

## Systemic root-shoot signaling drives jasmonate-based root defense against nematodes

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1 **Systemic root-shoot signaling drives**  
2 **jasmonate-based root defense against nematodes**

3  
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## SUMMARY

Shoot-root communication is crucial for plant adaptation to environmental changes. However, the extensive crosstalk between shoots and roots that controls the synthesis of jasmonates (JAs), in order to enhance defense responses against rhizosphere herbivores, remains poorly understood. Here, we report that the root-knot nematode (RKN) *Meloidogyne incognita* induced the systemic transmission of electrical and reactive oxygen species (ROS) signals from attacked tomato roots to the leaves leading to an increased accumulation of JAs in the leaves. Grafting of 1.0 cm stem sections from mutants lacking *GLUTAMATE RECEPTOR-LIKE 3.5* or the mutants deficient in *RESPIRATORY BURST OXIDASE HOMOLOG 1* abolished the RKN-induced electrical signals and associated ROS and JAs accumulation in the upper stems and leaves with attenuated resistance to RKN. Furthermore, the absence of systemic transmission of electrical and ROS signals compromised the activation of mitogen-activated protein kinases (MPK) 1/2 in leaves. Silencing *MPK1* or *MPK2* abolished RKN-induced accumulation of JAs and associated resistance. These findings reveal a systemic signaling loop that integrates electrical, ROS and JAs signals to enhance the resistance in distal organs via root-shoot-root communication.

## INTRODUCTION

Shoot and root processes are intimately interconnected through long-distance communication pathways that allow appropriate whole plant growth and resource allocation, as well as defense responses [1, 2]. Shoot-root communication is dependent on the vascular system for the transport of RNAs, peptides, phytohormones etc [3-7]. In addition, long-distance signal transmission also involves other systems including ROS, Ca<sup>2+</sup> and electrical signals around the vascular cells [8-12]. Adaptation to abiotic stresses such as high light, salt, nutrient deficiency, cold and water deficits, and to biotic threats such as pathogens and herbivores, as well as mutualistic and symbiotic microorganisms is largely achieved through the mediation of phytohormones [13-15]. Plants frequently accumulate jasmonates (JAs) in response to herbivores, leading to the induction

53 of defence responses [16]. JAs are formed from  $\alpha$ -linolenic acid in the chloroplast membranes  
54 via a light-regulated biosynthetic pathway. In spite of the absence of chloroplasts, the root  
55 system accumulates JAs in response to nematode attack [16, 17]. However, the mechanisms that  
56 lead to JA accumulation in roots are unknown [18]. Here, focusing on shoot-root communication  
57 in nematode resistance, we examined the role of a systemic signal transmission loop by which  
58 JAs biosynthesis in the leaves is linked to resistance in the roots. We show that nematode attack  
59 induced the systemic transmission of electrical signals and that together with ROS, these ‘SOS’  
60 signals serve to activate JAs synthesis in systemic leaves. This leads to increased JAs  
61 accumulation in roots and enhanced resistance to nematodes.

62

## 63 **RESULTS**

64

### 65 **Shoot JAs synthesis contributes to plant resistance against root nematodes**

66 JAs play a critical role in plant defenses against herbivores [19]. Inoculation with the root-knot  
67 nematode *Meloidogyne incognita* (RKN) at a density of 1000 infective second stage juveniles  
68 (J2s) per plant induced a significant increase in the accumulation of JA and JA-isoleucine  
69 (JA-Ile, an active form of JA in the defence response) in the leaves at 24 hours post inoculation  
70 (hpi) (Figure 1A). Such an increase in the accumulation of JA and JA-Ile in either the roots or  
71 the leaves was largely attenuated in the JA biosynthesis defective mutant, *suppressor of*  
72 *prosystemin-mediated responses2* (*spr2*) (Figure S1A) [20, 21]. To determine the respective  
73 contributions of JA synthesis in shoots and roots to nematode resistance, wild type (WT) plants  
74 at the 3-leaf stage were reciprocally grafted with *spr2* as scion or rootstock, respectively.  
75 Compared to the plants with the WT as scion (WT/WT and WT/*spr2*), plants with *spr2* as scion  
76 (*spr2*/WT, *spr2*/*spr2*) showed decreased resistance to nematode infestation, as demonstrated by  
77 the increased number of galls on the roots relative to WT/WT and WT/*spr2* at 28 days post  
78 inoculation (dpi) (Figure 1B). Interestingly, no significant differences in the resistance of  
79 WT/WT and WT/*spr2* plants were observed. Similarly, there were no significant differences in  
80 infestation between the *spr2*/WT and *spr2*/*spr2* plants. The lower resistance observed in the  
81 *spr2*/WT and *spr2*/*spr2* plants was in agreement with the lower JA accumulation observed in  
82 both the leaves or the roots of these lines relative to the WT/WT and WT/*spr2* plants (Figure

83 S1B). Therefore, the basal resistance of roots against the RKN is largely dependent on JAs  
84 synthesis in shoots, but not in roots.

85

## 86 **Nematode attack induces a systemic transmission of electrical and ROS** 87 **signals**

88 We next examined whether RKN infection induced JAs synthesis in leaves and whether leaf JAs  
89 synthesis was linked to systemic changes in electrical and ROS signals transmitted from roots to  
90 leaves. RKN induced an increase in the accumulation of JA and JA-Ile in both the leaves and  
91 roots, particularly at 24 hpi (Figure S1C). A 48 h continuous recording revealed that RKN induced  
92 intermittent changes in the surface potential of stems, petioles and leaf lamina and the  
93 cytoplasmic potential in the leaf cells of all plants with intervals of minutes to hours (n=6, Figure  
94 1C). When the surface potential of the stems was recorded for a duration of 20 min,  
95 RKN-induced changes in the surface potential were not observed in every plant at 3 hpi, 6 hpi,  
96 12 hpi and 24 hpi (Figures S1D and Table S1), suggesting that the random attack from RKN  
97 induced discontinuous and irregular changes in the electrical pulses. At 24 hpi, RKN infestation  
98 induced potential changes on the stem with a frequency of  $2.42 \pm 1.88$ , an amplitude of  
99  $-5.34 \pm 2.16$  mV and a duration of  $27.2 \pm 5.54$  seconds for each pulse during the 20 min recording  
100 (Figure 1D). While pulse duration decreased from the stems to the leaves, no significant  
101 differences in pulse frequency or amplitude were observed.

