds that enable rapid host cell
129 death and disease spreading. *B. cinerea* produces cell-death inducing proteins, but also manipulates
130 the plant regulated cell death to promote host cell death (27,28).
131 In this study, we explored how the ER could shape *Arabidopsis thaliana* immune response to
132 necrotrophic pathogens, with a specific emphasis on *Botrytis cinerea*. The role of UPR, ER-QC and
133 ERAD in this context was investigated using a reverse genetic approach in order to gain insights into
134 molecular events that govern plant susceptibility and defence
135

136

137 ## Results

138

139 **The IRE1-bZIP60 branch of Arabidopsis UPR restricts lesions induced by necrotrophic pathogens.**

140

141 To investigate a potential role of the UPR pathway in Arabidopsis response to necrotrophic
142 fungi, we inoculated mutants defective in canonical UPR actors (*bzip17-1*, *bzip28-2*, *bzip60-3*, *ire1a-2*
143 *ire1b-4*) with *B. cinerea* or *A. brassicicola.* Mutant susceptibility to *B. cinerea* was examined 3 days
144 upon drop-inoculation of leaves with a solution of conidia using the lesion diameter as proxy. The
145 necrotic lesion diameter was averaging 5 mm in the WT genetic background, as previously reported
146 (29), so was it in the *bzip28-2* mutant. By contrast, a significant decrease of 18 % in lesion size was
147 recorded in *bzip17-1* mutant when compared to WT. Interestingly, both the single *bzip60-3* and
148 double *ire1a-2 ire1b-4* mutants were more susceptible to the infection, displaying a 17 % increase in
149 lesion diameter with respect to the WT genotype (Figure 1A, Supplemental Figure S1A). *A.*
150 *brassicicola* susceptibility was also determined by inoculating leaves with a spore solution and lesion
151 diameter were measured at 5 days post-inoculation. In WT plants, *A. brassicicola* induced necrotic
152 lesions of 2.5 mm diameter in average. Both *bzip17-1* and *bzip28-2* mutations had no impact on
153 symptoms. Genetic inactivation of *bZIP60* or *IRE1A* and *IRE1B* led to a 37 % or 27 % increase in lesion
154 diameter with regard to WT, respectively (Figure 1B, Supplemental Figure S1B). Altogether, our data
155 suggest that the IRE1-bZIP60 branch of UPR could be activated and participate either in defence
156 mounting or PCD inhibition triggered by the two necrotrophic fungal pathogens tested.

157

158 **The IRE1-bZIP60 branch of UPR is activated in response to *Botrytis cinerea*.**

159

160 To get into molecular mechanisms involving UPR, we focussed on the interaction between *A.*
161 *thaliana* and *B. cinerea*. The expression of *IRE1A, IRE1B, bZIP17, bZIP28* and *bZIP60* was followed by
162 RT-qPCR over time upon infection. For that purpose, a solution of *B. cinerea* spores prepared in PDB
163 medium or PDB alone (mock) was sprayed on leaves and samples were collected at 16, 24, 48 and 72
164 h post-infection (hpi) for further processing. Those experimental conditions were validated by
165 checking the expression of defence genes previously reported to be markedly induced in response to
166 *B. cinerea*, *i.e. PAD3*, *PR1*, *PDF1.2a* and *PATATIN-LIKE PROTEIN 2 (PLP2)* (19,24,30). As expected, all
167 four genes were up-regulated during infection (Supplemental Figure S2). As for UPR actors-encoded
168 genes, *IRE*1A and *IRE1B* were not differentially expressed between mock and challenged conditions.
169 A slight, but significant accumulation of *bZIP17* and *bZIP28* transcripts was only observed at 48 hpi
170 (Figure 2). However, in accordance to mutant over-susceptibility to *B. cinerea* (Figure 1A), the
171 strongest transcriptional response was observed for *bZIP60* gene with the unspliced mRNAs
172 accumulating at early time points (16 and 24 hpi) whereas the spliced mRNA levels kept increasing
173 from 16 hpi to 72 hpi. In addition to confirming the activation of the IRE1-bZIP60 branch, these
174 results suggest that *B. cinerea* infection induces an ER stress.

175

176 **Default in ER-QC machinery leads to enhanced necrotic lesions caused by *Botrytis cinerea***

177

178 We next determined by RT-qPCR the expression kinetic of ER stress gene hallmarks (*BIP1*, *BiP2*, *BiP3*,
179 *ERDJ3A*, *ERDJ3B* and *SDF2*) in mock and inoculated WT plants (Figure 3A). Consistent with a proteo-
180 toxic ER stress induced by *B. cinerea*, all genes coding for ER-QC components were up-regulated upon
181 infection, yet with distinct expression profiles. While *BIP1* and *BIP2* mRNAs were over-accumulating
182 as early as 16 hpi, the expression of *BIP3* gene tends to increase at 48 hpi. The expression level of the
183 three BiP-encoding genes were back to that of mock conditions at 72hpi. When compared to mock

184 plants, the infection by *B. cinerea* was also stimulating the expression of *ERDJ3A*, *ERDJ3B* and *SDF2*
185 genes at 24 and/or 48 hpi depending on the gene tested. These results prompted us to investigate
186 whether SDF2 and ERDJ3B could be involved in Arabidopsis response to *B. cinerea*. For that purpose,
187 we used *erdj3b-1* and *sdf2-2* mutants (31) that we challenged with a spore solution and lesion
188 diameters were measured at 3 days post-inoculation (Figure 3B, Supplemental Figure S3). Significant
189 increases in lesion diameter were recorded for both mutants (24 % for *erdj3b-1* and 18 % for *sdf2-2*)
190 in comparison to WT plants, indicating that they are more susceptible to *B. cinerea*. These data
191 indicate that both SDF2 and ERDJ3B could be acting in the same response pathway as that of IRE1-
192 bZIP60.
193
194
195 **Mutation in ERAD-related *CDC48* genes decreases susceptibility to *Botrytis cinerea***
196
197 The ERAD utilizes proteasome to dispose of misfolded proteins which have been retro-translocated
198 from ER lumen to cytoplasm. With ER-QC system which is reinforced to increase ER folding capacity
199 under ER stress, ERAD is part of the pro-adaptive mode of UPR that aims at restoring ER homeostasis.
200 The CDC48 family of AAA+-ATPase proteins have been described as ERAD actors in yeast (32),
201 mammals (33) and plants (34). To investigate a potential involvement of ERAD in response to *B.*
202 *cinerea*, we first functionally characterized Arabidopsis CDC48 homologs. In Arabidopsis, CD48
203 proteins are, indeed, likely to be encoded by five genes (35). CDC48A, B and C are the closest
204 sequence orthologs of human and yeast proteins, with which they share approximately 90 %
205 similarity. By contrast, CDC48D and E are more divergent, exhibiting no or little conservation within
206 their N and C-terminal domains (35,36). In accordance with its role in ERAD, CDC48A was shown to
207 control the turnover of two immune receptors (36,37), as well as that of a mutated carboxypeptidase
208 Y protein which is an ERAD substrate (34). It was also capable of complementing yeast *cdc48*
209 conditional mutant (37). The four Arabidopsis homologs of *CDC48A* were thus tested by yeast
210 functional complementation using the *Saccharomyces cerevisiae cdc48* mutant strains KFY189 and
211 DBY2030. KFY189 is a temperature-sensitive mutant which does not grow at 37°C whereas DBY2030
212 is cold-sensitive and fail to grow at 16°C (38,39). Both strains were transformed with the yeast
213 expression vector pDRF1-GW containing either the cDNA coding for CDC48A, B, C, D or E or with the
214 empty vector alone. All transformed strains were able to grow under permissive conditions at 30°C
215 (Figure 4A, C). As previously described (38), CDC48A complemented both strains under non-
216 permissive conditions while the empty vector did not, validating our experimental setup (Figure 4B,
217 D). Whereas *CDC48B* or *CDC48C* were able to restore the growth of DBY2030 strain at 16°C and that
218 of KFY189 strain at 37°C (Figure 4B, D), *CDC48D* and *CDC48E* could not rescue the temperature-
219 dependent phenotypes of both strains (Figure 4B, D). These data demonstrate that only CDC48A, B
220 and C can functionally substitute to the yeast protein, and suggest a role for CDC48B and C in ERAD.
221 Focusing on *CDC48A*, *B* and *C*, we next found that only *CDC48A* and *CDC48B* showed an
222 increased expression in response to *B. cinerea*, at 48 and 72 hpi (Figure 5A). We then evaluated the
223 susceptibility of different *cdc48* mutants to *B. cinerea*. We used *cdc48a-4/muse8* which is a partial
224 loss-of-function EMS mutant previously described (36), the knock-out *cdc48b3* (GABI_104F08) and
225 knock-down *cdc48b4* (GABI_485G04) mutants that show no or reduced *CDC48B* expression,
226 respectively (Supplemental Figure S4A), and the two knock-out *cdc48c2* (SALK_102955C) and *cdc48c3*
227 (SALK_123409) that do not express *CDC48C* (Supplemental Figure S4B). When compared to
228 challenged WT plants, *cdc48a-4/muse8* and *cdc48b-4* mutants were slightly, but significantly more
229 resistant to *B. cinerea*, as judged by the lesion size (Figure 5B, Supplemental Figure S5). *cdc48b-3* and
230 *cdc48c-2* mutants were even more resistant, and *cdc48c-3* exhibited the strongest resistance with a
231 37% reduction in lesion diameter. We also produced Arabidopsis lines overexpressing either a WT

232 AtCDC48B protein or a mutated one (AtCDC48B^{E308QE581Q} further abbreviated AtCDC48B-QQ) under
233 the control of a CaMV35S promoter, the latter being a negative dominant allele (40). Two
234 overexpressing lines were selected for each construct (Supplemental Figure S4C). Upon challenged
235 with *B. cinerea*, plants overexpressing AtCDC48B-QQ were more resistant to the infection whereas
236 plants overexpressing AtCDC48B-WT were as susceptible as WT plants (Figure 5B, Supplemental
237 Figure S5). These data are consistent with results obtained using T-DNA insertion lines (Figure 5B),
238 and further indicate that CDC48 activity, likely though ERAD pathway, is required for full Arabidopsis
239 susceptibility to *B. cinerea.*
240
241 **Inactivation of IRE1/bZIP60 branch impacts neither the expression of *B. cinerea*-induced defence**
242 **genes, nor the expression of ER-stress-induced cell death genes.**
243
244 Following *B. cinerea* perception, plant cells activate a complex array of defence mechanisms
245 that relies, at least in part, on a transcriptional reprogramming. Among genuine defence gene
246 markers induced upon B. cinerea infection, one may cite *PR1*, *PDF1.2a*, *ORA59*, *FLG22-INDUCED*
247 *RECEPTOR-LIKE KINASE 1* (*FRK1)*, *PAD3* and *GLUTHATIONE S-TRANSFERASE 6* (*GSTF6)* (Supplemental
248 Figure S2; (24,41,42)). We reasoned that *bzip60-3 and ire1a-2 ire1b-4* mutants could have a reduced
249 defence response if bZIP60 transcription factor positively regulates defence gene expression. To test
250 this hypothesis, changes in the aforementioned gene expression was measured by qRT-PCR in the
251 single and double mutants and compared to WT. In a WT genetic background, infection triggered an
252 increase in expression for all genes at 24 and 48 hpi, except for *PR1* whose enhanced transcript level
253 was only detected at 48 hpi (Supplemental Figure S6). This expression pattern is consistent with
254 previous work (41). However, neither *ire1a-2 ire1b-4*, nor *bzip60-3* mutations impacted the
255 expression profiles of the tested defence marker genes in response to *B. cinerea* (Supplemental
256 Figure S6).
257 Evidence have been accumulating that necrotrophic pathogens, like *B. cinerea*, actively
258 induce host PCD for feeding on cell debris and subsequent colonization of dead tissues (27). With this
259 regard, we hypothesized that ERSID could be over-induced in the *bzip60-3 and ire1a-2 ire1b-4*
260 mutants, potentially explaining their enhanced susceptibility to the fungus (Figure 1A). ERSID was
261 found to be positively controlled by the NAC089 transcription factor (10) and the protease family of
262 cathepsins (11) under chemically-induced ER stress. Whereas *NAC089* and *CATHEPSIN B1, B2, B3*
263 gene expression was previously shown to be significantly increased during ERSID triggered by
264 tunicamycin (10,11), none of those genes were differentially expressed between mock and infection
265 (24 and 48 hpi) conditions in a WT genetic background (Supplemental Figure S7). In addition, no or
266 minor expression changes for those genes could be recorded in *bzip60-3 and* double *ire1a-2 ire1b-4*
267 mutants compared to WT, whether plants were inoculated or not (Supplemental Figure S7).
268
269 **The expression of NAC053, a negative regulator of Arabidopsis defence against *B. cinerea*, is**
270 **dependent on IRE1 proteins.**
271
272 To explain the susceptibility phenotype of *ire1a-2 ire1b-4* and *bzip60* mutants and the
273 resistant phenotype of *cdc48* mutants, we tested the hypothesis that the IRE1/bZIP60 branch of the
274 UPR negatively control *CDC48* gene expression and its regulators in response to *B. cinerea*. None of
275 the *CDC48A, B, C* genes were mis-regulated at 24hpi and 48hpi in *bzip60-3* and *ire1a-2-ire1b-4*
276 mutants (Supplemental Figure S8). The expression of the two NAC TF (NAC053/NTL4 and
277 NAC078/NTL11) that have been shown to regulate proteasome stress regulon-encoding genes,
278 including *CDC48A* (Gladman *et al.*, 2016), was next checked in the same genetic backgrounds (Figure
279 6). The expression level of *NAC078* was not altered by the infection in any of the genotypes for the

