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Short communication

# Do strigolactones contribute to plant defence?

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## SUMMARY

Strigolactones are multifunctional molecules involved in several processes outside and within the plant. As signalling molecules in the rhizosphere, they favour the establishment of arbuscular mycorrhizal symbiosis, but they also act as host detection cues for root parasitic plants. As phytohormones, they are involved in the regulation of plant architecture, adventitious rooting, secondary growth and reproductive development, and novel roles are emerging continuously. In the present study, the possible involvement of strigolactones in plant defence responses was investigated. For this purpose, the resistance/susceptibility of the strigolactone-deficient tomato mutant *Slccd8* against the foliar fungal pathogens *Botrytis cinerea* and *Alternaria alternata* was assessed. *Slccd8* was more susceptible to both pathogens, pointing to a new role for strigolactones in plant defence. A reduction in the content of the defence-related hormones jasmonic acid, salicylic acid and abscisic acid was detected by high-performance liquid chromatography coupled to tandem mass spectrometry in the *Slccd8* mutant, suggesting that hormone homeostasis is altered in the mutant. Moreover, the expression level of the jasmonate-dependent gene *PinII*, involved in the resistance of tomato to *B. cinerea*, was lower than in the corresponding wild-type. We propose here that strigolactones play a role in the regulation of plant defences through their interaction with other defence-related hormones, especially with the jasmonic acid signalling pathway.

Strigolactones (SLs) are plant hormones that were initially identified as signalling molecules in the rhizosphere. They are mainly produced in the roots and have been detected in the root exudates of a wide range of monocot and dicot plant species, which suggests their importance in nature (Xie *et al*., 2010). In the rhizosphere, SLs act as host detection cues for symbiotic arbuscular mycorrhizal fungi and root parasitic plants of the Orobanchaceae (reviewed in López-Ráez *et al*., 2011). They are derived from the carotenoids through sequential oxidative cleavage by *c*arotenoid *c*leavage *d*ioxygenases (CCD7 and CCD8) (Gomez-Roldan *et al*., 2008; López-Ráez *et al*., 2008; Matusova *et al*., 2005; Umehara *et al*., 2008), thus belonging to the apocarotenoid class, which includes the plant hormone abscisic acid (ABA).

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As phytohormones, it has been suggested that SLs play a pivotal role in plants as modulators of the coordinated development of roots and shoots in response to nutrient-deficient conditions (Koltai and Kapulnik, 2011). Accordingly, their biosynthesis and exudation into the rhizosphere is induced under such adverse nutritional conditions (López-Ráez *et al*., 2008; Yoneyama *et al*., 2007). They have been shown to regulate above-ground architecture by the inhibition of lateral shoot branching (Gomez-Roldan *et al*., 2008; Umehara *et al*., 2008). Since then, extensive efforts have been devoted to examine the novel roles and functions of SLs in plant physiology. Thus, it has been shown that they stimulate secondary stem growth, acting as positive regulators of cambial activity (Agusti *et al*., 2011). They also regulate below-ground plant architecture by affecting primary root length, lateral root initiation and root hair development (Kapulnik *et al*., 2011a; Ruyter-Spira *et al*., 2011). More recently, it has been shown that they repress adventitious rooting and positively affect reproductive development (Kohlen *et al*., 2012; Rasmussen *et al*., 2012). In all of these functions, a cross-talk between SLs and other signalling pathways regulated by phytohormones, such as auxin, ethylene (ET) and ABA, seems to play a prominent role (reviewed in Kohlen *et al*., 2011). Novel functions for SLs are emerging at a fast pace, broadening our understanding of their relevance in plant physiology. However, no relationship with defence responses has been addressed so far.

Plants are exposed to ever-changing and often unfavourable environmental conditions, which cause both abiotic and biotic stresses. Consequently, plants have evolved sophisticated mechanisms to flexibly adapt themselves to overcome such stress conditions. Most of these mechanisms—if not all—are regulated by plant hormones (Robert-Seilaniantz *et al*., 2011). In recent years, significant progress has been made in the identification of the key components in plant responses to biotic stress. The phytohormones salicylic acid (SA), jasmonic acid (JA) and derivatives—known as jasmonates (JAs)–and ABA are the major players in the signalling networks regulating plant defence responses against pathogens and pests, although others, such as ET, brassinosteroids, gibberellins and auxins, are now known to contribute to modulate the response (Robert-Seilaniantz *et al*., 2011; Ton *et al*., 2009). In the present work, the potential involvement of SLs in plant defence responses has been investigated.

