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

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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^{2+} 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

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

RESULTS

Shoot JAs synthesis contributes to plant resistance against root nematodes

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

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

Nematode attack induces a systemic transmission of electrical and ROS signals

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

Histochemical analysis with DAB staining revealed that RKN infection，which was shown by using acid fuchsin staining (Figures 1E1 and S1E1), induced an accumulation of H_2O_2 in the vascular systems of roots, stems and petioles (Figures 1E2-4 and S1E2-4). Quantitation of DAB staining intensity showed RKN-induced H_2O_2 accumulation was highest at 24 hpi and decreased from the roots to the petioles (Figure S1F). Consistent with this finding, RKN induced the greatest accumulation of H_2O_2 in the leaves at 24 hpi (Figure S1G). Subcellular localization studies using $CeCl_3$ showed H_2O_2 accumulated in the apoplast of the leaf cells as a result of RKN attack (Figures 1E5 and S1E5). In addition, this increase in H_2O_2 accumulation was associated with an increase in the activity of NADPH oxidase in the leaves (Figure S1H). Therefore, RKN infestation in the roots induced a systemic transmission of electrical and ROS signals to the leaves, as has also been observed in the systemic transmission of light signals from the shoots to the roots [22].

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

GLUTAMATE RECEPTOR-LIKE (*GLR)* genes encode putative cation channels that are responsible for electrical activity and can influence JA signaling [9]. Using virus-induced gene silencing (VIGS) approaches, plants were produced that were silenced for either *SlGLR3.3* (pTRV-*GLR3.3*) or *SlGLR3.5* (pTRV-*GLR3.5*). These are the analogues of *GLR3.3* and *GLR3.6* in Arabidopsis, which have roles in wound signaling [9, 23]. qRT-PCR showed that the expression of *GLR3.3* and *GLR3.5* was reduced by 70~80% in the pTRV-*GLR3.3* and pTRV-*GLR3.5* plants, respectively (Figure S2A). Importantly, pTRV-*GLR3.3* and pTRV-*GLR3.5* plants both showed significantly lower resistance to RKN, together with decreased JA accumulation in the leaves (Figures S2B and S2C). To explore the role of *GLRs* in systemic signal transmission from roots to leaves, we sought to generate CRISPR/Cas9 *glr3.3* and *glr3.5* mutants. However, only the *glr3.5* mutation was successful, which carries a 4-bp deletion in the open reading frame (ORF) resulting in the premature termination of the protein translation. Grafted plants produced between the WT and *glr3.5* lines, as rootstock or scion, respectively, were inoculated with RKN at the 4-leaf stage. The plants with *glr3.5* as rootstock or scion (WT/*glr3.5, glr3.5*/WT and *glr3.5*/*glr3.5*) showed decreased electrical activity, as demonstrated by the decreased pulse amplitude and duration of surface potentials on the scion stems at 24 hpi, together with reduced resistance against RKN relative to self-grafted WT plants (Figures 2A, S2D and Table S2). In addition, RKN-induced accumulation of JA and JA-Ile in the leaves and roots was attenuated (Figure S2E). Interestingly, when a segment of the *glr3.5* stem (ca. 1.0 cm in length) was inserted into the WT stem between the cotyledons and the 1st true leaf (WT/*glr3.5*/WT) of the graft, there was a significant decrease in the resistance to RKN. Meanwhile, it attenuated RKN-induced changes in electrical pulse amplitude and duration at 24 hpi and decreased the accumulation of JA and JA-Ile in the leaves and roots relative to self-grafted WT plants (WT/WT/WT) (Figures 2B-2E). Other experiments showed that artificial current injection on the stem surface (20 μA for 2 min with 10 min interval, for 60 or 10 cycles) significantly decreased the number of RKN galls and increased the accumultation of JAs in the plants (Figures S2F and S2G). These results strongly suggest that activation of *GLR3.5* in both

