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Research

The tomato *CAROTENOID CLEAVAGE DIOXYGENASE8* (*SlCCD8*) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis

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Summary

- Strigolactones are plant hormones that regulate both above- and belowground plant architecture. Strigolactones were initially identified as rhizosphere signaling molecules. In the present work, the tomato (*Solanum lycopersicum*) *CAROTENOID CLEAVAGE DIOXYGENASE 8* (*SlCCD8*) was cloned and its role in rhizosphere signaling and plant physiology assessed by generating knock-down lines.
- Transgenic *SlCCD8* plants were generated by RNAi-mediated silencing. Lines with different levels of strigolactone reduction – confirmed by UPLC-MS/MS – were selected and their phenotypes investigated.
- Lines exhibiting reduced *SlCCD8* levels displayed increased shoot branching, reduced plant height, increased number of nodes and excessive adventitious root development. In addition, these lines exhibited reproductive phenotypes such as smaller flowers, fruits, as well as fewer and smaller seeds per fruit. Furthermore, we show that strigolactone loading to the xylem sap is possibly restricted to orobanchol.
- Infestation by *Phelipanche ramosa* was reduced by 90% in lines with a relatively mild reduction in strigolactone biosynthesis and secretion while arbuscular mycorrhizal symbiosis, apical dominance and fruit yield were only mildly affected. This demonstrates that reduction of strigolactone biosynthesis could be a suitable tool in parasitic weed management. Furthermore, our results suggest that strigolactones are involved in even more physiological processes than so far assumed.

Introduction

Strigolactones are a group of carotenoid-derived plant hormones (Matusova *et al.*, 2005; López-Ráez *et al.*, 2008a). They were initially identified as germination stimulants for root parasitic plants of the Orobanchaceae (Cook *et al.*, 1966; Bouwmeester *et al.*, 2003) and pre-symbiotic signals inducing hyphal branching in arbuscular mycorrhizal (AM) fungi (Akiyama *et al.*, 2005; Besserer *et al.*, 2006; Bouwmeester *et al.*, 2007). Strigolactones have been detected in the root extracts and exudates of both monocot and dicot plant species (Yoneyama *et al.*, 2007; Goldwasser *et al.*, 2008; Gomez-Roldan *et al.*, 2008; López-Ráez *et al.*, 2008a; Umehara *et al.*, 2008) and were identified to be the branching/tillering inhibiting signal (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). This graft-transmissible signal was postulated to exist over 15 years ago and to originate – at least to a large extent – from the roots (Beveridge *et al.*, 1994; Napoli, 1996; Turnbull *et al.*, 2002). However, intergrafting of hypocotyl tissue is sufficient to restore shoot branching of strigolactone biosynthesis mutants in pea and *Arabidopsis thaliana* (Arabidopsis) to near wild-type levels, indicating that – in addition to roots – other tissues can contribute to the production of strigolactones that inhibit the outgrowth of axillary buds (Foo *et al.*, 2001). Nevertheless, strigolactones are transported acropetally to the parts of the plant where they exert their function. This is supported by the fact that in both Arabidopsis and *Solanum lycopersicum* (tomato) at least one

strigolactone – orobanchol – is present in the xylem (Kohlen *et al.*, 2011a). Recently, a strigolactone cellular exporter – PhPDR1 – was identified in *Petunia hybrida* (petunia). PhPDR1 belongs to the family of ABC transporters, and it is involved in strigolactone secretion into the rhizosphere. The fact that it is also expressed near axillary buds seems to suggest a role in strigolactone loading to the xylem/apoplast in the shoot as well but how that is involved in regulating shoot branching is still unclear (Kretzschmar *et al.*, 2012).

Strigolactone production is induced under phosphate-limiting conditions in several plant species (Yoneyama *et al.*, 2007; López-Ráez *et al.*, 2008a; Umehara *et al.*, 2008; Kohlen *et al.*, 2011a), likely to stimulate the establishment of AM symbiosis (Akiyama *et al.*, 2005; Bouwmeester *et al.*, 2007). More recently, it was proposed that these elevated strigolactone concentrations might also serve an additional function *in planta*, as they could be involved in reducing shoot branching under these unfavorable conditions (Umehara *et al.*, 2010; Kohlen *et al.*, 2011a). In agreement with this, the concentration of orobanchol in the xylem sap of Arabidopsis is elevated under phosphate deficiency (Kohlen *et al.*, 2011a). This could explain why strigolactone biosynthesis in Arabidopsis – a plant species which is not mycorrhized – shows a similar response to phosphate starvation (Kohlen *et al.*, 2011a).

In addition to regulating shoot architecture, novel biological functions for strigolactones are being discovered at a rapid pace, showing that they play a broader role in plant development. A small-molecule screen identified several putative functions for strigolactones in Arabidopsis, ranging from seed germination to hypocotyl elongation (Tsuchiya *et al.*, 2010). Strigolactones were also identified as positive regulators of secondary stem growth by stimulating cambium development in several species (Agusti *et al.*, 2011). In addition, strigolactones have been shown to be involved in the regulation of plant architecture belowground affecting the root system architecture. It was demonstrated that manipulation of the strigolactone concentration in the root – either by mutation or exogenous application of the synthetic strigolactone analog GR24 – leads to changes in the primary root length, root hair development and lateral root initiation (Koltai *et al.*, 2010a; Kapulnik *et al.*, 2011; Ruyter-Spira *et al.*, 2011). Similarly, strigolactones were recently shown to repress adventitious root development in Arabidopsis and *Pisum sativum* (pea; Rasmussen *et al.*, 2012). Interestingly, in most – if not all – of these newly discovered strigolactone functions a cross-talk with auxin seems to play a predominant role (Bennett *et al.*, 2006; Agusti *et al.*, 2011; Ruyter-Spira *et al.*, 2011; Rasmussen *et al.*, 2012).

As mentioned above, strigolactones are derived from the carotenoids and therefore belong to a chemical class called the apocarotenoids. Indeed, recently 9-*cis*-β-carotene was identified as the substrate for strigolactone biosynthesis (Alder *et al.*, 2012). Moreover, three of the four enzymes previously demonstrated to be involved in their biosynthesis (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008; Lin *et al.*, 2009; Kohlen *et al.*, 2011a) have now been functionally identified (Alder *et al.*, 2012). All-*trans*-β-carotene is first isomerised to 9-*cis*-β-carotene by DWARF27, and is subsequently sequentially cleaved by CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7) and CAROTENOID CLEAVAGE DIOXYGENASE 8 (CCD8) to give rise to carlactone (Alder *et al.*, 2012). This apocarotenoid closely resembles strigolactones as it already has the D-ring and enol ether bridge characteristic for strigolactones. Alder and co-workers have also postulated that the fourth biosynthetic enzyme, with so far unknown catalytic activity – the cytochrome P450 MORE AXILLARY GROWTH1 (MAX1; Stirnberg *et al.*, 2002; Booker *et al.*, 2005) – might catalyse the conversion of carlactone to 5-deoxystrigol (Alder *et al.*, 2012), proposed to be the first real strigolactone in the biosynthetic pathway (Matusova *et al.*, 2005; Rani *et al.*, 2008). Strigolactone biosynthesis seems to be tightly regulated as it was demonstrated that despite increased carotenoid accumulation in the tomato mutant *high pigment-2*^{darkgreen} (*hp-2*^{dg}) strigolactone biosynthesis and secretion into the rhizosphere were significantly reduced. As a result, this mutant was less susceptible to the root parasitic plant *Phelipanche aegypthyaca* (López-Ráez *et al.*, 2008b). Also the *Orobanche*-resistant and AM-deficient tomato mutant – *Sl-ORT1* – with a mildly branched phenotype was shown to produce fewer strigolactones (Koltai *et al.*, 2010b). However, the gene underlying *Sl-ORT1* and its function in regulating strigolactone biosynthesis so far remains unknown. This demonstrates that controlling strigolactone biosynthesis might be a good strategy to control these crop-damaging parasitic weeds as previously suggested (Bouwmeester *et al.*, 2003; López-Ráez *et al.*, 2009; Cardoso *et al.*, 2011). Remarkably, AM symbiosis in tomato also leads to a reduced germinating stimulatory activity for *Phelipanche ramosa* seeds, a reduction caused by a decrease in strigolactone production and exudation (López-Ráez *et al.*, 2011a).