For this purpose, leaves of the SL-deficient tomato mutant *Slccd8* L09 and the corresponding wild-type plants (cv. Craigella) were infected with the pathogenic fungi *Botrytis cinerea* CECT2100 (Spanish Collection of Type Cultures, Universidad de Valencia, Valencia, Spain) and *Alternaria alternata* (isolated from an infected tomato field) in a detached leaf assay. *Slccd8* L9 is an antisense CCD8 RNA interference (RNAi) line displaying a 95% reduction in SLs (Kohlen *et al*., 2012). *Botrytis cinerea* is a necrotrophic fungus causing grey mould in the leaves and fruits of many plants, including tomato, potato, grapes and strawberry (Dean *et al*., 2012). This pathogen is responsible for one of the most prominent, widespread and destructive diseases in agriculture at both pre- and post-harvest stages (Dean *et al*., 2012). *Alternaria alternata* is an opportunistic pathogen on a number of host plants, such as tomato, causing a range of diseases with a high economic impact in agriculture (Thomma, 2003). For the infection assay, plants were grown as described previously (López-Ráez *et al*., 2010a) and watered three times a week with Hewitt nutrient solution (Hewitt, 1966) containing 25% of the standard phosphorus concentration to promote SL production. Disease bioassays with *B. cinerea* and *A. alternata* were carried out using detached leaves from 10 plants per genotype. Conidia were collected from 10–15-day-old potato dextrose agar (PDA) plates supplemented with 20% of lyophilized tomato leaves. In the case of *B. cinerea*, suspensions of spores were incubated for 2 h in the dark in Gamborg's B5 medium (Gamborg *et al*., 1968) supplemented with 10 mM sucrose and 10 mM $KH_2PO_4$ (Vicedo *et al*., 2006). Detached leaves from 4-week-old tomato plants were challenged by the application of 5 μL of a suspension containing $10^6$ spores/mL. Leaves were maintained at 20 °C and 100% relative humidity. Fungal hyphae grew concentrically, resulting in visible necrosis 48 h after inoculation. A disease index from '0' (healthy leaf) to '3' (heavily infected leaf) was established for infection quantification (Fig. 1). Data were subjected to one-way analysis of variance (ANOVA) using the software SPSS Statistics v. 20 for Windows (IBM, Armonk, NY, USA). Fisher's least-significant difference (LSD) test was applied to determine statistical significance.

*Slccd8* showed more severe symptoms of *B. cinerea* infection than the corresponding wild-type. An increase in disease development was observed in *Slccd8* leaves relative to the wild-type 5 days after inoculation (Fig. 1A). Indeed, heavily infested leaves were only observed in *Slccd8* at this stage. This increased susceptibility in *Slccd8* was maintained over time. After 9 days, 41% of the leaves from *Slccd8* were heavily infested (disease index 3), whereas only 15% of wild-type leaves reached this stage (Fig. 1A). The same trend was observed after inoculation with *A. alternata*. In this case, 5 days after inoculation, 40% of *Slccd8* leaves were heavily infected, but only 12% of the wild-type (Fig. 1C). As SLs stimulate hyphal branching in germinating spores of arbuscular mycorrhizal fungi (Akiyama *et al*., 2005) and have been proposed to affect fungal pathogen development (Dor *et al*., 2011), we

