1   **Silencing *SmD1* in Solanaceae alters susceptibility to root-knot nematodes**

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28

29   Summary

30   Root-knot nematodes (RKNs) are among the most damaging pests of agricultural crops.

31   Indeed, *Meloidogyne* is an extremely polyphagous genus of nematodes that can infect

32   thousands of plant species. A few genes for resistance (R-genes) to RKNs suitable for use in

33   crop breeding have been identified, and new virulent strains and species of nematode emerge

34   rendering these R-genes ineffective. Effective parasitism is dependent on the secretion, by the

35   RKN, of effectors targeting plant functions, which mediate the reprogramming of root cells

36   into specialised feeding cells. These cells, the giant cells, are essential for RKN development

37   and reproduction. The EFFECTOR 18 protein (EFF18) from *M. incognita* interacts with the

38   spliceosomal protein SmD1 in Arabidopsis, disrupting its function in alternative splicing

39   regulation and modulating the giant cell transcriptome. We show here that EFF18 is a

40   conserved RKN-specific effector. We also show here that EFF18 effectors also target SmD1

41   in *Nicotiana benthamiana* and *Solanum lycopersicum*. The alteration of *SmD1* expression by

42   virus-induced gene silencing (VIGS) in Solanaceae affects giant cell formation and nematode

43   development. Thus, *SmD1* is a susceptibility gene and a promising target for the development

44   of broad resistance, especially in Solanaceae, for the control of *Meloidogyne* spp.

45

Introduction

Plant parasitic nematodes are major crop pests causing crop losses of several million dollars annually, through damage to almost all cultivated plants, and the transmission of plant viruses (Singh et al., 2013). Root-knot nematodes (RKNs) of the genus *Meloidogyne* are considered to be the most detrimental of these plant parasites, due to the magnitude of the economic losses they cause (Jones *et al.*, 2013). RKNs are widespread worldwide and can infect more than 5,500 different plant species, including many species of major agricultural interest. About 100 RKN species have been described, and those reproducing asexually by mitotic parthenogenesis (*M. incognita*, *M. javanica*, *M. arenaria* and *M. enterolobii*) are the most polyphagous and damaging pests. By contrast, those reproducing sexually or by meiotic parthenogenesis (*M. hapla*) have a smaller host range (Blok et al., 2008; Castagnone-Sereno, 2006).

All RKNs are sedentary endoparasites that induce the formation of specialised feeding structures and typical root deformations, known as galls or root knots, that deprive the plant of nutrients (Escobar *et al.*, 2015; Favery *et al.*, 2016). After hatching from eggs, the stage 2 juveniles (J2) of *M. incognita* penetrate the root apex and migrate between plant cells to reach the plant vascular system (Holbein *et al.*, 2019). Once there, the filiform J2 switch to a sedentary lifestyle, by selecting five to seven cells of the vascular parenchyma and inducing their reprogramming into specialised feeding cells, known as giant cells (Escobar *et al.*, 2015; Favery *et al.*, 2016; Olmo *et al.*, 2020). These hypertrophied and multinucleate cells act as metabolic sinks close to the xylem and phloem vessels that withdraw water and nutrients from the sap (Rodiuc *et al.*, 2014). The nematode uses these specific giant cells for feeding for the rest of its life. After successive moults, the sedentary swollen juveniles develop into an adult female that lays her egg masses on the root surface, thus completing the cycle. The giant cells are hypertrophied and multinucleate, harbouring hundreds of nuclei. They are produced by

71   successive nuclear divisions uncoupled from cytokinesis, followed by nuclear

72   endoreduplication (de Almeida Engler and Gheysen, 2013). RKN induce giant cells and gall

73   formation by recruiting the developmental pathways of post-embryonic organogenesis and

74   regeneration to promote transient pluripotency (Olmo *et al.*, 2020).

75   RKNs parasitise plants and induce the redifferentiation of vascular cells into giant cells by

76   secreting effectors, molecules that recruit/hijack plant functions (Mejias *et al.*, 2019; Toruño

77   *et al.*, 2016). RKN effectors, particularly those produced by the three oesophageal gland cells

78   and secreted into the host through a stylet, are involved in the four main functions underlying

79   parasitism: (i) the degradation and modification of plant cell walls during J2 migration within

80   the root; (ii) the suppression of host defences; (iii) the reprogramming of plant vascular cells

81   as giant cells and (vi) the maintenance of these feeding sites (Mitchum et al., 2013; Truong et

82   al., 2015). The profound morphological and metabolic changes associated with giant cell

83   induction by RKNs and the transcriptional reprogramming occurring during the formation of

84   these cells require the secretion of effectors targeting key nuclear functions (Hewezi and

85   Baum, 2013; Quentin *et al.*, 2013). With the exception of plant cell wall-degrading enzymes

86   (Danchin *et al.*, 2010), very few effectors have been shown to be conserved and functional in

87   multiple RKN species. For example, 16D10 encodes a conserved secretory peptide conserved

88   in five RKN species (*M. incognita*, *M. arenaria*, *M. hapla*, *M. javanica*, *M. chitwoodi*) that

89   stimulates root growth and functions as a ligand for a putative plant transcription factor

90   (Huang et al., 2006; Dinh, 2015). The silencing of *16D10* by RNA interference methods

91   confers broad resistance to RKNs (Huang et al., 2006; Dinh, 2015). The chorismate mutates,

92   MiCM3 (Wang *et al.*, 2018) and MjCM1 (Doyle and Lambert, 2003), and the transthyretin-

93   like proteins, MjTTL5 (Lin *et al.*, 2016) and MhTTL2 (Gleason *et al.*, 2017), also appear to

94   be effectors conserved among RKNs. Interestingly, MhTTL2 is expressed in the amphids

95   (Gleason *et al.*, 2017), whereas MjTTL5 is expressed specifically in the subventral glands,

96   suggesting different roles for these two molecules in parasitism, encoded by the same gene

97   family (Lin *et al.*, 2016).

98   We recently showed that MiEFF18, a nuclear effector from *M. incognita,* is secreted *in*

99   *planta*, targets the giant cell nuclei and interacts with the SmD1 protein, a core component of

100   the spliceosome (Mejias *et al.*, 2020). We show here that MiEFF18 is a specific and

101   conserved RKN effector and that orthologous genes are specifically expressed in the salivary

102   glands of RKNs. We also show that MiEFF18 and its orthologue in *M. enterolobii,* MeEFF18,

103   interact with SmD1 proteins from different plant species. Moreover, virus-induced gene

104   silencing (VIGS) approaches silencing the SmD1 genes of *N. benthamiana* and *S.*

105   *lycopersicum* greatly impair RKN infection. These results are consistent with the targeting, by

106   RKNs, of conserved spliceosomal functions, to drive the development of giant cells,

107   facilitating parasitism on a large spectrum of host plants.

108

Results

110   **EFF18 is a conserved RKN-specific effector targeting plant nucleus**

111   MiEFF18 was first described in the *M. incognita* genome (Mejias *et al.*, 2020; Nguyen *et al.*,

112   2018; Rutter *et al.*, 2014). Database queries showed that MiEFF18 displayed no sequence

113   homology or known domains , and that it was absent from nematodes of other genera, such as

114   cyst nematodes and free-living nematodes. By contrast, EFF18 orthologues were identified in

115   seven of the eight RKNs for which genome sequences were available: *M. incognita*, *M.*

116   *javanica*, *M. arenaria* (Blanc-Mathieu *et al.*, 2017), *M. hapla* (Opperman *et al.*, 2008), *M.*

117   *enterolobii* (syn. *M. mayaguensis*) (Koutsovoulos *et al.*, 2020), *M. floridensis* (Lunt *et al.*,

118   2014) and *M. luci* (Susič *et al.*, 2020) (Figure 1, Table S1, Figure S1). No EFF18 orthologue

119   was identified in *M. graminicola* (Somvanshi *et al.*, 2018). Three paralogous copies were

120   identified, in the *M. incognita*, *M. javanica* and in *M. luci* genomes. Four copies were detected

121 in *M. arenaria* and a single copy was detected in *M. hapla, M. floridensis* and *M. enterolobii*.