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

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

RKN may induce H_2O_2 accumulation in the leaf apoplast via a systemic induction of the activity of NADPH oxidase, which is encoded by the *Respiratory Burst Oxidase Homolog*（*RBOH*） genes. qRT-PCR analysis revealed that of the 8 *RBOHs* in the plants, *RBOH1* was the most highly expressed (Figure S3A). We generated CRISPR/Cas9 *rboh1* mutant (containing a T insertion in the *RBOH* ORF to generate a premature stop codon TGA) and produced reciprocally grafted plants, which were then exposed to RKN. Compared to the WT/WT plants, plants with *rboh1* as scion (*rboh1*/WT, *rboh1*/*rboh1*) or rootstock (WT/*rboh1*) showed decreased resistance to nematode infestation, as demonstrated by the increased number of galls on the roots relative to WT/WT at 28 dpi (Figure 3A). Histochemical analysis using DAB staining, followed by quantification of staining intensity revealed that RKN induced H_2O_2 accumuation in the vascular system throughout the stems of the WT/WT plants (Figures S3B and S3C). However, no substantial increases in H_2O_2 accumulation were observed in the stems of *rboh1*/*rboh1* plants in response to RKN attack. Interestingly, the RKN infection induced accumulation of H_2O_2 only in the rootstock stems but not scion stems of the *rboh1*/WT plants. In addition, H_2O_2 accumulation in the apoplast and/or in the leaf tissues was abolished in plants with *rboh1* as the rootstock or scion, together with the loss of induction of NADPH oxidase activity in the leaves (Figures S3D-S3F). Furthermore, RKN-induced accumulation of JA and JA-Ile in the leaves or roots was abolished in plants with *rboh1* as rootstock or scion (Figure S3G). Crucially, when a segment of *rboh1* stem (ca. 1.0 cm in length) was inserted into the WT stem between the cotyledons and the 1st true leaf (WT/*rboh1*/WT), the graft significantly reduced resistance to RKN infestation and compromised RKN-induced accumulations of H_2O_2 in the stem above the *rboh1* graft (Figures 3B, 3C and S3H). Moreover, H_2O_2 accumulation was not observed in the apoplast of the leaf cells and the leaf tissues above the *rboh1* graft (Figures 3D and S3I). Similarly, JA and JA-Ile accumulation was not observed in the leaves or roots (Figure 3E). In agreement with a putative role for H_2O_2 as a signal for the induction of JA synthesis, the foliar application of H_2O_2 induced JA accumulation in the leaves (Figure S3J). Maximal effects of H_2O_2 were observed at a

concentration of 1 mM. We conclude that a cell to cell activation of H_2O_2 production from the roots to the leaves is essential for the induction of JAs production in the leaves, together with JAs-mediated resistance to RKN in the tomato roots.

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

The evidence presented above suggests that the activation of either electrical signals or H_2O_2 production is critical for the systemic induction of JAs synthesis in leaves and the associated induction of resistance. To test this further, we examined the relationship between electrical activity and H_2O_2 signaling in the plant systemic response to RKN infestation. We found RKN-induced increases in NADPH oxidase activity in the leaves were compromised in plants co-silenced for *GLR3.3* and *GLR3.5* (pTRV-*GLR3.3/3.5*) (Figure S4A). In addition, the RKN-induced accumulation of H_2O_2 in the leaf tissues and in the apoplast of the leaves or in the stems was attenuated in the grafted plants with *glr3.5* as rootstock or scion (Figures S4B -S4E). Crucially, we found that RKN infestation induced H_2O_2 accumulation in the WT rootstock stems but not in the *glr3.5* stem segments or the WT scion stems of the WT/*glr3.5*/WT plants (Figures 4A and S4F). Moreover, H_2O_2 accumulation was not induced in the apoplast of the leaves or in the whole leaves in response to RKN infestation in the shoots of the WT/*glr3.5*/WT plants (Figures 4B and S4G). Conversely, the grafted plants with *rboh1* as rootstock or scion, or those with an inserted *rboh1* segment showed attenuated RKN-induced electrical activity with decreased pulse amplitude and duration (Figures 4C, 4D, S4H and Table S3). To further characterize the relationship between electrical activity and H_2O_2 production, we applied current injection (at 20 µA for 2 min with an interval of 10 min) to the stems. This treatment induced resistance, and accumulation of H_2O_2 in the the vascular system of the shoots, together with an accumulation of H_2O_2 in the aploplast of the leaves of the WT plants, but this was not observed in the *glr3.5* or *rboh1* plants (Figures 4E, 4F, S4I and S4J). These results strongly suggest that there is an inter-dependency between *GLR3.5* and *RBOH1*-mediated processes in the continuous transmission of signals from roots to leaves in order to activate JAs biosynthesis.

