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1 **The root-knot nematode effector MiEFF12 targets the host ER quality**
2 **control system to suppress immune responses and allow parasitism**

3

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23

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26

27

28 **Abstract**

29

30 Root-knot nematodes (RKN) are microscopic parasitic worms able to infest the roots of thousands of
31 plant species, causing massive crop yield losses worldwide. They evade the plant's immune system and
32 manipulate plant cell physiology and metabolism to transform a few root cells into giant cells, which
33 serve as feeding sites for the nematode. RKN parasitism is facilitated by the secretion *in planta* of
34 effector molecules, mostly proteins that hijack host cellular processes. We describe here a conserved
35 RKN-specific effector, EFFECTOR12 (EFF12), which is synthesized exclusively in the esophageal
36 glands of the nematode, and we demonstrate its function in parasitism. In the plant, MiEFF12 localizes
37 to the endoplasmic reticulum (ER). A combination of RNA-sequencing analysis and immunity-
38 suppression bioassays revealed the contribution of MiEFF12 to the modulation of host immunity. Yeast
39 two-hybrid, split luciferase and co-immunoprecipitation approaches identified an essential component
40 of the ER quality control system, the *Solanum lycopersicum* PLANT BAP-LIKE (PBL), and BASIC
41 LEUCINE ZIPPER 60 (BZIP60) proteins as host targets of MiEFF12. Finally, silencing the *PBL* genes
42 in *N. benthamiana* decreased susceptibility to *M. incognita* infection. Our results suggest that EFF12
43 manipulates PBL function to modify plant immune responses to allow parasitism.

44

45 **Keywords:** *Meloidogyne incognita*, effector, endoplasmic reticulum (ER), ER quality control, *Solanum*
46 *lycopersicum*, *Nicotiana benthamiana*

47

48

49 1 | INTRODUCTION

50

51 Root-knot nematodes (*Meloidogyne spp.*, RKN) are extremely polyphagous plant pathogens
52 responsible for huge losses in agriculture (Jones *et al.*, 2013). These obligate biotrophic root parasites
53 manipulate plant functions to induce a permanent feeding structure. Following root penetration, the
54 RKN second-stage juveniles (J2s) migrate between the cells to reach the vascular cylinder, where they
55 induce the redifferentiation of five to seven selected vascular parenchyma cells into hypertrophied
56 multinucleate feeding cells or "giant cells". These giant cells are the sole source of nutrients for the
57 nematode and are essential for RKN development and reproduction. Concomitantly, the surrounding
58 cells, xylem and phloem proliferate, inducing typical root deformations known as galls or root knots
59 (Favery *et al.*, 2020; Rutter *et al.*, 2022). After successive molts, the adult female RKN lays eggs on the
60 root surface. The success of RKNs as parasites depends on their ability to hijack essential host-cell to
61 induce and maintain a functioning feeding site. The formation of this feeding site is mediated by the
62 secretion into the host of effector proteins essential for RKN parasitism. These effectors hijack host cell
63 processes, including those involved in immune responses, thereby facilitating successful parasitism
64 (Vieira & Gleason, 2019; Rutter *et al.*, 2022).

65 Plants can detect RKN infestation, which, like infections with other pathogens, triggers immune
66 responses (Kaloshian & Teixeira, 2019; Sato *et al.*, 2019; Siddique *et al.*, 2022). Plants specifically
67 recognize RKN pathogen-associated molecular patterns (PAMPs) or damage-associated molecular
68 patterns (DAMPs) released by the RKN during host invasion, via plasma membrane-associated pattern-
69 recognition receptors (PRRs), which initiate an immune response known as pattern-triggered immunity
70 (PTI) (Sato *et al.*, 2019; Goode & Mitchum, 2022; Siddique *et al.*, 2022; Huang *et al.* 2023). PTI enables
71 the plant to respond to nematode attack by producing reactive oxygen species (ROS), antimicrobial
72 pathogenesis-related proteins (PR) and metabolites, and by reinforcing their cell walls (Sato *et al.*, 2019;
73 Goode & Mitchum, 2022). In response, pathogens have evolved effector proteins that they secrete into
74 the host to suppress PTI (Jones & Dangl, 2006). Plants have acquired resistance genes enabling them to
75 recognize such effectors specifically, leading to the initiation of effector-triggered immunity (ETI),
76 which results in localized cell death through the hypersensitive response (HR) (Jones & Dangl, 2006;

77 Sato *et al.*, 2019). More than 20 RKN effectors have been reported to be involved in suppression of the
78 PTI or ETI (Rutter *et al.*, 2022). These effectors include MjShKT, which has a *Stichodactyla* toxin
79 (ShKT) domain and is secreted by *Meloidogyne javanica* (Kumar *et al.*, 2023). MjShKT has been
80 implicated in suppression of the ETI and is the only RKN effector known to target the host-plant
81 endoplasmic reticulum (ER) (Kumar *et al.*, 2023).

82 The ER is a highly dynamic organelle consisting of a complex network of cisternae and tubules
83 (Kriechbaumer & Brandizzi, 2020). It is physically connected to the nucleus, the plasma membrane,
84 plastids and mitochondria, and is continuous between cells, via the plasmodesmata (Michaud & Jouhet,
85 2019; Kriechbaumer & Brandizzi, 2020). The ER, thus, participates in intracellular and intercellular
86 communications. It is responsible for the biosynthesis and quality control of ER-resident proteins and
87 proteins destined for transportation to the vacuole, plasma membrane or apoplast (Kriechbaumer &
88 Brandizzi, 2020). It is involved in lipid biosynthesis and storage (Kanehara *et al.*, 2022) and is an
89 important organelle for the storage of calcium (Ca^{2+}), an instrumental intracellular messenger (Costa *et*
90 *al.*, 2018) involved in plant immune responses (Köster *et al.*, 2022). The ER can therefore respond and
91 adapt to the biosynthetic requirements imposed on plant cells during plant growth and by environmental
92 stress (Brandizzi, 2021). The ER quality control system (ERQC) identifies misfolded proteins and
93 directs them to the ER-associated degradation (ERAD) machinery (Strasser, 2018). In the absence of
94 such control, the accumulation of unfolded proteins within the ER would trigger ER stress, inducing the
95 unfolded protein response (UPR), leading to the production of proteins of the ERAD pathway and of
96 chaperones responsible for protein refolding, to restore ER homeostasis (Liu & Howell, 2016). If
97 prolonged, the UPR eventually leads to plant cell death (Liu & Howell, 2016).

98 The plant ER and ERQC system components are targeted by several plant pathogen effectors
99 (reviewed by Jing & Wang, 2020; Breeze *et al.*, 2023). For example, proteins acting downstream from
100 ER stress sensors and responsible for inducing the UPR, such as the BZIP60 and NAC transcription
101 factors, have been shown to be targeted by pathogen effectors (Jing & Wang, 2020; Breeze *et al.*, 2023).
102 Similarly, effectors secreted by the oomycete *Phytophthora sojae* interact with ER-resident chaperones,
103 such as plant binding immunoglobulin proteins (BiPs), to suppress host immune responses (Jing *et al.*,

104 2016). Consistently, plants with an impaired ability to sense ER stress or to trigger the UPR are generally
105 more susceptible to pathogens (Moreno *et al.*, 2012; McLellan *et al.*, 2013; Zhang *et al.*, 2015).

106 We describe here EFF12, an RKN-specific effector expressed in the dorsal esophageal glands and
107 conserved in different *Meloidogyne* species. We show that MiEFF12 is involved in *M. incognita*
108 parasitism and associates with the host-cell ER. A combination of transcriptomic and molecular analyses
109 indicated that MiEFF12 was involved in suppressing host immune responses. We show that MiEFF12
110 interacts with the ER-resident PLANT BAP-LIKE (PBL), orthologs of the human B-CELL
111 RECEPTOR-ASSOCIATED PROTEIN 31 (BAP31 or BCAP31) and the BASIC LEUCINE ZIPPER
112 60 (BZIP60) proteins, two known components of ERQC systems. The silencing of *PBL* genes in *N.*
113 *benthamiana* decreased susceptibility to *M. incognita*, suggesting a role for this protein in plant defense
114 responses directed against RKN.

115

116 **2 | RESULTS**

117

118 **2.1 | EFF12 is an RKN-specific effector required for parasitism**

119 MiEFF12s are putative secreted effectors encoded by the *MiEFF12a/Minc3s00905g18741/Minc12754*
120 gene and its two paralogs, *MiEFF12b/Minc3s01322g22768/Minc13608* and
121 *MiEFF12c/Minc3s00876g18368/Minc01345* (Abad *et al.* 2008; Blanc-Mathieu *et al.*, 2017) in *M.*
122 *incognita*. MiEFF12a, MiEFF12b and MiEFF12c are 96- to 98-amino acid (aa) proteins with a 23- to
123 25-amino acid signal peptide for secretion (SP) (Figure 1a). Secreted 73-aa MiEFF12 proteins display
124 no similarity to any other sequence in the genus *Meloidogyne* and they carry none of the functional
125 domains listed in public databases. The conserved C-terminal regions are enriched in positively (lysine-
126 K) and negatively (asparagine-D and glutamic acid-E) charged residues. A protein blast search with
127 Wormbase (Kevin *et al.* 2016) identified one EFF12 homolog in *M. hapla* and four in *M. arenaria* and
128 *M. javanica* (Figure 1b and 1c). A phylogenetic tree constructed from an alignment of 15 EFF12 protein
129 sequences and a pairwise analysis of nucleotide sequence identity revealed variations between copies
130 within the same species and identified MhEFF12 as the most divergent of these effectors (Figure 1b and
131 1c; Figure S1 and S2). These results indicate that EFF12 is an RKN-specific effector. *MiEFF12* genes

132 are more strongly expressed at the juvenile parasitic stages than at the J2 pre-parasitic stage, suggesting
133 that EFF12 may play a role in parasitism (Nguyen *et al.*, 2018; Da Rocha *et al.*, 2021).