122 A sequence alignment and analysis of the RKN EFF18 protein sequences showed that they

123 were more than 60% identical, between 279 and 316 amino acids (aa) long and that they had

124 an N-terminal secretion signal peptide (SSP), a low-complexity acidic D/E-rich region and a

125 C-terminal lysine (K)-rich domain carrying direct repeats (Figure 1a, Figure S2). Only the C-

126 terminal K-rich domain displayed marked differences between copies.

127   A phylogenetic tree based on an alignment of the 17 RKN EFF18 protein sequences

128 showed divergences between copies among the same species (Figure 1a). EFF18 proteins

129 more closely related to MiEFF18a/Minc18636 harboured one monopartite NLS and one

130 bipartite NLS, whereas other copies are more divergent (*e.g.* MiEFF18b/Minc15401 and

131 MiEFF18c) and contained only one monopartite NLS (Figure 1a). MiEFF18a fused at its C-

132 or N-terminus to GFP (green fluorescent protein) and MeEFF18a fused at its N-terminus to

133 GFP were transiently expressed in *Nicotiana benthamiana* leaf epidermis. For EFF18

134 constructs, GFP fluorescence was only detected in the nucleus, with a strong GFP signal

135 accumulating in the nucleolus (Figure 1b). In contrast, GFP alone was detected in the

136 cytoplasm and the nucleus, but not in the nucleolus (Figure 1b). The EFF18s with bipartite

137 NLS were 98% to 100% identical to the MiEFF18a protein, whereas those with only

138 monopartite NLS were only 79 to 89% identical to this protein (Figure 1c). *M. hapla* had the

139 most divergent genome of the *Meloidogyne* species tested. It was found to have a single copy

140 of the gene, 63-65% identical to the closest copies and the most divergent copies, which

141 suggests that the ancestor of RKN species had an EFF18 gene, and providing support for the

142 role of EFF18 as a conserved effector.

143

144 **RKN EFF18s are specifically expressed in the subventral glands**

145   MiEFF18 have been shown to be more strongly expressed at parasitic stages and to be

146   expressed specifically in the subventral glands of *M. incognita* J2s (Rutter et al., 2014;

147   Nguyen et al., 2018; Mejias et al., 2020). We studied the pattern of expression of genes

148   encoding orthologous sequences of MiEFF18 in two other RKN species, by performing *in situ*

149   hybridisation (ISH) for the *M. enterolobii MeEFF18a* and the *M. arenaria MaEFF18a*

150   sequences. A specific signal was detected in the subventral oesophageal gland cells of pre-J2s

151   after hybridisation with digoxigenin-labelled MeEFF18a and MaEFF18a antisense probes

152   (Figure 2). No signal was detected in pre-J2s with sense negative controls. This finding

153   suggests that MaEFF18a and MeEFF18a, may, like MiEFF18a, be secreted and play an

154   important role in nematode parasitism.

155

156   **MiEFF18a and MeEFF18a interact with the SmD1 proteins of *A. thaliana*, *N.**

157   *benthamiana* and *S. lycopersicum***

158   We have demonstrated an interaction between MiEFF18 and the nuclear ribonucleoproteins

159   SmD1s from *S. lycopersicum* and *A. thaliana,* modulating the pattern of alternative splicing

160   and promoting the formation of giant cells (Mejias et al., 2020). Two genes, *AtSmD1a*

161   (AT3G07590) and *AtSmD1b* (AT4G02840), encode SmD1 proteins in *Arabidopsis* (Koncz et

162   al., 2012) and two genes encode 100% identical SmD1 proteins (SlSmD1) in *S. lycopersicum:*

163   *SlSmD1a* (Solyc06g084310) and *SlSmD1b* (Solyc09g064660). In *N. benthamiana*, we

164   identified three genes encoding SmD1s: *NbSmD1a* (*Niben101Scf01782g05006*), *NbSmD1b*

165   (*Niben101Scf05290g01011*), and *NbSmD1c* (*Niben101Scf04283g03011*). A multiple sequence

166   alignment showed that SmD1 was highly conserved in these species, with 93% identity

167   between SlSmD1 and the sequence from which it diverged most strongly, AtSmD1b (Figure

168   3a). Like all Sm proteins, SmD1s carry two conserved Sm motifs mediating protein-protein

169   interactions during small nuclear ribonucleoprotein (snRNP) biogenesis (Figure 3b). We

170   investigated the subcellular localisation of SmD1 in plant cells, by transiently expressing

171   constructs encoding GFP-SmD1 fusion proteins in *N. benthamiana*. We confirmed a strong

172   accumulation of SlSmD1a and AtSmD1b in the nucleolus and in Cajal bodies, and a weaker

173   accumulation in the nucleoplasm (Figure 3c).

174   We then investigated whether MeEFF18a was also able to interact with SmD1 proteins

175   from *S. lycopersicum* and *A. thaliana,* like MiEFF18 (Mejias et al., 2020). Using a pairwise

176   yeast-two hybrid approach, we showed that MiEFF18a and MeEFF18a interact with SmD1

177   proteins from plants of different clades, such as *A. thaliana*, *S. lycopersicum* and *N.*

178   *benthamiana* (Figure 3d). As a control, we tested SmD1 interactions with another *M.*

179   *incognita* effector, MiEFF16, encoded by the *Minc16401* gene and expressed in the

180   subventral glands, with the same nuclear location *in planta* as MiEFF18 (Mejias et al., 2020).

181   No interaction was observed between MiEFF16 and SmD1 proteins in yeast (Figure 3d).

182   These results demonstrate that EFF18 proteins are conserved among RKNs and that they

183   interact with SmD1 proteins, which are conserved among plant species.

184

185   ***SmD1* acts as a susceptibility gene for infection in plants of different clades**

186   We recently demonstrated an important role for the AtSmD1b protein in giant cell formation

187   and successful nematode infection (Mejias et al., 2020). We investigated whether *SmD1* is a

188   conserved susceptibility gene required to ensure infection, and essential for RKN parasitism

189   in Solanaceae species, by using a virus-induced gene silencing (VIGS) approach to alter the

190   expression of *SmD1* genes in *S. lycopersicum* and *N. benthamiana*.

191   We first performed a VIGS assay to silence *SmD1* genes in *S. lycopersicum* (Figure 4a). We

192   evaluated silencing efficiency by RT-qPCR on emerging leaves. Treated tomatoes had much

193   lower levels of *SmD1* transcripts (Figure 4b). Tomatoes in which SmD1 genes were silenced

194   displayed developmental defects on emerging leaves and had a shorter root system (Figure

195   S3). In tomato plants infected with *M. incognita,* in which *SmD1* genes were silenced, the

8

196   number of females producing egg masses was much smaller than that in control plants treated

197   with the TRV-GFP virus (Figure 4c).

198   Because of adverse effect of *SmD1* silencing on development in tomato, we then silenced

199   the *SmD1* genes in *N. benthamiana,* which allows performing a VIGS assay at a later

200   developmental stage when roots have already developed substantially (Figure 5a and 5b). An

201   evaluation of silencing efficiency by RT-qPCR showed that *N. benthamiana* roots subjected

202   to VIGS had much lower levels of *SmD1* transcripts, particularly for the most strongly

203   expressed gene, *NbSmD1b* (Figure 5c). We observed no significant decrease in the expression

204   of the two mostly weakly expressed genes, *NbSmD1a* and *NbSmD1c* (Figure 5c; Figure S4).

205   *N. benthamiana* plants in which *SmD1* was silenced produced a much smaller number of galls

206   (up to 80% fewer) following infection with *M. incognita* (Figure 5d).