Redox-dependent activation of MPK1/2 is involved in the induction of JAs synthesis

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

DISCUSSION

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

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

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

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

data presented here show that the discontinuous induction of electrical signaling is accompanied by continuous increase in the accumulation of H_2O_2 due to the auto-propagating characteristics of H_2O_2 production and subsequent activation of MPK1/2 in response to RKNs [28, 25]. Therefore, the crosstalk between electrical, ROS and Ca^{2+} signaling pathways is pivotal to the systemic transmission of signals from local tissue to distant tissues to activate MPK-dependent JA biosynthesis [24]. JA and SA are the two major players in plant defense responses to pests, such as herbivores and necrotrophic and biotrophic pathogens [30, 31]. They are often considered to function antagonistically in such defense responses [32]. Within this context, our results showing that incresaed JAs accumulation in the leaves of RKN-infested plants, indicate that altered resistance to susceptibility to root invasion may be highly dependent on defense responses in the leaves, through the mediation of systemic signaling pathways. Crosstalk between aboveground and belowground organs not only regulates physiological processes but also alters many rhizosphere processes with ecological significance [33-36]. A general ecological theory may need to be developed to explain why plants involve their shoots in root defenses and why they enhance leaf-resistance upon contact with root-feeding insects and soil-dwelling microorganisms. Future studies are required to establish whether such systemic signaling pathways are a wide spread phenomenon in the plant kingdom and whether roots respond in a similar manner to threats to the shoots by herbivores and pathogens. However, given the greater availability of carbon and nitrogen substrates, together with other resources in leaves compared to roots, it may be logical that shoot pathways are induced as parts of the triage strategy that prevents invasion of the roots. While further research is required to identify shoot–root and root–shoot signals, the present demonstration of effective communication between roots and shoots to prevent or limit RKN infestation offers potential applications for improved plant protection.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.Q.Y., Y.H.Z. and C.H.F.; Methodology, J.Q.Y., G.T.W. and C.Y.H.; Formal 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., C.Z.P. and X.D.W.; Writing – Original Draft, J.Q.Y.; Writing – Review & Editing, J.Q.Y., Y.H.Z. and C.H.F.; Visualization, G.T.W, C.Y.H, and J.Z.; Supervision, J.Q.Y. and C.H.F.; Funding Acquisition, J.Q.Y. and Y.H.Z.;

DECLARATION OF INTERESTS

The authors declare no competing interests.

MAIN-TEXT FIGURE/TABLE LEGENDS

Figure 1. *Meloidogyne incognita* infection induces systemic transmission of electrical and H_2O_2 signals leading to increased JAs accumulation

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

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

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

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

(E) RKN infection (E1) induces systemic accumulation of H_2O_2 from the roots (E2) to stems (E3), petioles (E4) and leaves (E5) at 24 hpi.

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

(±SD). For (D), 'n' is the total number of plants examined and 'x' is the number of plants with detectable potential changes. For (E), acid fuchsin staining was used for E1, DAB staining was used for E2~4 and $CeCl_3$ staining was used for E5. Arrows indicate the accumulation of H_2O_2 in E5. Means denoted by the same letter did not significantly differ at $p < 0.05$ according to Tukey's test. See also Figure S1 and Table S1.