Tomato has become an important model in strigolactone research and its major strigolactone composition (solanacol, orobanchol and didehydro-orobanchol isomers 1 and 2) has been elucidated (López-Ráez *et al.*, 2008a,b). Therefore, having full insight into the strigolactone biosynthetic pathway in tomato is vital, as this would make it an excellent model for combining both analytical and molecular tools for strigolactone research in an agronomically important crop. The first strigolactone biosynthetic gene characterised in tomato was *SlCCD7* (Vogel *et al.*, 2010), and the authors demonstrated that strigolactones also regulate the outgrowth of axillary bud in tomato. In the present study, we set out to clone the tomato *SlCCD8* gene, and characterise its role in strigolactone biosynthesis and tomato plant development using knock-down transgenic lines. Our results provide insight into a possible new function for strigolactones in reproductive development of tomato.

Materials and Methods

Plant material, growth conditions and chemicals

Seeds of tomato (*Solanum lycopersicum* L. cv Craigella (LA3247)) and the three independent transgenic *SlCCD8* knock-down lines (L16, L04, and L09) were surface-sterilised in 4% sodium hypochlorite containing 0.02% (v/v) Tween 20, rinsed thoroughly with sterile water and germinated for 48 h on moistened filter paper at 25°C in darkness. For strigolactone analysis, germinated

seeds were pre-grown on perlite for 7 d. Then, all perlite was removed from the roots and ten seedlings per biological replicate were transferred to an X-stream 20 aeroponics system (Nutriculture, Lancashire, UK) operating on 5 l modified half-strength Hoagland nutrient solution (López-Ráez *et al.*, 2008a) as previously described (Liu *et al.*, 2011). To induce strigolactone production, a 7 d phosphate-starvation stress was applied to the plants by replacing the nutrient solution by modified half-strength Hoagland solution without phosphate (López-Ráez *et al.*, 2008a; Liu *et al.*, 2011). Prior to exudate collection the nutrient solution was refreshed in order to remove all accumulated strigolactones. The exudates were collected 24 h later; purified and concentrated within 2 h. Roots were quick frozen in liquid nitrogen and stored at − 80°C for further analysis. For phenotypical analysis, plants were grown in soil-filled pots for 8 wk and irrigated with 300 ml of tap water twice a week and once with 300 ml of full-strength Hoagland solution (Hoagland & Arnon, 1950). For complementation studies and analysis of adventitious root primordia, pre-germinated tomato seeds were transferred to an *in vitro* system using 200-ml half-strength MS medium supplemented with 1× Gamborg's B5 vitamin mix, 1% agar, and either 0 (mock) or 5 μM GR24 (synthetic strigolactone analog). Cutting experiments were performed using the top 4–5 primary stem nodes of 4-wk-old tomato seedlings. All plants were grown under controlled conditions in a glasshouse at with 16/8 h photoperiod, 23/20°C, and 60% relative humidity. Additional light was provided when needed to achieve a 250 μmol m^{-2} s^{-2} minimum light intensity.

IAA was purchased from Sigma-Aldrich (St. Louis, USA). GR24 was kindly provided by Prof. dr. Zwanenburg (Radboud University, Nijmegen, The Netherlands).

Cloning of the full-length *SlCCD8*

A 1233-bp partial *SlCCD8* coding sequence was amplified from tomato (cv Moneymaker) by reverse-transcriptase polymerase chain reaction (RT-PCR) using primers (forward, 5′-GCTGAGT GGCACGTACCTAA-3′; reverse, 5′-TCATCTTCTTCGGTT GCAC-3′) designed to a highly conserved region of plant CCD8s. The *SlCCD8* 5′- and 3′-cDNA ends were obtained using the SMART RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA; 5′-RACE, 5′-GCGTCCGATTCGATTTC-3′ and 3′ RACE, 5′-TCCTGCTTATTTAGGCAAG-3′). The complete *SlCCD8* coding sequence (1674 bp) was PCR amplified from root cDNA (forward, 5′-ATGGCTTCTTTTGCTCA TTCAG-3′; reverse, 5′-CTATTTCTTTGGAACCCAGC-3′). Finally, the amplified cDNA fragment was modified using the A-tailing procedure and cloned into pGEM-T Easy vector (Promega) according to the manufacturer instructions, and both strains sequenced.

RNAi-mediated silencing of *SlCCD8* and tomato transformation

The silencing of *SlCCD8* in tomato was carried out by means of a 349 bp fragment plus the gateway CACC directional cloning sequence that was PCR amplified using specific primers (forward, 5′-CACCCAGGACAATGGCACATAGGT-3′; reverse, 5′-TCT AGGGTGTTCGGATCAA-3′). The PCR fragment was cloned into the pENTR/D vector (Gateway Technology; Invitrogen) and then introduced into the binary destination vector pHellsgate8 (Helliwell *et al.*, 2002) by recombination using the LR clonase II (Invitrogen). The pHellsgate8::CCD8 RNAi construct was transferred to *Agrobacterium tumefaciens* strain LB4404 and used to transform tomato (cv Craigella) plants as previously described (van Roekel *et al.*, 1993).

RNA isolation and gene expression analysis by real time quantitative RT-PCR (qPCR)

Total RNA was extracted using Tri-Reagent (Sigma-Aldrich St. Louis, 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 RNA Clean-up kit (Macherey-Nagel, Düren, Germany) and stored at − 80°C until use. For gene expression analysis by real-time quantitative PCR (qPCR) the iCycler iQ5 system (Bio-Rad Hercules, CA, USA) was used (Spinsanti *et al.*, 2006) using specific primers. For the tomato elongation factor-1α (household gene) *SlEF*: 5′- GATTGGTGGTATTGGAACTGTC-3′ and 5′-AGCTTC GTGGTGCATCTC-3′; for *SlCCD7*: 5′-AGCCAAGAATTCG AGATCCC-3′ and 5′-GGAGAAAGCCCACATACTGC-3′; for *SlCCD8*: 5′-CCAATTGCCTGTAATAGTTCC-3′ and 5′-GC CTTCAACGACGAGTTCTC-3′. The first-strand cDNA was synthesized with 1 μg of purified total RNA using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Three independent biological replicates were used and each PCR reaction was done in triplicate. Relative quantification of mRNA level was performed using the comparative C_t method (Livak & Schmittgen, 2001). Values were normalised using the C_t value for the tomato household gene *SlEF* (Rotenberg *et al.*, 2006). Values were used to determine the change in gene expression according to the following calculation: fold-change = $2^{-\Delta(\Delta C_t)}$, where $\Delta C_t = C_t$ (target) $- C_t$ (household) and $\Delta(\Delta C_t) = \Delta C_t$ (treatment) $- \Delta C_t$ (control).

Strigolactone and auxin analysis by multiple reaction monitoring liquid chromatography-tandem mass spectrometry (UPLC-MS/MS)

Root exudates were purified and concentrated as previously described (López-Ráez *et al.*, 2008b, 2010) with some modifications. Five liters of root exudates were loaded onto a pre-equilibrated C18 column (GracePure C18-Fast 5000 mg 20 ml^{-1}). Subsequently, columns were washed with 50 ml of demineralised water, and 50 ml of 30% acetone/water. Strigolactones were eluted with 50 ml of 60% acetone/water. All exudates were collected within 2 h and stored at − 20°C before measurements. Strigolactones were extracted from root material as previously described (López-Ráez *et al.*, 2010). Xylem sap was collected and purified as previously described (Kohlen *et al.*, 2011a). Analysis and quantification of strigolactones were performed using UPLC-MS/MS, as previously described (Kohlen *et al.*, 2011a). Auxin (IAA) was

extracted, purified and analysed as previously described (Ruyter-Spira *et al.*, 2011).

Phelipanche ramosa germination assay and infection study

Germination assays with *Phelipanche ramosa* (L.) Pomel seeds were conducted as previously reported by Matusova *et al.* (2005). GR24 (10^{-9} M) and demineralised water were included as positive and negative controls, respectively. To perform the *P. ramosa* infection assay, 3-l pots were filled with a soil:sand mixture (3 : 1) and seeds were added following the procedure previously described by Kroschel (2001) with some modifications. *P. ramosa* seeds were sown in a layer of *c.* 3–10 cm below the surface at a density of 15 mg · pot^{-1}. In order to keep a moist environment, pots were watered daily with 60 ml of tap water for 12 d. Then, 5-d-old tomato seedlings were introduced into the pots and watered for an additional 5 d as described before. Subsequently, pots were irrigated with 300 ml of tap water twice a week and once with 300 ml of full-strength Hoagland solution (Hoagland & Arnon, 1950). Emerged *P. ramosa* shoots were counted 10 wk after tomato planting.