207   We studied the effect on nematode and giant cell development in detail, by investigating

208   J2s *in planta* by the fuchsine acid staining method, to determine the proportions of migrating

209   filiform and sedentary swollen parasitic juveniles and their ratio. The percentage of migrating

210   filiform J2s was higher (90%) in plants in which *SmD1* was silenced, which had a lower

211   percentage of swollen juveniles, indicating a defect in the RKN development (Figure 6a). We

212   also investigated whether the giant cells formed on plants in which *SmD1* was silenced

213   displayed developmental defects. We observed these cells directly, under a confocal

214   microscope, after clearing in benzyl alcohol/benzyl benzoate (BABB ; Cabrera et al., 2018). A

215   comparison of the mean surface areas of the largest giant cells in each gall showed that giant

216   cells from plants in which *SmD1* was silenced were 36% smaller than those from control

217   plants (Figure 6b and 6c). These results confirm the important role of SmD1 in giant cell

218   formation in Solanaceae species and the requirement of this protein for successful nematode

219   development.

220

221 **Discussion**

222 The ability of plant pathogens to infect their hosts is generally dependent on the secretion of

223 effectors. Most pathogens secrete effectors to overcome host physical defences, such as the

224 plant cell wall, and to suppress plant immune responses (Toruño *et al.*, 2016). Other effectors

225 are more specific to the parasitic strategy of the pathogen and may regulate host gene

226 expression or trigger changes in host cell morphology and physiology to allow pathogen

227 feeding and development. Most obligatory biotrophs form specific feeding structures, such as

228 the haustoria of biotrophic filamentous pathogens, and produce sets of specific effectors

229 (Chaudhari *et al.*, 2014; O'Connell and Panstruga, 2006). RKNs are root endoparasites that

230 manipulate host cells to form specialised giant cells for feeding. These giant cells constitute

231 the sole source of nutrients for the nematode, and are, therefore, essential for nematode

232 survival. RKNs induce giant cells by manipulating root cell developmental programmes.

233 Indeed, massive transcriptomic reprogramming occurs during giant cell formation (Favery *et*

234 *al.*, 2016; Mitchum *et al.*, 2013). Genes associated with root meristem function, lateral root

235 formation and the establishment of the vasculature, in particular, are tightly regulated upon

236 giant cell induction (Cabrera *et al.*, 2014; Olmo *et al.*, 2020; Yamaguchi *et al.*, 2017).

237 Alternative splicing has recently been shown to occur in *Arabidopsis* following infection with

238 *M. incognita*, and this process contributes to transcriptome and proteome diversity (Mejias et

239 al., 2020).

240

241 **EFF18 is a nuclear conserved RKN-specific effector**

242 Nuclear effectors are thought to mediate the transcriptional reprogramming required for giant

243 cell formation (Mejias *et al.*, 2019; Quentin *et al.*, 2013). They may interfere with the function

244 of transcription factors, as described for Mi16D10, which interacts with SCARECROW-like

245 transcription factors (Huang *et al.*, 2006), or may themselves act as transcription factors, as

246   reported for Mi7H08 (Zhang *et al.*, 2015). MiEFF18 is another RKN effector that has been

247   shown to be secreted within host cells, in which it localises to the nucleus. MiEFF18 has been

248   shown to interact with the SmD1 protein, a core component of the spliceosome conserved in

249   all eukaryotes, thereby modulating alternative splicing and gene expression (Mejias et al.,

250   2020). MiEFF18 may corrupt the function of *Arabidopsis SmD1* function to modulate the

251   expression of various genes encoding proteins involved in giant cell formation through

252   processes such as DNA replication or cytokinesis (Mejias et al., 2020). We show here that the

253   manipulation of SmD1 function by MiEFF18 plays a key role in giant cell development in

254   other plant species, such as *Nicotiana benthamiana* and the tomato *Solanum lycopersicum.*

255   Genes encoding the MiEFF18 effector were found in all available *Meloidogyne* spp.

256   genomes other than the draft genome for the rice RKN *M. graminicola* (Somvanshi *et al.*,

257   2018). EFF18 is exclusive to RKN, being absent from all other parasitic nematodes and other

258   plant pathogens with parasitic strategies not involving the induction of giant feeding cells. At

259   least one orthologous copy of a MiEFF18 sequence was detected in each of the available

260   *Meloidogyne* genomes, demonstrating that MiEFF18 is a conserved effector. The multiple

261   copies identified in *M. incognita, M. javanica* and *M. arenaria* are consistent with the

262   polyploidy of these mitotic parthenogenetic species (Koutsovoulos *et al.*, 2020). The absence

263   of an EFF18 effector in *M. graminicola* may be explained by the particular host range and life

264   cycle of this nematode. *M. graminicola* may have lost the EFF18 effector during

265   specialisation on monocotyledonous hosts and adaptation to the infection of submerged roots

266   (Mantelin *et al.*, 2017). These growing conditions may have resulted in different requirements

267   for the modulation of gene expression for giant cell ontogenesis, dependent on effectors other

268   than EFF18.

269   The distribution of EFF18 orthologues in two major groups, with copies (e.g. MiEFF18a)

270   carrying two NLS, and those of the most divergent group (e.g. MiEFF18b) carrying only one

271 NLS, suggested a possible duplication of the ancestral MiEFF18 gene in the ancestor of RKN

272 species, with one of the duplicated genes either gaining or losing a bipartite NLS. The

273 proteins from the closest group to the MiEFF18a gene would be expected to function

274 similarly to MiEFF18a, through the modulation of SmD1 functions, due to the very high level

275 of sequence identity between these proteins (98% identity). *M. enterolobii* is an extremely

276 polyphagous species that reproduces through mitotic parthenogenesis, like *M. incognita*.

277 Therefore, we investigated the functionality of proteins MeEFF18 orthologue. We found that,

278 like MiEFF18, MeEFF18a was able to interact with SmD1 proteins from *A. thaliana*, *N.*

279 *benthamiana* and *S. lycopersicum*, suggesting that orthologous copies of MiEFF18a are

280 functional and target the same functions in different host plants. MiEFF18a and MeEFF18a

281 are the first examples of conserved RKN effectors able to target the same conserved plant

282 process in different plant species.

283

Targeting conserved effectors to engineer plant resistance

285   The identification of conserved effectors could lead to new strategies for developing broad

286 resistance (Huang *et al.*, 2006; Landry *et al.*, 2020; Peeters *et al.*, 2013; Roux *et al.*, 2015).

287 Only a few RKN effectors have been described to be conserved. The MAP (*Meloidogyne*

288 avirulence protein) effector family, which includes *M. incognita* Mi-MAP1.2, was shown to

289 be conserved in 13 of the 21 RKN species tested, and absent from other genera of plant-

290 parasitic nematodes (PPNs) (Tomalova *et al.*, 2012). The genes of the MAP effector family

291 harbour one or multiple CLE-like motifs, which may be involved in feeding site formation, as

292 demonstrated for cyst nematode CLE-like peptides, which promote syncytium formation

293 (Rutter et al., 2014; Mitchum et al., 2012). MjNULG1a, from *M. javanica,* is a nuclear

294 effector with a demonstrated role in parasitism. Southern blot experiments have revealed the

295 presence of MjNULG1a orthologues in *M. incognita* and *M. enterolobii*, but not in other

296 PPNs (Lin *et al.*, 2013). Similarly, the 16D10 effector is exclusive to RKNs (Huang et al.,

297 2006; Dinh, 2015). The use of host-induced gene silencing (HIGS) approaches to engineer

298 plant resistance to RKNs has excited considerable interest (Ali *et al.*, 2017; Banerjee *et al.*,

299 2017). The targeting of genes involved in nematode development or encoding effectors has

300 been considered. Silencing conserved effectors may allow specific resistance to RKNs with

301 no impact on non-targeted species. Studies of Mi16D10 have demonstrated the feasibility of

302 conferring RKN resistance in *Arabidopsis*, potato or grape through the targeting of this

303 effector (Huang et al., 2006; Yang et al., 2013; Dinh, 2015). However, this strategy is

304 constrained both by limited HIGS effectiveness, by the redundancy of the effector's function

305 and the difficulty in targeting the point in time when the effector plays a key role in the

306 interaction.