280 two time points tested (Figure 6A-B). By contrast, in response to *B. cinerea*, *NAC053* expression was
281 induced earlier in the *bzip60-3* mutant (24hpi, Figure 6A) when compared to WT (48hpi, Figure 6B),
282 and was totally abolished in the double *ire1a-2-ire1b-4* mutant at both time points (Figure 6A-B).
283 Infected *nac053-1* and *nac078-1* mutants showed smaller lesions at 3 dpi with respect to WT plants
284 (Figure 6C, Supplemental Figure S9), indicating that both mutants are more resistant to *B. cinerea*.
285 These results indicate that *NAC053* expression is dependent on IRE1 proteins, and that both NAC053
286 and NAC078 act as negative regulator of Arabidopsis defence against *B. cinerea*.
287
288
289
290 **Discussion**
291
292 The UPR pathway plays a crucial role in plant-pathogen interactions, irrespective of the
293 pathogen nature, *i.e.* bacteria, fungi and viruses (13,44). However, our understanding of its specific
294 role during plant infection by necrotrophic fungi remains elusive. Here we present a study that
295 identifies UPR pathway as a critical element in *Arabidopsis thaliana* response to two necrotrophic
296 fungi, *Botrytis cinerea* and *Alternaria brassicicola*. The RT-qPCR results demonstrated that *B. cinerea*
297 infection activated UPR pathways in WT plants. During infection, we observed an accumulation of
298 mRNAs coding the three BIP isoforms, along with its co-chaperones SDF2.2, ERDJ3A and ERDJ3B.
299 Additionally, we detected an increased level of spliced bZIP60 mRNA variants coding the active TF.
300 The importance of UPR has been confirmed by challenging plants mutated in the genes coding the
301 canonical UPR regulators IRE1A/IRE1B, bZIP60, as well as those of RIP-UPR bZIP17 and bZIP28, with a
302 spore solutions of *B. cinerea*. Mutation in the genes coding *IRE1A* and *IRE1B* or *bZIP60* results in a
303 greater plant susceptibility to *B. cinerea.* Similar observations were made when these mutants were
304 challenged with *Alternaria brassicicola* indicating that IRE1-bZIP60 pathway plays important role in
305 limiting the growth of both pathogens. However, it is worth noting that mutation in *bZIP28* did not
306 have a significant impact on the plant susceptibility to these fungi. Overall, our findings support the
307 hypothesis that the IRE1-bZIP60 branch is an important element in the establishment of plant
308 immunity to necrotrophic pathogens. This is in accordance with previous work on *Nicotiana*
309 *benthamiana*, where silencing of *NbbZIP60*, *NbIRE1a*, and *NbIRE1b* expression through a VIGS
310 approach led to increased plant susceptibility to the necrotrophic fungus *Alternaria alternata* (14).
311 Furthermore, infection of the double mutant *bzip28-2-bzip60-1* with *Drechslera gigantea*, a
312 necrotrophic fungus causing eyespot disease in crop plants, leads to increase disease symptoms
313 compared to WT plants in Arabidopsis (15). However, it is noteworthy to mention that neither the
314 knock down *bzip60.1* nor the double mutant *bzip28-2-bzip60-1* showed increased susceptibility when
315 infected with *B. cinerea* (Supplemental Figure S10). The significance of the IRE1-bZIP60 branch of the
316 UPR in controlling plant immunity is further demonstrated by the work of Tateda *et al.* (45), who
317 showed that silencing of *IRE1a/1b* or *bZIP60* resulted in disease symptoms in *Nicotiana tabacum*
318 caused by the non-host bacterial pathogen *Pseudomonas cichorri*. Additionally, concerning the
319 virulent bacteria *Pseudomonas syringae pv maculicola* ES4326, bacterial growth was substantially
320 more pronounced in *ire1a ire1b* or *bzip60* infected mutants compared to the infected WT plants (46).
321 It is must be pointed out that our study revealed distinct responses to *B. cinerea* and *A. Brassicicola*
322 depending on the genotype tested. Notably, the *bzip17* mutant demonstrated higher resistance to *B.*
323 *cinerea* compared to WT plants, while it exhibited a similar level of infection as WT plants when
324 exposed to *A. brassicicola*. Studies demonstrating a role for bZIP17 during biotic stresses are scarce.
325 One may cite the study of Li (47) where the silencing of both *NbbZIP17* and *NbbZIP28* delayed Rice
326 Stripe Virus infection and decreased the accumulation of viral RNA and proteins in *N. benthamiana*,

327 indicating that RIP-UPR promotes virus development. However, in Arabidopsis infected with the
328 *Plantago asiatica* mosaic virus, bZIP60 and bZIP17 acts synergistically to restrict viral infection (48).
329 Understanding the role of bZIP17 during *B. cinerea* infection will need further investigations.
330 Activation of UPR leads to an increase of the ER folding capacity, preventing an excessive
331 build-up of misfolded or aberrant proteins that may occur during stressful situations like pathogen
332 attacks. Unfolded and misfolded proteins can also be degraded through ERAD. During this process,
333 misfolded proteins are tagged with polyubiquitin chains and extracted from the ER membrane with
334 the assistance of the CDC48 AAA+ATPase complex (9). The number of gene coding CDC48 proteins in
335 Arabidopsis genome is unclear (35). Through yeast *cdc48* mutant complementation experiments, our
336 findings unequivocally establish that only three proteins - AtCDC48A (At3g09840), AtCDC48B
337 (At3g53230), and AtCDC48C (At5g03340) - are functional orthologs of ScCDC48 (as demonstrated in
338 our study and by Feiler *et al.* for CDC48A (38)). Thus, the two closely related AAA+-ATPase proteins
339 (At2g03670 and At3g01610), previously referred to as AtCDC48D and E (35,36), are not ScCDC48
340 functional orthologues and should not be categorized as such.
341 An increase in the accumulation of *AtCDC48A* and *AtCDC48B* mRNA have been detected
342 during *B. cinerea* infection. Interestingly, plants mutated in either *AtCDC48A*, *AtCD48B* or *AtCDC48C*
343 genes are more resistance to *B. cinerea* infection. Furthermore, the overexpression of an inactive
344 form of AtCDC48B (*eg* AtCDC48B^{QQ}) also results in an increased resistance phenotype toward *B.*
345 *cinerea*. The resistant phenotype of *atcdc48a-4* mutant to *B. cinerea* could be tentatively explained
346 by a constitutive immune response characterized by the stronger accumulation of *PR2* mRNA in this
347 mutant (36). Ao et *al.* (49) have proposed a model to explain this autoimmune phenotype related to
348 the role of AtCDC48A during plant pathogen interaction. AtCDC48A is recognized by the SFC^{SNIPER7}
349 complex and targeted for proteasomal degradation. It results in the accumulation of the NLR SNC1
350 (and likely other NLR) which may serve to enhance defence to the virulent oomycete pathogen *H.*
351 *arabidopsidis* NOCO2. The existence of such a mechanism in the context of *B. cinerea* infection
352 remains to be demonstrated. Regarding AtCDC48A paralogs, AtCDC48B and AtCDC48C, it is unlikely
353 that they have redundant functions with AtCDC48A. This is supported by the observations that
354 mutation in each of the *AtCDC48* gene results in resistant phenotypes against *B. cinerea.*
355 Nonetheless, mutations in *AtCDC48B* or *AtCDC48C* do not affect the resistance of Arabidopsis to *H.*
356 *Arabidopsis* NOCO2 (36). However, the importance of the IRE1-bZIP60 pathway in basal resistance to
357 *B. cinerea* infection is unlikely linked to CDC48 because *AtCDC48* mRNAs do not accumulate
358 differentially in *atire1a/ire1b* or *atbzip60* genetic backgrounds. Understanding the involvement of
359 each of the *AtCDC48* genes in plant immunity will necessitate additional in-depth investigations.
360 To identify potential mechanisms that play a role in the defence response against *B. cinerea*
361 via the IRE1-bZIP60 pathway, we have undertaken a RT-qPCR approach. None of the tested genes
362 had their expression altered in the *ire1a-2 ire1b-4* and *bzip60-3.* It concerns the SA (*PR1*) and JA
363 (*PDF1.2, ORA59*) responsive genes, indicating that the IRE1-bZIP60 pathway does not influence the
364 expression of SA and JA-dependant gene expression in response to *B. cinerea* infection. However, we
365 did not investigate the hypothesis whether SA or JA signalling pathways were necessary to regulate
366 the activation of UPR pathways as observed in other studies. In tomato, JA treatment induced the
367 expression of *BIP1* and tunicamycin-induced *BIP1* expression was strongly reduced in the JA signalling
368 mutant *jai1* indicating that JA pathway plays a role in ER stress signalling (50). Similar observations
369 were made in *N. attenuata* in which MeJA treatment induced the accumulation of mRNA coding
370 chaperone proteins such as BIP, PDI, CNX and CRT as well as the spliced form of *bZIP60* mRNA (14).
371 Using JA-deficient and JA-insensitive plants, these authors have also shown that JA controls
372 chaperone gene expression in response to *A. alternata*.
373 In addition, our data indicate that *PAD3* gene, which encode a cytochrome P450 enzyme that
374 catalyses the last step of camalexin biosynthesis is not regulated by UPR. To the contrary, the *F6´H1*