Analysis of AM colonization of *SlCCD8* knock-down lines

The AM fungus *Glomus intraradices* N.C. Schenck & G.S. Sm (BEG 121) was maintained as a soil:sand based inoculum containing a mix of diverse fungal propagules (spores, hyphae and chopped mycorrhizal roots). Tomato seeds of the *SLCCD8* knock-down lines and corresponding wild-type (cv Craigella) were surface-sterilised and germinated for 3 d on a container with sterile vermiculite at 25°C in darkness. Subsequently, individual seedlings were transferred to 0.5-l pots with a sterile sand:soil (4 : 1) mixture. Pots were inoculated by adding 10% (v : v) *G. intraradices* inoculum. The same amount of soil:sand mix but free from AM was added to control plants. For each treatment five replicate plants were used. Plants were randomly distributed and grown in a glasshouse at 24/16°C with 16/8 h photoperiod and 70% humidity and watered three times a week with Long Ashton nutrient solution (Hewitt, 1966) containing 25% of the standard phosphorous concentration. Plants were harvested after 8 wk of growth. Roots were stained with trypan blue (Phillips & Hayman, 1970) and examined using a Nikon Eclipse 50i microscope (Nikon Corporation, Tokyo, Japan) under bright-field conditions. The percentage of root length colonised by the AM fungus was determined by the gridline intersection method (Giovannetti & Mosse, 1980).

Sucrose analysis by high-performance liquid chromatography (HPLC)

Samples were extracted and analysed as previously described (Sergeeva *et al.*, 2000).

Statistical analysis

Data for strigolactone and auxin content were subjected to one-way analysis of variance (ANOVA) using GenStat for Windows (9th edition). To analyse the results of germination bioassays, ANOVA after arcsine(square root(X)) transformation was used. When appropriate, data were subjected to the Student's *t*-test.

Results

SlCCD8 cloning and characterization

A search of the available tomato EST libraries failed to identify ESTs with homology to any published *CCD8* sequence. Therefore, a PCR-based approach using primers designed against highly conserved regions in known plant *CCD8*s in combination with RACE was used to isolate the full-length coding sequence of the putative tomato *CCD8*/*MAX4*, hereafter designated as *SlCCD8* (JF831532). *SlCCD8* has an open reading frame (ORF) of 1674 bp (Supporting Information Table S1). A BLAST search of the *SlCCD8* sequence was performed on the tomato genome (Bombarely *et al.*, 2011) and the sequence aligned with a 4100-bp region located on chromosome 8. *SlCCD8* is predicted to contain six exons (Fig. 1a). The ORF encodes a 557 amino acid protein (Table S2) with 89% and 66% homology to petunia CCD8/DAD1 (PhCCD8/DAD1 (Snowden *et al.*, 2005)) and Arabidopsis CCD8/MAX4 (AtCCD8/MAX4 (Sorefan *et al.*, 2003)) proteins, respectively (Fig. 1b, Table S3). In a phylogenetic alignment, *SlCCD8* clustered closely together with *PhCCD8/DAD1* in what seems to constitute a sub-clade of dicot *CCD8s* (Fig. 1c). Monocot *CCD8s* of maize, rice and sorghum clustered separately from dicot *CCD8s*. *SlCCD8* expression was detected at low levels in all plant tissues. However, it was primarily expressed in tomato roots and stems with the highest expression in roots (Fig. 2a).

In order to address the biological function of *SlCCD8*, an RNA interference (RNAi) construct containing a specific region of 349 bp of the gene was created and introduced into tomato (cv Craigella) through *Agrobacterium tumefaciens*-mediated transformation. Three independent *SlCCD8* RNAi lines (L16, L04 and L09) displaying 64%, 91% and 97% reduction in *SlCCD8* mRNA levels in the roots, respectively (Fig. 2b), were selected and propagated to T_3 generation. Transcript levels of the other closely related carotenoid cleavage dioxygenase described in tomato – *SlCCD7* (Vogel *et al.*, 2010) – were not affected in any of the transgenic lines (Fig. S1), demonstrating the specificity of the RNAi construct for *SlCCD8*.

Role of *SlCCD8* in rhizosphere signaling

The concentrations of all strigolactones previously reported in tomato (López-Ráez *et al.*, 2008a,b) were reduced in the exudates of all three transgenic lines compared with wild-type plants. In L16 strigolactone concentrations were reduced by 52% and in L04 and L09 by 95% (Fig. 3a), correlating with the reduction in *SlCCD8* transcript (Fig. 2b). Colonisation by the mutualistic AM fungus *Glomus intraradices* in L16, L04 and L09 was reduced by 27%, 44% and 65%, respectively, compared to wild-type plants (Fig. 3b). This reduction in AM symbiosis correlated to some extent with the decrease in strigolactone exudation (Fig. 3a). In addition, a 90% reduction in shoot emergence of *Phelipanche ramosa* was observed