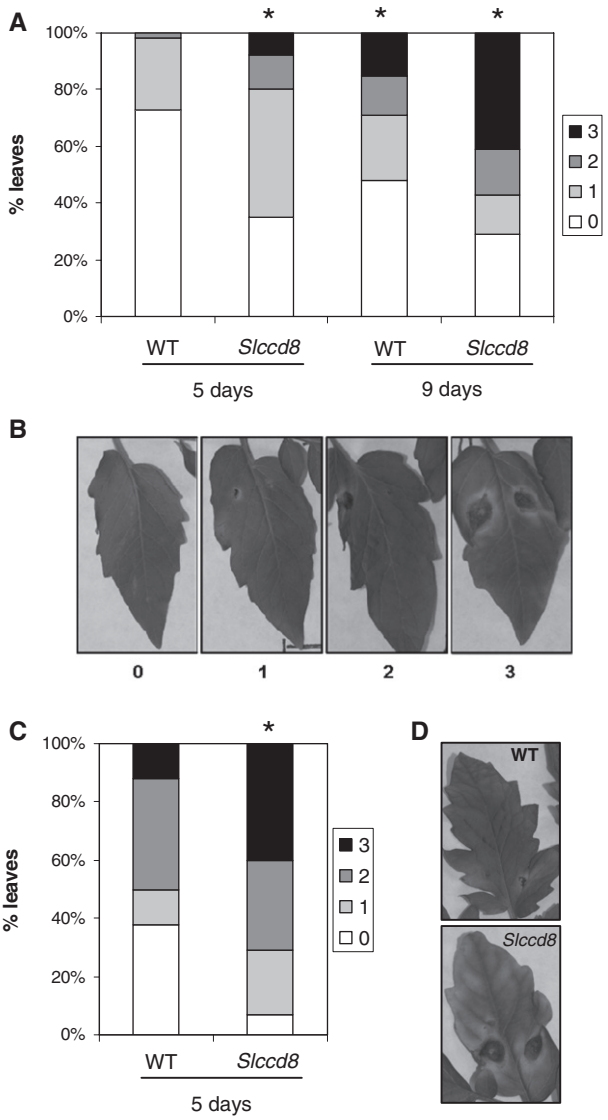


**Fig. 1** Effect of *Botrytis cinerea* and *Alternaria alternata* inoculation on the tomato mutant *Slccd8*. (A) Evaluation of *B. cinerea* lesions in leaves of *Slccd8* and corresponding wild-type (WT) 5 and 9 days after inoculation with $10^6$ spores/mL. Disease rating is expressed as the percentage of leaves in disease classes (B). (C) Evaluation of *A. alternata* lesions in leaves of *Slccd8* and corresponding WT 5 days after inoculation with $10^6$ spores/mL. (D) Close-up of *Slccd8* and WT leaves with *A. alternata* 5 days after inoculation. Asterisks (*) indicate statistically significant different contributions ($P < 0.01$) of the disease severity classes according to Fisher's least-significant difference (LSD) test.

aimed to test whether the results observed were dependent on the inoculum source. Detached leaves from *Slccd8* and wild-type plants were inoculated with 5-mm-diameter PDA plugs containing actively growing *B. cinerea* hyphae (1 week old). As for the previous experiment, leaves from 10 plants per genotype were used. Interestingly, the same disease pattern was observed (Fig. S1, see Supporting Information). The data confirm, therefore, that the

A

| GR24 (M) | Radial growth (cm) |
|---|---|
| Control | 3.2 ± 0.2 |
| $10^{-6}$ | 3.2 ± 0.2 |
| $10^{-7}$ | 3.1 ± 0.3 |
| $10^{-8}$ | 3.1 ± 0.2 |

B

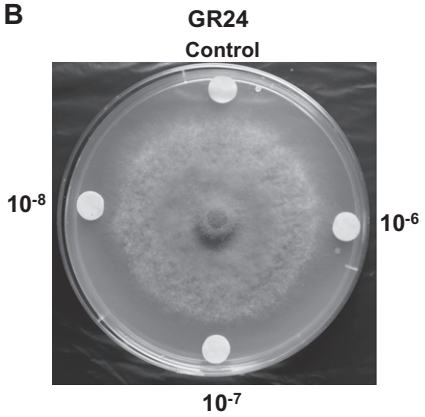


**Fig. 2** Effect of GR24 on fungal development of *Botrytis cinerea*. (A) Radial growth of *B. cinerea in vitro* determined 5 days after application of 10 μL of different concentrations ($10^{-6}$, $10^{-7}$ and $10^{-8}$ M) of the strigolactone (SL) analogue GR24. As a control, 10 μL of sterilized demiwater were used. Data represent the means of 10 independent replicates ± standard error (SE). (B) Representative plate showing no effect of GR24 on *B. cinerea* growth.

SL-deficient tomato line *Slccd8* is more susceptible to fungal pathogens, regardless of the inoculum source (germinating spores or actively growing hyphae), supporting an additional role of SLs in plant defence.