102 Histochemical analysis with DAB staining revealed that RKN infection, which was shown by  
103 using acid fuchsin staining (Figures 1E1 and S1E1), induced an accumulation of H<sub>2</sub>O<sub>2</sub> in the  
104 vascular systems of roots, stems and petioles (Figures 1E2-4 and S1E2-4). Quantitation of DAB  
105 staining intensity showed RKN-induced H<sub>2</sub>O<sub>2</sub> accumulation was highest at 24 hpi and decreased  
106 from the roots to the petioles (Figure S1F). Consistent with this finding, RKN induced the  
107 greatest accumulation of H<sub>2</sub>O<sub>2</sub> in the leaves at 24 hpi (Figure S1G). Subcellular localization  
108 studies using CeCl<sub>3</sub> showed H<sub>2</sub>O<sub>2</sub> accumulated in the apoplast of the leaf cells as a result of RKN  
109 attack (Figures 1E5 and S1E5). In addition, this increase in H<sub>2</sub>O<sub>2</sub> accumulation was associated  
110 with an increase in the activity of NADPH oxidase in the leaves (Figure S1H). Therefore, RKN  
111 infestation in the roots induced a systemic transmission of electrical and ROS signals to the  
112 leaves, as has also been observed in the systemic transmission of light signals from the shoots to  
113 the roots [22].

114

115 **GLR-dependent electrical activity is critical for leaf JAs synthesis and related**  
116 **defenses**

117 *GLUTAMATE RECEPTOR-LIKE (GLR)* genes encode putative cation channels that are  
118 responsible for electrical activity and can influence JA signaling [9]. Using virus-induced gene  
119 silencing (VIGS) approaches, plants were produced that were silenced for either *SIGLR3.3*  
120 (pTRV-*GLR3.3*) or *SIGLR3.5* (pTRV-*GLR3.5*). These are the analogues of *GLR3.3* and *GLR3.6*  
121 in Arabidopsis, which have roles in wound signaling [9, 23]. qRT-PCR showed that the  
122 expression of *GLR3.3* and *GLR3.5* was reduced by 70~80% in the pTRV-*GLR3.3* and  
123 pTRV-*GLR3.5* plants, respectively (Figure S2A). Importantly, pTRV-*GLR3.3* and  
124 pTRV-*GLR3.5* plants both showed significantly lower resistance to RKN, together with  
125 decreased JA accumulation in the leaves (Figures S2B and S2C). To explore the role of *GLRs* in  
126 systemic signal transmission from roots to leaves, we sought to generate CRISPR/Cas9 *glr3.3*  
127 and *glr3.5* mutants. However, only the *glr3.5* mutation was successful, which carries a 4-bp  
128 deletion in the open reading frame (ORF) resulting in the premature termination of the protein  
129 translation. Grafted plants produced between the WT and *glr3.5* lines, as rootstock or scion,  
130 respectively, were inoculated with RKN at the 4-leaf stage. The plants with *glr3.5* as rootstock or  
131 scion (WT/*glr3.5*, *glr3.5*/WT and *glr3.5*/*glr3.5*) showed decreased electrical activity, as  
132 demonstrated by the decreased pulse amplitude and duration of surface potentials on the scion  
133 stems at 24 hpi, together with reduced resistance against RKN relative to self-grafted WT plants  
134 (Figures 2A, S2D and Table S2). In addition, RKN-induced accumulation of JA and JA-Ile in the  
135 leaves and roots was attenuated (Figure S2E). Interestingly, when a segment of the *glr3.5* stem  
136 (ca. 1.0 cm in length) was inserted into the WT stem between the cotyledons and the 1<sup>st</sup> true leaf  
137 (WT/*glr3.5*/WT) of the graft, there was a significant decrease in the resistance to RKN.  
138 Meanwhile, it attenuated RKN-induced changes in electrical pulse amplitude and duration at 24  
139 hpi and decreased the accumulation of JA and JA-Ile in the leaves and roots relative to  
140 self-grafted WT plants (WT/WT/WT) (Figures 2B-2E). Other experiments showed that artificial  
141 current injection on the stem surface (20  $\mu$ A for 2 min with 10 min interval, for 60 or 10 cycles)  
142 significantly decreased the number of RKN galls and increased the accumulation of JAs in the  
143 plants (Figures S2F and S2G). These results strongly suggest that activation of *GLR3.5* in both

144 the shoots and roots is essential for the activation of JAs synthesis in leaves and subsequent RKN  
145 resistance in the roots.

146

### 147 **RBOH1-dependent ROS production is important in the regulation of leaf JAs** 148 **synthesis and RKN resistance**

149 RKN may induce H<sub>2</sub>O<sub>2</sub> accumulation in the leaf apoplast via a systemic induction of the activity  
150 of NADPH oxidase, which is encoded by the *Respiratory Burst Oxidase Homolog (RBOH)*  
151 genes. qRT-PCR analysis revealed that of the 8 *RBOHs* in the plants, *RBOH1* was the most  
152 highly expressed (Figure S3A). We generated CRISPR/Cas9 *rboh1* mutant (containing a T  
153 insertion in the *RBOH* ORF to generate a premature stop codon TGA ) and produced reciprocally  
154 grafted plants, which were then exposed to RKN. Compared to the WT/WT plants, plants with  
155 *rboh1* as scion (*rboh1*/WT, *rboh1*/*rboh1*) or rootstock (WT/*rboh1*) showed decreased resistance  
156 to nematode infestation, as demonstrated by the increased number of galls on the roots relative to  
157 WT/WT at 28 dpi (Figure 3A). Histochemical analysis using DAB staining, followed by  
158 quantification of staining intensity revealed that RKN induced H<sub>2</sub>O<sub>2</sub> accumulation in the vascular  
159 system throughout the stems of the WT/WT plants (Figures S3B and S3C). However, no  
160 substantial increases in H<sub>2</sub>O<sub>2</sub> accumulation were observed in the stems of *rboh1*/*rboh1* plants in  
161 response to RKN attack. Interestingly, the RKN infection induced accumulation of H<sub>2</sub>O<sub>2</sub> only in  
162 the rootstock stems but not scion stems of the *rboh1*/WT plants. In addition, H<sub>2</sub>O<sub>2</sub> accumulation  
163 in the apoplast and/or in the leaf tissues was abolished in plants with *rboh1* as the rootstock or  
164 scion, together with the loss of induction of NADPH oxidase activity in the leaves (Figures  
165 S3D-S3F). Furthermore, RKN-induced accumulation of JA and JA-Ile in the leaves or roots was  
166 abolished in plants with *rboh1* as rootstock or scion (Figure S3G). Crucially, when a segment of  
167 *rboh1* stem (ca. 1.0 cm in length) was inserted into the WT stem between the cotyledons and the  
168 1<sup>st</sup> true leaf (WT/*rboh1*/WT), the graft significantly reduced resistance to RKN infestation and  
169 compromised RKN-induced accumulations of H<sub>2</sub>O<sub>2</sub> in the stem above the *rboh1* graft (Figures  
170 3B, 3C and S3H). Moreover, H<sub>2</sub>O<sub>2</sub> accumulation was not observed in the apoplast of the leaf  
171 cells and the leaf tissues above the *rboh1* graft (Figures 3D and S3I). Similarly, JA and JA-Ile  
172 accumulation was not observed in the leaves or roots (Figure 3E). In agreement with a putative  
173 role for H<sub>2</sub>O<sub>2</sub> as a signal for the induction of JA synthesis, the foliar application of H<sub>2</sub>O<sub>2</sub> induced  
174 JA accumulation in the leaves (Figure S3J). Maximal effects of H<sub>2</sub>O<sub>2</sub> were observed at a



175 concentration of 1 mM. We conclude that a cell to cell activation of H<sub>2</sub>O<sub>2</sub> production from the  
176 roots to the leaves is essential for the induction of JAs production in the leaves, together with  
177 JAs-mediated resistance to RKN in the tomato roots.