134 We analyzed the possible secretion of EFF12 into the plant during parasitism, by using *in situ*
135 hybridization to localize *EFF12* expression in nematode J2s. *MiEFF12* expression was observed
136 exclusively in the dorsal esophageal gland of pre-parasitic *M. incognita* J2s, consistent with previous
137 findings (Nguyen *et al.*, 2018). A similar expression pattern, restricted to the dorsal gland cell, was
138 observed in *M. enterolobii* with an antisense *MeEFF12* probe (Figure 2). Sense probes were used as
139 negative controls and gave no staining in *M. incognita* or *M. enterolobii* (Figure 2).

140 We investigated the role of MiEFF12 in parasitism through a host-induced gene-silencing approach.
141 We silenced *MiEFF12* during *M. incognita* feeding on *Nicotiana benthamiana*, using the tobacco rattle
142 virus (TRV) for virus-induced gene silencing (VIGS). An empty VIGS construct and a construct
143 targeting the GFP transcript were used as controls. RT-qPCR analyses showed lower levels of *MiEFF12*
144 mRNA in nematodes collected from *N. benthamiana* roots (Figure 3a). Relative to the two controls, the
145 silencing of *MiEFF12* significantly decreased the numbers of both galls and egg masses produced by
146 *M. incognita* on *N. benthamiana* roots six weeks post-infection (Figure 3b). These results demonstrate
147 that MiEFF12 is an effector involved in parasitism.

148

149 **2.2 | MiEFF12 modulates plant immune responses**

150 Transgenic *Arabidopsis thaliana* lines overexpressing *MiEFF12a* were generated. A large-scale
151 investigation of the possible effects of MiEFF12 secretion on root physiology *in planta* was performed
152 by sequencing the transcripts of wild-type (Col-0) and MiEFF12-expressing *Arabidopsis* (line #C3;
153 Figure S3) roots. The RNA-sequencing data analysis identified 4,080 differentially expressed genes
154 (DEGs) (adjusted *P*-value ≤ 0.01). By analyzing DEGs with a \log_2 FoldChange of expression ≥ 1 or ≤ -1 ,
155 we found that 1,103 genes were upregulated and 1,126 were downregulated in the roots of the MiEFF12-
156 expressing line relative to the wild type (Table S1). A gene ontology (GO) enrichment analysis with
157 AgriGO v2.0 showed that genes associated with the GO biological process ‘response to decreased
158 oxygen level’ were overrepresented among the DEGs upregulated in the MiEFF12-expressing line
159 ($P=4.27e-7$; Figure 4a; Table S2 and S3). Thirteen of the 19 genes associated with this GO term are also

160 upregulated in galls induced by *M. incognita* in *A. thaliana* (Table S3; Yamaguchi et al., 2017). Genes
161 involved in ‘defense response’ were overrepresented among the 1,126 DEGs downregulated in the
162 MiEFF12a-expressing line ($P=2.16e-6$; Figure 4b; Table S4 and S5). Most of the 103 genes associated
163 with this GO term and downregulated in MiEFF12-expressing *Arabidopsis* encoded nucleotide-binding
164 site (NBS) leucine-rich repeat (LRR) proteins, LRR-receptor kinase like the EF-TU receptor (EFR),
165 receptor-like proteins (RLPs) and PR proteins. These 103 genes included 48 known to be downregulated
166 in galls induced by *M. incognita* (Table S5; Yamaguchi et al., 2017).

167 Given the role of reactive oxygen species (ROS) in cellular signaling to initiate plant immune
168 responses, we assessed the ability of MiEFF12a to affect the ROS burst induced by the bacterial PAMP
169 flg22 responsible for triggering PTI (Lee *et al.*, 2020). We quantified H₂O₂ in a previously described
170 luminol-based assay (Zhao *et al.*, 2021), in agroinfiltrated *N. benthamiana* leaves with and without
171 *MiEFF12a* expression, after treatment with flg22 or mock treatment. Almost no H₂O₂ production was
172 detected in plant leaves expressing MiEFF12a, whereas an ROS burst was observed in the negative
173 control following treatment with flg22 (Figure 4c). Thus, MiEFF12a abolished the H₂O₂ production
174 associated with PTI.

175 We also investigated whether MiEFF12a could suppress the programmed cell death physiologically
176 resembling the HR triggered by the mouse pro-apoptotic protein BAX (Lacomme & Santa Cruz, 1999).
177 BAX constructs were introduced into *N. benthamiana* leaves by agroinfiltration 24 h after MiEFF12 or
178 the control construct. As observed for the control, no inhibition of BAX-induced apoptosis was observed
179 when a GFP-MiEFF12a fusion was expressed in the plant (Figure 4d and Figure S3). ETI assays were
180 also performed with the *Globodera pallida* GpRBP-1 protein and the potato Gpa2 resistance protein.
181 GpRBP-1 is recognized by Gpa2 when co-expressed in *N. benthamiana*, triggering an HR (Sacco *et al.*,
182 2009; Figure 4d). The induction of the Gpa2/GpRBP-1-mediated HR was suppressed by the co-
183 expression of MiEFF12a in *N. benthamiana* leaves (Figure 4e and Figure S3). MiEFF12a suppressed
184 the HR as efficiently as the *Globodera rostochiensis* effector GrCEP12 used as a control (Figure 4e;
185 Chronis *et al.*, 2013). These findings indicate a possible role for MiEFF12 in suppressing plant immunity
186 during plant-nematode interactions.

187

188 **2.3 | MiEFF12 targets the host-cell endoplasmic reticulum**

189 The subcellular localization of proteins can help to elucidate their function. We localized EFF12 in plant
190 cells by performing transient expression assays in *N. benthamiana* leaves. The coding sequence (CDS)
191 of *MiEFF12a* without the SP was transiently expressed as an N- or C-terminal fusion to GFP under
192 control of the CaMV p35S promoter. The GFP-MiEFF12a fusion was detected in the ER — visualized
193 as a reticulated network at the cell periphery and around the nucleus (Figure 5a). Intriguingly, the signal
194 for the MiEFF12a-GFP fusion was different, displaying localization within large perinuclear structures
195 (Figure 5b). We studied the localization of MiEFF12a further, using an RFP-ER marker (Nelson et al.,
196 2007). With this marker, both the GFP fusions were found to colocalize with the ER marker (Figure 5c
197 and 5d). The use of the RFP-ER marker confirmed that the MiEFF12a-GFP signal associated with
198 perinuclear structures was indeed associated with the ER, the distribution of which was disturbed by the
199 presence of the effector (Figure 5d). These findings indicate that MiEFF12 is an effector targeting the
200 ER, the structure and/or function of which it is capable of altering.

201

202 **2.4 | MiEFF12a interacts with ER-associated proteins**

203 We investigated the function of the MiEFF12 effector in manipulating host cell physiology in more
204 detail by performing a yeast two-hybrid (Y2H) screen to identify direct interactors in tomato. We used
205 MiEFF12a without its signal peptide as a bait, and a tomato root cDNA library from healthy and *M.*
206 *incognita*-infected roots as the prey (Hybrigenics service, France), as previously described (Zhao *et al.*,
207 2020; Mejias *et al.*, 2021). We screened 100 million interactions between MiEFF12 and the cDNA
208 library. We identified two major effector targets, the *Solanum lycopersicum* PLANT BAP-LIKE
209 PROTEINS (SIPBL1 and SIPBL2) and the BASIC REGION/LEUCINE ZIPPER MOTIF 60
210 (SIBZIP60), which were captured 12 and 7 times, respectively (Table S6; Figure S4). Other selected
211 clones carried putative targets captured five times or less (Table S6). Both PBL and BZIP60, known
212 ER-associated proteins involved in ERQC, the ERAD and/or UPR systems (Liu & Howell, 2016;
213 Atabekova *et al.*, 2017), were considered in subsequent analyses.

214 Proteins present in the same subcellular compartment are more likely to be true interactors than those
215 found in different compartments. We therefore performed agroinfiltration experiments to investigate the

216 subcellular distribution of SIPBL1 and SIBZIP60 in *N. benthamiana* epidermal leaf cells. In *S.*
217 *lycopersicum*, five genes — *SIPBL1* (*Solyc12g005910*), *SIPBL2* (*Solyc10g053910*), *SIPBL3*
218 (*Solyc09g059570*), *SIPBL4* (*Solyc02g032930*) and *SIPBL5* (*Solyc02g080870*) — encode PBL proteins
219 (Atabekova *et al.*, 2017), whereas SIBZIP60 is encoded by a single gene, *Solyc04g082890* (Kaur &
220 Kaitheri Kandoth, 2021) (Figure S5). The full-length CDS of *SIPBL1* and *SIBZIP60* were cloned to
221 generate fusion proteins with GFP. Coexpression experiments confirmed the colocalization of RFP-
222 MiEFF12 with GFP-SIPBL1 and GFP-BZIP60 in the ER (Figure 6a and 6b). Unlike RFP-MiEFF12 and
223 GFP-SIPBL1, GFP-SIBZIP60 was also observed in the nucleoplasm (Figure 6b). For the SIPBL1-GFP
224 fusion, fluorescence was observed in large perinuclear structures similar to those observed for the
225 MiEFF12a-GFP fusion, while SIBZIP60-GFP was mostly detected in the nucleoplasm (Figure S6).
226 These results confirm that SIPBL and SIBZIP60 are located in the ER and could interact with MiEFF12
227 *in planta*.