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

(A) *Meloidogyne incognita* (RKN) resistance in grafted plants with *glr3.5* as rootstock or scion.

(B) RKN resistance in grafted plants inserted with *glr3.5* segment.

(C) Typical surface potential changes on the scion stems in grafted plants inserted with *glr3.5* segment at 24 hpi.

(D) Surface potential characteristics on the scion stems in grafted plants inserted with *glr3.5* segment at 24 hpi.

(E) Attenuated accumulation of JAs in grafted plants inserted with g*lr3.5* segment at 24 hpi.

For (A and B), resistance against RKN was determined at 28 dpi and data are the means of three replicates with 15 plants for each replicate (±SD). For (D), 'n' is the total number of plants examined and 'x' is the number of plants with detectable potential changes. For (E), four biological samples were used for the determination of JAs. Means denoted by the same letter did not significantly differ at $p < 0.05$ according to Tukey's test. See also Figure S2 and Table S2.

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

(A) *Meloidogyne incognita* (RKN) resistance in grafted plants with *rboh1* as rootstock or scion.

(B) RKN resistance in grafted plants inserted with *rboh1* segment.

(C) H_2O_2 accumulation in the stems determined with DAB staining at 24 hpi.

(D) H_2O_2 accumulation in the apoplast of leaves determined with $CeCl_3$ staining at 24 hpi.

(E) Accumulation of JAs in grafted plants inserted with *rboh1* segment at 24 hpi.

For (A and B), resistance against RKN was determined at 28 dpi and data are the means of three replicates with 15 plants for each replicate (±SD). For (C), S: scion; IS: inserted segment; R: rootstock. For (D), arrows indicate the accumulation of H_2O_2. For (E), four biological samples

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

Figure 4. Interdependency of ROS and electrical signals in systemic message transmission

(A) H_2O_2 accumulation on the stem in grafted plants determined with DAB staining at 24 hpi.

(B) H_2O_2 accumulation in the apoplast of leaves in grafted plants determined with $CeCl_3$ staining at 24 hpi.

(C) Typical surface potential changes on the scion stems in grafted plant at 24 hpi.

(D) Surface potential characteristics on the scion stems in grafted plants at 24 hpi.

(E) Current injection (CI, at 20 μA for 2 min with an interval of 10 min for 60 cycles) induced changes in the nematode resistance.

(F) Current injection (CI, at 20 μA for 2 min with an interval of 10 min for 10 cycles) induced accumulation of H_2O_2 in the apoplast of leaves determined with $CeCl_3$ staining.

For (A), S:scion; IS: inserted segment; R: rootstock. For (B and F), arrows indicate the accumulation of H_2O_2. For (D), 'n' is the total number of plants examined and 'x' is the number of plants with detectable potential changes. For (E), resistance against the nematode was determined at 28 dpi and data are the means of three replicates with 15 plants for each replicate (±SD). For (F), leaf samples were taken after the current injection. Means denoted by the same letter did not significantly differ at $p < 0.05$ according to Tukey's test. See also Figure S4 and Table S3.

Figure 5. Activation of MPK1/2 is involved in JAs biosynthesis and nematode resistance

(A) Time course of RKN-induced activation of MPK1/2.

(B) MPK1/2 activation in the leaves of grafted plants inserted with *rboh1* segment.

(C) MPK1/2 activation in the leaves of grafted plants inserted with *glr3.5* segment.

(D) MPK1/2 activation in the leaves of wild type plants after different cycles of current injection (CI, at 10 μA for 30 s with an interval of 9 min).

(E) MPK1/2 activation in the leaves after current injection (20 μA for 2 min with 10 min interval for 10 cycles) in the wild type plants and mutants.

(F) Nematode resistance in *MPK1/2*-silenced plants.

(G) Accumulation of JAs in leaves in *MPK1/2*-silenced plants.