As mentioned, an effect of SLs on fungal pathogen development has been proposed previously (Dor *et al*., 2011). In order to evaluate a possible direct effect of SLs on fungal development, an *in vitro* assay with GR24 and *B. cinerea* was performed. Five-millimetre-diameter PDA plugs containing actively growing *B. cinerea* hyphae were applied to the middle of a Petri dish with PDA medium. Four different concentrations of GR24 (Chiralix) were tested, 0, $10^{-6}$, $10^{-7}$ and $10^{-8}$ M, all within the physiological concentration range (Xie *et al*., 2010). Four sterilized 5-mm-diameter fibreglass discs were applied to each plate, and 10 μL of the different GR24 solutions were added to each disc (Fig. 2B). Plates were incubated at 25 °C and high humidity for 5 days, and 10 individual plates were used. No effect on the *B. cinerea* growth pattern was observed by any of the GR24 concentrations tested (Fig. 2). This observation is in agreement with a previous study in which no alterations in the growth pattern of *B. cinerea* after $10^{-6}$ M GR24 application were detected (Steinkellner *et al*., 2007).

**Table 1** Levels of defence-related hormones in the strigolactone (SL)-deficient tomato mutant *Slccd8* and the wild-type (WT).

| | Genotype | |
|---|---|---|
| Hormone (ng/g dry weight) | WT | *Slccd8* |
| OPDA | 4495 ± 473 | 3672 ± 557 |
| JA | 157 ± 37 | 41 ± 16** |
| SA | 2375 ± 165 | 1251 ± 60* |
| ABA | 2515 ± 79 | 1624 ± 118* |

Levels of oxo-phytodienoic acid (OPDA), jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) were measured by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) in leaves of the *Slccd8* mutant and corresponding wild-type (WT). Data represent the means of five independent replicates ± standard error (SE). Asterisks (*) indicate statistically significant differences according to Fisher's least-significant difference (LSD) test (*$P < 0.05$ and **$P < 0.01$).

No effect was detected when using higher concentrations ($10^{-4}$ and $10^{-5}$ M) of GR24 (Fig. S2, see Supporting Information). These results suggest that there is no direct effect of SLs on either spore germination or the development of this pathogenic fungus.

Plant resistance/susceptibility to pathogens is generally associated with changes in hormonal defence pathways (Robert-Seilaniantz *et al*., 2011). To determine whether the increased susceptibility of *Slccd8* to *B. cinerea* was related to alterations in the hormonal profiles, the contents of the defence-related phytohormones JA, SA, ABA and the JA precursor oxo-phytodienoic acid (OPDA), also biologically active (Wasternack, 2007), were analysed in the leaves of *Slccd8* and compared with those of the corresponding wild-type. The samples were analysed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), as described by Flors *et al*. (2008). Analysis by HPLC-MS/MS allowed the simultaneous quantification of all four compounds from each sample. A 100-mg aliquot of dried leaf tissue was used per sample, and five independent replicates per genotype were employed. Quantifications were carried out with MassLynx 4.1 software (Waters, Yvelines Cedex, France) using internal standards as a reference for extraction recovery and the standard curves as quantifiers. Hormone analysis was conducted at the Scientific Instrumentation Service at the Estación Experimental del Zaidín-CSIC (Granada, Spain). As for the pathogenicity bioassay, hormonal data were subjected to one-way ANOVA, and Fisher's LSD test was applied to determine the statistical significance.

The levels of JA in *Slccd8* were less than 30% (about 75% reduction) of those in the corresponding wild-type Craigella (Table 1, $P < 0.01$). A similar trend was observed for OPDA, although the reduction here was not significantly different. A significant reduction of about 50% was also observed in the levels of SA in the mutant leaves (Table 1, $P < 0.05$). As in the case of JA and SA, the ABA content was also reduced (about 35%) in the mutant compared with the wild-type (Table 1, $P < 0.05$), confirm-

**Table 2** Genes analysed, signalling pathways they represent and primer sequences used in the real-time quantitative polymerase chain reaction (qPCR) analysis.

| ID | Gene | Pathway | Primers (5'–3') | Reference |
|---|---|---|---|---|
| K03291 | Proteinase inhibitor II (*PinII*) | JA | GAAAATCGTTAATTTATCCCAC<br>ACATACAAACTTTCCATCTTTA | Uppalapati *et al*. (2005) |
| M69247 | Pathogenesis-related protein *PR1a* | SA | ATGTGTGTGTTGGGGTTGGT<br>ACTTTGGCACATCCAAGACG | López-Ráez *et al*. (2010b) |
| X51904 | *Le4* | ABA | ACTCAAGGCATGGGTACTGG<br>CCTTCTTTCTCCTCCCACCT | López-Ráez *et al*. (2010b) |
| X14449 | Elongation factor-1 (*SlEF*) | Housekeeping | GATTGGTGGTATTGGAACTGTC<br>AGCTTCGTGGTGCATCTC | Rotenberg *et al*. (2006) |