228 Co-immunoprecipitation assays (Co-IP) were then performed to validate these interactions. The full-
229 length SIPBL1 and its soluble fragment and the full length of SIBZIP60 were fused separately with GFP
230 to generate the SIPBL_{total}-GFP, SIPBL_{soluble}-GFP and GFP-SIBZIP60 constructs, respectively
231 (Figure 6c). These constructs were co-expressed together with MiEFF12a carrying an HA tag
232 (MiEFF12a-HA), in *N. benthamiana* leaves. The complete SIPBL1 and SIBZIP60 proteins were co-
233 immunoprecipitated with MiEFF12a (Figure 6d). Furthermore, because RKN calreticulin (CRT)
234 effectors also localize in the ER when expressed in plant cells (Jaouannet *et al.*, 2013; Liu *et al.*, 2024),
235 we used the *M. incognita* MiCRT1 effector as a negative control. MiCRT1 was unable to interact with
236 the full-length SIPBL1 or with its cytosolic fragment, nor with SIBZIP60 (Fig. 6d). These findings
237 demonstrate that MiEFF12a interacts with SIPBL1 and SIBZIP60 at the ER of the plant cell.

238

239 **2.5 | PBLs promote root-knot nematode parasitism**

240 We then investigated the possible role of PBL and BZIP60 proteins in the plant immune response and
241 RKN parasitism. A knockout *bzip60* Arabidopsis mutant line was challenged with *M. incognita*. Six
242 weeks after inoculation, we observed no significant effect of the *BZIP60* mutation on the number of
243 females producing egg masses (Figure S7a). Similarly, silencing the unique *NbBZIP60* gene in *N.*

244 *benthamiana* (*Niben101Scf24096g00018*) through a VIGS approach did not affect RKN development
245 or reproduction (Figure S8 and S7b). These results indicate that plant BZIP60 is not required for RKN
246 parasitism. We then silenced *PBL* genes in *N. benthamiana*, using the TRV for VIGS. Using the SGN
247 VIGS tool (Fernandez-Pozo *et al.*, 2015), we selected six genes encoding NbPBLs in *N. benthamiana*:
248 *NbPBL1a* (*Niben101Scf02543g02013*), *NbPBL1b* (*Niben101Scf08039g00007*), *NbPBL2a*
249 (*Niben101Scf00435g05003*), *NbPBL2b* (*Niben101Scf04477g03012*), *NbPBL3a*
250 (*Niben101Scf02516g00006*) and *NbPBL3b* (*Niben101Scf02145g09007*) (Figure S9). Using a Co-IP
251 approach, we could verify that MiEFF12a could indeed interact with NbPBL1a, NbPBL2a and
252 NbPBL3a (Figure S10). We then designed a chimeric TVR2 construct to silence all *NbPBL* genes. This
253 construct specifically targeted a 200-nucleotide region of each *NbPBL1a/b*, *NbPBL2a/b* and *NbPBL3a/b*
254 pair (Figure S11). A TRV2 targeting a GFP transcript was used as a negative control. The TRV1 and
255 TRV2 were introduced into three-week-old *N. benthamiana* plants with *A. tumefaciens*. Seven days after
256 inoculation (dai), root samples were harvested for RNA extraction, and the remaining plants were
257 inoculated with 200 *M. incognita* J2s (Figure 7a). RT-qPCR was performed to confirm that the targeted
258 *NbPBL* genes in the treated *N. benthamiana* plants were effectively silenced relative to the control
259 (Figure 7b). The plants were recovered six weeks post-infection with *M. incognita*. The plants displayed
260 no macroscopic developmental phenotype, and root weight was not altered by the silencing of *NbPBL*
261 genes (Figure S12). Roots were stained with eosin, and galls and egg masses were counted. In three
262 independent experiments, the numbers of galls and egg masses were found to be significantly smaller
263 in plants with silenced *PBL* genes than in controls (Figure 7c). To uncover PBLs involvement in
264 regulating plant response to RKN infection, we analyzed SIPBL1 function in suppressing plant defenses
265 as described earlier. SIPBL1 could suppress ROS burst triggered by *fgl22* in *N. benthamiana* (Figure
266 S13). These results suggest that RKNs hijack the function of the plant PBL proteins, negative regulators
267 of plant defense, to promote host susceptibility to *M. incognita*.

268

269 **3 | DISCUSSION**

270

271 **3.1 | MiEFF12 is an effector targeting the host ER and involved in defense suppression**

272 Numerous RKN effectors have been reported to suppress plant immunity (Vieira and Gleason; Rutter et
273 al., 2022). MiCRT, a calreticulin secreted into the host apoplast, was the first RKN effector shown to
274 suppress PTI-triggered callose deposition and the induction of PR genes (Jaouannet *et al.*, 2013). Several
275 PTI-suppressing effectors have since been reported, including *M. incognita* MiMIF-2, MiCTL1 and
276 MiPDCD6, and *M. graminicola* MgMO289 (Zhao *et al.*, 2020; Song *et al.*, 2021; Zhao *et al.*, 2021;
277 Kamaruzzaman *et al.*, 2022). RKN effectors have also been implicated in the suppression of ETI-type
278 cell death. The effectors involved include *M. enterolobii* MeTCTP, *M. javanica* Mj10A08 and Mimsp40,
279 which suppress the programmed cell death triggered by the pro-apoptotic Bcl-2 family protein BAX
280 (Hu *et al.*, 2022; Niu *et al.*, 2016; Zhuo *et al.*, 2017). The co-expression of a resistance gene with the
281 corresponding avirulence effector is often used to trigger an HR in such bioassays (Naalden *et al.*, 2018;
282 Nguyen *et al.*, 2018). Low levels of cell death via the HR are triggered by the recognition of *G. pallida*
283 RBP1 proteins by the potato Gpa2 (Carpentier *et al.*, 2013), which can be suppressed by some RKN
284 effectors, such as *M. graminicola* MgMO289 (Song *et al.*, 2021).

285 We describe here a conserved RKN effector, EFF12, which is produced in the dorsal gland. Despite
286 the absence of a known functional domain, MiEFF12 suppresses the ROS burst induced by the flg22
287 peptide and the HR triggered by co-expression of the GpRBP1 effector and the potato Gpa2 resistance
288 protein. However, like the HR-suppressing MiSGCR1 effector (Nguyen *et al.*, 2018), MiEFF12 was
289 unable to suppress the strong cell death response induced by BAX. A genome-scaled transcriptomic
290 analysis on an MiEFF12a-overexpressing *Arabidopsis* line further confirmed the function of EFF12 in
291 repressing defense-associated genes.

292 Transient expression experiments in *N. benthamiana* epidermal leaf cells demonstrated that
293 MiEFF12, while its not having an ER-retention signal, targets the ER. Several RKN effectors, such as
294 MiASP2 and 6D4 (Vieira *et al.*, 2011), have been reported to target the host apoplast, whereas others,
295 such as MiPDI1 and MiCTL-1 are secreted into the cytoplasm (Zhao *et al.*, 2020 and 2021), or target
296 the nucleus, as reported for MiEFF1 and MiEFF18 (Truong *et al.*, 2021; Mejias *et al.*, 2021). Only a few
297 have been reported to target the plant endomembrane system. Several pathogens are known to target the
298 ER to ensure the successful infestation of plants (Jing & Wang, 2020) and MiEFF12 is the third RKN
299 effector demonstrated to target this endomembrane compartment. Kumar *et al.* (2023) recently described

300 the *M. javanica* MjShKT effector, which localizes to the ER and Golgi compartments when transiently
301 expressed in *N. benthamiana* epidermal leaf cells. However, the host cell targets of MjShKT and the
302 mechanism of its suppression of plant cell death have not yet been identified. MgCRT1 is another RKN
303 effector, secreted into giant cells, that localize in the ER during parasitism (Liu et al., 2024). MgCRT1
304 was shown to interact with an ER-localised rice calmodulin-like protein OsCML31 to regulate rice
305 immunity and promote susceptibility to *M. graminicola*.

306

307 **3.2 | PBL is a new host-cell player in RKN parasitism**

308 We identified BZIP60 and PBLs, which are known to be resident in the ER, as the host-cell targets of
309 MiEFF12, consistent with the subcellular distribution reported here for this effector. The full length
310 BZIP60 is anchored in the ER membrane, where its function remains unknown. But when ER stress is
311 elevated, the stress sensor and activator INOSITOL-REQUIRING ENZYME 1 (IRE1) is splicing
312 BZIP60 mRNA, resulting in the production of a transcription factor that can be translocated in the
313 nucleus allowing the UPR to restore ER homeostasis. Roles for IRE1 and BZIP60 in plant immunity
314 have been documented (Bao & Howell, 2017; Jing & Wang, 2020). Our results indicate that suppressing
315 BZIP60 function does not alter plants susceptibility to RKN. The role of other UPR branches, having
316 overlapping functions with IRE1/BZIP60 pathway, in this biotrophic interaction remains, however, to
317 be investigated. B-CELL RECEPTOR-ASSOCIATED PROTEIN 31 (BAP31) is a conserved integral
318 ER-associated protein with three transmembrane domains in its N-terminal region and a C-terminal
319 cytosolic tail (Quistgaard, 2021). In animals, BAP31 has been described as an important chaperone or
320 quality control factor involved in ER membrane protein sorting, promoting protein retention in the ER,
321 transport from the ER to the downstream secretory pathway or other cellular compartments, or targeting
322 to the ERAD system for degradation (Quistgaard, 2021; Wakana et al., 2008). It is also cleaved by
323 caspase and plays a role in apoptosis (Breckenridge et al., 2003; Quistgaard, 2021). The equivalent
324 proteins to BAP31 in plants are probably BAP-like proteins (PBLs) and are encoded by multigene
325 families (Atabekova et al., 2017). The functions of PBLs remain largely undescribed. A PBL from *N.*
326 *tabacum*, NtPBL, was shown to interact with the tobacco protein Nt-4/1, a protein known to interact
327 with plant virus movement proteins and to affect the long-distance transport of potato spindle tuber

328 viroid (PSTVd) via the phloem (Pankratenko et al., 2017). Interestingly, NtPBL was shown to induce
329 the relocalization of Nt-4/1 to ER bodies and large aggregates with a granular structure (Pankratenko et
330 al., 2017) similar to those we observed when overexpressing MiEFF12a- or SIPBL1-GFP fusions. In
331 animal cells, the re-localization of BAP31 under stress conditions to a juxta-nuclear ER compartment
332 involved in the ER-associated degradation of misfolded proteins is well documented (Quistgaard, 2021).
333 In addition, the cytosolic tail of NtPBL has been shown to bind RNA (pre-miRNA and viroid RNA),
334 and the expression of *NtPBL* in *N. benthamiana* via a TRV-based approach strongly affects plant
335 development and/or the symptoms induced by TRV (Atabekova et al., 2017). We show here that
336 NbPBLs play a role in RKN parasitism, as plants in which *NbPBL* genes were silenced were less
337 susceptible to *M. incognita*. Furthermore, our finding indicates that PBLs negatively regulate host
338 immunity.