178

### 179 **Crosstalk between cytoplasmic electrical activity and ROS production is** 180 **intrinsic to long-distance signal transmission**

181 The evidence presented above suggests that the activation of either electrical signals or H<sub>2</sub>O<sub>2</sub>  
182 production is critical for the systemic induction of JAs synthesis in leaves and the associated  
183 induction of resistance. To test this further, we examined the relationship between electrical  
184 activity and H<sub>2</sub>O<sub>2</sub> signaling in the plant systemic response to RKN infestation. We found  
185 RKN-induced increases in NADPH oxidase activity in the leaves were compromised in plants  
186 co-silenced for *GLR3.3* and *GLR3.5* (pTRV-*GLR3.3/3.5*) (Figure S4A). In addition, the  
187 RKN-induced accumulation of H<sub>2</sub>O<sub>2</sub> in the leaf tissues and in the apoplast of the leaves or in the  
188 stems was attenuated in the grafted plants with *glr3.5* as rootstock or scion (Figures S4B -S4E).  
189 Crucially, we found that RKN infestation induced H<sub>2</sub>O<sub>2</sub> accumulation in the WT rootstock stems  
190 but not in the *glr3.5* stem segments or the WT scion stems of the WT/*glr3.5*/WT plants (Figures  
191 4A and S4F). Moreover, H<sub>2</sub>O<sub>2</sub> accumulation was not induced in the apoplast of the leaves or in  
192 the whole leaves in response to RKN infestation in the shoots of the WT/*glr3.5*/WT plants  
193 (Figures 4B and S4G). Conversely, the grafted plants with *rboh1* as rootstock or scion, or those  
194 with an inserted *rboh1* segment showed attenuated RKN-induced electrical activity with  
195 decreased pulse amplitude and duration (Figures 4C, 4D, S4H and Table S3). To further  
196 characterize the relationship between electrical activity and H<sub>2</sub>O<sub>2</sub> production, we applied current  
197 injection (at 20 μA for 2 min with an interval of 10 min) to the stems. This treatment induced  
198 resistance, and accumulation of H<sub>2</sub>O<sub>2</sub> in the the vascular system of the shoots, together with an  
199 accumulation of H<sub>2</sub>O<sub>2</sub> in the apoplast of the leaves of the WT plants, but this was not observed  
200 in the *glr3.5* or *rboh1* plants (Figures 4E, 4F, S4I and S4J). These results strongly suggest that  
201 there is an inter-dependency between *GLR3.5* and *RBOH1*-mediated processes in the continuous  
202 transmission of signals from roots to leaves in order to activate JAs biosynthesis.

203

### 204 **Redox-dependent activation of MPK1/2 is involved in the induction of JAs** 205 **synthesis**



206 MPKs play important roles in the regulation of JA synthesis through effects on the early steps of  
207 the biosynthetic pathway. Moreover, MPK1/2 activation is subject to RBOH-dependent redox  
208 regulation [24, 25]. RKNs induced MPK1/2 activation from 3~6 hpi and MPK1/2 activation  
209 reached a peak at 24 hpi (Figure 5A). This finding is in agreement with the point of highest  
210 accumulation of JAs in WT plants. While RKN infection induced the activation of MPK1/2 in  
211 the leaves of WT/WT/WT plants, this activation was, however, attenuated in the leaves of the  
212 WT/*rboh1*/WT plants and the WT/*glr3.5*/WT plants (Figures 5B and 5C). Moreover, 10 cycles  
213 of current injection with a electrical activity similar to RKN-induced electrical activity (at 10  $\mu$ A  
214 for 30 s with an interval of 9 min) was sufficient to activate MPK1/2 in WT leaves (Figure 5D).  
215 Again, current injection-induced activation of MPK1/2 was significantly attenuated in the leaves  
216 of the *rboh1* and *glr3.5* mutants (Figure 5E). We next examined whether MPK1/2-dependent  
217 pathways are involved in the regulation of JAs synthesis in relation to RKN resistance. Using  
218 independent silencing of each gene, as well as co-silencing of *MPK1* and *MPK2*, we found that  
219 suppressed expression of either *MPK1* (pTRV-*MPK1*) or *MPK2* (pTRV-*MPK2*) or both  
220 (pTRV-*MPK1/2*) was accompanied by an increased susceptibility to RKN infestation in the roots  
221 (Figures 5F and S5A). In addition, the roots of the pTRV-*MPK1/2* plants were more susceptible  
222 to RKN infection, as demonstrated by the increased number of galls on the roots, than either the  
223 pTRV-*MPK1* or the pTRV-*MPK2* plants. Meanwhile, down-regulation of *MPK1* or *MPK2*  
224 expression compromised RKN-induced accumulation of JA and JA-Ile in the leaves and roots  
225 (Figures 5G and S5B). Consistent with an earlier study [24], MPK1/2-induced changes in the  
226 abundance of transcript of several key JA-related genes (*LOXD*, *AOS*, *AOC* and *OPR3*) were not  
227 substantial (Figure S5C). The observed small differences are unlikely to be sufficient to induce  
228 large differences in JA accumulation.

229

## 230 **DISCUSSION**

231

232 The data presented here demonstrate the existence of a novel systemic signaling pathway that  
233 enables rapid communication between the aboveground and underground parts of the plant to  
234 induce defenses against nematode attack. We present a proof of the presence of extensive  
235 reciprocal crosstalk in the systemic transmission of electrical and redox signals from roots to  
236 leaves in response to the perception of RKN attack. The results also demonstrate that MPK1/2

237 activation is intrinsic to this signaling pathway that leads to increased JAs synthesis in the leaves.  
238 Directional transport of JAs produced in response to these signals occurs from the shoots to roots  
239 leading to the activation of appropriate defense responses to increase resistance against nematode  
240 attack (Figure 5H).