ABA, abscisic acid; JA, jasmonic acid; SA, salicylic acid.

ing an altered hormonal balance in the mutant. In addition to the hormonal profiles by HPLC-MS/MS, the expression of marker genes for the different signalling pathways regulated by these hormones was analysed in the leaves of *Slccd8* and the wild-type from noninoculated plants by real-time quantitative polymerase chain reaction (qPCR). qPCR was conducted using the iCycler iQ5 system (Bio-Rad, Hercules, CA, USA) and specific primers for each gene (Table 2). As JA marker, we used the responsive gene *PinII*, encoding for the proteinase inhibitor II involved in plant defence against insects and *B. cinerea* (El Oirdi *et al*., 2011; Wasternack, 2007). The genes *PR1a* and *Le4* were used as markers for SA- and ABA-regulated responses, respectively (López-Ráez *et al*., 2010b). *PR1a* encodes a pathogenesis-related protein which is inducible by SA, and *Le4* encodes for an ABA-inducible dehydrin. Total RNA from leaves was extracted using Tri-Reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The RNA was treated with RQ1 DNase (Promega, Madison, WI, USA), purified through a silica column using the NucleoSpin RBA Clean-up Kit (Macherey-Nagel, Düren, Germany) and stored at −80 °C until use. The first-strand cDNA was synthesized with 1 μg of purified total RNA using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Six independent biological replicates were analysed per genotype. Relative quantification of specific mRNA levels was performed using the comparative $2^{-\Delta(\Delta Ct)}$ method (Livak and Schmittgen, 2001). Expression values were normalized using the housekeeping gene *SlEF*, which encodes for the tomato elongation factor-1α. Interestingly, the expression of the SA and ABA response marker genes, *PR1a* and *Le4*, respectively, was not altered significantly in *Slccd8* relative to the wild-type. However, the expression of the JA marker *PinII* was reduced by more than four-fold in the mutant (Fig. 3). The data suggest that, despite the reduction in the basal level of the three phytohormones (SA, ABA and JA), only JA-dependent responses were affected significantly.

Overall, the reduced levels in *Slccd8* of SA, ABA and, more markedly, JA, together with the lack of a direct effect of SLs on *B. cinerea* observed in the *in vitro* assay with GR24, indicate an indirect involvement of SLs in the regulation of plant defence responses. The results suggest a cross-talk between SLs and some of the signalling pathways related to defence. An interaction

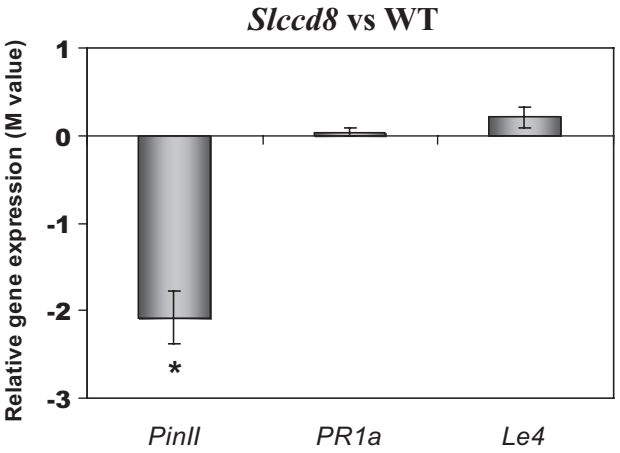


**Fig. 3** Expression levels in the tomato mutant *Slccd8* compared with the corresponding wild-type (WT) Craigella. Gene expression analysis by real-time quantitative polymerase chain reaction (qPCR) of molecular markers for different defence signalling pathways. M values ($\log_2$ ratio) are represented. The M value is zero if there is no change, and '+1' and '−1' indicate a two-fold induction and reduction, respectively. *PinII*, *PR1a* and *Le4* genes were the markers for the jasmonic acid (JA)-, salicylic acid (SA)- and abscisic acid (ABA)-dependent pathways, respectively. Bars represent the means of six independent replicates [±standard error (SE)]. Asterisk (*) indicates significantly different ($P < 0.01$) expression levels according to Fisher's least-significant difference (LSD) test.