339

340 **3.3 | MiEFF12 targets plant PBLs to suppress ER-mediated immunity**

341 The ER plays an important role in plant immunity. PRRs are synthesized in the ER and are subject to
342 ER quality control to ensure that they are transported in the correctly folded form from the ER to the
343 plasma membrane (Park & Seo, 2015). As an example, actors of the ER quality control were shown to
344 be involved in the EF-Tu receptor (EFR) biogenesis (Li et al., 2009; Nekrasov et al., 2009). Similarly,
345 antimicrobial PR proteins secreted following recognition of the infesting pathogens are produced and
346 folded at the ER and then processed by the Golgi apparatus for delivery to the apoplast. As a result, the
347 ER is frequently targeted by pathogens and several ER-associated proteins have been implicated in plant
348 responses to pathogen infections (Jing & Wang, 2020). Given the role of BAP31 as a chaperone
349 controlling the fate of the protein — retention in the ER, export, degradation by the ERAD or escape
350 from degradation (Quistgaard, 2021) — we suggest that these functions are conserved in the PBLs of
351 plants and are hijacked by MiEFF12 to prevent the initiation or full expression of PTI. We have shown
352 that MiEFF12a can suppress HR-cell death, possibly through its interaction with SIPBLs. In plants, the
353 ER, or more specifically ER stress, is a recognized initiator of programmed cell death (Eichmann &
354 Schäfer, 2012; Simoni *et al.*, 2022). The role of PBLs in modulating these pathways remains to be
355 elucidated, but the induction of plant cell death programs must be prevented if biotrophic interactions

356 are to be initiated and maintained throughout the nematode life cycle. Several molecules have already
357 been identified as involved in both PTI and ETI, and PBLs may play such a role at the interaction of the
358 PTI and ETI signaling pathways.

359 Further analysis would be required to define more precisely the functions of PBLs in regulating plant
360 immunity and the ways in which the effector affects them. This study has taken the first step in this
361 process by revealing a new pathogenic strategy used by RKN to hijack plant metabolism. In this strategy,
362 the MiEFF12 effector targets PBL corrupting their functions so as to promote RKN parasitism. PBL-
363 encoding genes, thus, emerge as promising susceptibility genes that could be targeted in innovative
364 breeding strategies with the aim of generating RKN-resistant crops.

365

366 **4 | EXPERIMENTAL PROCEDURES**

367

368 **4.1 | Nematode and plant materials**

369 *Meloidogyne incognita* strain “Morelos” was multiplied in tomato (*S. lycopersicum* cv. “Saint Pierre”)
370 grown in a growth chamber (25°C, with a 16 h photoperiod). Freshly hatched J2s were collected as
371 described by Caillaud & Favery (2020). The previously described *bzip60* mutant (SALK_050203; Lu
372 & Christopher, 2008) was obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio
373 State University, Columbus, OH, USA). For VIGS experiments, *N. benthamiana* seeds were sown on
374 soil and incubated at 4°C for two days. After germination, plantlets were transplanted into pots
375 containing soil and sand (1:1) and were grown at 24°C (photoperiod, 16 h light: 8 h dark). For
376 transcriptome analysis, *Arabidopsis* seeds were surface-sterilized and added to liquid MS medium (0.5
377 x MS salts, 1% sucrose, pH 6.4). They were incubated at 25°C, under a 12-hour period, with gentle
378 shaking (70 rpm), as previously described (Mejias *et al.*, 2021).

379

380 **4.2 | EFF12 sequence analysis, alignment, and phylogenetic tree**

381 The sequences of putative EFF12 paralogs and orthologs were obtained from *Meloidogyne* genomic
382 resources (http://www6.inra.fr/meloidogyne_incognita and Wormbase parasite). We used 15
383 *Meloidogyne* EFF12 sequences from *M. arenaria*, *M. enterolobii*, *M. incognita*, *M. floridensis*, and *M.*

384 *javanica* in this analysis. The sequences of the proteins encoded by these genes were analyzed with
385 PHOBIUS and Prosite to identify the SP and to search for putative functional domains, respectively
386 (<http://phobius.sbc.su.se/>; <https://prosite.expasy.org/>). EFF12 sequences were aligned with the ClustalW
387 algorithm (Thompson *et al.*, 2003) and their evolutionary history was inferred by maximum likelihood
388 methods, as previously described (Berger *et al.*, 2020). *Meloidogyne hapla* was used as the outgroup for
389 the phylogenetic tree based on putative orthologs of EFF12.

390

391 **4.3 | *In situ* hybridization (ISH)**

392 ISH was performed on freshly hatched *M. incognita* and *M. enterolobii* J2s, as previously described
393 (Jaouannet *et al.*, 2018). For probe production, the MiEFF12a, and MeEFF12 sequences were amplified
394 specifically from entry vectors with the primers MiEFF12_GW3 and MiEFF12_GW5 (for MiEFF12a)
395 or MeEFF12_GW3 and MeEFF12_GW5 (for MeEFF12) (Table S7). Sense probes for MiEFF12a and
396 MeEFF12 were used as negative controls. Photomicrographs were obtained with a Zeiss Axioplan2
397 microscope (Zeiss, Germany).

398

399 **4.4 | RKN infection assay, juveniles in the plant**

400 Three-week-old *Arabidopsis* seedlings were inoculated with 200 *M. incognita* J2s per plant. Roots were
401 collected six weeks after infection and stained with 0.5% eosin. The number of females forming egg
402 masses was then determined. *N. benthamiana* plants subjected to HIGS/VIGS were inoculated with 200
403 *M. incognita* J2s per plant, seven dpi with TRV, and incubated at 24°C. Infected *N. benthamiana* roots
404 were collected six weeks after infection. Galls or egg masses were counted under a binocular
405 microscope, and the root system was weighted.

406

407 **4.5 | Subcellular distribution in the plant**

408 The *M. incognita* MiEFF12 CDS lacking the signal peptide, the *S. lycopersicum* PBL (total and soluble
409 portion) and BZIP60 (unspliced form) were amplified by PCR with specific primers (Table S7) and
410 inserted into the pDON207 donor vector. They were recombined in pK2GW7 (P35S:MiEFF12),

411 pK7WGR2 (P35S:mRFP-MiEFF12a), pK7FGW2 (P35S:eGFP-SIPBL, P35S:eGFP-SIBZIP60 and
412 P35S:eGFP-MiEFF12a), or pK7FWG2 (P35S:SIPBL-eGFP, P35S:SIBZIP60-eGFP and P35S:
413 MiEFF12a-eGFP) (Karimi *et al.*, 2002) with Gateway technology (Invitrogen). All the constructs were
414 sequenced (GATC Biotech) and transferred into *A. tumefaciens* strain GV3101. Leaves from three- to
415 four-week-old *N. benthamiana* plants were subjected to agroinfiltration with recombinant strains of *A.*
416 *tumefaciens* containing GFP or RFP vectors, as described by Mejias *et al.* (2021). Leaves were imaged
417 48 hours after agroinfiltration, with an inverted confocal microscope (LSM 880; Zeiss) equipped with
418 an argon ion laser as the excitation source. Samples were excited at 488 nm for GFP and 543 nm for
419 RFP. GFP and RFP emissions were detected selectively with 505–530 nm and 560–615 nm band-pass
420 emission filters, respectively.

421

422 **4.6 | Yeast two-hybrid screen**

423 For the Y2H screen, the CDS of the MiEFF12 without the SP was used as a bait. The MiEFF12a
424 sequence was amplified (Table S2) and inserted into the pB27 vector (Hybrigenics Services, Paris,
425 France). The Y2H screen was performed with an infested tomato root cDNA library (Hybrigenics
426 Services, Paris, France), as previously described (Mejias *et al.*, 2021).

427

428 **4.7 | Host-induced silencing of the MiEFF12a gene and virus-induced silencing of PBL and BZIP60** 429 **genes**

430 VIGS/HIGS assays were performed on *N. benthamiana*. For HIGS experiments, fragments of
431 *MiEFF12a* (291 bp) and *GFP* (298 bp) were amplified by PCR with the primers listed in Table S7 and
432 inserted into the TRV2 vector. HIGS experiments were performed as described by Zhao *et al.* (2021),
433 and RT-qPCR were performed to validate the silencing of the MiEFF12 genes 10 dai with the TRV. We
434 used the VIGS-Tool (<https://vigs.solgenomics.net/>) to design the best sequence for silencing *BZIP60*
435 and *PBL* genes in *N. benthamiana* (Figure S7 and S9). A 300 nucleotides fragment of *NbBZIP60* was
436 PCR-amplified using specific primers (Table S7) before ligation into the TVR2 plasmid. The nucleotide
437 sequences for the following pairs of genes — *NbPBL1a/NbPBL1b*, *NbPBL2a/NbPBL2b*,
438 *NbPBL3a/NbPBL3b* — were sufficiently similar for the design of a chimeric construct targeting 200

439 nucleotides for each pair synthesized with pUC57 (Gene Universal Inc, Newark DE, USA). The insert
440 was then inserted into the TRV2 plasmid (Figure S9) and the resulting construct was used to transform
441 *A. tumefaciens* strain GV3101. VIGS experiments were performed as previously described (Mejias et
442 al., 2022). Each treatment involved at least 20 *N. benthamiana* plants, and the entire experiment was
443 performed at least twice.