375 gene was found less induced in *N. attenuata* plants impaired in the IRE1-bZIP60 pathway during *A.*
376 *alternata* infection (14). It encodes the key enzyme which catalyzes the synthesis of scopoletin and
377 scopolin, two phytoalexin previously shown to be crucial for *N. attenuata* resistance to *A. alternata*.
378 These results indicate that the role of UPR in regulating plant resistance to one particular class of
379 pathogen is different from one plant-pathogen interaction model to another.
380 Necrotrophic pathogens induce plant cell death to their own benefits either by secreting
381 plant cell death inducers or by manipulating host regulated cell death (27,28). Genes known to
382 orchestrate ERSID, such as *NAC089* and *CATHEPSIN B*, are not induced after *B. cinerea* infection
383 whereas these genes are up-regulated following plant treatment with the ER stress-inducing agent
384 tunicamycin (10,11). We can formulate two hypotheses. Firstly, increased susceptibility of *ire1a-2*
385 *atire1b-4* and *atbzip60-3* to *B. cinerea* is unlikely the consequence of an increase ERSID phenomenon
386 because *NAC089* or *Cathepsin B* genes are not upregulated in these mutant genotypes. It suggests
387 that a NAC089- and CATHEPSIN B-dependant ERSID is unlikely to be involved during Arabidopsis-*B.*
388 *cinerea* interaction. Secondly, we can hypothesized that ER stress is not involved in *B. cinerea*-
389 induced cell death. In any case, further experiments are needed to clarify the role of ER stress in *B.*
390 *cinerea*-induced cell death.
391 Our data indicate that, in *bzip60* mutant infected with *B. cinerea*, the expression of
392 NAC053/NTL4 is upregulated. Infected-*bzip60* mutant accumulates much more *NAC053* mRNA than
393 WT plant and this accumulation occurs earlier in *bzip60* (at 24 hpi) than in WT plant (at 48 hpi).
394 However, the expression of the NAC053 closest relative NAC078/NTL11 is not modulated by *B.*
395 *cinerea* in any of the genotypes tested. Together, both transcription factors are required to control
396 the expression of the proteasome stress regulon including *CDC48A* (43). Nevertheless, the expression
397 of genes coding CDC48 A, B or C proteins are not modulated in Botrytis-infected *bzip60* mutant. Very
398 surprisingly, results are different when using *ire1a-ire1b* double mutant. *NAC053* mRNA does not
399 accumulate neither at 24 hpi nor at 48 hpi in this mutant infected with *B. cinerea* in comparison to
400 WT plants in which *NAC053* accumulate at 48 hpi. It indicates that *NAC053* expression depends on
401 IRE1 proteins during *B. cinerea* infection. NAC053 is anchored to the plasma membrane (51) and
402 activated following stress perception in a ROS-dependant mechanism (52). Once activated, NAC053
403 control ROS production through the activation of *RBOH* genes (53). In our model, it is reasonable to
404 suggest that a disturbance in ROS homeostasis is likely present in the *bzip60* mutant, whereas such a
405 disturbance might not exist in the *ire1a/ire1b* mutant. Further research is necessary to substantiate
406 this hypothesis.
407 Summarizing, the infection of wild type Col-0 plant by *Botrytis cinerea* induces ER stress and
408 activates the UPR pathways (Figure 7). The activation of the IRE1-bZIP60 branch and ERQC is crucial
409 for the plant's response to the pathogen, as mutations in genes encoding key players in these
410 pathways result in increased plant susceptibility. Further work is however necessary to understand
411 how the IRE1/bZIP60 branch contributes to defence against this necrotrophic fungus, particularly its
412 potential involvement in the secretion of defence proteins. On the other hand, our results indicate
413 that mutations in the bZIP17 branch of the UPR, as well as mutations in genes encoding the ERAD-
414 involved proteins CDC48, lead to greater resistance of the plant. This suggests either that these
415 proteins act as negative regulators of immunity against this pathogen, or that these pathways might
416 be manipulated by the pathogen during infection. Similarly, further work is needed for a detailed
417 understanding of these mechanisms.

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426 ## Experimental procedures
427
428 **Plant materials**
429 *A. thaliana* seeds were grown on jiffy-7 pellets in a growth chamber under controlled conditions (10h
430 light/14h dark, 22°C/18°C, 60–70% humidity, light intensity of 100 and 120 μE.m^{-2}.sec^{-1}). All the
431 mutants used are listed in Supplemental table S1. To screen T-DNA homozygous plant mutants by
432 PCR, genomic DNA was isolated using Phire Plant Direct PCR kit (Thermoscientific) and PCR was
433 performed using primers pairs as indicated in Supplemental table S2.
434
435 **Pathogen infection**
436 *Botrytis cinerea* strain BMM (29) was grown 10 days on sterile V8 agar plate (50% Campbells original
437 V8 juice (v/v), 0.5 % KH2PO4 (m/v), 1.5 % agar (m/v), pH 6) at 20°C in the dark. Spores were
438 harvested by scraping the plate with sterile milliQ water and filtered through sterile gaze. Infection
439 tests are carried out by placing 6 µL of a solution of botrytis spores (5.10^{4} spores.mL^{-1} diluted in
440 quarter-strength Difco potato dextrose broth PDB) on 4 leaves of four weeks old plants. Lesion
441 diameters were measured after 3 days. For qPCR analysis, plants were either sprayed with a spore
442 solution (2.5.10^{5} spores.mL^{-1}) or with quarter-strength PDB as described previously (41). For both
443 experiments, the inoculated plants are placed in a tray closed with a transparent lid to maintain a
444 high-humidity environment.
445
446 *Alternaria brassicicola* strain MIAE01824 was provided by Dr. Christian Steinberg (INRAe, Dijon,
447 France) and grown 15-20 days on PDA medium (19 g.L^{-1}) supplemented with sucrose (20 g.L^{-1}) and
448 $CaCO_3$ (30 g.L^{-1}) at 20°C in the dark. Spores were harvested by scraping the plate with sterile infection
449 medium (GamborB5 medium (Duchefa), sucrose 10 mM, KH_2PO_4 10 mM) and filtered through sterile
450 gaze. Four leaves of four weeks old plants were drop inoculated (6 µL) with a solution of 1.10^{6}
451 spores.mL^{-1} in infection medium. Inoculated plants are placed in a tray closed with a transparent lid
452 to maintain a high-humidity environment and lesion diameters were scored after five days.
453
454 **RNA extraction and RT-qPCR analysis**
455 Inoculated and mock-treated leaves were harvested at different time points, flash frozen and finely
456 grinded in liquid nitrogen. Total RNA extraction and DNAse treatment were performed using SV Total
457 RNA Isolation System (Promega) as described by the manufacturer. RNA integrity was analysed on a
458 1% agarose gel and concentration was measured using a nanodrop system (Thermoscientific). RNA
459 (500 ng) were retrotranscribed into cDNA (High-Capacity cDNA Reverse Transcription Kit,
460 Thermoscientific) using random hexamer and 17-mer oligodT primers. mRNA accumulation was
461 assessed by real-time qPCR (GoTaq® qPCR Master Mix, Promega) and expression values were
462 normalized to the expression of plant genes *At4g26410* and *At3g01150* as previously described as a
463 stable reference genes (54). Levels of transcripts were calculated using efficiency-weighted $\Delta\Delta Cq^{\omega}$
464 method (55). All primers are listed in Supplemental table S3.
465
466 **Statistical methods**
467 For infection tests, significant differences from WT plant were determined by a Kruskal–Wallis one-
468 way anova on ranks followed by a comparison with the Dunnett's method. For qPCR analysis, ΔCq^{ω}
469 value from one infection time-point is compared to the data of the control condition at the same

470 time point using by a one-way ANOVA followed by a Tukey HSD Test (55). Then, data are represented
471 on the figures as fold induction to the control condition ($\Delta\Delta Cq^{(0)}$).
472
473 **AtCDC48B cloning procedures and transgenic lines generation**
474 Arabidopsis lines overexpressing either AtCDC48B or AtCDC48B mutated in its two ATPase sites
475 (AtCDC48B^{E308QE581Q}) under the control of a CaMV35S promoter were produced as follows.
476 PDONR/Zeo containing either AtCDC48B^{WT} or AtCDC48B^{E308QE581Q} cDNA were kindly provided by Drs.
477 Annette Niehl and Manfred Heinlein (IBMP, Strasbourg, France). pDNOR/Zeo were BP-recombined
478 (Thermoscientific) in pB2GW7 vector (56) and resulting vectors were introduced in *Agrobacterium*
479 *tumefaciens* strain GV3101. WT *A. thaliana* Columbia plants (N60,000, Eurasian Arabidopsis Stock
480 Center) were transformed using the floral dipping method (57). T1 and T2 transgenic plants were
481 grown on jiffy-7 pellets and selected using BASTA herbicide after 10 days of growth. To identify
482 homozygous plants overexpressing *AtCDC48B^{WT}* or *AtCDC48B^{E308QE581Q}*, seeds from T2 plants were
483 surface-sterilized and sown on MS Agar plates (4.4 g.L^{-1} of Duchefa MS powder M0222.0050, 1%
484 sucrose, 10 mM MES, 1% agar pH 5.7) containing 50 µg/mL of glufosinate ammonium (Sigma-
485 Aldrich). Expression of mRNA coding AtCDC48B^{WT} or AtCDC48B^{E308QE581Q} in transgenics plants were
486 analysed by RT-qPCR using primers described in supplemental table S3.
487
488 **CDC48 expression in *cdc48* yeast mutant**
489 CDC48 cDNA were cloned from an Arabidopsis thaliana cDNA pools made from total RNA extracted
490 from Arabidopsis Col-0 plants. AtCDC48A (At3g09840; forward primer
491 ATGTCTACCCCAGCTGAATCTTC; reverse primer CTAATTGTAGAGATCATCATCGTCCC), AtCDC48C
492 (At5g03340; forward primer ATGTCAAACGAACCGGAATC; reverse primer
493 CTAACTGTAGAGATCGTCGTCATC), AtCDC48D (At2g03670; forward primer ATGTTGGAAACCGAAAGC;
494 reverse primer TCATGTAGCAGAAGCTACTAGTAA), AtCDC48E (At3g01610; forward primer
495 ATGGGGAGGAGAGGTCGC; reverse primer TTACTCGAGGGTAAAAGATGGCC) were amplified by PCR
496 using Phusion DNA polymerase (Thermoscientific) and cloned into PCR8 vector (Thermoscientific).
497 PDONR/Zeo containing CDC48B (At3g53230) was kindly provided by Drs. Annette Niehl and Manfred
498 Heinlein (Institut de biologie moléculaire des plantes, Strasbourg, France; (40)). PCR8 and
499 pDNOR/Zeo vectors were then recombined in pDRF1-GW (58) and resulting vectors or empty vector
500 were introduced in *cdc48* deficient *Saccharomyces cerevisiae*. Two *cdc48 S. cerevisiae* mutants (39)
501 were kindly provided by K.-U. Fröhlich (University of Graz, Austria): the cold-sensitive strain DBY2030
502 (MATa ade2-101 lys2-801 ura3-52 cdc48-1;) or the temperature-sensitive strain KFY189 (MATa lys2
503 leu2 ura3 cdc48-8). Transformed *S. cerevisiae* cells were plated on minimal selective medium
504 containing 2% glucose, 0,77g/L SD-URA drop out mix (Clontech), 0.67 g/L Yeast Nitrogen Base (Sigma-
505 Aldrich), 15 g/L agar (Sigma-Aldrich) and grown at 30°C. Transformed colonies were checked by
506 colony PCR and then grown overnight at 30°C under shaking in YPD medium (10 g/L yeast extract, 20
507 g/L peptone, 2% glucose). Yeast cells were then streaked out on YDP agar plate (YPD medium
508 containing 15g/L agar) and grown at 30°C and 16 °C for DBY2030 transformed strain or 30°C and 37°C
509 for KFY189 transformed strain.
510

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512
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683 Figure legends

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688 **induced by *B. cinerea* and *A. brassicicola***
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691 infection and four times for *A. brassicicola* infection depending on the genotype tested. Seven plants
692 per genotype were infected in each experiment. Violin plots display the distribution of lesion
693 diameters for all experiments and diamonds indicate the median. Stars indicate significant
694 differences compared to the wild type and were assessed using Kruskal-Wallis' method followed by
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696

697 **Figure 2: Expression kinetic of UPR genes in response to *B. cinerea*.**
698 Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B.*
699 *cinerea* spores (black bars). For each experiment, four leaves of three plants were collected at 16, 24,
700 48 and 72 hours post-inoculation. Level of transcripts coding IRE1A, IRE1B, bZIP17, bZIP28 and bZIP60
701 (unspliced or spliced forms) were quantified by RT-qPCR and normalized to those of two reference
702 genes *AT4G26410* and *AT3G01150* (54). Experiments were repeated five times and mean expression
703 for all experiments are presented. Significant differences between non-infected and infected plants
704 were determined by a one-way ANOVA followed by a Tukey HSD Test (*** $p<0.005$; ** $p<0.01$; *
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708 **Figure 3: Defect in ER-QC machinery enhances *B. cinerea*-induced symptoms.**
709 (A) Expression of genes coding ER-QC machinery over *B. cinerea* infection. Plants were sprayed with
710 ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars).
711 For each experiment, four leaves of three plants were collected at 16, 24, 48 and 72 hours post-
712 inoculation and used for total RNAs extraction. Transcript levels were quantified by RT-qPCR and
713 normalized to those of two reference genes, *AT4G26410* and *AT3G01150* (54). The represented fold
714 change is the mean of six independent experiments. Significant differences between non-infected
715 and infected plants were determined by one-way ANOVA followed by a Tukey HSD post-hoc test (***
716 $p<0.005$; ** $p<0.01$; * $p<0.05$; • $p<0.1$).