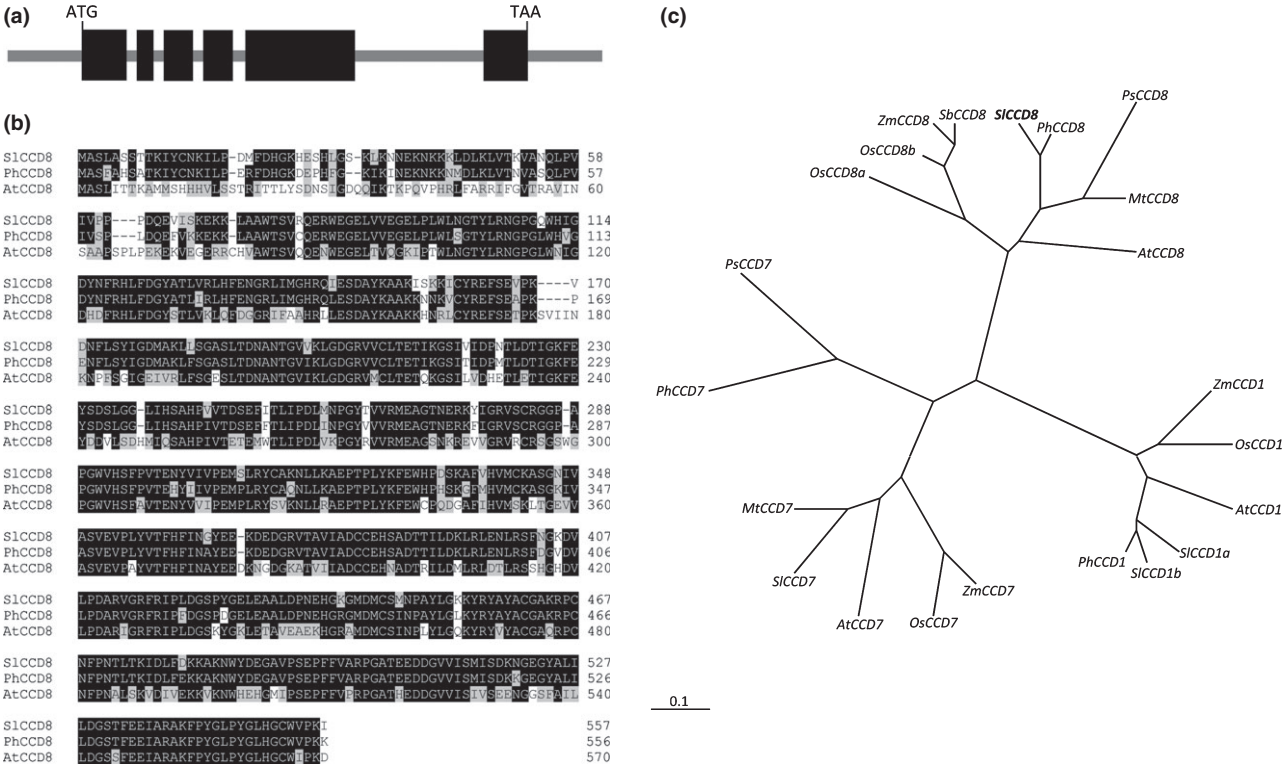

Fig. 1 Tomato (*Solanum lycopersicum*) *CCD8* (*SlCCD8*). (a) The postulated intron/exon structure for *SlCCD8* (4100 nt). (b) Alignment of the putative SlCCD8 amino acid sequence with those from known CCD8 proteins; Sl, *Solanum lycopersicum*; Ph, *Petunia hybrida*; At, *Arabidopsis thaliana*. Identical and similar amino acids are shaded in black and grey, respectively (c) Phylogenetic tree of known *CCD1*, *CCD7* and *CCD8* nucleotide sequences; Sl, *Solanum lycopersicum*; Ph, *Petunia hybrida*; At, *Arabidopsis thaliana*; Ps, *Pisum sativum*; Os, *Oryza sativa*; Zm, *Zea mays*; Mt, *Medicago truncatula*; Sb, *Sorghum bicolor* (*AtCCD1* (AT3G63520), *OsCCD1* (Os12g0640600), *PhCCD1* (AY576003), *SlCCD1a* (AY576001), *SlCCD1b* (AY576002), *ZmCCD1* (GRMZM2G057243), *AtCCD7* (AT2G44990), *MtCCD7* (Medtr7g040830), *OsCCD7* (Os04g0550600), *PsCCD7* (DQ403160), *PhCCD7* (FJ790878), *SlCCD7* (GQ468556), *ZmCCD7* (GRMZM2G158657), *AtCCD8* (AT4G32810), *MtCCD8* (Medtr3g127920), *OsCCD8a* (Os01g0566500), *OsCCD8b* (Os01t0746400), *PsCCD8* (AY557342), *PhCCD8* (AY743219), *SbCCD8* (Sb03g034400), *SlCCD8* (JF831532), *ZmCCD8* (GRMZM2G446858).

for all three transgenic lines (Fig. 3c). *Phelipanche ramosa* seed germination using root exudates was significantly lower for all transgenic lines (Fig. 3d), and this decrease correlated well with the reduction in strigolactone exudation (Fig. 3a).

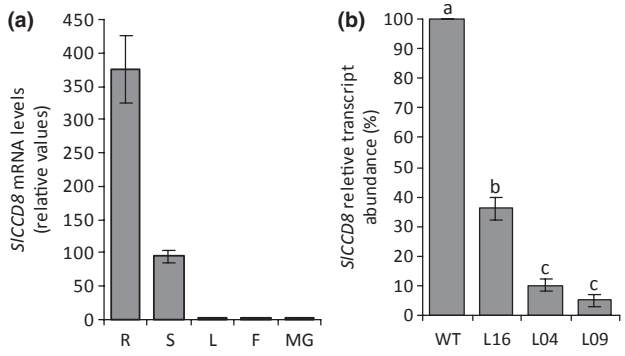

Fig. 2 *SlCCD8* transcript accumulation (normalised to the tomato (*Solanum lycopersicum*) household gene *SlEF*). (a) Relative gene expression of *SlCCD8* in tomato cv Craigella (WT) in different plant tissues: R, root; S, stem; L, leaf; F, flower; MG, mature green fruit. (n = 3) (b) Relative gene expression of *SlCCD8* in the roots of cv Craigella (WT) and three independent *SlCCD8* RNAi lines (L16, L04, and L09) The expression in wild-type tomato is set at 100% (n = 3). Error bars represent means ± SE. Bars with different letters differ significantly at $P < 0.05$.

SlCCD8 knock-down alters strigolactone concentrations *in planta*

Strigolactone concentrations in root extracts of L16, L04 and L09 were also analysed. As in exudates, a clear reduction in the concentration of all strigolactones was detected for all three transgenic lines (Fig. 4a). On average, the strigolactone concentration in root extracts of L16, which showed the mildest reduction in *SlCCD8* expression, was reduced by 53%, whereas strigolactone concentrations in L04 and L09 were reduced by 92% and 94%, respectively (Fig. 4a). This decrease in strigolactone production was almost identical to the reduction observed in the root exudates (Fig. 3a).

As mentioned above, strigolactone production is induced under phosphate deficiency (Yoneyama *et al.*, 2007; López-Ráez *et al.*, 2008a), just as the concentration of orobanchol in the xylem sap of Arabidopsis (Kohlen *et al.*, 2011a). Indeed, a five-fold increase in the concentration of orobanchol was also detected in the xylem sap of phosphate-starved wild-type tomato plants (Fig. 4b, Fig. S2). The three knock-down lines L16, L04 and L09 showed a clear reduction in xylem sap orobanchol concentrations (26%, 55% and 84%, respectively) compared with wild-type plants (Fig. 4c). Interestingly, neither solanacol nor any of the

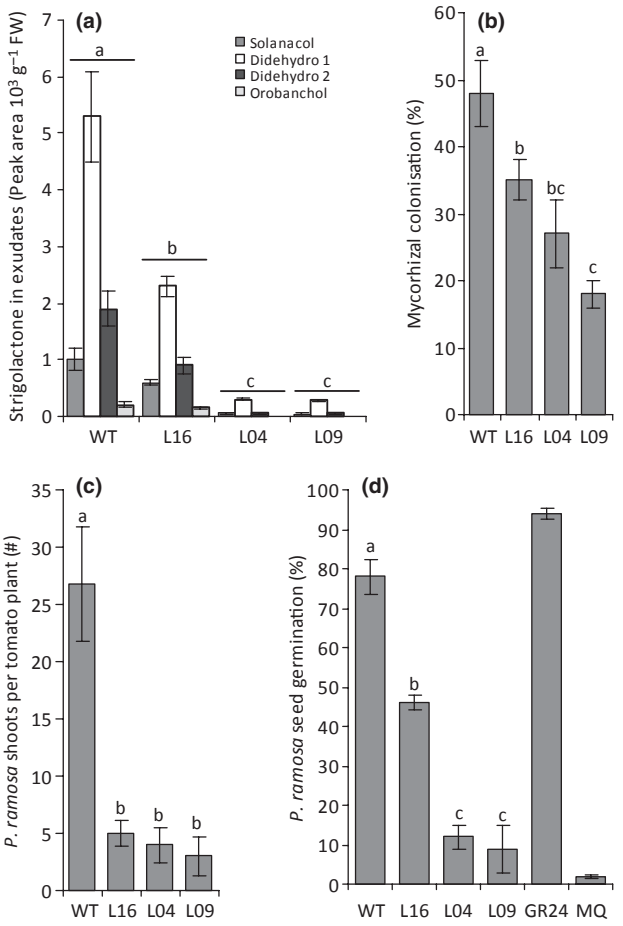

Fig. 3 Strigolactone content of root exudates and rhizosphere interaction analysis of tomato (*Solanum lycopersicum*) cv Craigella (WT) and three independent *SlCCD8* RNAi lines (L16, L04, and L09). (a) MRM-LC-MS/MS quantification of the major tomato strigolactones (solanacol, didehydro-orobanchol isomers 1 and 2, and orobanchol) in root exudates according to the peak area (n = 3). (b) Percentage of root colonisation by the arbuscular mycorrhizal fungi *Glomus intraradices* (n = 6). (c) *Phelipanche ramosa* infestation counted as emerged shoots per tomato plant (n = 6). (d) *P. ramosa* seed germination induced by root exudates. Synthetic strigolactone analog GR24 (10^{-9} M) and demineralised water (MQ) are positive and negative control, respectively (n = 3). Error bars represent means ± SE. Bars with a different letter differ significantly; $P < 0.05$.

didehydro-orobanchol isomers were detected in the xylem sap. A second strigolactone – orobanchyl acetate – was also detected in tomato xylem sap (Fig. S2), but its concentration was too low to accurately quantify its reduction in the transgenic lines or to assess the effect of phosphate starvation on the concentration of this compound in the xylem. No sucrose was detected in any of the xylem sap samples analysed (Fig. S3), showing the samples are not contaminated with phloem sap. Furthermore, hypocotyls were also analysed for their strigolactone content, but strigolactones were undetectable in these samples (Fig. S4). These data confirm that the orobanchol detected is from the xylem sap and not from contaminating phloem sap or hypocotyl tissue.