241 Prior to this study, the general consensus of opinion was that local resistance was determined  
242 by the capacity of phytohormone synthesis, leading to an accumulation of salicylic acid (SA) and  
243 JA in attacked tissues [26]. The data presented here demonstrates that the local ability to produce  
244 JAs in the roots alone is insufficient to induce an effective defence against RKN infestation.  
245 Moreover, these findings reveal an important and previously unrecognized role for other organs  
246 particularly leaves in enabling root resistance through intensive and continuous shoot-root  
247 communication pathways. This systemic signaling pathway is distinct from the known systemic  
248 acquired resistance (SAR) or systemic acquired acclimation (SAA) responses. Our findings  
249 regarding RKN-induced systemic transmission of electrical activity and ROS signals are in  
250 agreement with previous reports demonstrating the presence of electrical and ROS signaling  
251 pathways in the distal activation of key pathways required for the stress responses [27, 28].  
252 However, the intermittent and mild attack from RKN induced a larger number of electrical  
253 pulses but with less amplitude and shorter duration than those induced by wounding or  
254 herbivores [9]. Importantly, the series of grafting experiments reported here provide strong  
255 evidence for the propagation characteristics of electrical signaling and ROS regeneration  
256 responses. These findings are consistent with the concept of stimuli-induced waves of  $\text{Ca}^{2+}$ , ROS  
257 and electrical signaling in systemic communication as suggested by other researchers [8, 22, 27].  
258 We present the first genetic evidence in support of this concept by demonstrating an  
259 interdependency between ROS production and electrical activity in the elicitation of appropriate  
260 RKN defences in the roots of tomato plants.

261 Our data demonstrate the involvement of multiple-signaling pathways in the transmission of  
262 systemic signals between roots and shoots. These findings support the consensus view that plants  
263 orchestrate effective specific responses to perceived threats through a repertoire of signaling  
264 pathways including electrical, ROS,  $\text{Ca}^{2+}$  and phytohormone-based processes[28]. Wounding  
265 triggers the long-distance transmission of  $[\text{Ca}^{2+}]_{\text{cyt}}$  increases and systemic defense responses,  
266 which are *GLRs*-dependent [12]. Consistent with the roles of the vascular system in the  
267 transmission of electrical signaling and of  $\text{Ca}^{2+}$  in the activation of NADPH oxidase [29, 10], the

268 data presented here show that the discontinuous induction of electrical signaling is accompanied  
269 by continuous increase in the accumulation of H<sub>2</sub>O<sub>2</sub> due to the auto-propagating characteristics  
270 of H<sub>2</sub>O<sub>2</sub> production and subsequent activation of MPK1/2 in response to RKNs [28, 25].  
271 Therefore, the crosstalk between electrical, ROS and Ca<sup>2+</sup> signaling pathways is pivotal to the  
272 systemic transmission of signals from local tissue to distant tissues to activate MPK-dependent  
273 JA biosynthesis [24]. JA and SA are the two major players in plant defense responses to pests,  
274 such as herbivores and necrotrophic and biotrophic pathogens [30, 31]. They are often  
275 considered to function antagonistically in such defense responses [32]. Within this context, our  
276 results showing that increased JAs accumulation in the leaves of RKN-infested plants, indicate  
277 that altered resistance to susceptibility to root invasion may be highly dependent on defense  
278 responses in the leaves, through the mediation of systemic signaling pathways. Crosstalk  
279 between aboveground and belowground organs not only regulates physiological processes but  
280 also alters many rhizosphere processes with ecological significance [33-36]. A general ecological  
281 theory may need to be developed to explain why plants involve their shoots in root defenses and  
282 why they enhance leaf-resistance upon contact with root-feeding insects and soil-dwelling  
283 microorganisms. Future studies are required to establish whether such systemic signaling  
284 pathways are a wide spread phenomenon in the plant kingdom and whether roots respond in a  
285 similar manner to threats to the shoots by herbivores and pathogens. However, given the greater  
286 availability of carbon and nitrogen substrates, together with other resources in leaves compared  
287 to roots, it may be logical that shoot pathways are induced as parts of the triage strategy that  
288 prevents invasion of the roots. While further research is required to identify shoot–root and  
289 root–shoot signals, the present demonstration of effective communication between roots and  
290 shoots to prevent or limit RKN infestation offers potential applications for improved plant  
291 protection.

292

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294

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302

## 303 **AUTHOR CONTRIBUTIONS**

304

305 Conceptualization, J.Q.Y., Y.H.Z. and C.H.F.; Methodology, J.Q.Y., G.T.W. and C.Y.H.; Formal  
306 Analysis, K.S. and X.J.X.; Investigation, G.T.W, C.Y.H, J.Z, Y.L. and J.X.C.; Resources, Y.W.,  
307 C.Z.P. and X.D.W.; Writing – Original Draft, J.Q.Y.; Writing – Review & Editing, J.Q.Y., Y.H.Z.  
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309 Acquisition, J.Q.Y. and Y.H.Z.;

310

## 311 **DECLARATION OF INTERESTS**

312

313 The authors declare no competing interests.

314

## 315 **MAIN-TEXT FIGURE/TABLE LEGENDS**

316

317 **Figure 1. *Meloidogyne incognita* infection induces systemic transmission of electrical and**  
318 **H<sub>2</sub>O<sub>2</sub> signals leading to increased JAs accumulation**

319 (A) *Meloidogyne incognita* (RKN) infection induces accumulation of JAs in the leaves at 24 hpi.

320 (B) Shoot JAs biosynthesis contributes to the resistance of RKN.

321 (C) Typical surface potential changes on stems, petioles and leaf lamina and cytoplasmic potential  
322 changes in leaf cells after RKN infection.

323 (D) Potential characteristics of RKN infection induced systemic transmission of electrical signals  
324 from the roots to the leaves at 24 hpi.

325 (E) RKN infection (E1) induces systemic accumulation of H<sub>2</sub>O<sub>2</sub> from the roots (E2) to stems (E3),  
326 petioles (E4) and leaves (E5) at 24 hpi.

327 For (A), JAs were determined with four biological samples. For (B), resistance against RKN was  
328 determined at 28 dpi and data are the means of three replicates with 15 plants for each replicate

329 ( $\pm$ SD). For (D), ‘n’ is the total number of plants examined and ‘x’ is the number of plants with  
330 detectable potential changes. For (E), acid fuchsin staining was used for E1, DAB staining was  
331 used for E2~4 and CeCl<sub>3</sub> staining was used for E5. Arrows indicate the accumulation of H<sub>2</sub>O<sub>2</sub> in  
332 E5. Means denoted by the same letter did not significantly differ at  $p < 0.05$  according to Tukey’s  
333 test. See also [Figure S1](#) and [Table S1](#).