717 (B) Disease phenotype of *erdj3b-1* and *sdf2-2* mutants infected by *B. cinerea*. Lesion diameters
718 observed in wild-type (Col-0) and mutants were measured 3 days after *B. cinerea* infection.
719 Experiments were repeated four times. Seven plants per genotype were infected in each experiment.
720 Violin plots display the distribution of lesion diameters for all experiments and diamonds indicate the
721 median. Stars indicate significant differences compared to the wild type and were using Kruskal-
722 Wallis' method followed by Dunnett's post-hoc test (*** $p < 0.005$).
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725 **Figure 4: Functional complementation of yeast *cdc48* mutants.**
726 The yeast *cdc48* mutant strains which are cold-sensitive (strain DBY2030) or heat-sensitive (strain
727 KFY189) were transformed with pDRF1-GW vector containing or not the cDNA coding Arabidopsis
728 CDC48A-E proteins. Yeast strains were then streaked out on YPD agar plates and grown at 30°C (A, C)
729 or 16°C for DBY2030 (B) or 37°C for KFY189 (D). Experiments were repeated three times with similar
730 results.
731
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733 **Figure 5: CDC48 proteins are involved in disease susceptibility caused by *B. cinerea***
734 (A) Expression of *CDC48A*, *CDC48B and CDC48C genes* in response to *B. cinerea* infection. Plants were
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739 represented fold change is the mean of 6 independent experiments. Significant differences between
740 non-infected and infected plants were assessed by one-way ANOVA followed by a Tukey HSD post-
741 hoc test (*** $p<0.005$; * $p<0.05$; • $p<0.1$).
742 (B) Disease phenotypes of *cdc48* mutants, *CDC48B-WT* overexpressors and *CDC48B-QQ* dominant
743 negative overexpressors. Lesion diameters observed in wild-type (Col-0) and transgenics were
744 measured 3 days after *B. cinerea* infection. Experiments were repeated seven to nine times. Seven
745 plants per genotype were infected in each experiment. The stars indicate significant differences
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747 post-hoc test (*** $p<0.005$; * $p<0.05$; • $p<0.1$).
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751 **Figure 6: Defect in NAC053 and NAC078 transcription factors reduces *B. cinerea*-induced**
752 **symptoms**
753 (A-B) Expression of *NAC053* and *NAC078* genes in response to *B. cinerea* infection in WT, *ire1a/ire1b*
754 and *bzip60* mutants. Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB
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757 levels were quantified by RT-qPCR and normalized to those of two reference genes, *AT4G26410* and
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759 Different letters represent groups which were significantly different from one another as determined
760 by a one-way ANOVA followed by a multiple comparison with a Fisher's Least Significant Difference
761 (LSD) test.
762 (C) Disease phenotype of *nac053* and *nac078* mutants infected with *B. cinerea*. Lesion diameters
763 observed in wild-type (Col) and mutants were measured 3 days after *B. cinerea* infection.
764 Experiments were repeated four times. Seven plants per genotype were infected in each experiment.

765 The stars indicate significant differences compared to the wild type. Significant differences between
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767 post-hoc test (*** $p < 0.005$; ns : non-significant difference).
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771 **Figure 7: schematic representation of the role of UPR, ERQC and ERAD during *Botrytis cinerea***
772 **infection of *Arabidopsis thaliana* plants.**
773 *Botrytis cinerea* infection induces an ER stress which activates the canonical IRE1-bZIP60 branch of
774 the UPR and the expression of the genes coding the ERQC machinery (BIP, ERDJ3, SDF2). We
775 hypothesized the activation of these signalling pathways might control the secretion of defence
776 proteins which are necessary to establish defence against *B. cinerea*. Our data also indicate that
777 active bZIP60 suppresses *NAC053* expression, a negative regulator of defence against *B. cinerea*.
778 However, *NAC053* expression depends on IRE1. Regarding the bZIP17 arm of the UPR or the ERAD
779 proteins CDC48, we hypothesized that they act as negative regulators of immunity against B. cinerea
780 or might be the target of *B. cinerea* effectors (purple diamonds) thus facilitating infection process.
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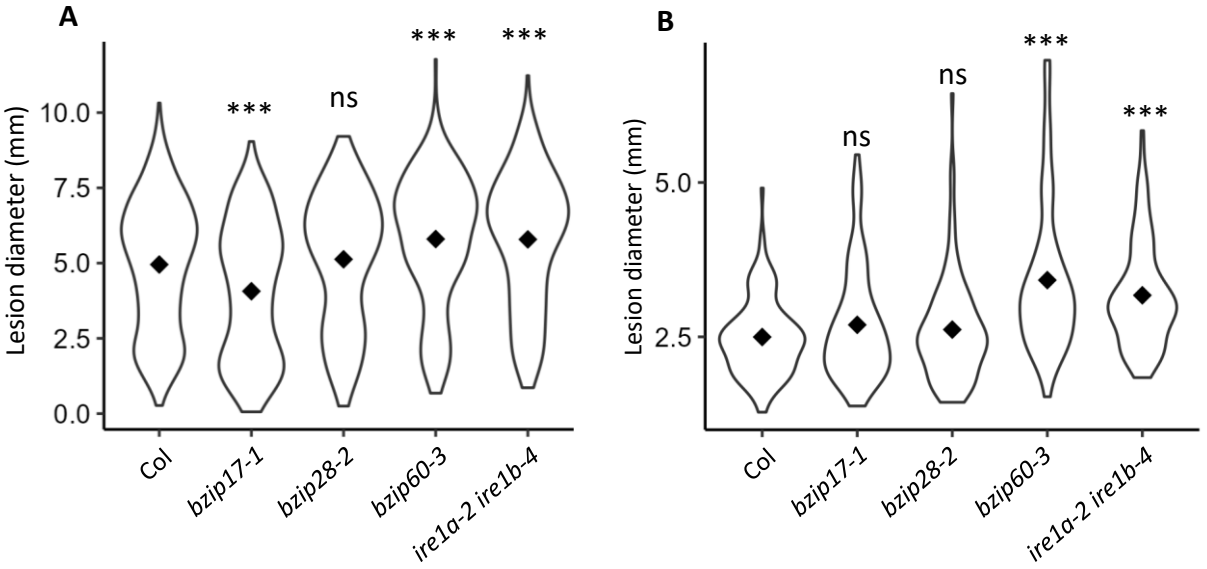

Figure 1 : Mutants deficient for IRE1-bZIP60 branch of UPR show enhanced necrotic symptoms induced by *B. cinerea* and *A. brassicicola*

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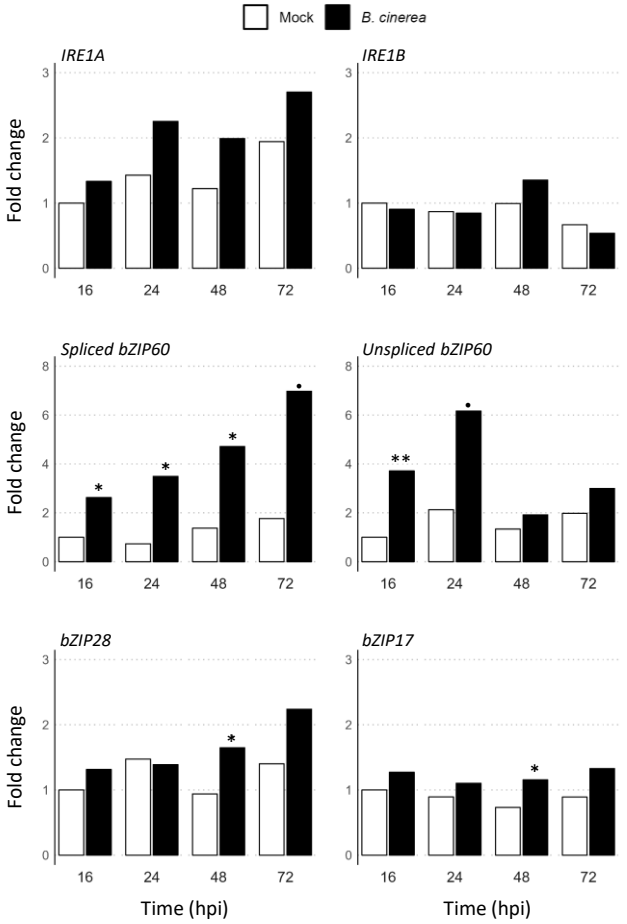

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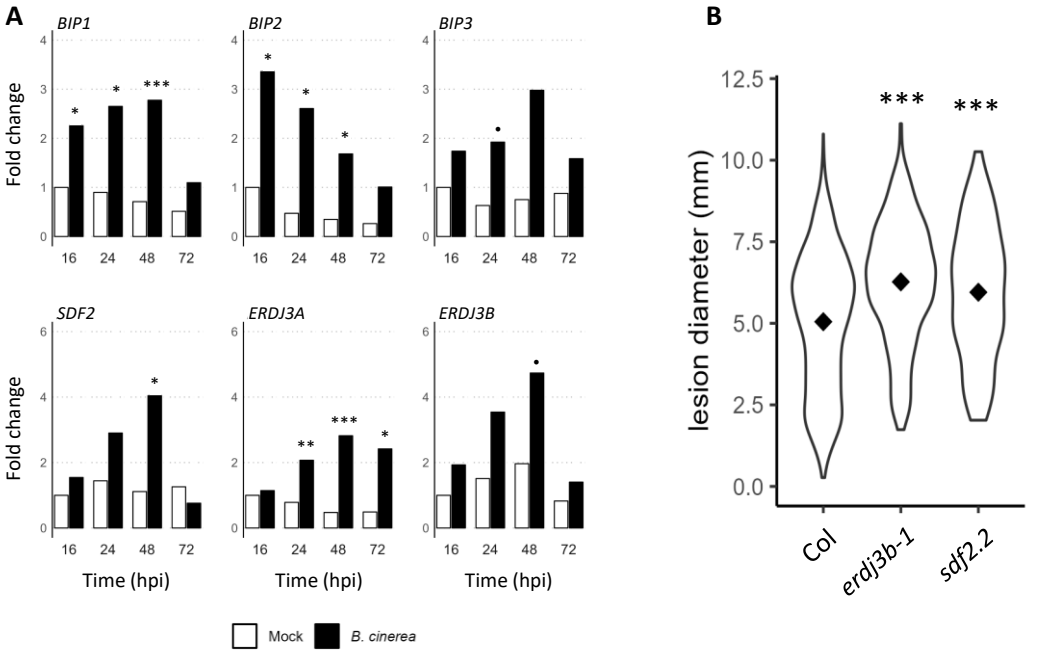

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(A) Expression of genes coding ER-QC machinery over *B. cinerea* infection. Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars). For each experiments, four leaves of three plants were collected at 16, 24, 48 and 72 hours post-inoculation and used for total RNA extraction. Transcript levels were quantified by RT-qPCR and normalized to those of two reference genes, *AT4G26410* and *AT3G01150* (Czechowski et al., 2005). The represented fold change is the mean of six independent experiments. Significant differences between non-infected and infected plants were determined by one-way ANOVA followed by a Tukey HSD post-hoc test (*** $p<0.005$; ** $p<0.01$; * $p<0.05$; • $p<0.1$).