307

308 **Targeting essential conserved effector targets to induce a loss of susceptibility**

309 The use of resistant cultivars or rootstocks is an efficient and non-polluting method for

310 controlling RKNs. Very few natural resistance genes (R-genes) have been identified to date,

311 in a limited number of plant species. Furthermore, some RKN species or populations are not

312 controlled by these genes, e.g. *M. enterolobii* (Elling, 2013; Kiewnick *et al.*, 2009) or can

313 overcome these resistances, e.g. populations of *M. incognita* virulent against tomato *Mi1.2*

314 (Castagnone-Sereno, 2006). One alternative would be to target conserved plant genes

315 encoding proteins involved in host processes that are hijacked by the biotrophic pathogens for

316 settlement and feeding, and that are essential for disease development. These susceptibility

317 genes (S-genes) represent an alternative to R-genes for the deployment of pathogen resistance,

318 and they may be more durable in the field (Dong and Ronald, 2019; Engelhardt *et al.*, 2018;

319 van Schie and Takken, 2014). Well-characterised examples of S-genes include the genes

320 encoding eukaryotic translation initiation factors, the sugar transporter SWEET14 or PMR6,

321   which are required for viral, bacterial and oomycete infections, respectively (Langner *et al.*,

322   2018; van Schie and Takken, 2014; Schmitt-Keichinger, 2019).

323   In recent decades, transcriptomic approaches have been widely used to identify genes

324   regulated upon RKN infection, and, thus, host functions manipulated by RKNs. However, as

325   thousands of genes are differentially regulated during a compatible interaction, the

326   identification of S-genes from these data is a very time-consuming process, probably

327   explaining why only a few genes to date have been shown to be important for the

328   establishment of giant cells (Favery *et al.*, 2016). Interactomics approaches have recently been

329   used to identify the direct plant targets manipulated by PPN effectors. Only a few targets of

330   RKN effectors have been identified, but most have been shown to be instrumental in

331   promoting nematode parasitism (Mejias *et al.*, 2019). SmD1 is a host target of an effector

332   required for host susceptibility to RKNs in several plant clades. It exerts a conserved plant

333   function targeted by a core effector in *Arabidopsis* and Solanaceae, common to diverse

334   *Meloidogyne* species that have adopted the same successful parasitic strategy based on the

335   induction of giant feeding cells in the root in several host species. SmD1 is thus a good

336   candidate S-gene for targeting in the engineering of crop resistance to RKN. As SmD1

337   functions is required for plant development knockout mutations of this gene have adverse

338   effects, it will be necessary to identify mutant alleles that can evade recognition by MiEFF18

339   whilst remaining competent to perform the functions of SmD1 in the regulation of plant

340   development in a continually changing environment. This strategy has proven to be effective

341   for potyvirus susceptibility *eIF4E* genes (Bastet *et al.*, 2019).

342

343   **Experimental procedures**

344   **Nematode and plant materials**

345   *M. incognita* (Morelos strain), *M. arenaria* (Guadeloupe strain) and *M. enterolobii* (Godet

346   strain) were multiplied on tomato (*Solanum lycopersicum* cv. "Saint Pierre") growing in a

347   growth chamber (25°C, 16 h photoperiod). Freshly hatched J2s were collected as previously

348   described (Caillaud and Favery, 2016). For VIGS experiments, *Nicotiana benthamiana* and

349   *Solanum lycopersicum* (cv M82) seeds were sown on soil and incubated at 4°C for two days.

350   After germination, *N. benthamiana* and tomato plantlets were transplanted into pots

351   containing soil and sand (1:1), and were grown at 24°C and 16°C, respectively (photoperiod,

352   16 h: 8 h, light: dark).

353

354   **Sequence analysis, alignment and phylogenetic tree**

355   The sequences of EFF18 paralogues and orthologues were obtained from *Meloidogyne*

356   genomic resources http://www6.inra.fr/meloidogyne_incognita and Wormbase parasite.

357   Protein sequences were aligned with the MAFFT tool on the EBI server

358   (https://www.ebi.ac.uk/Tools/msa/mafft/). The alignment was then used as input for the

359   IQTree Web server http://iqtree.cibiv.univie.ac.at/ (Trifinopoulos et al., 2016) to generate the

360   maximum likelihood phylogenetic tree. The model chosen by the inbuilt model test was

361   HIVb+F+G4. Support for the nodes was calculated with 100 bootstrap replicates. *M. hapla*

362   was used as the outgroup in the phylogenetic tree for MiEFF18 orthologues. The tree was

363   visualised in iTOL https://itol.embl.de/. The sequence alignment were coloured with

364   Boxshade (https://embnet.vital-it.ch/software/BOX_form.html). The pairwise sequence

365   identity matrix of RKN EFF18 protein sequences was generated with Sequence Demarcation

366   Tool version 1.2 software (Muhire *et al.*, 2014) (http://web.cbio.uct.ac.za/~brejnev/).

367

368   *In situ* **hybridisation (ISH)**

369   ISH was performed on freshly hatched *M. arenaria* and *M. enterolobii* pre-J2s, as previously

370   described (Jaouannet *et al.*, 2018). The MaEFF18, and MeEFF18 gene fragments were

15

371   amplified with specific primers (Table S2). A sense probe for MeEFF18 was used as a

372   negative control. Images were obtained with a microscope (Zeiss Axioplan2, Germany).

373

374   **Pairwise yeast two-hybrid assays**

375   For pairwise yeast two-hybrid (Y2H) assays, the coding sequences (CDS) of the MiEFF16,

376   MiEFF18 and MeEFF18 effectors without their secretion signals were amplified (Table S1)

377   and inserted into pB27 as C-terminal fusions with LexA. Full-length SmD1 CDS sequences

378   (*SlSmD1*, *NbSmD1* and AtSmD1b) were amplified (Table S1) and inserted into pP6 as C-

379   terminal fusions with Gal4-AD. The pB27 and pP6 constructs were verified by sequencing

380   and used to transform the L40ΔGal4 (MATa) and Y187 (MATα) yeast strains, respectively.

381   Y187 and L40ΔGal4 were crossed and diploids were selected on medium lacking tryptophan

382   and leucine. Interactions were investigated on medium lacking tryptophan, leucine and

383   histidine and supplemented with 0.5 mM 3-aminotriazole (3-AT).

384

385   *N. benthamiana* **agroinfiltration**

386   Transient expression was achieved by infiltrating *N. benthamiana* leaves with *A. tumefaciens*

387   GV3101 strains harbouring GFP-fusions, as previously described (Caillaud *et al.*, 2008).

388   Leaves were imaged 48 hours after agroinfiltration, with an inverted confocal microscope

389   equipped with an argon ion and HeNe laser as the excitation source. For simultaneous GFP

390   imaging, samples were excited at 488 nm and GFP emission was detected selectively with a

391   505-530 nm band-pass emission filter.

392

393   **Virus-induced gene silencing in Solanaceae**

394   VIGS assays were performed on *N. benthamiana* and *S. lycopersicum*. We used the Sol

395   Genomics Network VIGS-Tool (https://vigs.solgenomics.net/) to design the best sequence for

396   silencing SlSmD1a transcripts, and selected the full-length *SlSmD1a* (without the ATG and

397   STOP codons) for amplification by PCR with the TRV2-SlSmD1-F/TRV2-SlSmD1-R primer

398   pairs (Table S2). The PCR products were digested with *Eco*RI and *Xho*I and ligated to the

399   tobacco rattle virus RNA 2 vector (TRV2) for the transformation of *A. tumefaciens* strain

400   GV3101. VIGS assays were performed, as previously described, by the co-infiltration of

401   leaves of three-week-old *N. benthamiana* plants (Lange *et al.*, 2013; Velasquez *et al.*, 2009)

402   or 10-days-old tomato plants (Cox *et al.*, 2019) with agrobacterial strains containing the RNA

403   1 vector (TRV1) and TRV2. Tomato plants were incubated at 16°C for four weeks. Three

404   independent biological replicates were established for each set of conditions ($n = 15$ per

405   replicate). Two *N. benthamiana* root systems per set of conditions and per replicate, or *S.*

406   *lycopersicum* leaves were collected upon RKN infection and frozen in liquid nitrogen for

407   subsequent RNA extraction and the assessment of silencing efficiency by RT-qPCR.