334  
335 **Figure 2. GLR3.5-dependent electrical signaling is essential for JAs biosynthesis and**  
336 **nematode resistance**

337 (A) *Meloidogyne incognita* (RKN) resistance in grafted plants with *glr3.5* as rootstock or scion.  
338 (B) RKN resistance in grafted plants inserted with *glr3.5* segment.  
339 (C) Typical surface potential changes on the scion stems in grafted plants inserted with *glr3.5*  
340 segment at 24 hpi.  
341 (D) Surface potential characteristics on the scion stems in grafted plants inserted with *glr3.5*  
342 segment at 24 hpi.  
343 (E) Attenuated accumulation of JAs in grafted plants inserted with *glr3.5* segment at 24 hpi.  
344 For (A and B), resistance against RKN was determined at 28 dpi and data are the means of three  
345 replicates with 15 plants for each replicate ( $\pm$ SD). For (D), ‘n’ is the total number of plants  
346 examined and ‘x’ is the number of plants with detectable potential changes. For (E), four  
347 biological samples were used for the determination of JAs. Means denoted by the same letter did  
348 not significantly differ at  $p < 0.05$  according to Tukey’s test. See also [Figure S2](#) and [Table S2](#).

349  
350 **Figure 3. ROS are essential for JAs biosynthesis and nematode resistance**

351 (A) *Meloidogyne incognita* (RKN) resistance in grafted plants with *rboh1* as rootstock or scion.  
352 (B) RKN resistance in grafted plants inserted with *rboh1* segment.  
353 (C) H<sub>2</sub>O<sub>2</sub> accumulation in the stems determined with DAB staining at 24 hpi.  
354 (D) H<sub>2</sub>O<sub>2</sub> accumulation in the apoplast of leaves determined with CeCl<sub>3</sub> staining at 24 hpi.  
355 (E) Accumulation of JAs in grafted plants inserted with *rboh1* segment at 24 hpi.  
356 For (A and B), resistance against RKN was determined at 28 dpi and data are the means of three  
357 replicates with 15 plants for each replicate ( $\pm$ SD). For (C), S: scion; IS: inserted segment; R:  
358 rootstock. For (D), arrows indicate the accumulation of H<sub>2</sub>O<sub>2</sub>. For (E), four biological samples

359 were used for the determination of JAs. Means denoted by the same letter did not significantly  
360 differ at  $p < 0.05$  according to Tukey's test. See also [Figure S3](#).

361  
362 **Figure 4. Interdependency of ROS and electrical signals in systemic message transmission**  
363 (A)  $H_2O_2$  accumulation on the stem in grafted plants determined with DAB staining at 24 hpi.  
364 (B)  $H_2O_2$  accumulation in the apoplast of leaves in grafted plants determined with  $CeCl_3$  staining  
365 at 24 hpi.  
366 (C) Typical surface potential changes on the scion stems in grafted plant at 24 hpi.  
367 (D) Surface potential characteristics on the scion stems in grafted plants at 24 hpi.  
368 (E) Current injection (CI, at 20  $\mu A$  for 2 min with an interval of 10 min for 60 cycles) induced  
369 changes in the nematode resistance.  
370 (F) Current injection (CI, at 20  $\mu A$  for 2 min with an interval of 10 min for 10 cycles) induced  
371 accumulation of  $H_2O_2$  in the apoplast of leaves determined with  $CeCl_3$  staining.  
372 For (A), S:scion; IS: inserted segment; R: rootstock. For (B and F), arrows indicate the  
373 accumulation of  $H_2O_2$ . For (D), 'n' is the total number of plants examined and 'x' is the number of  
374 plants with detectable potential changes. For (E), resistance against the nematode was determined  
375 at 28 dpi and data are the means of three replicates with 15 plants for each replicate ( $\pm SD$ ). For (F),  
376 leaf samples were taken after the current injection. Means denoted by the same letter did not  
377 significantly differ at  $p < 0.05$  according to Tukey's test. See also [Figure S4](#) and [Table S3](#).

378  
379 **Figure 5. Activation of MPK1/2 is involved in JAs biosynthesis and nematode resistance**  
380 (A) Time course of RKN-induced activation of MPK1/2.  
381 (B) MPK1/2 activation in the leaves of grafted plants inserted with *rboh1* segment.  
382 (C) MPK1/2 activation in the leaves of grafted plants inserted with *glr3.5* segment.  
383 (D) MPK1/2 activation in the leaves of wild type plants after different cycles of current injection  
384 (CI, at 10  $\mu A$  for 30 s with an interval of 9 min).  
385 (E) MPK1/2 activation in the leaves after current injection (20  $\mu A$  for 2 min with 10 min interval  
386 for 10 cycles) in the wild type plants and mutants.  
387 (F) Nematode resistance in *MPK1/2*-silenced plants.  
388 (G) Accumulation of JAs in leaves in *MPK1/2*-silenced plants.  
389 (H) A model for the basal resistance by shoot-root communication.

390 For (B, C and G), samples were taken at 24 hpi. For (D and E), samples were taken after the  
391 current injection. For (A-E), the protein loading was shown by Ponceau staining. For (F),  
392 resistance against the nematode was determined at 28 dpi and data are the means of three replicates  
393 with 15 plants for each replicate ( $\pm$ SD). For (G), four biological samples were used for the  
394 determination of JAs. For (H), ES: electrical signaling; ROS: reactive oxygen species; MPKs:  
395 mitogen-activated protein kinases; JAs: jasmonates. Means denoted by the same letter did not  
396 significantly differ at  $p < 0.05$  according to Tukey's test. See also [Figure S5](#).

397

## 398 **STAR★Methods**

399

## 400 **LEAD CONTACT AND MATERIALS AVAILABILITY**

401

402 Transgenic tomato plants generated in this study are available on request. Requests for reagents  
403 should be directed to and will be fulfilled by the Lead Contact, Jingquan Yu ([jqyu@zju.edu.cn](mailto:jqyu@zju.edu.cn)). This  
404 study did not generate new unique reagents.

405

## 406 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

407

408 Wild-type tomato (*Solanum lycopersicum* L. cv. 'Ailsa Craig', 'Castlemart', 'Condine Red'),  
409 and *spr2* mutants in the Castlemart background were used. *RBOH1* CRISPR/Cas9 vector  
410 and *GLR3.5* CRISPR/Cas9 vector were constructed as described by Pan *et al.* [37]. The ta  
411 rget sequence (ACGTCGGATACGGTGTCTTC) for *RBOH1* and the target sequence (TAG  
412 CAGATCAGCTGGCCAAG) for *GLR3.5* were designed using a web tool of CRISPR-P [3  
413 8]. The synthesized sequences were annealed and inserted into *BbsI* site of AtU6-sgRNA-A  
414 tUBQ-Cas9 vector, and the AtU6-sgRNA-AtUBQ-Cas9 cassette was inserted into the *HindI*  
415 II and *KpnI* sites of pCAMBIA1301 binary vector. The resulting plasmids were transforme  
416 d into *Agrobacterium tumefaciens* strain EHA105, and then introduced into tomato of Cond  
417 ine Red and Ailsa Craig respectively [39]. CRISPR/Cas9-induced mutations were genotyped  
418 by PCR amplification and DNA sequencing. Cas9-free T2 homozygotes with mutation we  
419 re identified for further experiments. Virus-induced gene silencing (VIGS) was used for sil