Effect of decreased *SlCCD8* expression on shoot architecture

In order to assess the consequence of the reduced strigolactone concentrations on shoot architecture, L16, L04 and L09 lines were grown in pots for 8 wks and their phenotypes compared with wild-type plants (Fig. 5a). All three knock-down lines were significantly more branched, displaying a 1.7-, 4.4- and 7.3-fold increase in lateral shoot branch number, respectively (Fig. 5b), which inversely correlated with the level of *SlCCD8* transcript (Fig. 2b) and strigolactone production (Figs 3a, 4a). GR24 application complemented the branching phenotype (Fig. 5c), further confirming its relation to strigolactone depletion. To get a more detailed insight into the effect of the reduction in *SlCCD8* expression on branching, the distribution of first- and second-order branches was analysed. In wild-type plants *c.* 50% of the primary stem nodes were carrying a visible lateral branch, most of which were shorter than 5 cm (Fig. S5). All *SlCCD8* knock-down lines had a significantly ($P < 0.05$) higher number of branches of the first order (Fig. S5). Moreover, these branches were longer than in the wild-type plants (Fig. S5). No branches of the second order were observed in any of the wild-type plants at this stage of development (Figs 5d, S5), whereas all knock-down lines displayed multiple lateral branches of the second order (Figs 5e, S5). The transgenic lines – L16, L04 and L09 – also displayed a reduction in the primary stem height of 19%, 52% and 60%, respectively, compared with wild-type plants (Fig. 5f). In addition, the total number of nodes in the transgenic lines increased slightly, but significantly ($P < 0.05$; Fig. 5g).

SlCCD8 reduction alters adventitious rooting

In addition to these phenotypes, all transgenic plants, but not the wild-type, displayed a larger number of root primordia and adventitious roots on their stems (Fig. 6a,b). The severity of this phenotype was less pronounced in L16 than in L04 and L09, correlating with the stronger reduction in strigolactone biosynthesis in these latter two lines (Fig. 4a). Cuttings from the *SlCCD8* knock-down lines produced 34%, 77% and 89% (L16, L04 and L09, respectively) more adventitious roots compared with wild-type plants (Fig. 6c), a phenotype that could be rescued by GR24 application (Fig. 6c). Moreover, application of GR24 also reduced the number of adventitious roots (Fig. 6c) and adventitious root primordia (Fig. 6d) in wild-type plants. Conversely, IAA application led to an increase in the number of adventitious root primordia (Fig. 6d), while combined GR24 and IAA application restored this to near untreated levels (Fig. 6d). The free IAA concentration in the lower stem (first internode above the cotyledons) was 1.9-, 2.3- and 3.2-fold higher in L16, L04 and L09, respectively, than in wild-type plants (Fig. 6e), correlating with the increase in adventitious rooting.

Effect of *SlCCD8* knock-down on tomato reproductive development

An unexpected phenotype observed in the *SlCCD8* knock-down lines was that their flowers were significantly smaller than in

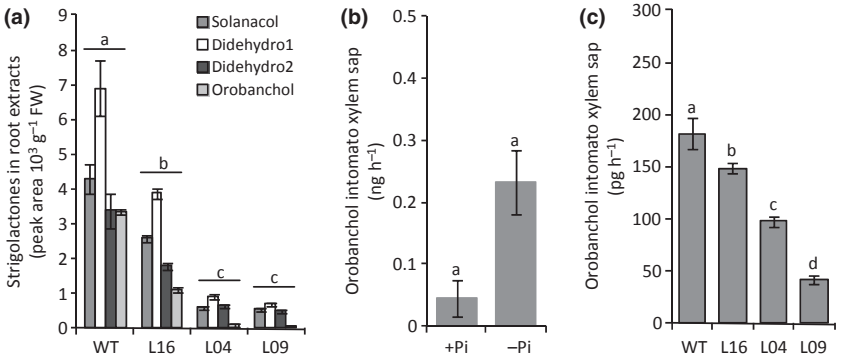

Fig. 4 MRM-LC-MS/MS quantification of strigolactone content *in planta* of tomato (*Solanum lycopersicum*) cv Craigella (WT) and three independent *SlCCD8* RNAi lines (L16, L04, and L09). (a) Quantification of the major tomato strigolactones (solanacol, didehydro-orobanchol isomers 1 and 2, and orobanchol) in root extracts of 5-wk-old plants according to the peak area (n = 3). (b) Quantification of the effect of treatment with sufficient phosphate (+ Pi) and limiting phosphate (− Pi) on orobanchol content in tomato xylem sap (n = 3). (c) Quantification of orobanchol in tomato xylem sap of 8-wk-old plants (n = 5). Error bars represent means ± SE. Bars with different letters differ significantly (P < 0.05).

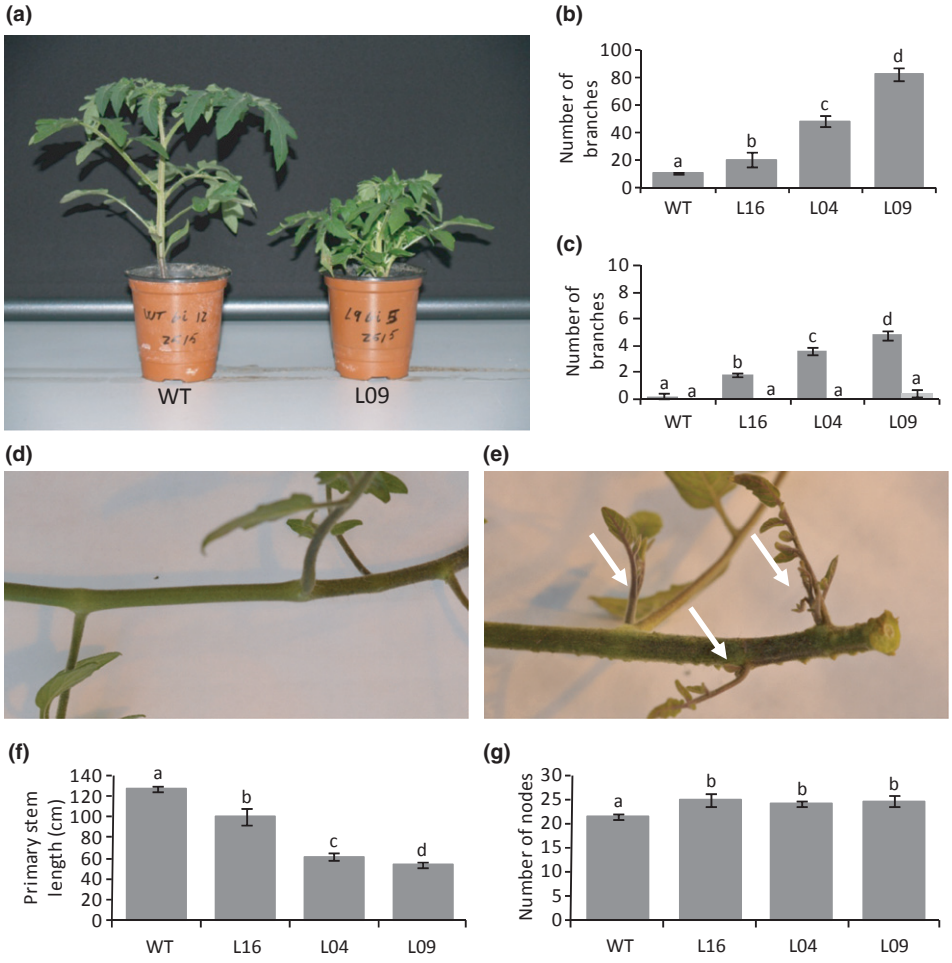

Fig. 5 Analysis of plant architecture of tomato (*Solanum lycopersicum*) cv Craigella (WT) and three independent *SlCCD8* RNAi lines (L16, L04, and L09). (a) Photograph of 4-wk-old wild type and transgenic *SlCCD8* RNAi line L09 plants. (b) Average number of visible branches (1st and 2nd order, > 2 mm) on 8-wk-old plants (n = 5). (c) The effect of GR24 on branching in 4-wk-old plants (n = 5). Light grey bars, 5 μM GR24; dark grey bars, control. (d) Close-up of 8-wk-old wild-type primary branch. (e) Close-up of 8-wk-old L09 primary branch, arrows indicate secondary branches. (f) Average main stem length of 8-wk-old plants (n = 5). (g) Average number of nodes per main stem of 8-wk-old plants (n = 5). Error bars represent means ± SE. Bars with different letters differ significantly (P < 0.05).

wild-type plants (Fig. 7a). In order to quantify this effect and elucidate a possible role of strigolactones in flower development, the length of sepals, petals and anthers were measured at anthesis. The average length of all these organs was significantly (P < 0.05) reduced in all three transgenic lines (Fig. 7b). The diameter of the ovaries was also significantly reduced (Fig. 7c). This effect persisted throughout fruit development (Fig. 7d), leading to significantly smaller fruits in all transgenic lines compared with the wild-type in different ripening stages, mature green (40 DAP), breaker and ripe red (Fig. 7e,f). Although fruit size of all transgenic lines was reduced, only in L04 and L09 was the total fruit yield slightly decreased (Fig. 7g).