between SLs and other phytohormones has been shown for their other physiological functions described so far (reviewed in Kohlen *et al*., 2011). For instance, a cross-talk between SLs, auxin and cytokinins has been proposed to control shoot branching (Domagalska and Leyser, 2011). Similarly, an interaction of SLs with auxin and ET seems to regulate root system architecture and root hair formation (Kapulnik *et al*., 2011b; Ruyter-Spira *et al*., 2011). A regulatory role for ABA in SL biosynthesis has been proposed as tomato ABA-deficient mutants and tomato plants treated with the ABA inhibitor abamine-SG show a reduced capacity to produce SLs (López-Ráez *et al*., 2010a). Here, we show that SLs may also alter ABA levels. ABA has emerged as an important regulator of biotic defence responses (Ton *et al*., 2009). Therefore, it seems likely that the effect of SLs on ABA content may impact on the plant's ability to cope with stresses. In this sense, it has been

shown that the tomato ABA-deficient mutant *sitiens* is more resistant than wild-type plants to *B. cinerea* (Audenaert *et al*., 2002; Curvers *et al*., 2010), suggesting a role of ABA in tomato susceptibility to this pathogen. Here, we show that, despite a moderate reduction in ABA content, the SL-deficient line *Slccd8* was more susceptible to *B. cinerea*. Although a positive effect of ABA in early defence responses against *B. cinerea* has also been reported (Abuqamar *et al*., 2009), our data do not support a major contribution of the changes in ABA levels to the enhanced susceptibility of *Slccd8* to *B. cinerea* and *A. alternata*.

Unlike ABA, as far as we know, no relationship between SLs and the SA- and JAs-related signalling pathways has been reported to date. Among the hormonal changes observed in the mutant *Slccd8*, those produced in the JA-dependent pathway were the most pronounced. Moreover, this was correlated with a strong reduction in the level of the JA-responsive gene *PinII*. *Botrytis cinerea* and *A. alternata* are necrotrophic fungi that colonize senescent or dead plant tissues (Dean *et al*., 2012). Necrotrophic pathogens are generally sensitive to defence responses regulated by JA (Pieterse *et al*., 2009). In this sense, JA is known to be the key signalling pathway regulating defences against *B. cinerea*, and *PinII* has been proven to be important for tomato resistance to *B. cinerea* (El Oirdi *et al*., 2011). PinII is a proteinase inhibitor which accumulates on wounding and damage caused by herbivores and pathogens or by treatment with exogenous JA (Wasternack, 2007). El Oirdi *et al*. (2011) reported that tomato plants silenced in *PinII* were more susceptible to this pathogenic fungus, showing that this enzyme is required for resistance against *B. cinerea* in tomato. Therefore, the reduction in JA content and *PinII* basal levels could well explain the increased susceptibility of *Slccd8* to *B. cinerea* and *A. alternata*.

We can conclude that the SL-deficient mutant *Slccd8* is more susceptible to necrotrophic fungal pathogens. The reduced levels of the phytohormones JA, SA and ABA in *Slccd8* suggest a role of SLs in the regulation of plant defence responses through their interaction with other signalling pathways, especially with the JA-related pathway, rather than a direct effect on fungal development. The results provide insights into a possible new role for SLs and open up a new area of research related to the involvement of SLs in the regulatory network controlling plant responses to stresses. Unravelling the role of SLs in defence is definitely exciting and promising. However, further research is needed to define the mechanisms that may regulate this new function and to assess whether this effect can be extended to other pathosystems.

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## REFERENCES

**Abuqamar, S., Luo, H., Laluk, K., Mickelbart, M.V. and Mengiste, T.** (2009) Crosstalk between biotic and abiotic stress responses in tomato is mediated by the AIM1 transcription factor. *Plant J.* **58**, 347–360.

**Agusti, J., Herold, S., Schwarz, M., Sanchez, P., Ljung, K., Dun, E.A., Brewer, P.B., Beveridge, C.A., Sieberer, T., Sehr, E.M. and Greb, T.** (2011) Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. *Proc. Natl. Acad. Sci. USA*, **108**, 20 242–20 247.

**Akiyama, K., Matsuzaki, K. and Hayashi, H.** (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorhrizal fungi. *Nature*, **435**, 824–827.

**Audenaert, K., De Meyer, G.B. and Höfte, M.M.** (2002) Abscisic acid determines basal susceptibility of tomato to *Botrytis cinera* and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiol.* **128**, 491–501.