encing the target genes with the tobacco rattle virus (TRV)-based vectors (pTRV1/2) [40]. Sequences of primer pairs used for VIGS lines were: *GLR3.3* forward, 5'-CCGgaattcATGAATGTGGTTTGGATTAT-3'; reverse, 5'-AGCggatccTACTGCAACAACATCAGTCT-3'. *GLR3.5* forward, 5'-CCGgaattcCCAATCCAGATGTTCTTGGA-3'; reverse, 5'-AGCggatccATTTCAGCTATAGCTTCCAT-3'. *MPK1* forward, 5'-GGCCGtctagaATAATTGCTGACAGATTGTT-3'; reverse, 5'-CGCGCggatccCATTTTCAGTCTAAAATAAAA-3'. *MPK2* forward, 5'-GGCCGtctagaGTACTCGCTCGTTTGCTGTTG-3'; reverse, 5'-CGCGCggatccAGCAGAAAAAATT-3'. *MPK1/2* forward, 5'-GGCGCgagctcCATGGTGGCAGGTTTCATTC-3'; reverse, 5'-CGGCgctcGCTCAGGTGGACGATACCAT-3'. The cDNA fragments of target genes were PCR-amplified and the amplified fragments were digested and ligated into the corresponding sites of the pTRV2 vector. Empty pTRV2 vector was used as a control. All constructs were confirmed by sequencing and subsequently transformed into *Agrobacterium tumefaciens* strain GV3101. VIGS was performed by infiltration of germinated seeds, followed by infiltration into the fully expanded cotyledons of 8-d-old tomato seedlings with *A. tumefaciens* harboring a mixture of pTRV1 and pTRV2-target gene in a 1:1 ratio. Plants were grown at 23/21°C (day/night) in a growth chamber with a 12 h day length for 30 d, and qRT-PCR was performed to determine the gene silencing efficiency [41]. Tomato seeds were sown in pots with a mixture of sand and vermiculite (v: v=1:1), receiving Hoagland's nutrient solution. The growth conditions were as follows: 12 h photoperiod, temperature of 25/20 °C (day/night), and photosynthetic photo flux density (PPFD) of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

## METHOD DETAILS

### Grafting experiment

To determine the respective role of *SPR2*, *GLR3.5* and *RBOH1* expression in the shoots and roots in the nematode resistance and JAs biosynthesis, shoots of wild type (WT), *spr2*, *glr3.5* and *rboh1* plants at 3-leaf stage were self-grafted or reciprocally grafted onto rootstocks of WT, *spr2*, *glr3.5* and *rboh1*, respectively, which resulted in three lines of grafted plants: 1), WT/WT, *spr2/spr2*, *spr2*/WT and WT/*spr2*; 2), WT/WT, *rboh1/rboh1*, *rboh1*/WT and WT/*rboh1*; 3), WT/WT, *glr3.5/glr3.5*, *glr3.5*/WT and WT/*glr3.5*. Meanwhile, WT plant was grafted by inserting a 1 cm stem segment from WT or *rboh1* or *glr3.5* plants into the WT stem between cotyledons and 1<sup>st</sup> true

451 leaf, which resulted in two lines of grafted plants: 1) WT/WT/WT, WT/*glr3.5*/WT; 2)  
452 WT/WT/WT, WT/*rboh1*/WT. After adaptation under dark for 3 days, the grafted plants were  
453 gradually exposed to light up to a PPFD of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at temperatures of 25/20 °C.

454

#### 455 **Root-knot nematode infection and resistance assay**

456 The root-knot nematode was cultured on tomato plants grown with sand and vermiculite (v:v=1:1)  
457 at 22-26 °C in a greenhouse. Nematodes were extracted from 3-month-old infected plants. Briefly,  
458 eggs were extracted from infected roots by processing in 0.5% NaClO in a Warring blender, for 2  
459 min at high speed [42]. Eggs and root debris were passed through 80, 200, 325-mesh sieves in turn  
460 and the eggs were collected on 500-mesh sieve. The second stage juveniles (J2s) were obtained by  
461 hatching the eggs in a petri dish with eight layers of paper towels. The dish was incubated at 28 °C  
462 and J2s were collected after 2 days and used immediately. J2 nematode number in the solution was  
463 determined under a microscope (BX61; Olympus Co., Tokyo, Japan). Tomato plants at the  
464 four-leaf stage were inoculated with 1000 J2s of *M. incognita* per plant in 5 ml of water applied  
465 with a pipette over the surface of the growth media around the primary roots. Later, plants were  
466 maintained in a growth chamber with the growth conditions as follows: 12 h photoperiod,  
467 temperature of 25/20 °C (day/night), and PPFD of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After 4 weeks, the roots of  
468 plants were washed off all the growth substrates. The fresh root weights of plants were measured.  
469 Nematode susceptibility of the plants was evaluated by counting the number of galls per plant and  
470 calculating the number of galls  $\text{g}^{-1}$  fresh root weight [43]. Nematode colonization was detected by  
471 staining the roots with 3.5% acid fuchsin [44].

472

#### 473 **Pharmacological treatments**

474 To determine the effects of  $\text{H}_2\text{O}_2$  on the biosynthesis of JA,  $\text{H}_2\text{O}_2$  was foliar applied onto leaves at  
475 a concentration of 0~10 mM. Leaves were taken 1d after the application of  $\text{H}_2\text{O}_2$ .

476

#### 477 **Electric potential recordings and current injection**

478 For the determination of surface potential recordings, silver electrodes (0.5 mm in diameter, World  
479 Precision Instruments, USA) were chloridized with 0.1 M HCl before their usage. The  
480 electrode–plant (stem or petiole) interface was a drop (10  $\mu\text{l}$ ) of 1M KCl in 1% (w/v) agar placed  
481 to avoid direct contact with plant cells and damage the cuticle. The ground electrode was placed in

482 the soil [45]. The glass microelectrodes with a tip diameter approximately 0.5  $\mu\text{m}$  for intracellular  
483 cytoplasmic potential measurements were prepared from the borosilicate glass capillaries with an  
484 outer diameter of 1.0 mm and an inner diameter of 0.58 mm (Hilgenberg GmbH, Germany).  
485 Pulling was performed after heating with a PE-2 vertical micropipette puller (Narishige Co.,  
486 Tokyo, Japan). Microelectrodes were filled with 1M KCl, and inserted into the mesophyll cells of  
487 a leaf. The reference electrode was immersed into artificial pond water (APW, composed of 5 mM  
488 MES, 0.5 mM  $\text{CaSO}_4$ , 0.05 mM KCl, pH 6.0) where the leaf was also submerged [46]. Both  
489 electrodes were connected to a differential amplifier. Potentials were detected at 3~24 h post  
490 inoculation. Two 2-channel amplifiers (FD 223 and Duo 773, World Precision Instruments, USA)  
491 were simultaneously used to record the potential at stem, petiole and lamina. Frequency is the  
492 times with the changes in potential within 20 minutes. Amplitude is potential difference relative to  
493 the baseline before the changes. Duration is the length of time for each amplitude change. 'n' is the  
494 total number of plants observed and 'x' is the number of plants with detectable potential changes.  
495 For current injection two platinum wire electrodes (Qiushi Electric Co., Hangzhou, China, 0.1mm  
496 diameter) were circled around the stems with 1 cm apart one day before the current injection was  
497 applied. Current injection was applied at 20  $\mu\text{A}$  for 2 min with an interval of 10 min for 10 or 60  
498 cycles for biochemical analysis and resistance assay respectively, unless other described. Control  
499 plants were circled with Pt wires in all current injection experiments.