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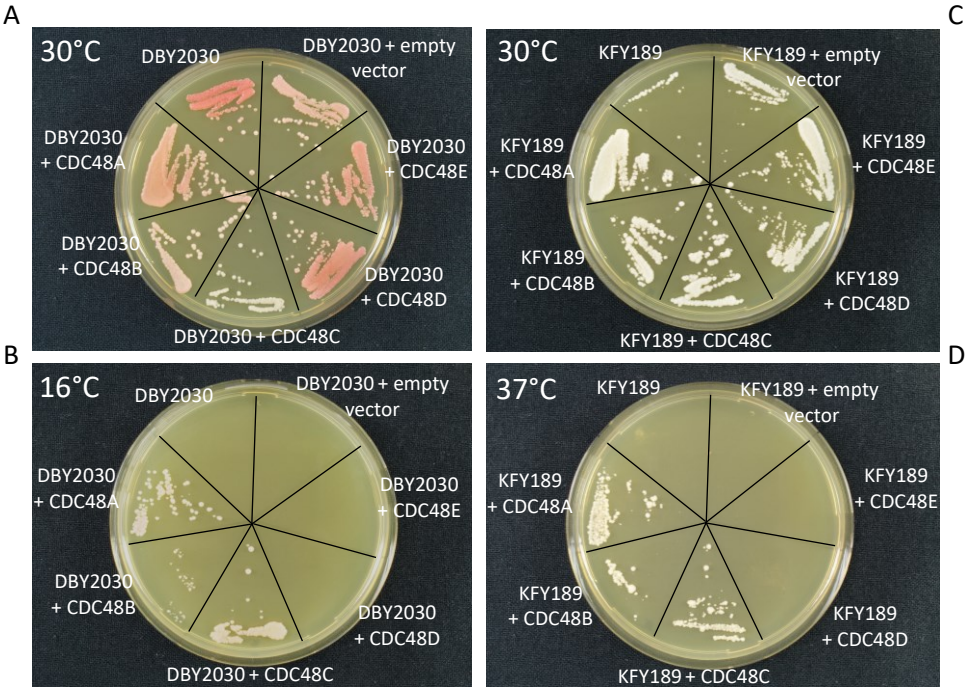
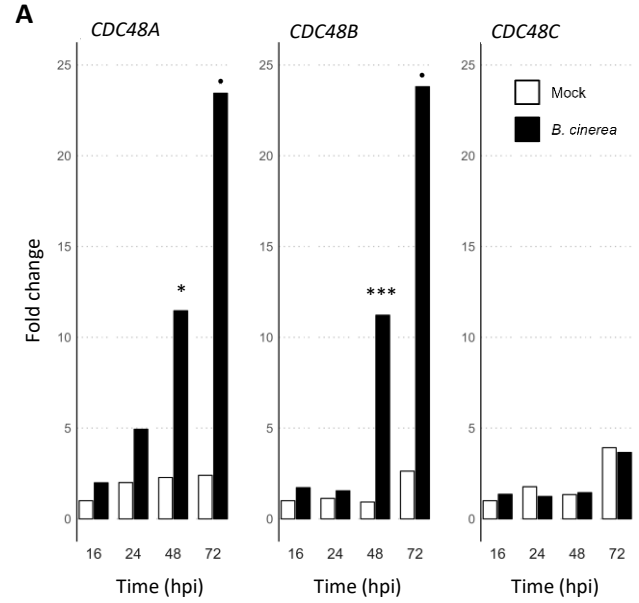
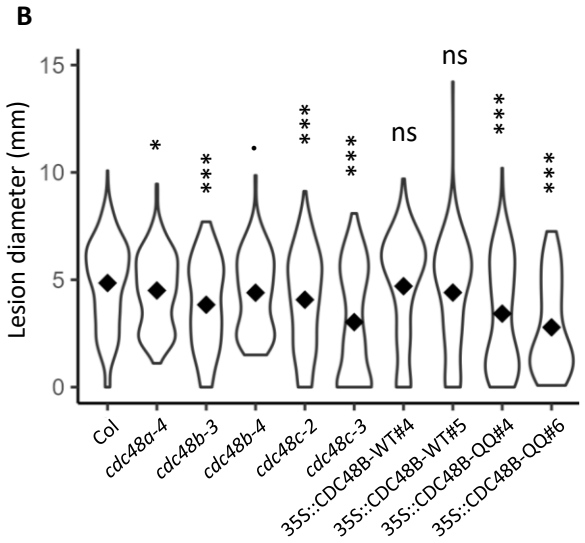


Figure 4: Functional complementation of yeast *cdc48* mutants.
The yeast *cdc48* mutant strains which are cold-sensitive (strain DBY2030) or heat-sensitive (strain KFY189) were transformed with pDRF1-GW vector containing or not the cDNA coding Arabidopsis CDC48A-E proteins. Yeast strains were then streaked out on YPD agar plates and grown at 30°C (A, C) or 16°C for DBY2030 (B) or 37°C for KFY189 (D). Experiments were repeated three times with similar results.





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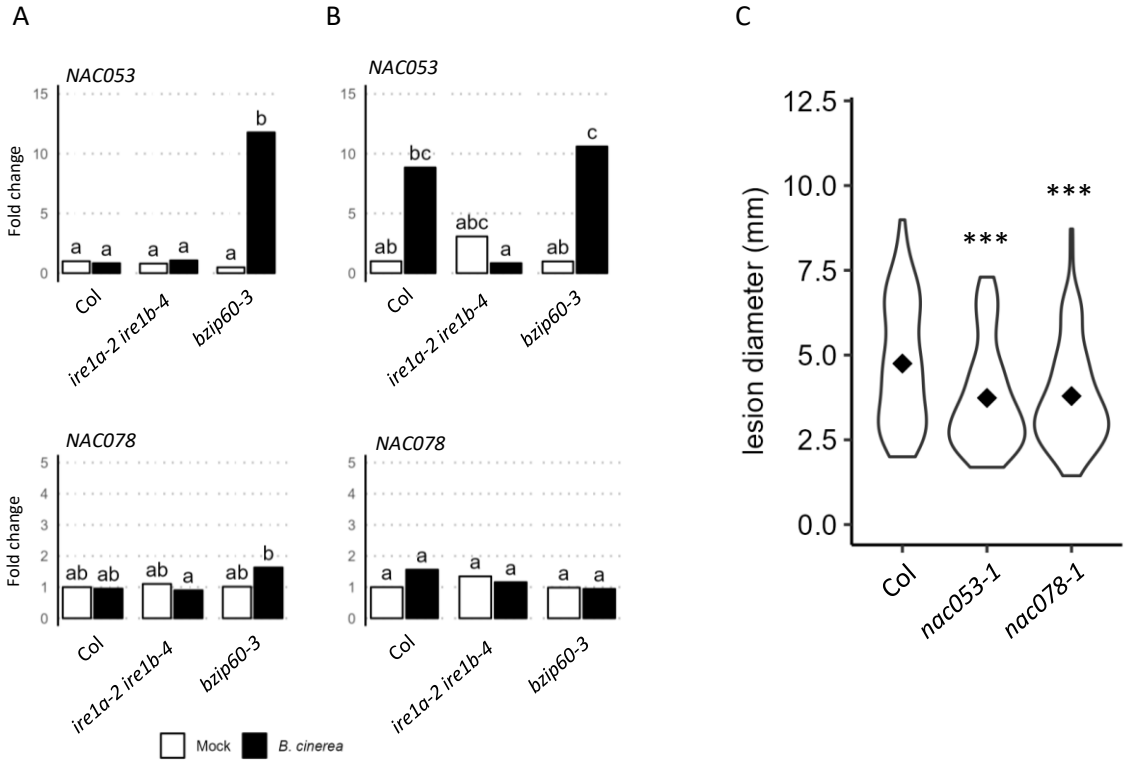


Figure 6 : Defect in NAC053 and NAC078 transcription factors reduces *B. cinerea*-induced symptoms

(A-B) Expression of *NAC053* and *NAC078* genes in response to *B. cinerea* infection in WT, *ire1a/ire1b* and *bzip60* mutants. Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars). For each experiments, four leaves of three plants were collected at 24 h (A) or 48 h (B) post infection and used for total RNA extraction. Transcript levels were quantified by RT-qPCR and normalized to those of two reference genes, *AT4G26410* and *AT3G01150* (Czechowski et al., 2005). The represented fold changes are the mean of six independent experiments. Different letters represent groups which were significantly different from one another as determined by a one-way ANOVA followed by a multiple comparison with a Fisher's Least Significant Difference (LSD) test.

(C) Disease phenotype of *nac053* and *nac078* mutants infected with *B. cinerea*. Lesion diameters observed in wild-type (Col) and mutants were measured 3 days after *B. cinerea* infection. Experiments were repeated four times. Seven plants per genotype were infected in each experiment. The stars indicate significant differences compared to the wild type. Significant differences between a mutant genotype and WT plants were assesed using Kruskal-Wallis' method followed by Dunnett's post-hoc test (*** $p < 0.005$; ns : non significant difference).

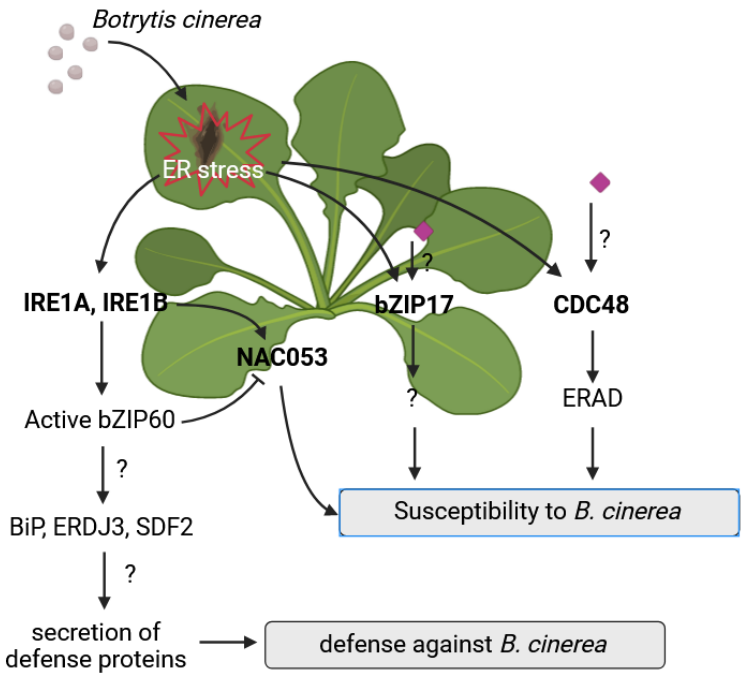
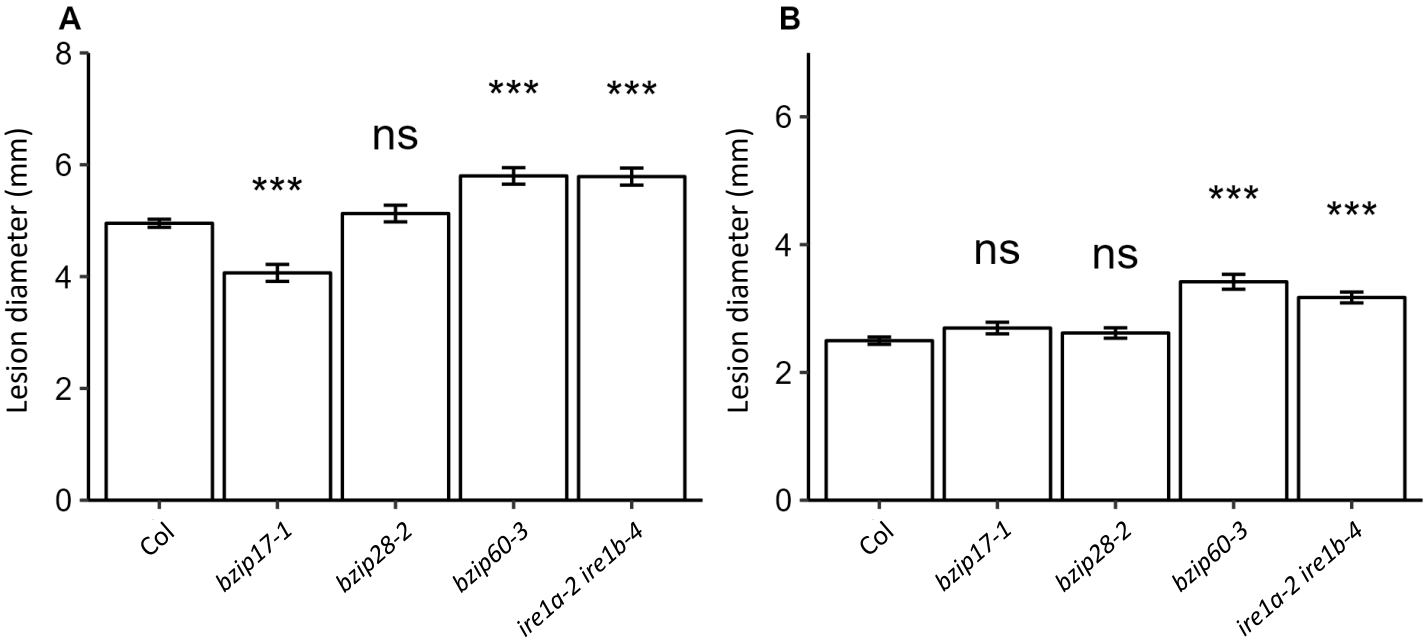