**Curvers, K., Seifi, H., Mouille, G., de Rycke, R., Asselbergh, B., Van Hecke, A., Vanderschaeghe, D., Höfte, H., Callewaert, N., Van Breusegem, F. and Höfte, M.** (2010) Abscisic acid deficiency causes changes in cuticle permeability and pectin composition that influence tomato resistance to *Botrytis cinerea*. *Plant Physiol.* **154**, 847–860.

**Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J. and Foster, G.D.** (2012) The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **13**, 414–430.

**Domagalska, M.A. and Leyser, O.** (2011) Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* **12**, 211–221.

**Dor, E., Joel, D.M., Kapulnik, Y., Koltai, H. and Hershenhorn, J.** (2011) The synthetic strigolactone GR24 influences the growth pattern of phytopathogenic fungi. *Planta*, **234**, 419–427.

**El Oirdi, M., El Rahman, T.A., Rigano, L., El Hadrami, A., Rodríguez, M.C., Daayf, F., Vojnov, A. and Bouarab, K.** (2011) *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *Plant Cell*, **23**, 2405–2421.

**Flors, V., Ton, J., van Doorn, R., Jakab, G., García-Agustín, P. and Mauch-Mani, B.** (2008) Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant J.* **54**, 81–92.

**Gamborg, O.L., Miller, R.A. and Ojima, K.** (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* **50**, 151–158.

**Gomez-Roldan, V., Fermas, S., Brewer, P.B., Puech-Pagés, V., Dun, E.A., Pillot, J.P., Letisse, F., Matusova, R., Danoun, S., Portais, J.C., Bouwmeester, H., Bécard, G., Beveridge, C.A., Rameau, C. and Rochange, S.F.** (2008) Strigolactone inhibition of shoot branching. *Nature*, **455**, 189–194.

**Hewitt, E.J.** (1966) Sand and water culture methods used in the study of plant nutrition. In: *Technical Communication No. 22* (Hudson, J.P., ed.), pp. 547. London: Commonwealth Agriculture Bureau.

**Kapulnik, Y., Delaux, P.M., Resnick, N., Mayzlish-Gati, E., Wininger, S., Bhattacharya, C., Séjalon-Delmas, N., Combier, J.P., Bécard, G., Belausov, E., Beeckman, T., Dor, E., Hershenhorn, J. and Koltai, H.** (2011a) Strigolactones affect lateral root formation and root-hair elongation in Arabidopsis. *Planta*, **233**, 209–216.

**Kapulnik, Y., Resnick, N., Mayzlish-Gati, E., Kaplan, Y., Wininger, S., Hershenhorn, J. and Koltai, H.** (2011b) Strigolactones interact with ethylene and auxin in regulating root-hair elongation in Arabidopsis. *J. Exp. Bot.* **62**, 2915–2924.

**Kohlen, W., Ruyter-Spira, C. and Bouwmeester, H.J.** (2011) Strigolactones: a new musician in the orchestra of plant hormones. *Botany*, **89**, 827–840.

**Kohlen, W., Charnikhova, T., Lammers, M., Pollina, T., Toth, P., Haider, I., Pozo, M.J., de Maagd, R.A., Ruyter-Spira, C., Bouwmeester, H.J. and López-Ráez, J.A.** (2012) The tomato *CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8)* regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. *New Phytol.* **196**, 535–547.

**Koltai, H. and Kapulnik, Y.** (2011) Strigolactones as mediators of plant growth responses to environmental conditions. *Plant Signal. Behav.* **5**, 1–5.

**Livak, K.J. and Schmittgen, T.D.** (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2\_DDC_t$ method. *Methods*, **25**, 402–408.

**López-Ráez, J.A., Charnikhova, T., Gómez-Roldán, V., Matusova, R., Kohlen, W., De Vos, R., Verstappen, F., Puech-Pages, V., Bécard, G., Mulder, P. and Bouwmeester, H.** (2008) Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. *New Phytol*. **178**, 863–874.

**López-Ráez, J.A., Kohlen, W., Charnikhova, T., Mulder, P., Undas, A.K., Sergeant, M.J., Verstappen, F., Bugg, T.D., Thompson, A.J., Ruyter-Spira, C. and Bouwmeester, H.** (2010a) Does abscisic acid affect strigolactone biosynthesis? *New Phytol*. **187**, 343–354.

**López-Ráez, J.A., Verhage, A., Fernández, I., García, J.M., Azcón-Aguilar, C., Flors, V. and Pozo, M.J.** (2010b) Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. *J. Exp. Bot*. **61**, 2589–2601.