500

### 501 Measurement of JAs Levels

502 Extraction and quantification of JAs were performed using previously reported procedures with  
503 minor modifications [47]. Briefly, 100 mg of frozen leaf or root material was homogenized in 1  
504 mL of ethyl acetate which had been spiked with D6-JA (OlChemIm Ltd., Czechoslovakia) and  
505 D6-JA-Ile (QUALITY CONTROL CHEMICALS INC., USA) as internal standards with a final  
506 concentration of 100  $\text{ng mL}^{-1}$  and 40  $\text{ng mL}^{-1}$ , respectively. The samples were shook at 180 rpm  
507 in the dark at 4 °C for 12 h and then centrifuged at 18,000 g for 10 min at 4°C. The pellet was  
508 re-extracted with 1 mL of ethyl acetate. Both supernatants were combined and evaporated to  
509 dryness under  $\text{N}_2$ . The residue was re-suspended in 0.5 ml of 70% methanol (v/v) and centrifuged.  
510 The supernatants were then analyzed in a liquid chromatography tandem mass spectrometry  
511 system (Varian 320-MS LC/MS, Agilent Technologies, Amstelveen, the Netherlands). LC analysis  
512 was performed using an Agilent Zorbax XDB C18 column (150 mm  $\times$  2.1 mm, 3.5  $\mu\text{m}$ ). The

513 mobile phase consisted of a mixture of solvent A (0.1% formic acid in water; E. Merck, Darmstadt,  
514 Germany) and solvent B (methanol; E. Merck) at a flow rate of 0.3 ml min<sup>-1</sup> with the following  
515 gradient: 0-1.5 min, A: B at 60: 40; followed by 6.5 min solvent A: B at 0: 100; subsequently  
516 returning to solvent A: B to 60: 40 for 5 min until the end of the run. The column temperature was  
517 kept at 40 °C, and the injection volume was 20 µL. A negative electrospray ionization mode was  
518 used for detection. The JAs were detected in MRM mode by monitoring the transitions 209.1 >  
519 59.1 for JA; 214.3 > 62.1 for D6-JA; 322.0 > 130.0 for JA-Ile; 328.5 > 130.1 for D6-JA-Ile.

520

### 521 **Quantification, histochemical analysis, and cytochemical detection of H<sub>2</sub>O<sub>2</sub>**

522 The concentration of H<sub>2</sub>O<sub>2</sub> in leaves was measured by monitoring the absorbance of the  
523 titanium-peroxide complex at 415 nm using the method of Brennan and Frenkel [48]. The  
524 histochemical staining of H<sub>2</sub>O<sub>2</sub> was performed by using DAB staining as previously [49]. Stems  
525 and petioles were cut into 0.5 mm thick sections. The intensity of DAB staining in the vascular  
526 systems of roots, stems and petioles was quantificated with Image-Pro Plus 6.0 (Media  
527 Cybernetics, Inc., USA) [50]. H<sub>2</sub>O<sub>2</sub> in the leaves was visualized at the subcellular level using  
528 CeCl<sub>3</sub> for localization [51]. Electron-dense CeCl<sub>3</sub> deposits are formed in the presence of H<sub>2</sub>O<sub>2</sub> and  
529 are visible by transmission electron microscopy at an accelerating voltage of 75 kV (H7650;  
530 Hitachi, Tokyo, Japan). The concentration of H<sub>2</sub>O<sub>2</sub> in leaves was measured by monitoring the  
531 absorbance of the titanium-peroxide complex at 415 nm [52].

532

### 533 **Isolation of plasma membrane and the determination of NADPH oxidase activity**

534 Isolation of plasma membrane and the determination of NADPH oxidase activity were carried out  
535 as described previously [53]. Briefly, leaf samples were homogenized in four volumes of the  
536 extraction buffer (50 mM Tris-HCl, pH 7.5, 0.25 M Suc, 1 mM ascorbic acid (AsA), 1 mM EDTA,  
537 0.6% PVP, and 1 mM PMSF). The homogenate was filtered through four layers of cheesecloth,  
538 and the resulting filtrate was centrifuged at 10,000 g for 15 min. Microsomal membranes were  
539 pelleted from the supernatant by centrifugation at 50, 000 g for 30 min. The pellet was suspended  
540 in 0.33 M Suc, 3 mM KCl, and 5 mM potassium phosphate, pH 7.8. The plasma membrane  
541 fraction was isolated by adding the microsomal suspension to an aqueous two-phase polymer  
542 system to give a final composition of 6.2% (w/w) Dextran T500, 6.2% (w/w) polyethylene glycol  
543 3350, 0.33 M Suc, 3 mM KCl, and 5 mM potassium phosphate, pH 7.8. Three successive rounds

544 of partitioning yielded the final upper phase. The upper phase produced was diluted 5-fold in  
545 Tris-HCl dilution buffer (10 mM, pH 7.4) containing 0.25 M Suc, 1 mM EDTA, 1 mM DTT, 1  
546 mM AsA, and 1 mM PMSF. The fractions were centrifuged at 120, 000 g for 30 min. The pellets  
547 were then resuspended in Tris-HCl dilution buffer and used immediately for further analysis. All  
548 procedures were carried out at 4 °C. Protein content of plasma membranes was determined with  
549 BSA as standard [54]. The NADPH-dependent  $O_2^{\cdot-}$  generating activity in isolated plasma  
550 membrane vesicles was determined by following the reduction of XTT by  $O_2^{\cdot-}$ . The assay mixture  
551 of 1 mL contained 50 mM Tris-HCl buffer (pH 7.5), 0.5 mM XTT, 100  $\mu$ M NADPH and 15–20  $\mu$ g  
552 of membrane proteins. The reaction was initiated with the addition of NADPH, and XTT reduction  
553 was determined at 470 nm. Corrections were made for background production in the presence of  
554 50 units SOD. Rates of  $O_2^{\cdot-}$  generation were calculated using an extinction coefficient of  $2.16 \times 10^4$   
555  $M^{-1} \text{ cm}^{-1}$ .