444

445 **4.8 | Cell death and PTI suppression assay in *N. benthamiana***

446 The CDS of MiEFF12 and GFP were amplified and inserted into the super1300 vector. The resulting
447 constructs were then used to transform *A. tumefaciens* GV3101. *Agrobacterium* cells carrying BAX or
448 GpRBP1/Gpa2 were used to trigger cell death in *N. benthamiana* leaves (Sacco *et al.*, 2009; Jing *et al.*,
449 2016). GrCEP12 was used as a positive control to suppress GpRBP1/Gpa2-induced cell death (Chronis
450 *et al.*, 2013). Agro-infiltrations into four-week-old *N. benthamiana* leaves were performed as described
451 elsewhere (Nguyen *et al.*, 2018). Photographs were taken 5 dpi for the assessment of cell death.

452 For the ROS assays the CDS of MiEFF12 without its native SP and SIPBL1 were inserted into the
453 super 1300 vector. Four-weeks old *N. benthamiana* leaves were infiltrated with *A. tumefaciens* carrying
454 the plasmid, and buffer was used for control. Two days after infiltration, leaf discs were collected and
455 prepared for the ROS assay (luminol-based method) as previously described (Chen *et al.*, 2013).

456

457 **4.9 | Quantitative RT-PCR**

458 Total RNA was extracted with the RNeasy® Mini kit (Qiagen), and cDNA was synthesized with the
459 SuperScript III First Strand Synthesis system (Invitrogen, Thermo Fisher Scientific, Waltham, MA,
460 USA) according to the manufacturer's instructions. At least three separate biological replicates were
461 performed for each experiment. PCR was performed with Maxima SYBR Green qPCR Master Mix
462 (29; Fermentas, Thermo Fisher Scientific, Waltham, MA, USA) on an I-Cycler (Bio-Rad, CA, USA)
463 with gene-specific primers (Table S1). Quantifications and statistical analyses were performed with
464 SATqPCR (Rancurel *et al.*, 2019).

465

466 **4.10 | Co-immunoprecipitation assays**

467 For the Co-IP assay, the CDS of MiCRT and MiEFF12a (without the signal peptide sequence) or
468 SIPBL1total, SIPBL1soluble, NbPBL1a, NbPBL2a and NbPBL3a were inserted into the super1300
469 vectors with a HA-tag and a GFP-tag, respectively, fused to the C-terminal end of the sequence, and
470 SIBZIP60 was cloned into pBIN vector with a GFP-tag fused to the N-terminal of the sequence. Total
471 protein was extracted from four-week-old *N. benthamiana* leaves co-expressing SIPBL1 or SIBZIP60
472 and MiEFF12a or MiCRT, after 48 h of infiltration. Co-IPs were performed with BeyoMag™ anti-HA
473 Magnetic Beads (Beyotime, China), and the eluted proteins were identified by western blotting with
474 anti-GFP (Beyotime, China) and anti-HA (Beyotime, China) antibodies as described by Zhao *et al.*
475 (2023).

476

477 **4.11 | Transcriptome analysis**

478 For RNA-seq experiments, seeds were surface-sterilized and sown in liquid MS medium (½MS salts,
479 1% sucrose, pH 6.4) with gentle shaking (70 rpm), under a 12 h : 12 h, light : dark photoperiod, at 25°C.
480 Roots were collected after 11 days and immediately frozen in liquid nitrogen. Total RNA was extracted
481 with TriZol (Invitrogen), according to the Invitrogen protocol. The RNA was treated with DNase
482 (Ambion), and its quality and integrity were assessed with a Bioanalyzer (Agilent). Library construction,
483 paired-end sequencing and data analysis were performed as described by Mejias *et al.* (2021). Gene
484 ontology enrichment analysis was performed with the agriGO v2.0 toolkit
485 (<http://systemsbiology.cau.edu.cn/agriGOv2/>; Tian *et al.*, 2017), using default parameters.

486

487 **4.12 | Statistical analysis**

488 Graphs and plots were created with R and Microsoft® Office Excel® 2019. Statistical analyses were
489 performed with R software (R Development Core Team, version 4.1.0). and SATqPCR
490 (<https://satqpcr.sophia.inrae.fr/cgi/home.cgi>) (Rancurel *et al.*, 2019).

491

492 **4.13 | Accession numbers**

493 Sequence data from this article can be found in Solgenomics (<https://solgenomics.net/>) and
494 GenBank/EMBL databases under the following accession numbers: *Meloidogyne incognita*

495 MiEFF12a/Minc12754 (KX907763), MiCRT/Minc06693 (AF402771.1); *Arabidopsis thaliana*
496 AtBZIP60 (AT1G42990); *Nicotiana benthamiana* NbBZIP60 (Niben101Scf24096g00018), NbPBL1a
497 (Niben101Scf02543g02013), NbPBL1b (Niben101Scf08039g00007), NbPBL2a
498 (Niben101Scf00435g05003), NbPBL2b (Niben101Scf04477g03012), NbPBL3a
499 (Niben101Scf02516g00006), NbPBL3b (Niben101Scf02145g09007); *Solanum lycopersicum*
500 SIBZIP60 (Solyc04g082890), SIPBL1 (Solyc12g005910), SIPBL2 (Solyc10g053910), SIPBL3
501 (Solyc09g059570), SIPBL4 (Solyc02g032930), SIPBL5 (Solyc02g080870). The transcriptome data are
502 available from the Sequence Read Archive (SRA) under accession number PRJNA641665 and
503 PRJNA719908 (*A. thaliana* Col-0 and P35S:MiEFF12 roots, respectively).

504

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520

521 **AUTHOR CONTRIBUTIONS**

522 S.S., J.Z., B.F. and M.Q. designed the experiments. S.S., K.H., K.M., J.M., J.B, T.N.M., J.L.K. and
523 M.Q. performed the experiments. S.S., J.B., S.J., P.A., J.Z., B.F. and M.Q. analyzed the data. S.J., P.A.,
524 J.Z., B.F. and M.Q. obtained funding, and supervised experiments and data analysis. SS., P.A., B.F.
525 and M.Q. wrote the manuscript. All the authors read and edited the final manuscript.

526

527 **CONFLICT OF INTEREST STATEMENT**

528 The authors have no conflict of interest to declare.

529

530 **DATA AVAILABILITY STATEMENT**

531 The data supporting the findings of this study are available from the corresponding author upon
532 reasonable request.

533

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733

734 **SUPPORTING INFORMATION**

735 **Table S1.** Differentially expressed genes identified in the MiEFF12-expressing *Arabidopsis* line.

736 **Table S2.** Gene ontology (GO) analyses of the 1,103 genes upregulated in the MiEFF12a-expressing
737 line with a $\log_2fd \geq 1$.

738 **Table S3.** List of 19 genes upregulated in the EFF12-expressing line and associated with GO term '
739 GO:0036293 response to decreased oxygen levels'.

740 **Table S4.** Gene ontology (GO) analyses of the 1,126 genes downregulated in the MiEFF12a-expressing
741 line with $\log_2fd \leq -1$.

742 **Table S5.** List of 103 genes downregulated in the EFF12-expressing line and associated with GO term
743 'GO:0006952 defense response'.

744 **Table S6.** Results of the yeast two-hybrid screen using MiEFF12a as a bait against the tomato root
745 cDNA library.

746 **Table S7.** Primers used in this study.

747 **Figure S1.** Amino acid sequences of EFF12 effector proteins identified in RKN species.

748 **Figure S2.** Nucleotide sequences of *EFF12*-encoding genes identified in RKN species.

749 **Figure S3.** Ectopic expression of MiEFF12a in *Arabidopsis thaliana* does not affect root development.

750 **Figure S4.** MiEFF12a interacts with SIPBL1, SIPBL2 and SIBZIP60 in yeast.

751 **Figure S5.** Nucleotide sequences of SIPBL1 and SIBZIP60

752 **Figure S6.** MiEFF12a and SIPBL1 colocalize in *N. benthamiana* epidermal leaf cells and both
753 MiEFF12a- and SIPBL1-GFP fusions were localized in large subcellular structures mostly juxtannuclear.

754 **Figure S7.** Nucleotide sequences of *N. benthamiana* BZIP60 and design of the VIGS construct.

755 **Figure S8.** Plant BZIP60 is not required for *M. incognita* parasitism.

756 **Figure S9.** Nucleotide sequence of *N. benthamiana* PBL genes targeted by the VIGS approach.

757 **Figure S10.** MiEFF12a physically interacts in planta with NbPBL1a, NbPBL2a and NbPBL3a.

758 **Figure S11.** A chimeric sequence was introduced into the TRV2 RNA to silence the NbPBL genes
759 through VIGS.

760 **Figure S12.** The silencing of *PBL* genes by VIGS does not affect *N. benthamiana* root development.

761 **Figure S13.** MiEFF12a and SIPBL1a suppress flg22-mediated reactive oxygen species (ROS)
762 production in *Nicotiana benthamiana*.