408

409   **RKN infection assay, juveniles in the plant and giant cell area measurements**

410   *N. benthamiana* plants subjected to VIGS were inoculated with 200 *M. incognita* J2s per

411   plant, 10 days post inoculation (dpi) with TRV, and incubated at 24°C. *S. lycopersicum* plants

412   subjected to VIGS were inoculated with 150 *M. incognita* J2s per plant, 30 dpi with TRV, and

413   incubated at 16°C for two weeks before transfer to 24°C. *N. benthamiana* infected roots were

414   collected two weeks after infection whereas *S. lycopersicum* infected roots were collected six

415   weeks after infection. Galls, and egg masses stained with 4.5% eosin were counted under a

416   binocular microscope, and root system was weighted (*n*=12 to 19 and n=18 to 21 plants per

417   replicates for *N. benthamiana* and *S. lycopersicum*, respectively). Three and two independent

418   biological replicates were established for each set of conditions in *N. benthamiana* and *S.*

419   *lycopersicum*, respectively. The impact of the plant lines on the number of galls per mg of

420   root in *N. benthamiana* and the number of egg masses per plant in *S. lycopersicum* were

421   analyzed using Kruskal Wallis test since the dependent variable did not follow a Normal

422   distribution using a Shapiro-Wilk Test. The different replicates of the numbers of galls per mg

423   of roots in *N. benthamiana* were pooled for the analyzes because there was no difference

424   between the replications (X^2_2= 2.8, P = 0.248). By contrast, the different replicates of the

425   number of egg masses per plant in *S. lycopersicum* varied depending on the replication (X^2_1=

426   5.3, P = 0.022), and they were analyzed separately. Thus, both the number of galls per mg of

427   root in *N. benthamiana* and the number of egg masses per plant in *S. lycopersicum* varied

428   significantly between the two plant lines tested (X^2_1= 57.2, P < 0.001; X^2_1 > 25.6, P < 0.001,

429   respectively). For determination of the ratio of filiform-to-swollen nematodes, infected roots

430   were collected 14 dpi, parasitic nematodes were stained with fuchsine acid, as previously

431   described (Karssen and Moens, 1983), and nematodes were examined under a binocular

432   microscope (model LSM 880; Zeiss) (*n*= 3 plants per replicate for TRV-empty lines and n = 5

433   plants per replicate for TRV-SmD1 lines, with a mean of 75 nematodes observed per

434   condition and per replicate). Three independent biological replicates were established for each

435   set of conditions. Statistical analyses were carried out with R software (R Development Core

436   Team, version 3.1.3). For giant cell area measurements, galls were cleared in BABB, as

437   previously described (Cabrera *et al.*, 2018), and examined under an inverted confocal

438   microscope (model LSM 880; Zeiss). The mean areas of the biggest giant cell in each gall

439   from *N. benthamiana*, for each genotype, were measured with Zeiss ZEN software (*n* = 32

440   and 26 galls for control and SmD1-VIGSed plants, respectively). One biological experiment

441   was performed for giant cells measurement. The data were analysed with a *t*-test since the

442   data followed a normal distribution (t = 3.5, P < 0.001).

443

444   **Reverse transcription-quantitative PCR**

445 We assessed silencing efficiency in Solanaceae species, by extracting total RNA with TriZol

446 (Invitrogen) and subjecting 1 µg of total RNA to reverse transcription with the Superscript IV

447 reverse transcriptase (Invitrogen). qPCR analyses were performed as described by Nguyen et

448 al., 2016. Primers were designed to amplify both SlSmD1 transcripts or to discriminate

449 between the three copies of SmD1 present in *N. benthamiana* specifically according to their

450 UTR, to prevent the amplification of TRV2-SlSmD1 constructs (Table S2). We performed

451 RT-qPCR in triplicate on cDNA samples from three independent biological replicates. The

452 *EF-1α* and Actin genes were used for the normalisation of RT-qPCR data (Liu *et al.*, 2012).

453 Quantifications and statistical analyses were performed with SATqPCR (Rancurel *et al.*,

454 2019), and the results are expressed as normalised absolute quantities.

455

456 **Graphs and statistical analysis**

457 Graphs and plots were created with Microsoft® Office Excel® 2016 and statistical

458 calculations were performed in R.

459

460 **Accession numbers**

461 The sequence data from this article can be found in the *Arabidopsis* Information Resource

462 (https://www.arabidopsis.org/),   Solgenomics   (https://solgenomics.net/)   and/or

463 GenBank/EMBL databases. All RKN EFF18 protein sequences are presented in the Figure S1

464 The accession numbers are summarised in Table S1 including MiEFF18a (KX907770),

465 MeEFF18a (MW272456), NbSmD1a (MT683762), NbSmD1b (MT683763) and NbSmD1c

466 (MT683764).

467

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487

488   **Conflict of interest**

489   The authors have no conflict of interest to declare.

490

491   **Author contributions**

492   J.M. designed and performed experiments, and interpreted data; J.M., YC and N.M.T.

493   generated constructs and performed yeast two-hybrid analysis; YC performed *in situ*

494 hybridisation and *in planta* subcellular localisation experiments; KM performed VIGS in

495 tomato and nematode infection tests; J.M., P.A., S.J.P., B.F. and M.Q. wrote the manuscript;

496 B.F. and M.Q. obtained funding, designed the work and supervised the experiments and data

497 analyses; all the authors read and edited the manuscript.

498

499 **References**

500

501 Ali, M.A., Azeem, F., Abbas, A., Joyia, F.A., Li, H., and Dababat, A.A. (2017) Transgenic

502   Strategies for Enhancement of Nematode Resistance in Plants. *Frontiers in Plant*

503   *Science*, **8**, 1–13.

504 de Almeida Engler, J. and Gheysen, G. (2013) Nematode-induced endoreduplication in plant

505   host cells: why and how? *Molecular Plant-Microbe Interactions*, **26**, 17–24.

506 Banerjee, S., Banerjee, A., Gill, S.S., Gupta, O.P., Dahuja, A., Jain, P.K., and Sirohi, A.

507   (2017) RNA Interference: A Novel Source of Resistance to Combat Plant Parasitic

508   Nematodes. *Frontiers in Plant Science*, **8**:834, doi: 10.3389/fpls.2017.00834.

509 Bastet, A., Zafirov, D., Giovinazzo, N., Guyon-Debast, A., Nogué, F., Robaglia, C., and

510   Gallois, J.L. (2019) Mimicking natural polymorphism in eIF4E by CRISPR-Cas9 base

511   editing is associated with resistance to potyviruses. *Plant Biotechnology Journal*, **17**,

512   1736–1750.

513 Blanc-Mathieu, R., Perfus-Barbeoch, L., Aury, J.-M.M., Da Rocha, M., Gouzy, J., Sallet, E.,

514   et al. (2017) Hybridization and polyploidy enable genomic plasticity without sex in the

515   most devastating plant-parasitic nematodes. *PLoS Genetics*, **13**, e1006777.

516 Blok, V.C., Jones, J.T., Phillips, M.S., and Trudgill, D.L. (2008) Parasitism genes and host

517   range disparities in biotrophic nematodes: The conundrum of polyphagy versus

518   specialisation. *Bioessays*, **30**, 249–259.

519 Cabrera, J., Bustos, R., Favery, B., Fenoll, C., and Escobar, C. (2014) NEMATIC: a simple

520   and versatile tool for the in silico analysis of plant-nematode interactions. *Molecular*

521   *Plant Pathology*, **15**, 627–636.

522   Cabrera, J., Olmo, R., Ruiz-Ferrer, V., Abreu, I., Hermans, C., Martinez-Argudo, I., et al.

523   (2018) A Phenotyping Method of Giant Cells from Root-Knot Nematode Feeding Sites

524   by Confocal Microscopy Highlights a Role for CHITINASE-LIKE 1 in Arabidopsis.

525   *International Journal of Molecular Sciences*, **19**, 429.

526   Caillaud, M.-C., Abad, P., and Favery, B. (2008) Cytoskeleton reorganization. *Plant*

527   *Signaling & Behavior*, **3**, 816–818.