556

#### 557 **MPK1/2 activation assay**

558 For the determination of activated MPK1 and MPK2, the frozen leaf tissue (0.3 g) was ground in  
559 liquid nitrogen in 1 ml of extraction buffer. The extracts were centrifuged at 12000 g for 20 min at  
560 4 °C. Protein content was determined with BSA as standard and total protein was separated by  
561 SDS-PAGE and blotted onto nitrocellulose membranes (Millipore, Saint-Quentin, France) [55].  
562 Immunoblots were blocked in TBS buffer containing 5% (w/v) BSA (Sigma) for 1h at room  
563 temperature and then incubated overnight in 1% (w/v) BSA (Sigma) in TBS buffer containing the  
564 anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/tyr204)(D13.12.4E)XP Rabbit mAb (Cell Signaling  
565 Technology, Boston, USA) as primary antibody, which recognizes both MPK1 and MPK2 [25].  
566 After, immunoblots were incubated for 1.5 h with HRP (horseradish peroxidase) linked antibody  
567 (Cell Signaling Technology, Boston, USA) as secondary antibody. The complexes on the blot  
568 were visualized using an enhanced chemiluminescence kit (Fdbio, Hangzhou, China), following  
569 the manufacturer's instructions. Rubisco was as loading control. The assay was replicated three  
570 times with independent biological samples.

571

#### 572 **qRT-PCR analysis**

573 Total RNA was extracted from leaf tissues using RNA simple Total RNA Kit (TIANGEN,  
574 Beijing, China) according to the instructions. Total RNA (0.5  $\mu$ g) was reverse transcribed

575 to cDNA using HiScript II Q RT SuperMix for qPCR (Vazyme, Nanjing, China). qRT-P  
576 CR was performed using a Light Cycler 480 II Real-Time PCR detection system (Roche).  
577 Each reaction consisted of 10 µl qPCR SYBR Green Master Mix, 1 µl cDNA, and forward  
578 and reverse primers at 0.1 µM according to the instructions of qPCR SYBR Green Master  
579 Mix (Vazyme, Nanjing, China). The housekeeping gene *SIACTIN2* was used as internal  
580 reference to calculate the relative expression of target genes [41]. Sequences of primer pairs:  
581 *ACTIN2* forward 5'-TGTCCCTATTTACGAGGGTTATGC-3' and reverse 5'-CAGTTA  
582 AATCACGACCAGCAAGAT-3'; *GLR3.3* forward 5'-ATGTGGGATTGCATGCTTTA-3' and  
583 reverse 5'-CTGACCATCCGAATCAACTG-3'; *GLR3.5* forward 5'-GGCTTTCTGGAATAG  
584 CTTGC-3' and reverse 5'-TGCCAACCCACATAGAAAGA-3'; *MPK1* for pTRV-*MPK1* and  
585 pTRV-*MPK2* plants forward 5'-TCGTCCACCTGAGCTGTTGTT-3' and reverse 5'-ACAT  
586 GCGGGAACCTTTTCAGT-3'; *MPK2* for pTRV-*MPK1* and pTRV-*MPK2* plants forward 5'-  
587 AGGGTTTACTATTTACGG-3' and reverse 5'-TGGAGGCTTATACTTCG-3'; *MPK1* for pTRV-  
588 *MPK1/2* plants forward 5'-GCTGACAGATTGTTGCAGGT-3' and reverse 5'-TCCACC  
589 CCATAAAGATACATCA-3'; *MPK2* for pTRV-*MPK1/2* plants forward 5'-TACTCGCTCGT  
590 TTGCTGTTG-3' and reverse 5'-TTGGAGTACAGGAAAACAATGG-3'; *RBOHA* forward  
591 5'-TACATGCCACGGATGAGGAA-3' and reverse 5'-CATCACAACACCGGTCCATC-3'; *RBOHB*  
592 forward 5'-TTATCGGCCTTAGTGCGTCT-3' and reverse 5'-CCGTTTGATTTGGTG  
593 CTTGC-3'; *RBOHC* forward 5'-TGAGCCACAGTACGCCTTTA-3' and reverse 5'-TAGCA  
594 AGCAACCACAGCAAG-3'; *RBOHD* forward 5'-CAGGTCAAGCGTCAAGGATG-3' and reverse  
595 5'-TGCAGCACAGTTGACAAACA-3'; *RBOHE* forward 5'-AGCAACTTCGACTACC  
596 ACCA-3' and reverse 5'-GCCTGTTACACCTGGAATGG-3'; *RBOHF* forward 5'-TGCTTG  
597 GCAACTGCTAAAGG-3' and reverse 5'-GGCCCTAGTAGACCGTAACC-3'; *RBOHI* forward  
598 5'-TCCAGCACAAGATTACCG-3' and reverse 5'-CCTCCATTGCGACGAT-3'; *RBOHH*  
599 forward 5'-CCACGGCTGCTTCATATTCC-3' and reverse 5'-CGTGGTAGCGGTTCTCATT  
600 G-3'; *AOC (ALLENE OXIDE CYCLASE)* forward 5'-CCGTTTCAGGGAGCGTACTTA-3' and  
601 reverse 5'-ACCGCCGTACACAACAATTC-3'; *AOS (ALLENE OXIDE SYNTHASE)* forward  
602 5'-GATCCTCCGGTAGCTTCACA-3' and reverse 5'-TTCTTCTCCGACGAACCGAT-3'; *L*  
603 *OXD (LIPOXYGENASE D)* forward 5'-TGTGCCACTGGTAACTGGAT-3' and reverse 5'-  
604 TCCAAGCTTGCATGTGTACG-3'; *OPR3 (12-OXO-PHYTODIENOIC ACID REDUCTASE)*

605 forward 5'-ATAGGAGCTGATCGCGTAGG-3' and reverse 5'-TAGGCAAGCTTGGAACCA  
606 GA-3'.

607

## 608 **QUANTIFICATION AND STATISTICAL ANALYSIS**

609

### 610 **Image quantification**

611 The intensity of DAB staining was quantificated with Image-Pro Plus 6.0.

612

### 613 **Statistical analysis**

614 A completely randomized block design with three replicates was used for the nematode resistance  
615 assay in each experiment. Each replicate involved 15 plants. For the measurements, four biological  
616 samples were used. Data were statistically analyzed by analysis of variance (ANOVA). The  
617 significance of treatment differences was analyzed using Tukey's test ( $p < 0.05$ ). Means denoted  
618 by the same letter in the figure did not significantly differ at  $p < 0.05$ . All of the statistical  
619 parameters of experiments can be found in the figure legends, figures and tables.

620

## 621 **DATA AND CODE AVAILABILITY**

622 This study did not generate/analyze any datasets/code.

623

## 624 **REFERENCES**

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