763

764 **FIGURE LEGENDS**

765

766 **Figure 1.** Effector 12 (EFF12) is a conserved effector in root-knot nematodes. (a) Alignment
767 of the MiEFF12 protein sequences. The green box indicates the position of the signal peptide
768 for secretion. The conserved C-terminal region is enriched in positively (lysine-K) and
769 negatively (asparagine-D and glutamic acid-E) charged residues. (b) Phylogenetic tree of
770 *Meloidogyne* spp. EFF12 amino-acid sequences. The percentages displayed next to each branch
771 represent the number of tree replicates in which the associated taxa were assembled together in
772 1,000 bootstraps. The lengths of the branches are not proportional to phylogenetic distance. (c)
773 Pairwise sequence identity matrix for RKN *EFF12* nucleotide sequences.

774

775 **Figure 2.** The *Meloidogyne* *EFF12* genes are specifically expressed in the dorsal esophageal
776 gland. *In situ* hybridization with specific antisense probes localized *EFF12* transcripts
777 exclusively in the dorsal gland cell of pre-parasitic juveniles of *M. incognita* and *M. enterolobii*.
778 Sense probes for *MiEFF12* and *MeEFF12* transcripts were used as a negative control and gave
779 no signal. DG, dorsal gland. Bars = 20 μ m.

780

781 **Figure 3.** The silencing of *MiEFF12* genes by host-induced gene silencing affects *M. incognita*
782 parasitism. (a) Transcript quantification by RT-qPCR confirmed the effective silencing of
783 *MiEFF12* genes in parasitic nematodes extracted from *N. benthamiana* roots infected with
784 TRV2-MiEFF12 relative to controls (TRV2-empty and TRV2-GFP). Normalized relative
785 transcript levels for three independent biological replicates are shown. (b) Infection test on *N.*
786 *benthamiana* control plants (TRV2-empty and TRV2-GFP) and plants producing siRNA for
787 the silencing of *MiEFF12* genes in *M. incognita* (TRV2-MiEFF12). Galls were counted six
788 weeks after inoculation with 200 *M. incognita* second-stage juveniles per plant. Results from
789 two independent experiments are shown (n=15 and n=17 plants for test N°1 and N°2,
790 respectively). The cross represents average value. Box indicates interquartile range (25th to the

791 75th percentile). The central line within the box represents mean value. Whiskers indicate the
792 minimum and maximum values for the normal values present in the dataset. Statistical
793 significance was assessed in Student's *t* tests. Significant differences were observed between
794 controls and TRV-MiEFF12 plants (* $p < 0.05$).

795

796 **Figure 4.** MiEFF12 suppress host defense responses. (a-b) Gene ontology (GO) enrichment
797 analysis of DEGs in the *Arabidopsis* MiEFF12a-expressing line with AgriGO v2.0. (a) GO
798 enrichment analysis for the 1,103 genes upregulated in the MiEFF12a-expressing line with
799 $\log_2fd \geq 1$, indicating an enrichment in genes related to the response to decreased oxygen levels.
800 (b) GO enrichment analysis on the 1,126 genes downregulated in the MiEFF12a-expressing
801 line with $\log_2fd \leq -1$, indicating an enrichment in genes related to the defense response. (c)
802 MiEFF12 suppresses flg22-mediated reactive oxygen species (ROS) production in *Nicotiana*
803 *benthamiana*. *Agrobacterium tumefaciens* strain GV3101 carrying MiEFF12a was used to
804 infiltrate the leaves of *N. benthamiana* plants. Infiltrated leaf discs were collected 48 h post-
805 agroinfiltration and assayed for ROS production in response to treatment with the flg22 elicitor.
806 ROS production was monitored for 40 min, and the values shown are the mean relative
807 luminescence units \pm SD for 28 leaf discs. (d) BAX-triggered cell death was not suppressed by
808 MiEFF12a. Photographs for assessment of the cell-death phenotype were taken five days after
809 the last infiltration. (e) Gpa2/RBP-1-triggered cell death was suppressed by MiEFF12a.
810 Photographs showing the suppression of cell death were taken five days after the last
811 infiltration. Each cell death suppression bioassay was performed at least three times; results
812 from a representative experiment are shown.

813

814 **Figure 5.** MiEFF12a was localized to the endoplasmic reticulum (ER) of epidermal *Nicotiana*
815 *benthamiana* leaf cells. (a) Single-plane confocal images of *N. benthamiana* leaf cells infiltrated

816 with *Agrobacterium tumefaciens* and producing MiEFF12a without its signal peptide, fused to
817 the C-terminal end of the green fluorescent protein (GFP) reporter (GFP-MiEFF12a; green
818 signal; left pictures). Overlays of differential interference contrast and fluorescence images are
819 shown (right pictures). (b) Single-plane confocal images of *N. benthamiana* leaf cells infiltrated
820 with *A. tumefaciens* and producing MiEFF12a without the signal peptide, fused to the N-
821 terminal end of the green fluorescent protein (GFP) reporter (MiEFF12a-GFP; green signal; left
822 pictures). Overlays of differential interference contrast and fluorescence images are shown
823 (right pictures). (c) The monomeric red fluorescent protein (mRFP) signal (RFP-ER; red signal)
824 of an ER marker was used to analyze colocalization of the GFP-MiEFF12a fusion (green signal)
825 and the ER. (d) The mRFP signal (red signal) of the ER marker was used to analyze its co-
826 localization with the MiEFF12a-GFP fusion (green signal). Both fusions between the GFP and
827 MiEFF12a colocalized with the ER marker in *N. benthamiana* leaf cells. Asterisk; nucleus.
828 Scale bars: 20 μm .

829

830 **Figure 6.** MiEFF12a physically interacts *in planta* with SIPBL1 and SIBZIP60. SIPBL1 and
831 SIBZIP60 co-localized localized with MiEFF12a in the endoplasmic reticulum (ER) of
832 epidermal *Nicotiana benthamiana* leaf cells. (a) Single-plane confocal images of *N.*
833 *benthamiana* leaf cells infiltrated with *Agrobacterium tumefaciens* and producing SIPBL1,
834 fused to the C-terminal end of the green fluorescent protein (GFP) reporter (GFP-SIPBL1; green
835 signal) and MiEFF12a fused to the c-terminal end of the red fluorescent protein (RFP) reporter
836 (RFP-MiEFF12a; red signal). (b) Single-plane confocal images of *N. benthamiana* leaf cells
837 infiltrated with *Agrobacterium tumefaciens* and producing SIBZIP60, fused to the C-terminal
838 end of the green fluorescent protein (GFP) reporter (GFP-SIBZIP60; green signal) and the RFP-
839 MiEFF12a recombinant protein (RFP-MiEFF12a: red signal). Overlays of fluorescence images
840 are shown (merge). Scale bars: 20 μm . (c) Schematic representation of the full-length (tot, total)

841 and truncated (sol, soluble) SIPBL1 and SIBZIP60 proteins used for interactomic assays. (d)
842 Co-immunoprecipitation experiments confirmed that MiEFF12a interacted with the full-length
843 SIPBL1 and BZIP60. SIPBL_{tot}-GFP, SIPBL_{sol}-GFP or SIBZIP60 were transiently co-
844 expressed with MiEFF12a-HA or MiCRT in tobacco leaves. The Co-IP experiment was
845 performed with anti-HA affinity gel, and the protein isolated was analyzed by immunoblotting
846 with anti-GFP antibodies to detect SIPBL_{tot} and SIPBL_{sol}, and with anti-HA antibodies to
847 detect MiEFF12a and MiCRT. Three independent experiments were performed, with similar
848 results.

849

850 **Figure 7.** The silencing of *PBL* genes in *N. benthamiana* affects susceptibility to *M. incognita*.

851 (a) Timeline used for virus-induced gene silencing (VIGS) experiments. (b) RT-qPCR showing
852 the efficient silencing of the *NbPBL1a/b*, *NbPBL2a/b* and *NbPBL3a/b* gene pairs in *N.*
853 *benthamiana* control plants (TRV-GFP) and plants in which *NbPBLs* were silenced (TRV2-
854 PBLs). The data shown are normalized relative transcript levels for three independent biological
855 replicates obtained with SatqPCR software. The *NbEF1a* and *NbGADPH* housekeeping genes
856 were used for data normalization. Error bars indicate the SEM. (c) Infection test on *N.*
857 *benthamiana* TRV-GFP or TRV2-PBLs plants. Galls were counted six weeks after inoculation
858 with 200 *M. incognita* second-stage juveniles (J2s) per plant. Results from three independent
859 experiments are shown (n=19, n=15 and n= 21 plants for test N°1, N°2 and N°3, respectively).
860 The cross represents average value. Box indicates interquartile range (25th to the 75th
861 percentile). The central line within the box represents mean value. Whiskers indicate the
862 minimum and maximum values for the normal values present in the dataset. Statistical
863 significance was determined in Student's *t* test and significant differences were observed
864 between TRV-GFP control and TRV-PBL plants (* $p < 0.01$).