(H) A model for the basal resistance by shoot-root communication.

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

STAR★Methods

LEAD CONTACT AND MATERIALS AVAILABILITY

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

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Wild-type tomato (*Solanum lycopersicum* L. cv. 'Ailsa Craig', 'Castlemart', 'Condine Red'), and *spr2* mutants in the Castlemart background were used. *RBOH1* CRISPR/Cas9 vector and *GLR3.5* CRISPR/Cas9 vector were constructed as described by Pan *et al.* [37]. The target sequence (ACGTCGGATACGGTGTCTTC) for *RBOH1* and the target sequence (TAGCAGATCAGCTGGCCAAG) for *GLR3.5* were designed using a web tool of CRISPR-P [38]. The synthesized sequences were annealed and inserted into *Bbs*I site of AtU6-sgRNA-AtUBQ-Cas9 vector, and the AtU6-sgRNA-AtUBQ-Cas9 cassette was inserted into the *Hind*III and *Kpn*I sites of pCAMBIA1301 binary vector. The resulting plasmids were transformed into *Agrobacterium tumefaciens* strain EHA105, and then introduced into tomato of Condine Red and Ailsa Craig respectively [39]. CRISPR/Cas9-induced mutations were genotyped by PCR amplification and DNA sequencing. Cas9-free T2 homozygotes with mutation were 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'-CCGgaattcATGA ATGTGGTTTGGATTAT-3'; reverse, 5'-AGCggatccTACTGCAACAACATCAGTCT-3'. *GLR 3.5* forward, 5'-CCGgaattcCCAATCCAGATGTTCTTGGA-3'; reverse, 5'-AGCggatccATTTC AGCTATAGCTTCCAT-3'. *MPK1* forward, 5'-GGCCGtctagaATAATTGCTGACAGATTGTT -3'; reverse, 5'-CGCGCggatccCATTTCAGTCTAAAATAAAA-3'. *MPK2* forward, 5'-GGCC GtctagaGTACTCGCTCGTTTGCTGTTG-3'; reverse, 5'-CGCGCggatccAGCAGAAAAAAATT CATTTC-3'. *MPK1/2* forward, 5'-GGCGCgagctcCATGGTGGCAGGTTCATTC-3'; reverse, 5'-CGGCgctcgagGCTCAGGTGGACGATACCAT-3'. The cDNA fragments of target genes were PCR-amplified and the amplified fragments were digested and ligated into the corresp onding sites of the pTRV2 vector. Empty pTRV2 vector was used as a control. All constr ucts were confirmed by sequencing and subsequently transformed into *Agrobacterium tumef aciens* strain GV3101. VIGS was performed by infiltration of germinated seeds, followed b y infiltration into the fully expanded cotyledons of 8-d-old tomato seedlings with *A. tumef aciens* 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 we re sown in pots with a mixture of sand and vermiculite (v: v=1:1), receiving Hoagland's n utrient 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 μmol m^{-2} 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 1st true

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

Root-knot nematode infection and resistance assay

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

Pharmacological treatments

To determine the effects of H_2O_2 on the biosynthesis of JA, H_2O_2 was foliar applied onto leaves at a concentration of 0~10 mM. Leaves were taken 1d after the application of H_2O_2.