528   Caillaud, M.-C.C. and Favery, B. (2016) In Vivo Imaging of Microtubule Organization in

529   Dividing Giant Cell. In: *Methods in Molecular Biology* (Caillaud,M.-C., ed) , pp. 137–

530   144. New York: Springer New York.

531   Castagnone-Sereno, P. (2006) Genetic variability and adaptive evolution in parthenogenetic

532   root-knot nematodes. *Heredity*, **96**, 282–289.

533   Chaudhari, P., Ahmed, B., Joly, D.L., and Germain, H. (2014) Effector biology during

534   biotrophic invasion of plant cells. *Virulence*, **5**, 703–709.

535   Cox, D.E., Dyer, S., Weir, R., Cheseto, X., Sturrock, M., Coyne, D., et al. (2019) ABC

536   transporter genes ABC-C6 and ABC-G33 alter plant-microbe-parasite interactions in the

537   rhizosphere. *Scientific Reports*, **9**, 19899.

538   Danchin, E.G.J., Rosso, M.-N.N., Vieira, P., de Almeida-Engler, J., Coutinho, P.M.,

539   Henrissat, B., and Abad, P. (2010) Multiple lateral gene transfers and duplications have

540   promoted plant parasitism ability in nematodes. *Proc Natl Acad Sci U S A*, **107**, 17651–

541   17656.

542   Dong, O.X. and Ronald, P.C. (2019) Genetic Engineering for Disease Resistance in Plants:

543   Recent Progress and Future Perspectives. *Plant Physiology*, **180**, 26–38.

544   Doyle, E.A. and Lambert, K.N. (2003) Meloidogyne javanica chorismate mutase 1 alters plant

545   cell development. *Mol Plant Microbe Interact*, **16**, 123–131.

546   Elling, A. a (2013) Major emerging problems with minor meloidogyne species.

547   *Phytopathology*, **103**, 1092–1102.

548   Engelhardt, S., Stam, R., and Hückelhoven, R. (2018) Good Riddance? Breaking Disease

549   Susceptibility in the Era of New Breeding Technologies. *Agronomy*, **8**, 114.

550   Escobar, C., Barcala, M., Cabrera, J., and Fenoll, C. (2015) Overview of Root-Knot

551   Nematodes and Giant Cells. In: *Advances in Botanical Research* , pp. 1–32.

552   Favery, B., Quentin, M., Jaubert-Possamai, S., and Abad, P. (2016) Gall-forming root-knot

553   nematodes hijack key plant cellular functions to induce multinucleate and hypertrophied

554   feeding cells. *Journal of Insect Physiology*, **84**, 60–69.

555   Gleason, C., Polzin, F., Habash, S.S., Zhang, L., Utermark, J., Grundler, F.M.W., and

556   Elashry, A. (2017) Identification of two Meloidogyne hapla genes and an investigation

557   of their roles in the plant-nematode interaction. *Molecular Plant-Microbe Interactions*,

558   **30**, 101–112

559   Hewezi, T. and Baum, T.J. (2013) Manipulation of Plant Cells by Cyst and Root-Knot

560   Nematode Effectors. *Molecular Plant-Microbe Interactions*, **26**, 9–16.

561   Holbein, J., Franke, R.B., Marhavý, P., Fujita, S., Górecka, M., Sobczak, M., et al. (2019)

562   Root endodermal barrier system contributes to defence against plant‐parasitic cyst and

563   root‐knot nematodes. *The Plant Journal*, **100**, 221–236.

564   Huang, G.Z., Allen, R., Davis, E.L., Baum, T.J., and Hussey, R.S. (2006) Engineering broad

565   root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential

566   root-knot nematode parasitism gene. *Proceedings of the National Academy of Sciences of*

567   *the United States of America*, **103**, 14302–14306.

568   Jaouannet, M., Nguyen, C.-N., Quentin, M., Jaubert-Possamai, S., Rosso, M.-N., and Favery,

569   B. (2018) In situ Hybridization (ISH) in Preparasitic and Parasitic Stages of the Plant-

570   parasitic Nematode Meloidogyne spp. *Bio-Protocol*, **8**, 1–13.

571   Jones, J.T., Haegeman, A., Danchin, E.G.J.J., Gaur, H.S., Helder, J., Jones, M.G.K.K., et al.

572   (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant*

573   *Pathology*, **14**, 946–961.

574   Karssen, G. and Moens, M. (1983) Root-knot nematodes. In: *Plant nematology* , pp. 59–90.

575   Wallingford: CABI.

576   Kiewnick, S., Dessimoz, M., and Franck, L. (2009) Effects of the Mi-1 and the N root-knot

577   nematode-resistance gene on infection and reproduction of Meloidogyne enterolobii on

578   tomato and pepper cultivars. *Journal of nematology*, **41**, 134–9.

579   Koutsovoulos, G.D., Poullet, M., Elashry, A., Kozlowski, D.K.L., Sallet, E., Da Rocha, M., et

580   al. (2020) Genome assembly and annotation of Meloidogyne enterolobii, an emerging

581   parthenogenetic root-knot nematode. *Scientific Data*, **7**, 324.

582   Landry, D., González‑Fuente, M., Deslandes, L., and Peeters, N. (2020) The large, diverse,

583   and robust arsenal of Ralstonia solanacearum type III effectors and their in planta

584   functions. *Molecular Plant Pathology*, **21**, 1377–1388.

585   Lange, M., Yellina, A.L., Orashakova, S., and Becker, A. (2013) Virus-Induced Gene

586   Silencing (VIGS) in Plants: An Overview of Target Species and the Virus-Derived

587   Vector Systems. In: *Methods in Molecular Biology* , pp. 1–14. Humana Press Inc.

588   Langner, T., Kamoun, S., and Belhaj, K. (2018) CRISPR Crops: Plant Genome Editing

589   Toward Disease Resistance. *Annual Review of Phytopathology*, **56**, 479–512.

590   Lin, B., Zhuo, K., Chen, S., Hu, L., Sun, L., Wang, X., et al. (2016) A novel nematode

591   effector suppresses plant immunity by activating host reactive oxygen species-

592   scavenging system. *New Phytologist*, **209**, 1159–1173.

593   Lin, B., Zhuo, K., Wu, P., Cui, R., Zhang, L.-H., and Liao, J. (2013) A novel effector protein,

594   MJ-NULG1a, targeted to giant cell nuclei plays a role in Meloidogyne javanica

595 parasitism. *Molecular plant-microbe interactions*, **26**, 55–66.

596 Liu, D., Shi, L., Han, C., Yu, J., Li, D., and Zhang, Y. (2012) Validation of Reference Genes

597 for Gene Expression Studies in Virus-Infected Nicotiana benthamiana Using

598 Quantitative Real-Time PCR. *PLoS ONE*, **7**, e46451.

599 Lunt, D.H., Kumar, S., Koutsovoulos, G., and Blaxter, M.L. (2014) The complex hybrid

600 origins of the root knot nematodes revealed through comparative genomics. *PeerJ*, **2**,

601 e356.

602 Mantelin, S., Bellafiore, S., and Kyndt, T. (2017) Meloidogyne graminicola□: a major threat

603 to rice agriculture. *Molecular Plant Pathology*, **18**, 3–15.

604 Mejias, J., Bazin, J., Truong, N., Chen, Y., Marteu, N., Bouteiller, N., et al. (2020) The

605 root‐knot nematode effector MiEFF18 interacts with the plant core spliceosomal

606 protein SmD1 required for giant cell formation. *New Phytologist*, **in press**, doi:

607 10.1111/nph.17089.

608 Mejias, J., Truong, N.M., Abad, P., Favery, B., and Quentin, M. (2019) Plant Proteins and

609 Processes Targeted by Parasitic Nematode Effectors. *Frontiers in Plant Science*, **10**, 970.

610 Mitchum, M.G., Hussey, R.S., Baum, T.J., Wang, X., Elling, A.A., Wubben, M., and Davis,

611 E.L. (2013) Nematode effector proteins: an emerging paradigm of parasitism. *New*

612 *Phytologist*, **199**, 879–894.