865

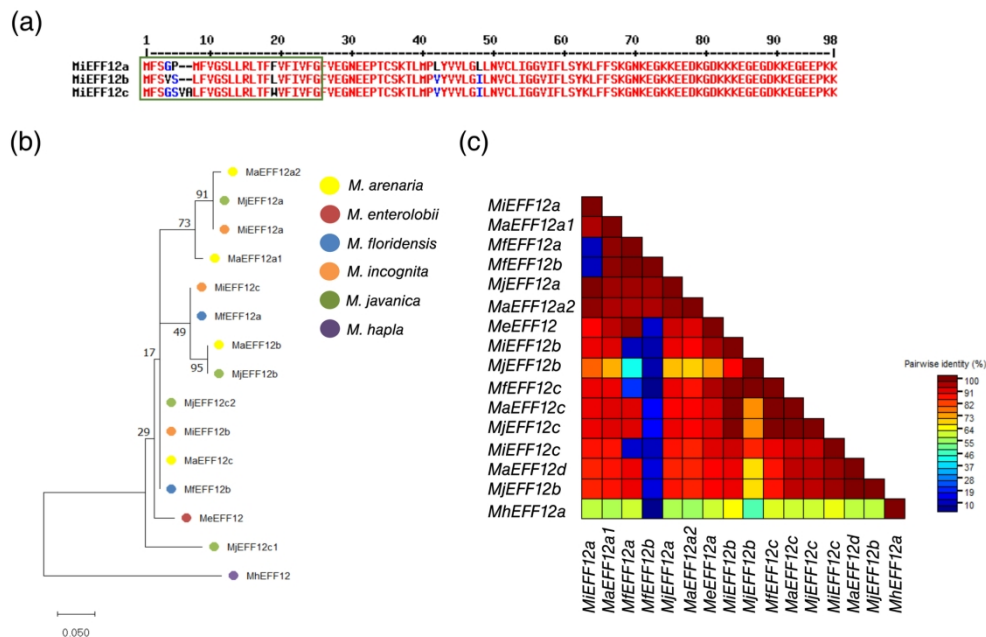


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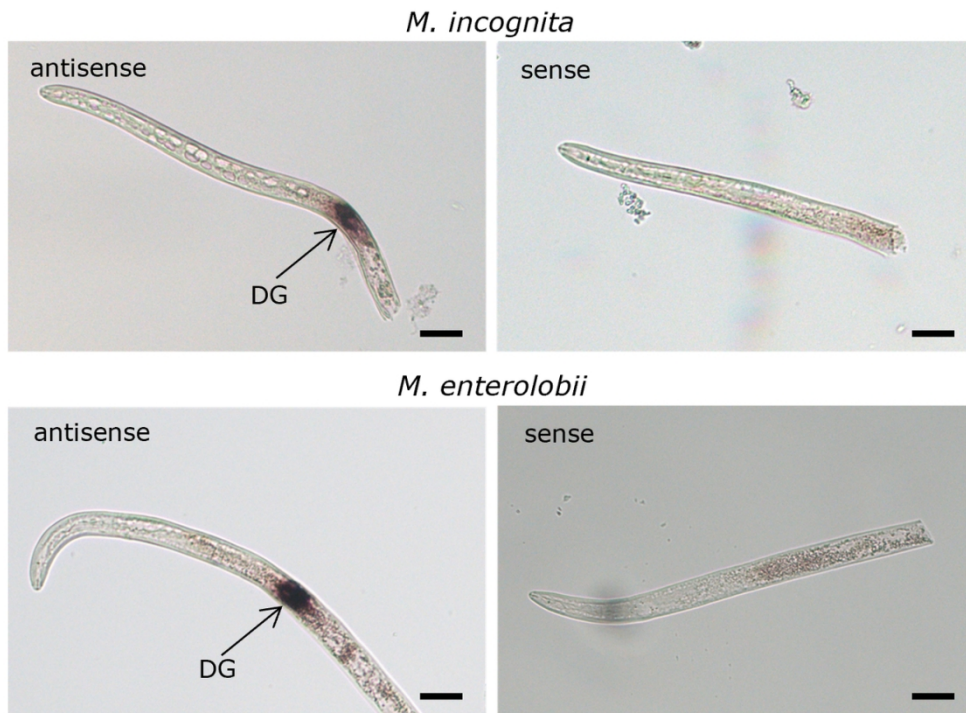


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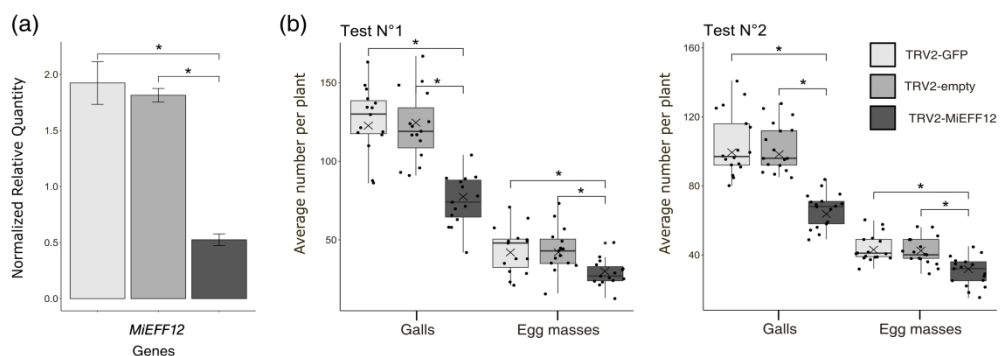


Figure 3. The silencing of MiEFF12 genes by host-induced gene silencing affects *M. incognita* parasitism. (a) Transcript quantification by RT-qPCR confirmed the effective silencing of MiEFF12 genes in parasitic nematodes extracted from *N. benthamiana* roots infected with TRV2-MiEFF12 relative to controls (TRV2-empty and TRV2-GFP). Normalized relative transcript levels for three independent biological replicates are shown. (b) Infection test on *N. benthamiana* control plants (TRV2-empty and TRV2-GFP) and plants producing siRNA for the silencing of MiEFF12 genes in *M. incognita* (TRV2-MiEFF12). Galls were counted six weeks after inoculation with 200 *M. incognita* second-stage juveniles per plant. Results from two independent experiments are shown ($n=15$ and $n=17$ plants for test N°1 and N°2, respectively). The cross represents average value. Box indicates interquartile range (25th to the 75th percentile). The central line within the box represents mean value. Whiskers indicate the minimum and maximum values for the normal values present in the dataset. Statistical significance was assessed in Student's t tests. Significant differences were observed between controls and TRV-MiEFF12 plants (* $p < 0.05$).

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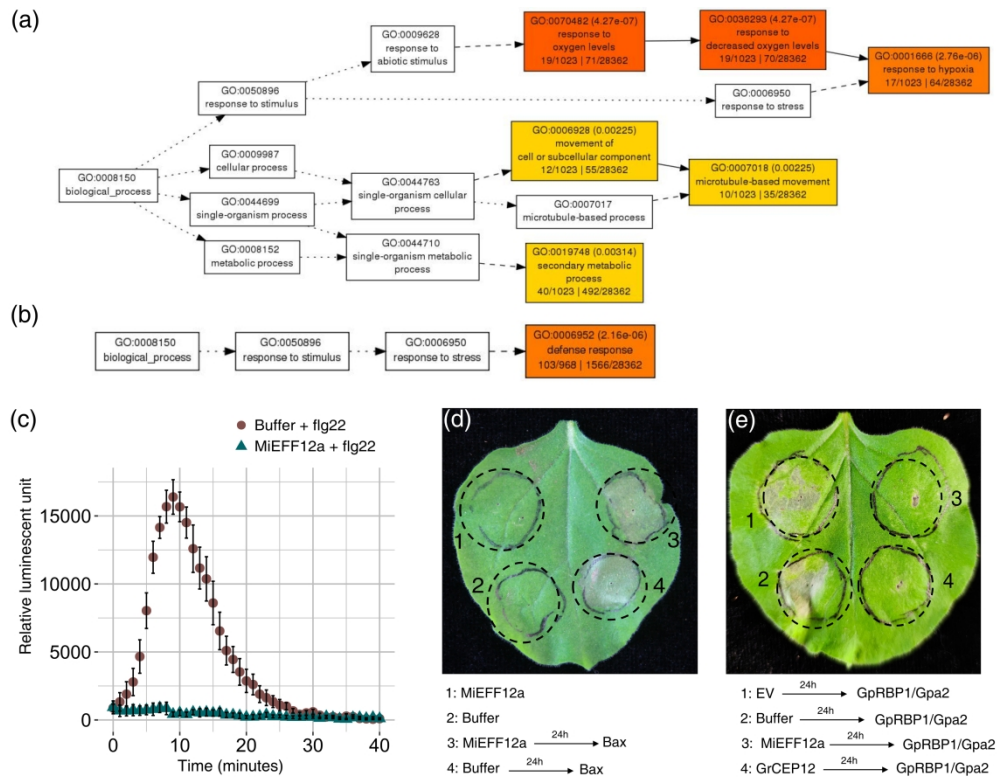


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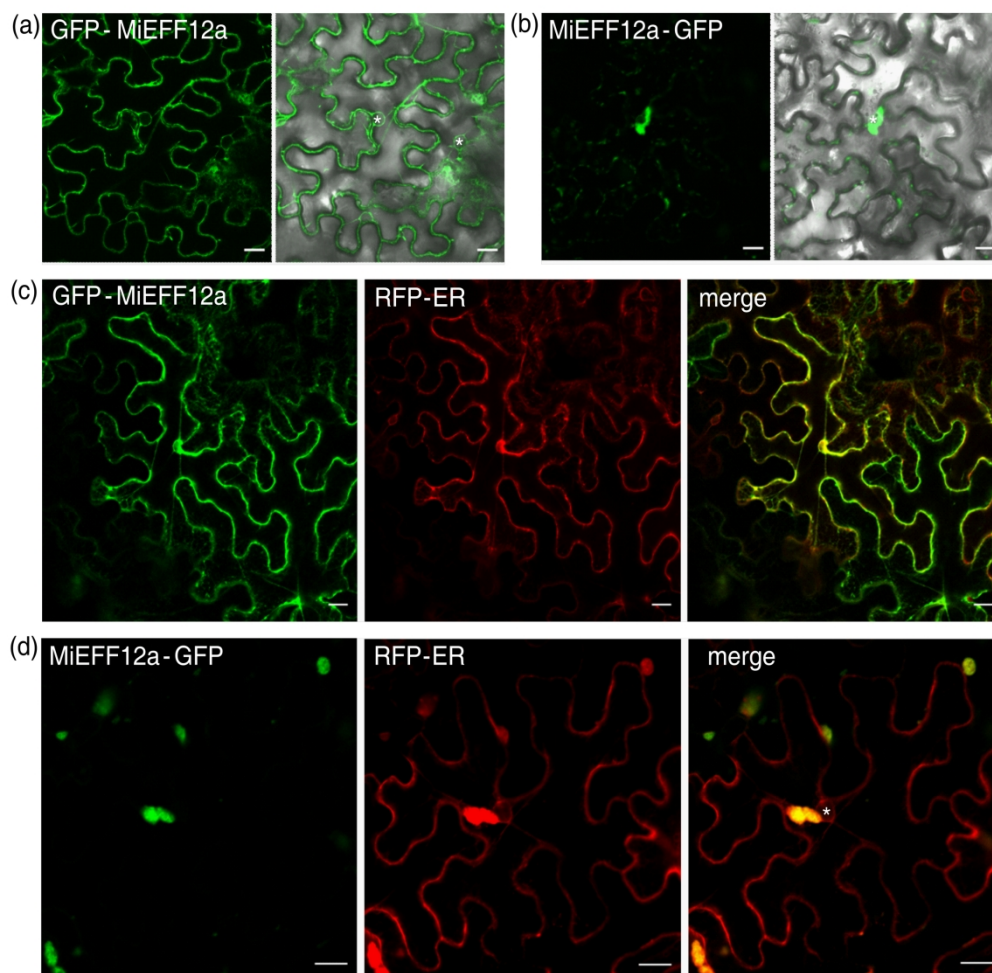