Electric potential recordings and current injection

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

the soil [45]. The glass microelectrodes with a tip diameter approximately 0.5 Λm for intracellular cytoplasmic potential measurements were prepared from the borosilicate glass capillaries with an outer diameter of 1.0 mm and an inner diameter of 0.58 mm (Hilgenberg GmbH, Germany). Pulling was performed after heating with a PE-2 vertical micropipette puller (Narishige Co., Tokyo, Japan). Microelectrodes were filled with 1M KCl, and inserted into the mesophyll cells of a leaf. The reference electrode was immersed into artificial pond water (APW, composed of 5 mM MES, 0.5 mM $CaSO_4$, 0.05 mM KCl, pH 6.0) where the leaf was also submerged [46]. Both electrodes were connected to a differential amplifier. Potentials were detected at 3~24 h post inoculation. Two 2-channel amplifiers (FD 223 and Duo 773, World Precision Instruments, USA) were simultaneously used to record the potential at stem, petiole and lamina. Frequency is the times with the changes in potential within 20 minutes. Amplitude is potential difference relative to the baseline before the changes. Duration is the length of time for each amplitude change. 'n' is the total number of plants observed and 'x' is the number of plants with detectable potential changes. For current injection two platinum wire electrodes (Qiushi Electric Co., Hangzhou, China, 0.1mm diameter) were circled around the stems with 1 cm apart one day before the current injection was applied. Current injection was applied at 20 μA for 2 min with an interval of 10 min for 10 or 60 cycles for biochemical analysis and resistance assay respectively, unless other described. Control plants were circled with Pt wires in all current injection experiments.

Measurement of JAs Levels

Extraction and quantification of JAs were performed using previously reported procedures with minor modifications [47]. Briefly, 100 mg of frozen leaf or root material was homogenized in 1 mL of ethyl acetate which had been spiked with D6-JA (OlChemIm Ltd., Czechoslovakia) and D6-JA-Ile (QUALITY CONTROL CHEMICALS INC., USA) as internal standards with a final concentration of 100 ng mL^{-1} and 40 ng mL^{-1}, respectively. The samples were shook at 180 rpm in the dark at 4 ºC for 12 h and then centrifuged at 18,000 *g* for 10 min at 4°C. The pellet was re-extracted with 1 mL of ethyl acetate. Both supernatants were combined and evaporated to dryness under N_2. The residue was re-suspended in 0.5 ml of 70% methanol (v/v) and centrifuged. The supernatants were then analyzed in a liquid chromatography tandem mass spectrometry system (Varian 320-MS LC/MS, Agilent Technologies, Amstelveen, the Netherlands). LC analysis was performed using an Agilent Zorbax XDB C18 column (150 mm × 2.1 mm, 3.5 μm). The

mobile phase consisted of a mixture of solvent A (0.1% formic acid in water; E. Merck, Darmstadt, Germany) and solvent B (methanol; E. Merck) at a flow rate of 0.3 ml min^{-1} with the following gradient: 0-1.5 min, A: B at 60: 40; followed by 6.5 min solvent A: B at 0: 100; subsequently returning to solvent A: B to 60: 40 for 5 min until the end of the run. The column temperature was kept at 40 °C, and the injection volume was 20 µL. A negative electrospray ionization mode was used for detection. The JAs were detected in MRM mode by monitoring the transitions 209.1 > 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.

Quantification, histochemical analysis, and cytochemical detection of H_2O_2

The concentration of H_2O_2 in leaves was measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm using the method of Brennan and Frenkel [48]. The histochemical staining of H_2O_2 was performed by using DAB staining as previously [49]. Stems and petioles were cut into 0.5 mm thick sections. The intensity of DAB staining in the vascular systems of roots, stems and petioles was quantificated with Image-Pro Plus 6.0 (Media Cybernetics, Inc., USA) [50]. H_2O_2 in the leaves was visualized at the subcellular level using $CeCl_3$ for localization [51]. Electron-dense $CeCl_3$ deposits are formed in the presence of H_2O_2 and are visible by transmission electron microscopy at an accelerating voltage of 75 kV (H7650; Hitachi, Tokyo, Japan). The concentration of H_2O_2 in leaves was measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm [52].