613 Mitchum, M.G., Wang, X., Wang, J., and Davis, E.L. (2012) Role of Nematode Peptides and

614 Other Small Molecules in Plant Parasitism. *Annual Review of Phytopathology*, **50**, 175–

615 195.

616 Muhire, B.M., Varsani, A., and Martin, D.P. (2014) SDT: A Virus Classification Tool Based

617 on Pairwise Sequence Alignment and Identity Calculation. *PLoS ONE*, **9**, e108277.

618 Nguyen, C.-N., Perfus-Barbeoch, L., Quentin, M., Zhao, J., Magliano, M., Marteu, N., et al.

619 (2018) A root-knot nematode small glycine and cysteine-rich secreted effector,

620   MiSGCR1, is involved in plant parasitism. *New Phytologist*, **217**, 687–699.

621   O'Connell, R.J. and Panstruga, R. (2006) Tete a tete inside a plant cell: establishing

622   compatibility between plants and biotrophic fungi and oomycetes. *New Phytologist*, **171**,

623   699–718.

624   Olmo, R., Cabrera, J., Díaz-Manzano, F.E., Ruiz-Ferrer, V., Barcala, M., Ishida, T., et al.

625   (2020) Root‑knot nematodes induce gall formation by recruiting developmental

626   pathways of post‑embryonic organogenesis and regeneration to promote transient

627   pluripotency. *New Phytologist*, **227**, 200–215.

628   Opperman, C.H., Bird, D.M., Williamson, V.M., Rokhsar, D.S., Burke, M., Cohn, J., et al.

629   (2008) Sequence and genetic map of Meloidogyne hapla: A compact nematode genome

630   for plant parasitism. *Proc Natl Acad Sci U S A*, **105**, 14802–14807.

631   P. T. Y. Dinh (2015) Plant-nematode interactions and the application of RNA interference for

632   controlling root-knot nematodes. *PhD*, **3**, 54–67.

633   Peeters, N., Carrère, S., Anisimova, M., Plener, L., Cazalé, A.-C., and Genin, S. (2013)

634   Repertoire, unified nomenclature and evolution of the Type III effector gene set in the

635   Ralstonia solanacearum species complex. *BMC Genomics*, **14**, 859.

636   Quentin, M., Abad, P., and Favery, B. (2013) Plant parasitic nematode effectors target host

637   defense and nuclear functions to establish feeding cells. *Frontiers in Plant Science*, **4**, 53.

638   Rancurel, C., van Tran, T., Elie, C., and Hilliou, F. (2019) SATQPCR: Website for statistical

639   analysis of real-time quantitative PCR data. *Molecular and Cellular Probes*, **46**, 101418.

640   Rodiuc, N., Vieira, P., Banora, M.Y., and de Almeida Engler, J. (2014) On the track of

641   transfer cell formation by specialized plant-parasitic nematodes. *Frontiers in Plant*

642   *Science*, **5**, 1–14.

643   Roux, B., Bolot, S., Guy, E., Denancé, N., Lautier, M., Jardinaud, M.-F., et al. (2015)

644   Genomics and transcriptomics of Xanthomonas campestris species challenge the concept

645   of core type III effectome. *BMC Genomics*, **16**, 975.

646   Rutter, W.B., Hewezi, T., Abubucker, S., Maier, T.R., Huang, G., Mitreva, M., et al. (2014)

647   Mining Novel Effector Proteins from the Esophageal Gland Cells of Meloidogyne

648   incognita. *Molecular plant-microbe interactions*, **27**, 965–974.

649   van Schie, C.C.N. and Takken, F.L.W. (2014) Susceptibility Genes 101: How to Be a Good

650   Host. *Annual Review of Phytopathology*, **52**, 551–581.

651   Schmitt-Keichinger, C. (2019) Manipulating Cellular Factors to Combat Viruses: A Case

652   Study From the Plant Eukaryotic Translation Initiation Factors eIF4. *Frontiers in*

653   *Microbiology*, **10**:17. doi: 10.3389/fmicb.2019.00017

654   Singh, S.K., Hodda, M., and Ash, G.J. (2013) Plant-parasitic nematodes of potential

655   phytosanitary importance, their main hosts and reported yield losses. *EPPO Bulletin*, **43**,

656   334–374.

657   Somvanshi, V.S., Tathode, M., Shukla, R.N., and Rao, U. (2018) Nematode Genome

658   Announcement: A Draft Genome for Rice Root-Knot Nematode, Meloidogyne

659   graminicola. *Journal of Nematology*, **50**, 111–116.

660   Susič, N., Koutsovoulos, G.D., Riccio, C., Danchin, E.G.J., Blaxter, M.L., Lunt, D.H., et al.

661   (2020) Genome sequence of the root-knot nematode Meloidogyne luci. *Journal of*

662   *Nematology*, **52**, e2020-25.

663   Tomalova, I., Iachia, C., Mulet, K., and Castagnone-Sereno, P. (2012) The map-1 gene family

664   in root-knot nematodes, Meloidogyne spp.: a set of taxonomically restricted genes

665   specific to clonal species. *PloS one*, **7**, e38656.

666   Toruño, T.Y., Stergiopoulos, I., and Coaker, G. (2016) Plant-Pathogen Effectors: Cellular

667   Probes Interfering with Plant Defenses in Spatial and Temporal Manners. *Annual Review*

668   *of Phytopathology*, **54**, 419–441.

669   Truong, N.M., Nguyen, C.-N., Abad, P., Quentin, M., and Favery, B. (2015) Function of

670   Root-Knot Nematode Effectors and Their Targets in Plant Parasitism. In: *Elsevier*

671   (Escobar,C. and Fenoll,C., eds) , pp. 293–324. Academic Press.

672   Velasquez, A., Chakravarthy, S., and Martin, G.B. (2009) Virus-induced Gene Silencing

673   (VIGS) in Nicotiana benthamiana and Tomato. *Journal of Visualized Experiments*, 28:

674   1292. doi: 10.3791/1292.

675   Wang, X., Xue, B., Dai, J., Qin, X., Liu, L., Chi, Y., et al. (2018) A novel Meloidogyne

676   incognita chorismate mutase effector suppresses plant immunity by manipulating the

677   salicylic acid pathway and functions mainly during the early stages of nematode

678   parasitism. *Plant Pathology*, **67**, 1436–1448.

679   Yamaguchi, Y.L., Suzuki, R., Cabrera, J., Nakagami, S., Sagara, T., Ejima, C., et al. (2017)

680   Root-Knot and Cyst Nematodes Activate Procambium-Associated Genes in Arabidopsis

681   Roots. *Frontiers in Plant Science*, **8**, 1195.

682   Yang, Y., Jittayasothorn, Y., Chronis, D., Wang, X., Cousins, P., and Zhong, G.-Y. (2013)

683   Molecular characteristics and efficacy of 16D10 siRNAs in inhibiting root-knot

684   nematode infection in transgenic grape hairy roots. *PloS one*, **8**, e69463.

685   Zhang, L., Davies, L.J., and Elling, A. a (2015) A Meloidogyne incognita effector is imported

686   into the nucleus and exhibits transcriptional activation activity in planta. *Molecular Plant*

687   *Pathology*, **16**, 48–60.

688

689   **Figure legends**

690

691   **Figure 1** Effector 18 (EFF18) is a conserved effector in root-knot nematodes. (a)

692   Phylogenetic tree and schematic diagram of root-knot nematode EFF18 protein sequences.

693   The tree scale corresponds to the number of substitutions per site based on the amino-acid

694   matrix (JTT). In the schematic diagram of EFF18 proteins, the predicted secretion signal

695   peptide (SP; grey boxes), the aspartic acid and glutamic acid (D-E)-rich region (red boxes),

696   the lysine (K)–rich C-terminal region (blue boxes) and the nuclear mono- (purple boxes) or

697   bi- (orange boxes) partite localisation signals (NLS) are shown. EFF18 proteins from the

698   closest group to MiEFF18a carry one mono- and one bipartite NLS, whereas the most

699   divergent copies have only a single monopartite NLS. (b) MiEFF18 localised to the nucleus

700   and nucleolus of plant cells. The MiEFF18 sequence was fused to that encoding GFP in an N-

701   or C-terminal position and expressed in *N. benthamiana* leaves by agroinfiltration. GFP was

702   used as a control and gave fluorescence in the cytoplasm and the nucleus (n), but not the

703   nucleolus (arrowhead). Bars = 10 µm. (c) Pairwise sequence identity matrix for RKN EFF18

704   protein sequences.