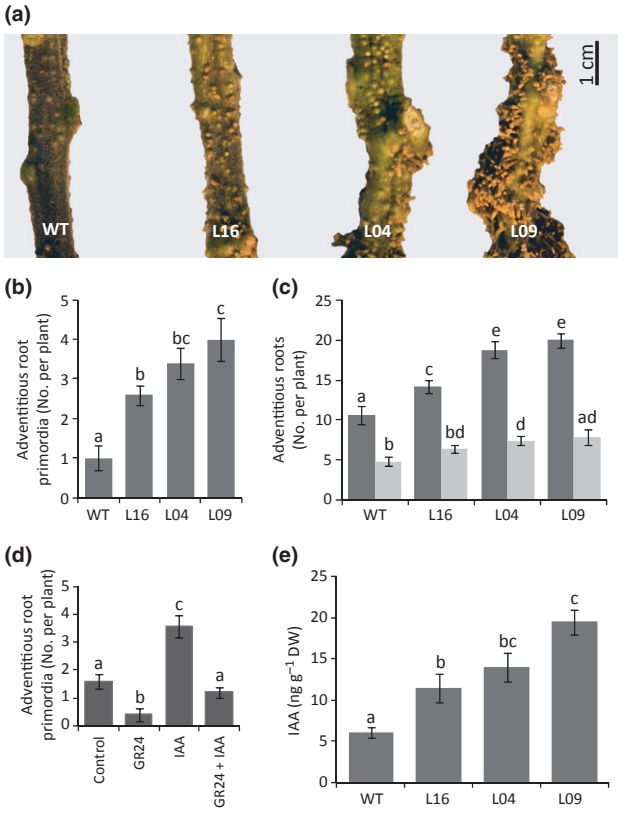

Fig. 6 Analysis of adventitious root development on tomato (*Solanum lycopersium*) cv Craigella (WT) and three independent *SlCCD8* RNAi lines (L16, L04, and L09). (a) Photograph of adventitious root primordia on 8-wk-old plants. (b) Average number of visible adventitious root primordia on 4-wk-old plants ($n = 5$). (c) The effect of GR24 (light grey bars) on adventitious root development in cuttings ($n = 5$). Dark grey bars, control. (d) The effect of 5 μM GR24 and 2.5 μM IAA on adventitious root development in 4-wk-old plants (e) Concentration of free IAA in the lower stem ($n = 3$). Error bars represent means ± SE. Bars with different letters differ significantly ($P < 0.05$).

As fruit size usually correlates with seed quantity (Mapelli *et al.*, 1978), the effect of *SlCCD8* knock-down on seed set was investigated. A reduction of about 65% in seed quantity (Fig. 7h) and a reduction in seed size (Fig. S6) was observed in all three knock-down lines. Despite their reduced number and size, the quality of the seeds appeared to be unaffected because no obvious differences in germination rate were observed in any of these lines when germinated under normal conditions (Fig. S7).

Auxin plays an important role in several strigolactone-related phenotypes and it has been shown to be one of the major regulators of fruit development (Mapelli *et al.*, 1978; Gillaspy *et al.*, 1993). Moreover, it has recently been demonstrated that during fruit development an auxin gradient between developing seeds, placenta and the pericarp is established during phase III, with relatively high auxin concentrations in the developing seeds (Pattison & Catalá, 2012). For this reason the concentration of free auxin was assessed in fruits harvested during this phase of fruit development. Indeed, in wild-type fruits a 4.5-fold higher concentration of IAA was detected in the fruit parts containing the seeds compared with the pericarp (Fig. 7i). This auxin gradient between the different tissues was absent in the *SlCCD8* knock-down fruits (Fig. 7i).

Discussion

In the present study we identified and characterised a second strigolactone biosynthetic gene in tomato – *SlCCD8* – encoding a carotenoid cleavage dioxygenase. SlCCD8 showed highest homology to the petunia PhDAD1/CCD8, belonging to a dicot subclade within the CCD8 cluster, clearly separated from the monocots. *SlCCD8* is expressed in all tissues examined, but predominantly in root and stem tissue. This expression pattern is in agreement with CCD8 expression in petunia, Arabidopsis, pea and rice (Napoli, 1996; Sorefan *et al.*, 2003; Bainbridge *et al.*, 2005; Arite *et al.*, 2007), indicating a conserved pattern across plant species. In the Arabidopsis *max4/CCD8* mutant, *MAX3/CCD7* expression was shown to be downregulated (Mashiguchi *et al.*, 2009). This feedback seems not to be present in tomato as the expression of SlCCD7 was not reduced. It has been reported that CCD7 is involved in processes other than strigolactone biosynthesis such as the production of mycorradicin in mycotrophic plants such as tomato (Walter *et al.*, 2010). Therefore, the regulation of *CCD7* may differ between tomato and other nonmycorrhizal plant species. By generating *SlCCD8* knock-down lines with a reduction in strigolactone levels, we showed that SlCCD8, through strigolactone biosynthesis, is involved in the regulation of multiple processes relevant for plant physiology and rhizosphere signaling.

Strigolactones were initially identified as germination stimulants for root parasitic plants of the Orobanchaceae (Cook *et al.*, 1966; Bouwmeester *et al.*, 2003) and hyphal branching factors for AM fungi (Akiyama *et al.*, 2005). As expected, reduced strigolactone concentrations in all the *SlCCD8* knock-down tomato lines resulted in reduced AM colonisation (Fig. 3a,b). However, there was a nonlinear correlation between AM colonisation and strigolactone reduction, with the reduction in mycorrhization being less severe than the reduction in strigolactone concentrations. This is probably due to the use of a mixed inoculum containing mycelium, colonised roots and spores. Because strigolactones are hyphal branching factors for AM fungi that seem to be particularly important in germinating spores, AM colonisation could be more compromised by strigolactone reduction if only spores are used, as was previously demonstrated (Koltai *et al.*, 2010b). In the present study a mixed inoculum was used as it more closely resembles the natural situation in the rhizosphere (Klironomos & Hart, 2002). Interestingly, the infection of all *SlCCD8* knock-down lines by *P. ramosa* was reduced by 90% compared with wild-type plants (Fig. 3c). The observed reduction in emerging parasite shoots in L16 cannot be explained exclusively by its reduced strigolactone exudation (*c.* 60%), also considering that germination of *P. ramosa* seeds when using root exudates was only reduced by *c.* 50%. Possibly, the increased shoot branching of the host and the associated resource requirement inhibits *P. ramosa* development. Alternatively, it might be possible that strigolactones produced by the host plant are also required in later phases of the *P. ramosa* lifecycle after seed germination. Nevertheless, our results show that a mild reduction in strigolactone exudation could be sufficient to