Isolation of plasma membrane and the determination of NADPH oxidase activity

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

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

MPK1/2 activation assay

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

qRT-PCR analysis

Total RNA was extracted from leaf tissues using RNA simple Total RNA Kit (TIANGEN, Beijing, China) according to the instructions. Total RNA (0.5 μg) was reverse transcribed

to cDNA using HiScript II Q RT SuperMix for qPCR (Vazyme, Nanjing, China). qRT-PCR was performed using a Light Cycler 480 II Real-Time PCR detection system (Roche). Each reaction consisted of 10 µl qPCR SYBR Green Master Mix, 1 µl cDNA, and forward and reverse primers at 0.1 µM according to the instructions of qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). The housekeeping gene *SlACTIN2* was used as internal reference to calculate the relative expression of target genes [41]. Sequences of primer pairs: *ACTIN2* forward 5'-TGTCCCTATTTACGAGGGTTATGC-3' and reverse 5'-CAGTTAAATCACGACCAGCAAGAT-3'; *GLR3.3* forward 5'-ATGTGGGATTGCATGCTTTA-3' and reverse 5'-CTGACCATCCGAATCAACTG-3'; *GLR3.5* forward 5'-GGCTTTCTGGAATAGCTTGC-3' and reverse 5'- TGCCAACCCACATAGAAAGA-3'; *MPK1* for pTRV-*MPK1* and pTRV-*MPK2* plants forward 5'-TCGTCCACCTGAGCTGTTGTT-3' and reverse 5'-ACATGCGGGAACTTTTCAGT-3'; *MPK2* for pTRV-*MPK1* and pTRV-*MPK2* plants forward 5'-AGGGTTTACTATTTACGG-3' and reverse 5'-TGGAGGCTTATACTTCG-3'; *MPK1* for pTRV-*MPK1/2* plants forward 5'-GCTGACAGATTGTTGCAGGT-3' and reverse 5'-TCCACCCCATAAAGATACATCA-3'; *MPK2* for pTRV-*MPK1/2* plants forward 5'-TACTCGCTCGTTTGCTGTTG-3' and reverse 5'- TTGGAGTACAGGAAAACAATGG-3'; *RBOHA* forward 5'-TACATGCCACGGATGAGGAA-3' and reverse 5'-CATCACAACACCGGTCCATC-3'; *RBOHB* forward 5'-TTATCGGCCTTAGTGCGTCT-3' and reverse 5'-CCGTTTGATTTGGTGCTTGC-3'; *RBOHC* forward 5'-TGAGCCACAGTACGCCTTTA-3' and reverse 5'-TAGCAAGCAACCACAGCAAG-3'; *RBOHD* forward 5'-CAGGTCAAGCGTCAAGGATG-3' and reverse 5'-TGCAGCACAGTTGACAAACA-3'; *RBOHE* forward 5'-AGCAACTTCGACTACCACCA-3' and reverse 5'-GCCTGTTACACCTGGAATGG-3'; *RBOHF* forward 5'-TGCTTGGCAACTGCTAAAGG-3' and reverse 5'-GGCCCTAGTAGACCGTAACC-3'; *RBOH1* forward 5'-TCCAGCACAAGATTACCG-3' and reverse 5'-CCTCCATTGCGACGAT-3'; *RBOHH* forward 5'-CCACGGCTGCTTCATATTCC-3' and reverse 5'-CGTGGTAGCGGTTCTCATTG-3'; *AOC* (*ALLENE OXIDE CYCLASE*) forward 5'-CCGTTCAGGGAGCGTACTTA-3' and reverse 5'-ACCGCCGTACACAACAATTC-3'; *AOS* (*ALLENE OXIDE SYNTHASE*) forward 5'-GATCCTCCGGTAGCTTCACA-3' and reverse 5'-TTCTTCTCCGACGAACCGAT-3'; *LOXD* (*LIPOXYGENASE D*) forward 5'- TGTGCCACTGGTAACTGGAT-3' and reverse 5'-TCCAAGCTTGCATGTGTACG-3'; *OPR3* (*12-OXO-PHYTODIENOIC ACID REDUCTASE*)

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

QUANTIFICATION AND STATISTICAL ANALYSIS

Image quantification

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

Statistical analysis

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

DATA AND CODE AVAILABILITY

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

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