705

706   **Figure 2** RKN EFF18s are specifically expressed in the subventral glands. *In situ*

707   hybridisation, showing EFF18 transcripts in the subventral glands of J2s of *M. incognita*, *M.*

708   *enterolobii* and *M. arenaria*, respectively. A sense probe for the MeEFF18 transcript was used

709   as a negative control. SvG, subventral glands. Bar = 50 µm.

710

711   **Figure 3** Conserved SmD1 proteins are targeted by EFF18. (a) MAFFT protein sequence

712   alignment of the *S. lycopersicum* (Sl), *N. benthamiana* (Nb) and *A. thaliana* (At) SmD1

713   proteins. (b) Schematic representation of Sm1 and Sm2 motif in SmD1 proteins. (c) GFP-

714   AtSmD1b and GFP-SlSmD1a accumulate in the nucleus and particularly in the nucleolus

715   when transiently expressed in *N. benthamiana* epidermal leaf cells. GFP was used as a

716   nucleocytoplasmic control. n= nucleoplasm; Black arrows show nucleolus. Bars = 5 µm. (d)

717   Pairwise yeast two-hybrid assays showed that the MiEFF18 and MeEFF18 proteins were able

718   to interact with the SmD1 proteins of *A. thaliana*, *S. lycopersicum* and *N. benthamiana*. We

719   used MiEFF18 and MiEFF16 as a positive and negative control, respectively. Diploid yeasts

720   containing the bait and prey plasmids carrying controls, effectors or SmD1 were serially

721   diluted and spotted on plates. The 10-2 dilution is shown. SD-WL corresponds to the non-

722   selective medium without tryptophan (W) and leucine (L). Only yeasts carrying a protein-

723   protein interaction can survive on the SD-WLH (H, histidine) + 0.5 mM 3-aminotriazole (3-

724   AT) selective medium.

725

726   **Figure 4** The silencing of SmD1 genes by VIGS affects susceptibility to *M. incognita* in *S.*

727   *lycopersicum.* (a) Timeline used for the VIGS experiments in S. lycopersicum. (b) RT-qPCR

728   demonstrating the effective silencing of *SlSmD1* in TRV-SlSmD1 line when compared to the

729   control TRV-GFP. RPN7 was used for data normalisation. (c) Infection test on tomato plants

730   in which SmD1 was silenced (TRV-SlSmD1) and control tomato plants (TRV-GFP). Females

731   producing egg masses were counted seven weeks after inoculation with 150 *M. incognita* J2s

732   per plant. Statistical significance was determined on the basis of three independent biological

733   replicates, by Kruskal-Wallis tests. Clearly significant differences were observed between

734   TRV-GFP control and TRV-SmD1 plants (*p ≤ 0.05).

735

736   **Figure 5** The silencing of *SmD1* genes by VIGS affects susceptibility to *M. incognita* in *N.*

737   *benthamiana.* (a) Timeline used for VIGS experiment in *N. benthamiana.* (b) *N. benthamiana*

738   plants with silenced SmD1 genes (TRV-SmD1, right panel) and TRV2-empty control plants

739   (TRV-empty, left panel), showing some developmental defects of the leaves (upper panel)

740   and a shorter root system (lower panel). Red arrow point-out galls on these pictures. (c) RT-

741   qPCR showing that the NbSmD1b gene, the most strongly expressed and closest orthologue

742   to the SlSmD1 gene, was effectively silenced. The data shown are the means of three

743   independent biological replicates. EF1a and actin were used for data normalisation. (d)

744   Infection test on *N. benthamiana* control plants (TRV-empty) and plants in which SmD1 was

745   silenced (TRV-SlSmD1). Galls were counted and root weight was measured two weeks after

746   inoculation with 200 *M. incognita* J2s per plant. Statistical significance was determined on the

747   basis of three independent biological replicates, by Kruskal-Wallis tests. Clear significant

748   differences were found between the TRV-GFP control and the TRV-SmD1 plants (*p ≤ 0.05;

749   **p ≤ 0.01).

750

751   **Figure 6** *SmD1* plays an important role in the formation of giant cells. (a) The filiform

752   J2s/swollen juveniles (Js) ratio obtained by fuchsine acid staining in the *N. benthamiana* root

753   system with (TRV-SmD1) and without (TRV-empty) silencing with the TRV-SlSmD1

754   construct, following infection with *M. incognita*. (b) Galls of negative control plants and

755   plants with SmD1 silencing collected two weeks post infection for measurement of the area

756   of giant cells (dotted line) by the BABB clearing method (Cabrera et al., 2018). The biggest

757   giant cell measured is shown by a surrounding dashed white line. Bar = 100 µm. (c) Box-and-

758   whisker plot of giant cell size (µm2) measurements (n = 32 and 26 galls).

759

760   **Supporting information**

761

762   **Figure S1** Identified EFF18 sequences in RKN species.

763   **Figure S2** EFF18 is a conserved RKN effector.

764   **Figure S3** Tomato phenotypes associated with VIGS of *SlSmD1* genes.

765   **Figure S4** Semi-quantitative RT-PCR expression analysis of *NbSmD1s* in *N. benthamiana*

766   roots VIGS experiments.

767   **Table S1** Sequences used in this study and accession numbers

768   **Table S2** Primers used in this study

(a) Tree scale: 0.01

MhEFF18
MaEFF18b
MjEFF18a
MjEFF18c
MfEFF18
MiEFF18a
MaEFF18a
MlucEFF18a
MeEFF18a
MeEFF18b
MaEFF18c
MaEFF18d
MjEFF18b
MlucEFF18c
MiEFF18b
MiEFF18c
MlucEFF18b

Signal peptide
D/E-rich domain
K-rich domain
Monopartite NLS motif
Bipartite NLS motif

(b)
MiEFF18a-GFP

GFP-MiEFF18a

GFP-MeEFF18a

GFP

(c) Pairwise identity (%)

100
97
94
91
88
85
82
79
76
73
70

M. incognita	*M. arenaria*
antisense	antisense

M. enterolobii

| antisense | sense |

(a)

		Sm1 motif			Sm2 motif

NbSmD1a/c
NbSmD1b
SlSmD1a/b
AtSmD1a
AtSmD1b

NbSmD1a/c   91
NbSmD1b   91
SlSmD1a/b   91
AtSmD1a   91
AtSmD1b   91

(b) SmD1 proteins

Sm1 motif   Sm2 motif

(c)

GFP-AtSmD1b   GFP-SlSmD1a   GFP

(d)

	MiEFF16	MiEFF18a		MeEFF18a		
	NbSmD1b	NbSmD1b	SlSmD1	SlSmD1	NbSmD1b	AtSmD1b
SD-WL						
SD-WHL + 3AT						

(a) timeline: *S. lycopersicum* germination — 16°C — 24°C — female with egg masses; VIGS; 150 Mi J2s; 10 days; 4 weeks; 2 weeks; 5 weeks

(b) Relative SlSmD1 expression; S1, S6, S17, S21, S25, S30 TRV-SlSmD1; G3, G7, G13, G15, G19, G8 TRV-GFP

(c) TRV-GFP / TRV-SlSmD1; females producing egg masses per plant; experiment 1 (n=20, n=21); experiment 2 (n=20, n=18)

(a) filiform J2s ▪ swollen Js

swollen J

filiform J2

TRV-empty
n=238

TRV-SmD1
n=232

(b) TRV-empty

TRV-SmD1

(c) size of the giant cells (μm²)

**

TRV-empty
n=32

TRV-SmD1
n=26