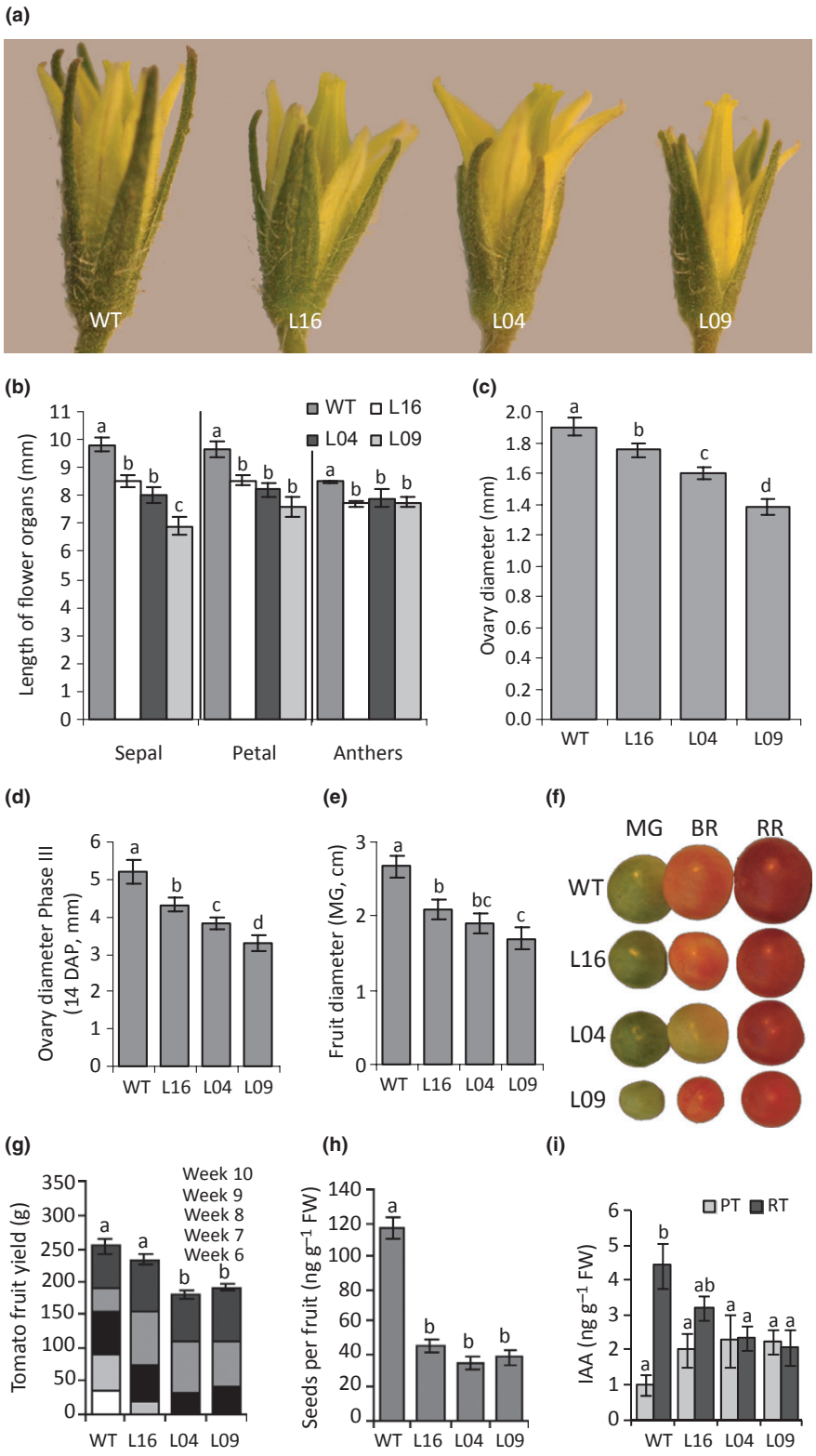

Fig. 7 Analysis of flowers, fruits and seeds of tomato (*Solanum lycopersium*) cv Craigella (WT) and three independent *SlCCD8* RNAi lines (L16, L04, and L09). (a) Photograph of flowers at anthesis. (b) Flower organs length ($n = 5$). (c) Diameter of un-pollinated ovaries ($n = 5$). (d) Diameter of fruits at phase III tomato fruit development (14 DAP, days after pollination; $n = 10$). (e) Diameter of mature green (MG) fruits (40 DAP; $n = 10$). (f) Photograph of tomato fruits; mature green (MG), breaker (BR) and mature red (MR). (g) Fruit yield in grams fresh weight over a 10-wk period. (h) Number of seeds per fruit ($n = 10$). (i) Auxin distribution at phase III (14 DAP); PT, pericarp tissue; RT, remaining tissue. ($n = 3$). Error bars represent means ± SE. Bars with a different letter differ significantly ($P < 0.05$).

significantly reduce parasitic weed infection without severely compromising AM symbiosis or apical dominance (Figs 3b, 5). Moreover, fruit yield of L16 was hardly affected, even though initial fruit set was delayed (Fig. 7i). These findings make strigolactone biosynthesis an attractive target for controlling root parasitism, as previously postulated (Bouwmeester *et al.*, 2003; López-Ráez *et al.*, 2009, 2011b; Cardoso *et al.*, 2011). However, more research is needed to further assess the consequences of a mild reduction in strigolactone content on fruit quality and parasitic weed resistance under field conditions.

The concentrations of strigolactones in root extracts in all *SlCCD8* knock-down lines were reduced to the same extent as in their exudates. This shows that, as expected, strigolactone biosynthesis and not secretion is compromised in these transgenic lines. All transgenic lines displayed a reduced primary stem height and an increase in the total number of lateral branches. Both phenotypes inversely correlated – to some extent – with the strigolactone concentrations. Surprisingly, in roots of L04 the reduction in *SlCCD8* expression and strigolactone content were more severe than expected considering the moderate increase in lateral shoot outgrowth when compared with L09. Interestingly, a better inverse correlation with lateral branching across the knock-down lines was observed with the concentrations of orobanchol present in the xylem sap (Fig. 4c). It seems that the orobanchol concentration in the xylem sap was less affected than in the exudates, which suggests a preferential loading into the xylem instead of secretion to the rhizosphere. The ratio of orobanchol and the other strigolactones was much higher in root extracts than in root exudates. Furthermore, neither solanacol nor any of the didehydro-orobanchol isomers were detected in the xylem sap, whereas they were abundantly present in root exudates and extracts (Figs 3a, 4a). This suggests that a selective mechanism of localised strigolactone biosynthesis and/or transport ensures that orobanchol is less well secreted into the rhizosphere and is preferentially transported through the xylem to the shoot. Therefore, indicating that this strigolactone (and/or its derivatives) might be the one that is regulating shoot branching, while solanacol and the didehydro-orobanchol isomers could have a role as signaling molecules in the rhizosphere (Fig. 8). As strigolactones also co-regulate root system architecture (Kapulnik *et al.*, 2011; Ruyter-Spira *et al.*, 2011), it is possible that all are involved in this belowground function (Fig. 8). However, it is also possible that solanacol and the didehydro-orobanchol isomers have no function in the plant and merely act as rhizosphere signaling molecules. It was previously proposed that solanacol is derived from orobanchol through didehydro-orobanchol (Xie *et al.*, 2010; Kohlen *et al.*, 2011b). Therefore, orobanchol might play a double role as the transmissible signal regulating root and shoot architecture, and as the precursor to signaling molecules in the rhizosphere.

When strigolactone production was assessed under phosphate-limiting conditions, a strong increase in all tomato strigolactones was detected in root extracts (Fig. S8), which is in line with previous findings (López-Ráez *et al.*, 2008a). In addition, we show that phosphate limitation also induced a five-fold increase in orobanchol concentrations in the xylem sap (Fig. 4b). Therefore, it seems that this upregulation under phosphate deficiency is a more conserved trade as it has also been demonstrated in Arabidopsis (Kohlen *et al.*, 2011a).

A reduction in main stem height was also observed for all transgenic lines, again correlating with xylem sap orobanchol concentrations. The reduction in plant height inversely correlated to the increased number of shoot branches. This suggests that perhaps the observed dwarfism in the strigolactone-deficient knock-down lines is a secondary effect of the increased lateral shoot growth. However, the total number of internodes slightly increased in all transgenic lines (Fig. 5g). Our results show that the reduction in plant height in the *SlCCD8* knock-down lines is due to shorter internodes maybe because of increased competition for nutrients between the primary stem and the axillary branches. However, the increase in node number suggests that strigolactones are not only involved in the regulation of bud outgrowth, but possibly also in the timing of organ development. Additional research will be needed to study this in more detail.

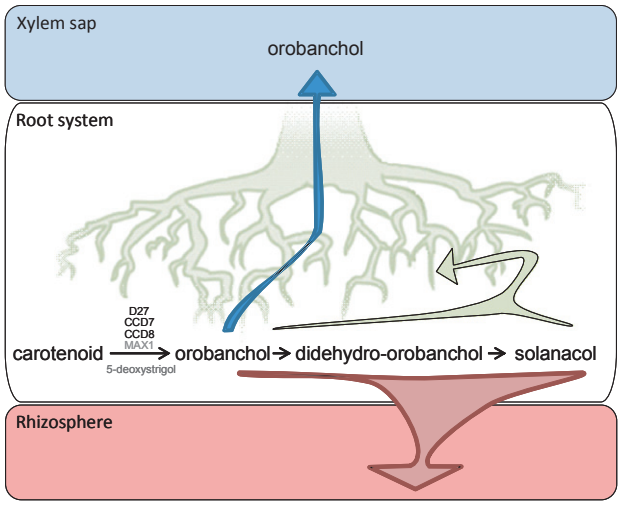

Fig. 8 Proposed model for strigolactone movement in- and outside the plant.