Figure 5. MiEFF12a was localized to the endoplasmic reticulum (ER) of epidermal *Nicotiana benthamiana* leaf cells. (a) Single-plane confocal images of *N. benthamiana* leaf cells infiltrated with *Agrobacterium tumefaciens* and producing MiEFF12a without its signal peptide, fused to the C-terminal end of the green fluorescent protein (GFP) reporter (GFP-MiEFF12a; green signal; left pictures). Overlays of differential interference contrast and fluorescence images are shown (right pictures). (b) Single-plane confocal images of *N. benthamiana* leaf cells infiltrated with *A. tumefaciens* and producing MiEFF12a without the signal peptide, fused to the N-terminal end of the green fluorescent protein (GFP) reporter (MiEFF12a-GFP; green signal; left pictures). Overlays of differential interference contrast and fluorescence images are shown (right pictures). (c) The monomeric red fluorescent protein (mRFP) signal (RFP-ER; red signal) of an ER marker was used to analyze colocalization of the GFP-MiEFF12a fusion (green signal) and the ER. (d) The mRFP signal (red signal) of the ER marker was used to analyze its co-localization with the MiEFF12a-GFP fusion (green signal). Both fusions between the GFP and MiEFF12a colocalized with the ER marker in *N. benthamiana* leaf cells. Asterisk; nucleus. Scale bars: 20 μm.

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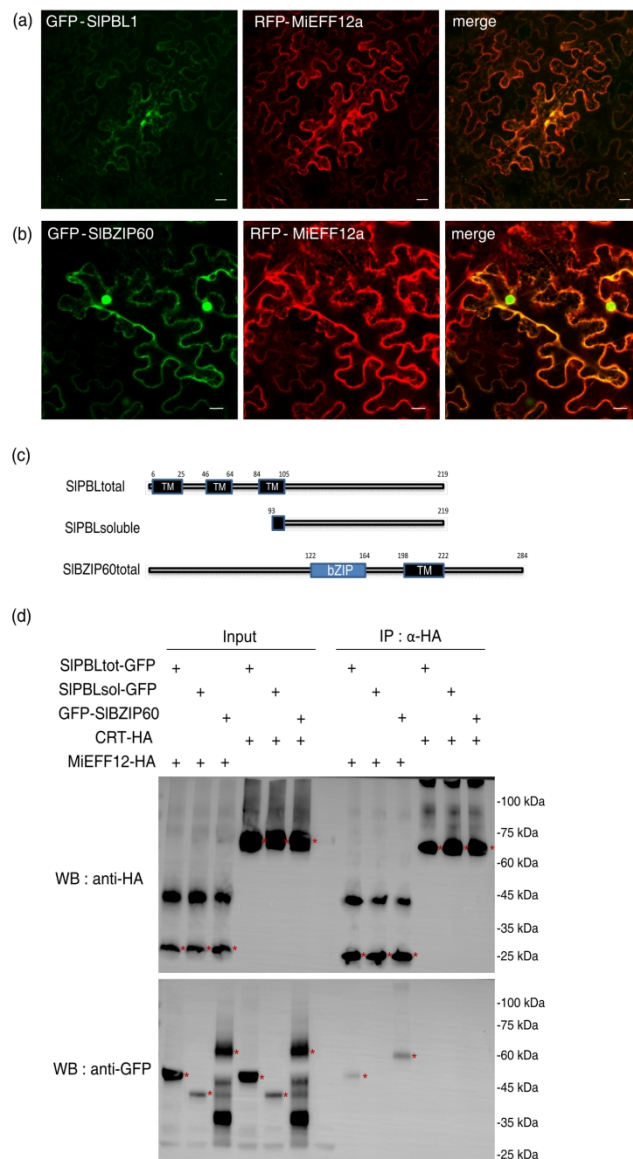


Figure 6. MiEFF12a physically interacts in planta with SIPBL1 and SIBZIP60. SIPBL1 and SIBZIP60 co-localized localized with MiEFF12a in the endoplasmic reticulum (ER) of epidermal *Nicotiana benthamiana* leaf cells. (a) Single-plane confocal images of *N. benthamiana* leaf cells infiltrated with *Agrobacterium tumefaciens* and producing SIPBL1, fused to the C-terminal end of the green fluorescent protein (GFP) reporter (GFP-SIPBL1; green signal) and MiEFF12a fused to the c-terminal end of the red fluorescent protein (RFP) reporter (RFP-MiEFF12a; red signal). (b) Single-plane confocal images of *N. benthamiana* leaf cells infiltrated with *Agrobacterium tumefaciens* and producing SIBZIP60, fused to the C-terminal end of the green fluorescent protein (GFP) reporter (GFP-SIBZIP60; green signal) and the RFP-MiEFF12a recombinant protein (RFP-MiEFF12a; red signal). Overlays of fluorescence images are shown (merge). Scale bars: 20 μ m. (c) Schematic representation of the full-length (tot, total) and truncated (sol, soluble) SIPBL1 and SIBZIP60 proteins used for interactomic assays. (d) Co-immunoprecipitation experiments confirmed that MiEFF12a interacted with the full-length SIPBL1 and BZIP60. SIPBLtot-GFP, SIPBLsol-GFP or SIBZIP60 were transiently co-expressed with MiEFF12a-HA or MiCRT in tobacco leaves. The Co-IP experiment was performed with anti-HA affinity gel, and the protein isolated was analyzed by immunoblotting with anti-GFP antibodies to detect

SIPBLtot and SIPBLsol, and with anti-HA antibodies to detect MiEFF12a and MiCRT. Three independent experiments were performed, with similar results.

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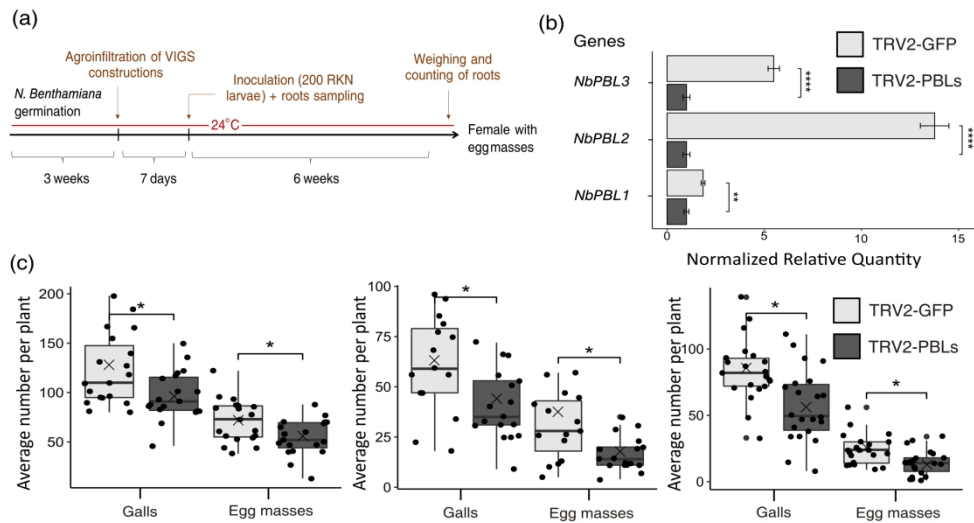


Figure 7. The silencing of PBL genes in *N. benthamiana* affects susceptibility to *M. incognita*. (a) Timeline used for virus-induced gene silencing (VIGS) experiments. (b) RT-qPCR showing the efficient silencing of the *NbPBL1a/b*, *NbPBL2a/b* and *NbPBL3a/b* gene pairs in *N. benthamiana* control plants (TRV-GFP) and plants in which *NbPBLs* were silenced (TRV2-PBLs). The data shown are normalized relative transcript levels for three independent biological replicates obtained with SatqPCR software. The *NbEF1a* and *NbGADPH* housekeeping genes were used for data normalization. Error bars indicate the SEM. (c) Infection test on *N. benthamiana* TRV-GFP or TRV2-PBLs plants. Galls were counted six weeks after inoculation with 200 *M. incognita* second-stage juveniles (J2s) per plant. Results from three independent experiments are shown (n=19, n=15 and n=21 plants for test N°1, N°2 and N°3, respectively). The cross represents average value. Box indicates interquartile range (25th to the 75th percentile). The central line within the box represents mean value. Whiskers indicate the minimum and maximum values for the normal values present in the dataset. Statistical significance was determined in Student's t test and significant differences were observed between TRV-GFP control and TRV-PBL plants (* p < 0.01).

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