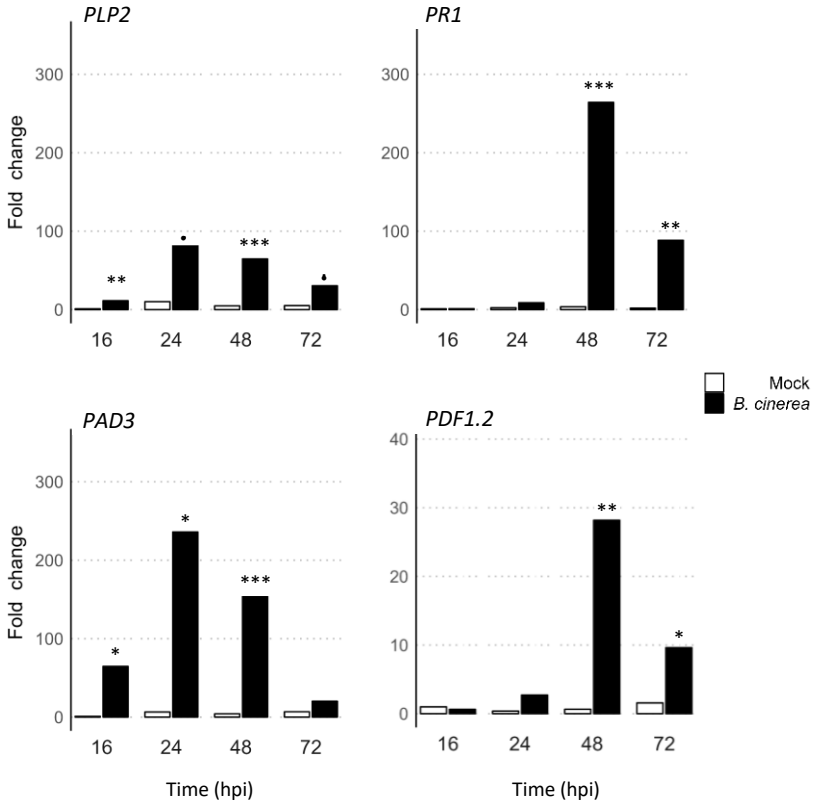
Figure 7 : schematic representation of the role of UPR, ERQC and ERAD during *Botrytis cinerea* infection of *Arabidopsis thaliana* plants.

Botrytis cinerea infection induces an ER stress which activates the canonical IRE1-bZIP60 branch of the UPR and the expression of the genes coding the ERQC machinery (BIP, ERDJ3, SDF2). We hypothesized the activation of these signalling pathways might control the secretion of defence proteins which are necessary to establish defence against *B. cinerea*. Our data also indicate that active bZIP60 suppresses *NAC053* expression, a negative regulator of defence against *B. cinerea*. However, *NAC053* expression depends on IRE1. Regarding the bZIP17 arm of the UPR or the ERAD proteins CDC48, we hypothesized that they act as negative regulators of immunity against B. cinerea or might be the target of *B. cinerea* effectors (purple diamonds) thus facilitating infection process.



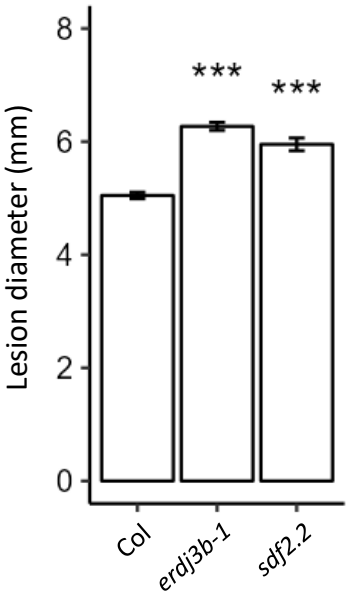
Supplemental Figure S1 : Mean lesion diameters observed on UPR-deficient mutants infected by *B. cinerea* or *A. brassicicola*.

Lesion diameters were measured 3 days after *B. cinerea* infection (A) and 5 days after *A. brassicicola* infection (B). Experiments were repeated seven to eight times for *B. cinerea* infection depending on the genotype tested and four times for *A. brassisicola* infection. Seven plants per genotype were infected in each experiment. The stars indicate significant differences compared to the wild type which were identified using a Kruskal-Wallis' method followed by Dunnett's post-hoc test (*** $p < 0.005$; ns : non significant difference).

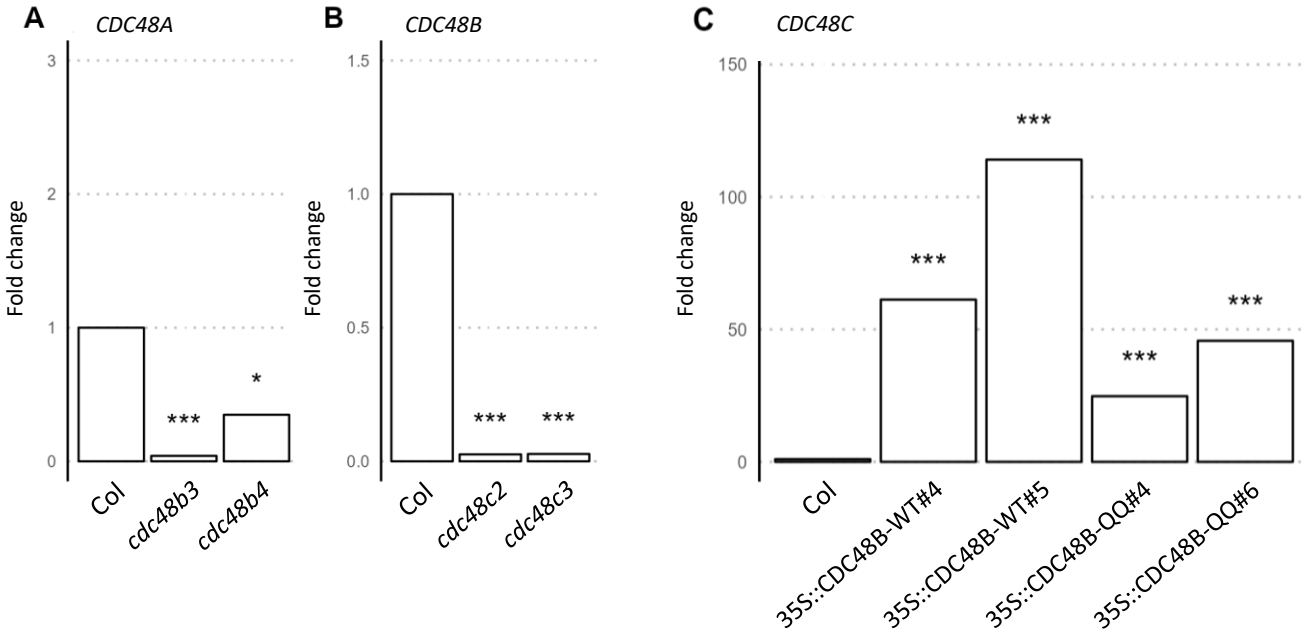


Supplemental Figure S2: Expression kinetic of the classical Botrytis-induced defense genes *PLP2*, *PR1*, *PAD3* and *PDF1.2* in our experimental conditions.

Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars). For each experiments, four leaves of three plants were collected at 16, 24, 48 and 72 hours post-inoculation. Transcript levels were quantified by RT-qPCR and normalized to the plant reference genes *AT4G26410* and *AT3G01150* transcript levels (Czechowski et al., 2005). The represented fold change is the mean of five independent experiments. Significant differences between non-infected and infected plants were determined by a one-way ANOVA followed by a Tukey HSD post-hoc test (*** $p<0.005$; ** $p<0.01$; * $p<0.05$; • $p<0.1$).

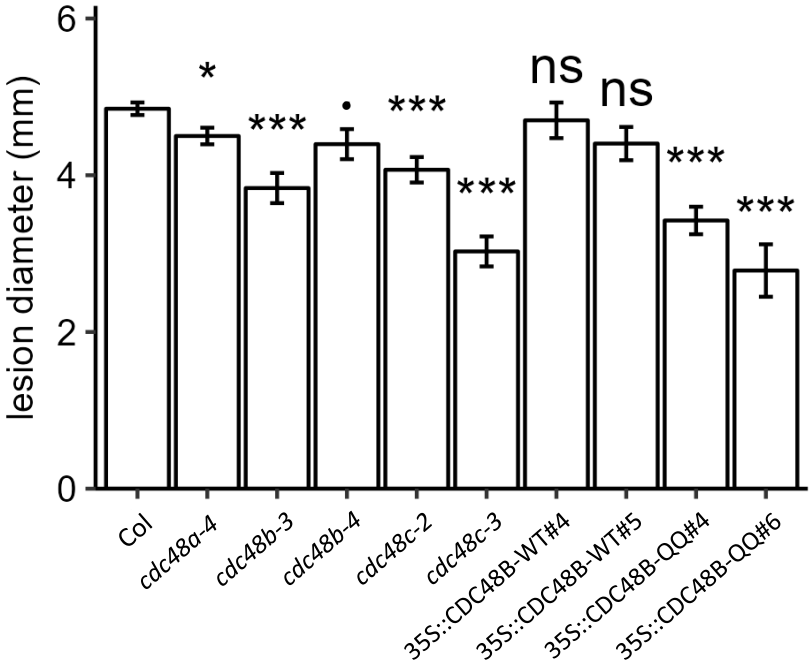


Supplemental Figure S3: Mean lesion diameters observed on ERQC mutants infected by *B. cinerea*.
Lesion diameters observed on wild-type Col plants and ERQC mutants were measured 3 days after *B. cinerea* infection. Experiments were repeated three to four times. Seven plants per genotype were infected in each experiment. The stars indicate significant differences compared to the wild type and were identified using Kruskal-Wallis' method followed by Dunnett's post-hoc test (*** $p < 0.005$).