**López-Ráez, J.A., Pozo, M.J. and García-Garrido, J.M.** (2011) Strigolactones: a cry for help in the rhizosphere. *Botany*, **89**, 513–522.

**Matusova, R., Rani, K., Verstappen, F.W.A., Franssen, M.C.R., Beale, M.H. and Bouwmeester, H.J.** (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiol*. **139**, 920–934.

**Pieterse, C.M., León-Reyes, A., Van der Ent, S. and Van Wees, S.C.** (2009) Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol*. **5**, 308–316.

**Rasmussen, A., Mason, M.G., De Cuyper, C., Brewer, P.B., Herold, S., Agusti, J., Geelen, D., Greb, T., Goormachtig, S., Beeckman, T. and Beveridge, C.A.** (2012) Strigolactones suppress adventitious rooting in Arabidopsis and pea. *Plant Physiol*. **158**, 1976–1987.

**Robert-Seilaniantz, A., Grant, M. and Jones, J.D.G.** (2011) Hormone crosstalk in plant disease and defense: more than just JASMONATE-SALICYLATE antagonism. *Annu. Rev. Phytopathol*. **49**, 317–343.

**Rotenberg, D., Thompson, T.S., German, T.L. and Willis, D.K.** (2006) Methods for effective real-time RT-PCR analysis of virus-induced gene silencing. *J. Virol. Methods*, **138**, 49–59.

**Ruyter-Spira, C., Kohlen, W., Charnikhova, T., van Zeijl, A., van Bezouwen, L., de Ruijter, N., Cardoso, C., López-Ráez, J.A., Matusova, R., Bours, R., Verstappen, F. and Bouwmeester, H.** (2011) Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? *Plant Physiol*. **155**, 721–734.

**Steinkellner, S., Lendzemo, V., Langer, I., Schweiger, P., Khaosaad, T., Toussaint, J.P. and Vierheilig, H.** (2007) Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant–fungus interactions. *Molecules*, **12**, 1290–1306.

**Thomma, B.P.** (2003) *Alternaria* spp.: from general saprophyte to specific parasite. *Mol. Plant Pathol*. **4**, 225–236.

**Ton, J., Flors, V. and Mauch-Mani, B.** (2009) The multifaceted role of ABA in disease resistance. *Trends Plant Sci*. **14**, 310–317.

**Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J. and Yamaguchi, S.** (2008) Inhibition of shoot branching by new terpenoid plant hormones. *Nature*, **455**, 195–200.

**Uppalapati, S.R., Ayoubi, P., Weng, H., Palmer, D.A., Mitchell, R.E., Jones, W. and Bender, C.L.** (2005) The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. *Plant J.* **42**, 201–217.

**Vicedo, B., Leyva, M., Flors, V., Finiti, I., Del Amo, G., Walters, D., Real, M.D., García-Agustín, P. and González-Bosch, C.** (2006) Control of the phytopathogen *Botrytis cinerea* using adipic acid monoethyl ester. *Arch. Microbiol*. **184**, 316–326.

**Wasternack, C.** (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot*. **100**, 681–697.

**Xie, X.N., Yoneyama, K. and Yoneyama, K.** (2010) The strigolactone story. *Annu. Rev. Phytopathol*. **48**, 93–117.

**Yoneyama, K., Xie, X., Kusumoto, D., Sekimoto, H., Sugimoto, Y., Takeuchi, Y. and Yoneyama, K.** (2007) Nitrogen deficiency as well as phosphorous deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. *Planta*, **227**, 125–132.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1** Effect of *Botrytis cinerea* inoculation on the tomato mutant *Slccd8*. Evaluation of lesions in leaves of *Slccd8* and corresponding wild-type (WT) 4 and 8 days after inoculation with 5-mm-diameter plugs containing actively growing hyphae. Disease rating is expressed as the percentage of leaves in disease classes. Asterisks (*) indicate statistically significant different contributions ($P < 0.01$) of the disease severity classes according to Fisher's least-significant difference (LSD) test.

**Fig. S2** Effect of GR24 on fungal development of *Botrytis cinerea*. Radial growth of *B. cinerea in vitro* determined 5 days after application of 10 μL of different concentrations ($10^{-4}$, $10^{-5}$ and $10^{-6}$ M) of the strigolactone (SL) analogue GR24 (A). As control, 10 μL of sterilized demiwater were used. Data represent the means of 10 independent replicates ± standard error. Representative plate showing no effect of GR24 on *B. cinerea* growth (B).

 