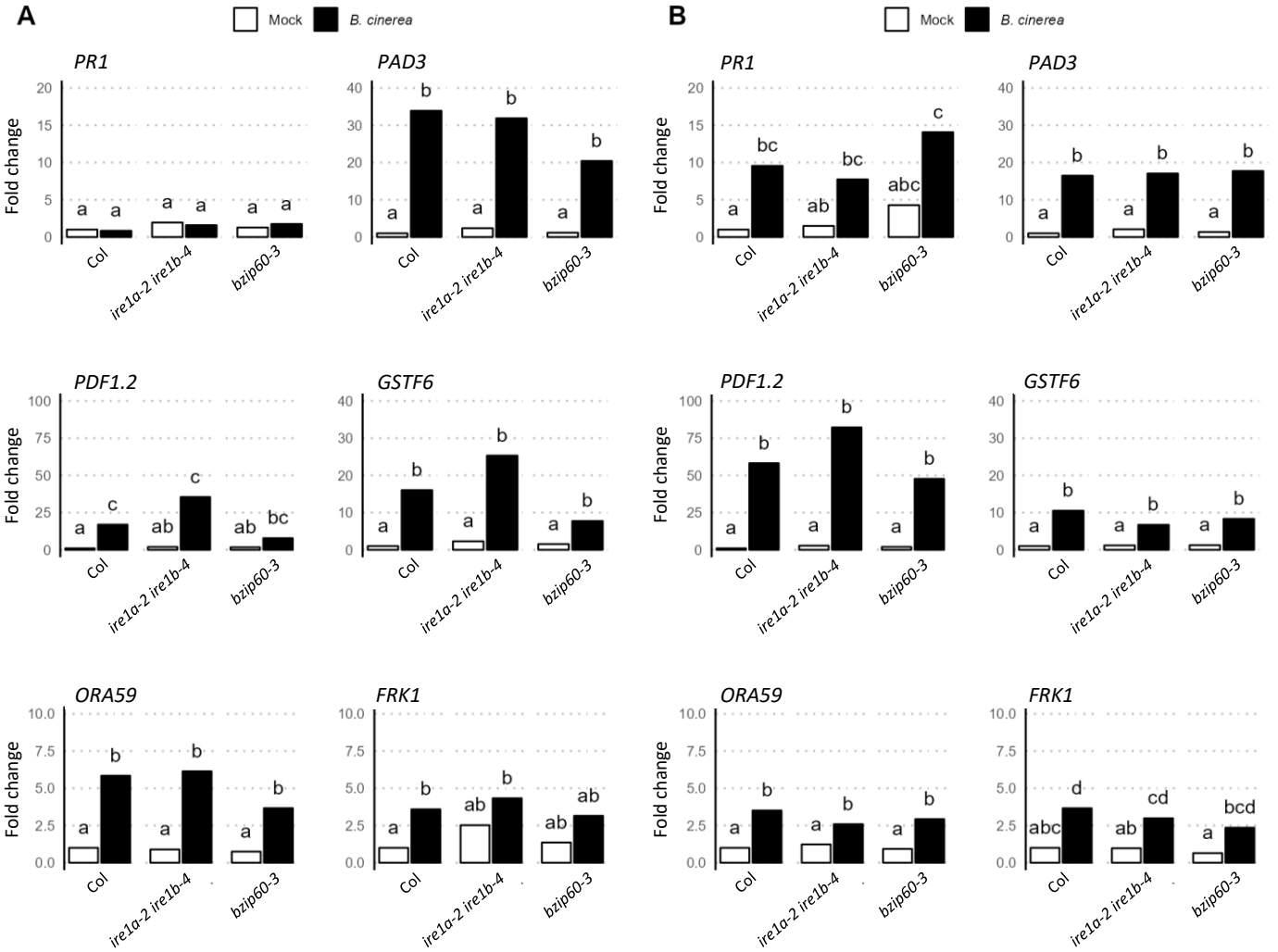
Supplemental Figure S4: Expression of *CDC48* in *cdc48* mutants

A, B, C. Expression of CDC48 mRNAs in *cdc48b* and *cdc48c* mutants and in CDC48B overexpressing lines. Four leaves of three plants were collected and *CDC48B* or *CDC48C* transcript levels were quantified by RTqPCR in *cdc48b3* and *cdc48b4* (A) or in *cdc48c2* and *cdc48c3* (B) mutants, respectively. *CDC48B* transcript level was quantified in transgenic lines overexpressing CDC48B-WT or CDC48B^{E308QE581Q} (CDC48-QQ). All transcript level were normalized to the plant reference genes *AT4G26410* and *AT3G01150* transcript levels (Czechowski et al., 2005). The represented fold change is the mean of five independent experiment. Significant differences between non-infected and infected plants were determined by a one-way ANOVA followed by a Tukey HSD post-hoc test (*** $p<0.005$; * $p<0.05$).



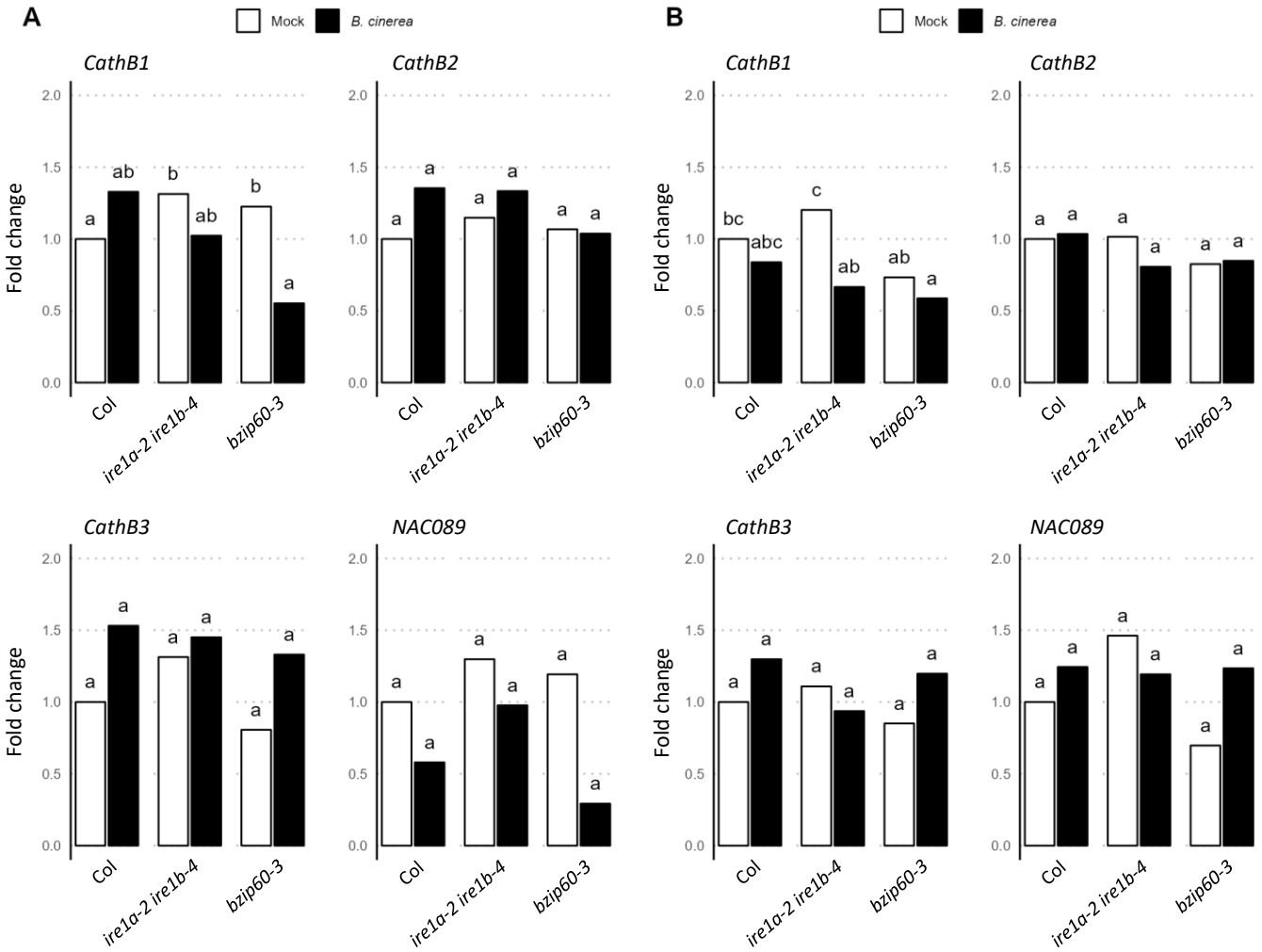
Supplemental Figure S5: Mean lesion diameters observed on CDC48 mutants and plants expressing a WT (CDC48B-WT) or an inactive isoforms of CDC48B (CDC48B-QQ) infected by *B. cinerea*.

Lesion diameters observed on wild-type Col plants and mutants were measured 3 days after *B. cinerea* infection. Experiments were repeated seven to nine times. Seven plants per genotype were infected in each experiment. The stars indicate significant differences compared to the wild type which were identified using Kruskal-Wallis' method followed by Dunnett's post-hoc test(*** $p<0.005$; * $p<0.05$; • $p<0.1$).



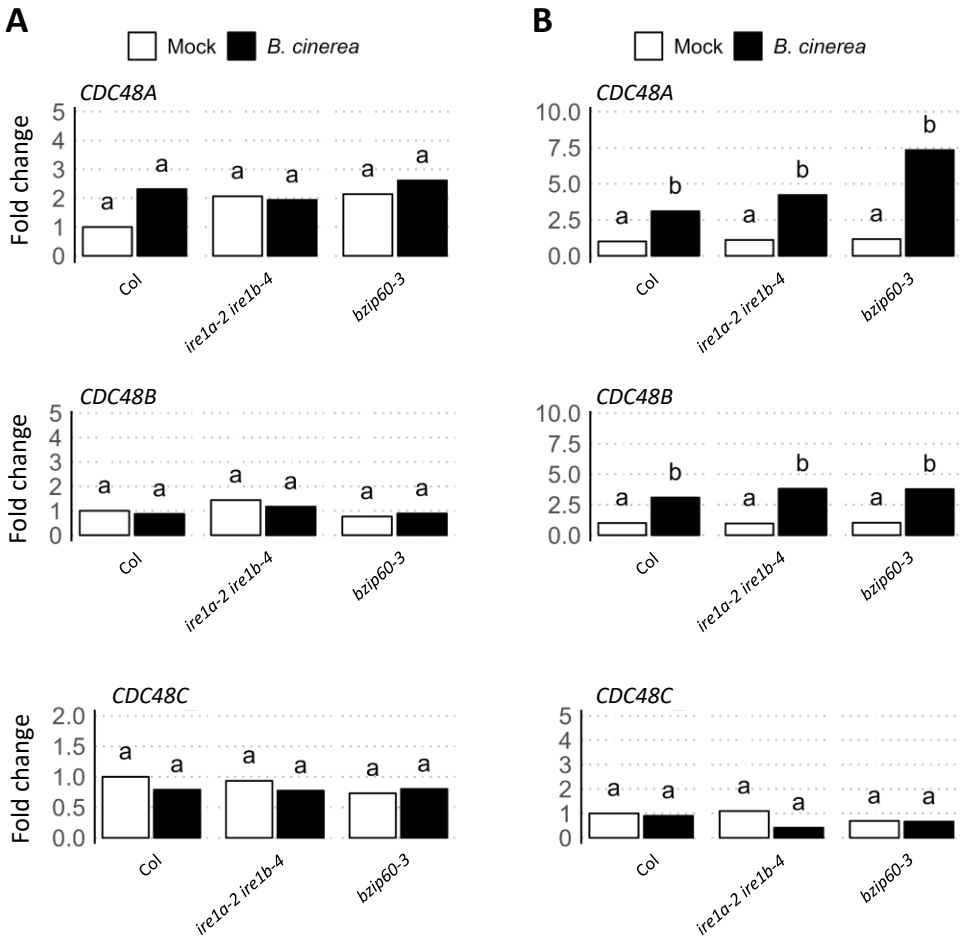
Supplemental Figure S6 : Accumulation of mRNA coding classical defense genes in response to *B. cinerea* infection in WT, *ire1a/ire1b* and *bzip60* mutants.

Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars). For each experiments, four leaves of three plants were collected at 24 h (A) or 48 h (B) post-treatment. Transcript levels were quantified by RT-qPCR and normalized to the plant reference genes *AT4G26410* and *AT3G01150* transcript levels (Czechowski et al., 2005). The represented fold change is the mean of six independent experiments. Different letters represent groups which were significantly different from one another as determined by a one-way ANOVA followed by a multiple comparison with a Fisher's Least Significant Difference (LSD) post-hoc test.



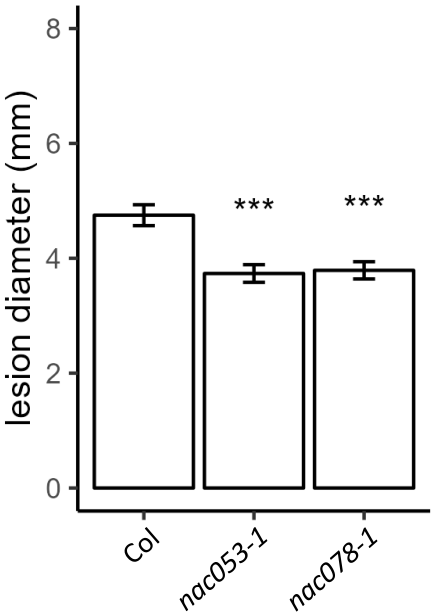
Supplemental Figure S7 : Accumulation of mRNA coding cathepsin and NAC089 transcription factor in response to *B. cinerea* infection in WT, *ire1a/ire1b* and *bzip60* mutants.

Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars). For each experiments, four leaves of three plants were collected at 24 h (A) or 48 h (B) post-treatment. Transcript levels were quantified by RT-qPCR and normalized to the plant reference genes *AT4G26410* and *AT3G01150* transcript levels (Czechowski et al., 2005). The represented fold change is the mean of six independent experiments. Different letters represent groups which were significantly different from one another as determined by a one-way ANOVA followed by a multiple comparison with a Fisher's Least Significant Difference (LSD) post-hoc test.



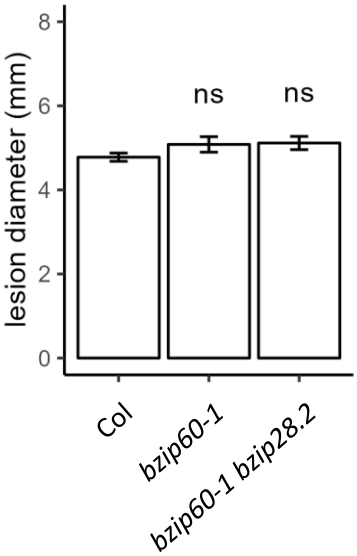
Supplemental Figure S8: Accumulation of mRNA coding CDC48 in response to *B. cinerea* infection in WT, *ire1a/ire1b* and *bzip60* mutants.

Plants were sprayed with ¼ PDB solution (Mock, white bars) or with ¼ PDB solution containing *B. cinerea* spores (black bars). For each experiments, four leaves of three plants were collected at 24 h (A) or 48 h (B) post-treatment. Transcript levels were quantified by RT-qPCR and normalized to the plant reference genes *AT4G26410* and *AT3G01150* transcript levels (Czechowski et al., 2005). The represented fold change is the mean of six independent experiments. Different letters represent groups which were significantly different from one another as determined by a one-way ANOVA followed by a multiple comparison with a Fisher's Least Significant Difference (LSD) post-hoc test.



Supplemental Figure S9: Mean lesion diameters observed on *nac053* and *nac078* mutants infected by *B. cinerea*.

Lesion diameters observed on wild-type Col plants and mutants were measured 3 days after *B. cinerea* infection. Experiments were repeated four times. Seven plants per genotype were infected in each experiment. The stars indicate significant differences compared to the wild type which were identified using Kruskal-Wallis' method followed by Dunnett's post-hoc test(*** $p<0.005$).



Supplemental Figure S10: Mean lesion diameters observed on *bzip60.1-bzip28.2 double mutant* infected by *B. cinerea*.

Lesion diameters observed on wild-type Col plants and mutants were measured 3 days after *B. cinerea* infection. Experiments were repeated six times. Seven plants per genotype were infected in each experiment. Significant differences compared to the wild type were identified using Kruskal-Wallis' method followed by Dunnett's post-hoc test (ns: not significant).

Supplemental table S1 : list of mutants used in this study

mutant name	gene locus	mutant reference	references	origin	
Atbzip17-1	At2g40950	SALK_104326	Liu et al., 2007a	Pr. Steven H Howell	https://doi.org/10.1111/j.1365-313X.2007.03195.x
Atbzip28-2	At3g10800	SALK_132285C	Liu et al., 2007b	Pr. Steven H Howell	https://doi.org/10.1105/tpc.106.050021
Atbzip60-3	At1g42990	GABI_326A12	Bao et al., 2012	Pr. Steven H Howell	https://doi.org/10.1080/15548627.2018.1462426
Atire1a-2	At2g17520	SALK_018112	Deng et al., 2011	Pr. Steven H Howell	https://doi.org/10.1073/pnas.1102117108
Atire1b-4	At5g24360	SAIL_238_F07	Deng et al., 2011	Pr. Steven H Howell	https://doi.org/10.1073/pnas.1102117108
Atire1a-2/Atire1b-4		SALK_018112 x SAIL_238_F07	Moreno et al., 2012	Pr. Steven H Howell	https://doi.org/10.1371/journal.pone.0031944
Aterdj3b-1	At3g62600	SALK_113364	Nekrasov et al, 2009	Pr. Cyril Zipfel	https://doi.org/10.1038/emboj.2009.262
Atsdf2.2	At2g25110	SALK_141321	Nekrasov et al, 2009	Pr. Cyril Zipfel	https://doi.org/10.1038/emboj.2009.262
Atcdc48a-4/muse8	At3g09840	EMS mutant	Copeland et al., 2016	Pr. Xin Li	https://doi.org/10.1111/tpj.13251
Atcdc48b-3	At3g53230	Gabi_104F08	our study	NASC	
Atcdc48b-4	At3g53230	Gabi_485G04	our study	NASC	
Atcdc48c-2	At5g03340	SALK_102955C	our study	NASC	
Atcdc48c-3	At5g03340	SALK_123409	our study	NASC	
Atnac53-1	At3g10500	SALK_009578C	Gladman et al., 2016	NASC	https://doi.org/10.1105/tpc.15.01022
Atnac78-1	At5g04410	SALK_025098	Gladman et al., 2016	NASC	https://doi.org/10.1105/tpc.15.01022

NASC : Nottingham Arabidopsis Stock Centre (https://arabidopsis.info/)

Supplemental table S2 : Primers used for mutant genotyping

mutant name	line	LB primer name	LB primer sequence	gene specific primer 1	gene specific primer 2
Atcdc48b1	GABI_104F08	GABI-LB-o8474	ATAATAACGCTGCGGACATCTACATTTT	TTCTCATCCCTCCTCTAACTAGGA	TAAATATGAACACCGAGCTCCTCA
Atcdc48b2	GABI_485G04	GABI-LB-o8474	ATAATAACGCTGCGGACATCTACATTTT	TTCTCATCCCTCCTCTAACTAGGA	TAAATATGAACACCGAGCTCCTCA
Atcdc48c1	SALK_123409	Lbe	GGAACAACACTCAACCCTATCTCG	GGTTGCTCTCACTCTCACCAG	TATGTCAAACGAACCGGAATC
Atcdc48c2	SALK_102955C	Lbe	GGAACAACACTCAACCCTATCTCG	GGTTGCTCTCACTCTCACCAG	TATGTCAAACGAACCGGAATC
Atnac053-1	SALK_009578C	Lbe	GGAACAACACTCAACCCTATCTCG	TCAAGAAGCAAACATGTGGTG	GATGACGACGCTTCTTTTCAG
Atnac078-1	SALK_025098	Lbe	GGAACAACACTCAACCCTATCTCG	TCTTTCGCATTTGCGATATTC	TTCAAGTTCTGGTTTTCACCG
Atbzip17-1	SALK_104326	Lbe	GGAACAACACTCAACCCTATCTCG	AAGGAGCAGCCTCCTCCAC	GCCTTTCTCGAAGCCACGG

Supplemental table S3 : RT-qPCR primers used in this work

name	locus		RT-qPCR primers	ref	
AtCDC48A	At3g09840	forward	AAACTCGAATTCTCTACTTTTCDC48 A	this work	
		reverse	TTTTTCCATTTGTAATGAACGACC		
AtCDC48B	At3g53230	forward	GGTTGTGGATGAAGCCATTACDC48 B	this work	
		reverse	CGGTTCCCATACTTGACATCAG		
AtCDC48C	At5g03340	forward	GGCGATACAATTCTCATCAACCDC48 C	this work	
		reverse	GACGGGGCATTCTTCTCAGC		
reference gene	*At4g26410*	forward	GAGCTGAAGTGGCTTCCATGAC	Czechowski *et al.* , 2005	https://doi.org/10.1104/pp.105.063743
		reverse	GGTCCGACATACCCATGATCC		
reference gene	*At3g01150*	forward	GATCTGAATGTTAAGGCTTTTAGCG	Czechowski *et al.* , 2005	https://doi.org/10.1104/pp.105.063743
		reverse	GGCTTAGATCAGGAAGTGTATAGTCTCTG		
AtIRE1A	AT2G17520	forward	GCTTCAGACCTCATATCCCG	Afrin *et al.* , 2020	https://doi.org/10.1038/s41598-020-76114-1
		reverse	AGCATCACGAAGGAAAGACAG		
AtIRE1B	AT5G24360	forward	GGTGGGATGAGAAACTGGATA	Afrin *et al.* , 2020	https://doi.org/10.1038/s41598-020-76114-1
		reverse	AGTTTGTTCCGTATGACCCG		
AtBzip60	AT1G42990	forward	GGAGACGATGATGCTGTGGCT	Afrin *et al.* , 2020	https://doi.org/10.1038/s41598-020-76114-1
AtBzip60 unspliced Rev		reverse	CAGGGATTCCAACAAGAGCACAG		
AtBzip60 spliced Rev		reverse	CAGGGAACCCAACAGCAGACT		
AtbZIP28	AT3G10800	forward	GCCAGTGATCCTCTCTTTGC	Qiang *et al.* , 2012	https://doi.org/10.1105/tpc.111.093260
		reverse	CAGAAGACAGTGCACCAGGA		
AtbZIP17	AT2G40950	forward	ACAGGAGATCGGGAGAGGAT	Qiang *et al.* , 2012	https://doi.org/10.1105/tpc.111.093260
		reverse	GCTCCTCGACGTAATGCTTC		
AtBIP1	AT5G28540	forward	GCTCGTTTGGAGCTAACAGTACC	this work	
		reverse	GAGGACAACGCAAATAAACATCCG		
AtBIP2	AT5G42020	forward	TTGCAGAGGAAGACAAGAAGGTG	this work	
		reverse	CACGTATGTCTCCAGGGCATTC		
AtBIP3	AT1G09080	forward	CACGGTTCCAGCGTATTTCAAT	Liu *et al.* , 2007b	https://doi.org/10.1105/tpc.106.050021
		reverse	ATAAGCTATGGCAGCACCCGTT		
AtSDF2	AT2G25110	forward	TCAAGAGTGGAGCAACCATTAG	Afrin *et al.* , 2020	https://doi.org/10.1038/s41598-020-76114-1
		reverse	CCAAAGCAGCTAACCTCTAAGT		
AtERDJ3A	AT3G08970	forward	GGTAGCTCATCGAATGCTGAA	Afrin *et al.* , 2020	https://doi.org/10.1038/s41598-020-76114-1
		reverse	GGTCCACAACGTCCTTCTTATAG		
AtERDJ3B	AT3G62600	forward	CAAATACGAACGGGAGGGATAC	Afrin *et al.* , 2020	https://doi.org/10.1038/s41598-020-76114-1
		reverse	GGTTCGCCGTCTTCATAGAAA		
AtPLP2	AT2G26560	forward	GTAGCTGGTTGGGGACTATTGA	La Camera *et al.* , 2005	https://doi.org/10.1111/j.1365-313X.2005.02578.x
		reverse	CGGTAGCGATATCAACAGAAGC		
AtPR1	AT2G14610	forward	AAGGGTTCACAACCAGGCAC	Anderson *et al.* , 2004	https://doi.org/10.1105/tpc.104.025833
		reverse	CACTGCATGGGACCTACGC		
AtPDF1.2a	AT5G44420	forward	TTTGCTGCTTTCGACGCAC	Anderson *et al.* , 2004	https://doi.org/10.1105/tpc.104.025833
		reverse	CGCAAACCCCTGACCATG		
AtNAC053/AtNTL4	AT3G10500	forward	TGTTGGGTGCTATTCCTGCT	Gladman *et al.* , 2016	https://doi.org/10.1105/tpc.15.01022
		reverse	CTGATGATTGTGCTGCGTGT		
AtNAC078/AtNTL11	AT5G04410	forward	TTGAAGAAAGCTGGTGTGCC	Gladman *et al.* , 2016	https://doi.org/10.1105/tpc.15.01022