It has already been shown that strigolactones play a role in more plant development processes than just axillary bud outgrowth (Tsuchiya *et al.*, 2010; Kapulnik *et al.*, 2011; Ruyter-Spira *et al.*, 2011). Strigolactones were also shown to co-regulate adventitious root development in pea (Rasmussen *et al.*, 2012). Our results corroborate this new function because all transgenic lines showed a marked increase in adventitious root development and rooting of cuttings (Fig. 6). Interestingly, the increase in adventitious rooting in the transgenic lines correlated with elevated auxin concentrations in the lower region of the stem, possibly a result of elevated transport as was previously reported (Bennett *et al.*, 2006). It is known that auxin induces lateral root formation (Klerk *et al.*, 1999), therefore it is plausible that the observed repressive effect of strigolactones on adventitious root formation is a consequence of their assumed control over auxin transport. However, it was recently postulated that the increase in adventitious root initiation in pea and Arabidopsis is not due to elevated auxin concentrations but to increased auxin sensitivity (Rasmussen *et al.*, 2012). The fact that the combined application of IAA and GR24 suppressed the increase in adventitious rooting induced by IAA (Fig. 6d) seems to support this hypothesis. If so, the elevated IAA concentrations detected in the lower part of the stem might be a secondary effect of reduced auxin sensitivity. Nevertheless, our results indicate that a link between strigolactones and auxin exists in adventitious root initiation. However, to determine the precise underlying mechanism of strigolactones-auxin cross-talk in relation to this phenotype further research will be needed.

A putative function for strigolactones in fruit development was already postulated as *SlCCD7* is highly expressed in mature green and turning tomato fruits (Vogel *et al.*, 2010), and the expression

of *AcCCD7* and *AcCCD8* in *Actinidia chinensis* (kiwifruit) was shown to be relatively high in young fruits and seeds (Ledger *et al.*, 2010). The petunia *ccd8/dad1* mutant was reported to have smaller flowers (Snowden *et al.*, 2005). In agreement with these observations, we observed that in the *SlCCD8* knock-down lines sepals, petals and anthers were smaller than in wild-type plants (Fig. 7), suggesting that strigolactone deficiency affects flower development. It might be that this phenotype is the result of increased competition for resources in these highly branched plants. However, the possibility that strigolactones have a more direct role in controlling flower development cannot be excluded. In addition to smaller flowers, the fruit size in the transgenic lines was also reduced, a reduction which correlated inversely with the strigolactone content in these lines. The relative reduction in fruit size was similar to the reduction in the diameter of un-pollinated ovaries. This implies that the initial reduction in ovary size is not compensated during later stages of fruit development, but also that the severity of the phenotype does not increase, which would be expected if the reduced fruit size is only caused by competition for resources. Moreover, as fruit yield was not strongly reduced in any of the transgenic lines, resource limitation seems not to be exclusively responsible for these reproductive phenotypes. It has been reported that auxin produced in developing seeds is required for fruit growth (Mapelli *et al.*, 1978). Seed number, as well as seed size in the transgenic lines were clearly reduced compared with wild-type plants (Fig. 7g), and it is hence possible that this reduction – resulting in a lower auxin production – is responsible for the observed reduction in fruit size. However, it cannot explain the reduction in ovary size before pollination, because no auxin producing seeds are present at this stage. As mentioned above, cross-talk between strigolactones and auxin seems to play an important role in several strigolactone-related phenotypes (Bennett *et al.*, 2006; Agusti *et al.*, 2011; Ruyter-Spira *et al.*, 2011; Rasmussen *et al.*, 2012). It is plausible that a similar mechanism forms the basis for the observed reproductive phenotypes, linking the effect of strigolactones on reproductive development to the co-regulation of auxin sensitivity and/or distribution. Auxin is known to be an important regulator of both flower and fruit development (Mapelli *et al.*, 1978; Gillaspy *et al.*, 1993) and it was demonstrated that auxin biosynthesis loss-of-function mutants display severe reproductive defects including aberrant flower morphology and male/female sterility (Cheng *et al.*, 2006; Gallavotti *et al.*, 2008). The relative mild phenotypes observed in all transgenic lines are hence not a consequence of auxin loss-of-function, but of auxin reduce-of-function. However, more research will be needed to further explore this possible interaction between strigolactones and auxin in reproductive development.

Overall, our results demonstrate that SlCCD8 is required for strigolactone biosynthesis in tomato and therefore is crucial in regulating several processes in plant development and rhizosphere signaling. We showed that a relatively small reduction in strigolactone secretion has a profound impact on *P. ramosa* infection, whereas AM interaction, apical dominance and fruit yield are only mildly affected, demonstrating that strigolactone reduction can be a suitable strategy against root parasitic weeds. In addition, specificity in strigolactone transport *in* and *ex planta* is proposed, as only orobanchol is preferentially loaded into the xylem. Finally, the phenotype of *SlCCD8* knock-down plants confirms a role for strigolactones in branching and adventitious root development and hints at a new role for strigolactones in reproductive development. Further research is required to establish whether this is a direct or an indirect effect.

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References

Agusti J, Herold S, Schwarz M, Sanchez P, Ljung K, Dun EA, Brewer PB, Beveridge CA, Sieberer T, Sehr EM *et al.* 2011. Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. *Proceedings of the National Academy of Sciences, USA* **108**: 20 242–20 247.

Akiyama K, Matsuzaki K-I, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824–827.

Alder A, Jamil M, Marzorati M, Bruno M, Vermathen M, Bigler P, Ghisla S, Bouwmeester H, Beyer P, Al-Babili S. 2012. The path from β-carotene to carlactone, a strigolactone-like plant hormone. *Science* **335**: 1348–1351.

Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyozuka J. 2007. DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. *Plant Journal* **51**: 1019–1029.

Bainbridge K, Sorefan K, Ward S, Leyser O. 2005. Hormonally controlled expression of the Arabidopsis *MAX4* shoot branching regulatory gene. *Plant Journal* **44**: 569–580.

Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O. 2006. The Arabidopsis MAX pathway controls shoot branching by regulating auxin transport. *Current Biology* **16**: 553–563.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology* **4**: 1239–1247.

Beveridge CA, Ross JJ, Murfet IC. 1994. Branching mutant *rms-2* in *Pisum sativum* (grafting studies and endogenous indole-3-acetic acid levels). *Plant Physiology* **104**: 953–959.

Bombarely A, Menda N, Tecle IY, Buels RM, Strickler S, Fischer-York T, Pujar A, Leto J, Gosselin J, Mueller LA. 2011. The Sol Genomics Network (solgenomics.net): growing tomatoes using Perl. *Nucleic Acids Research* **39**(Suppl 1): D1149–D1155.

Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, Turnbull C, Srinivasan M, Goddard P, Leyser O. 2005. MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. *Developmental Cell* **8**: 443–449.

Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH. 2003. Secondary metabolite signalling in host-parasitic plant interactions. *Current Opinion in Plant Biology* **6**: 358–364.

Bouwmeester HJ, Roux C, López-Ráez JA, Bécard G. 2007. Rhizosphere communication of plants, parasitic plants and AM fungi. *Trends in Plant Science* **12**: 224–230.

Cardoso C, Ruyter-Spira C, Bouwmeester HJ. 2011. Strigolactones and root infestation by plant-parasitic Striga, Orobanche and Phelipanche spp. *Plant Science* **180**: 414–420.

Cheng YF, Dai XH, Zhao YD. 2006. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes & Development* **20**: 1790–1799.

Cook CE, Whichard LP, Turner B, Wall ME, Egley GH. 1966. Germination of witchweed (*Striga lutea Lour.*): isolation and properties of a potent stimulant. *Science* **154**: 1189–1190.

Foo E, Turnbull CGN, Beveridge CA. 2001. Long-distance signaling and the control of branching in the *rms1* mutant of pea. *Plant Physiology* **126**: 203–209.

Gallavotti A, Barazesh S, Malcomber S, Hall D, Jackson D, Schmidt RJ, McSteen P. 2008. sparse inflorescence1 encodes a monocot-specific YUCCA-like gene required for vegetative and reproductive development in maize. *Proceedings of the National Academy of Sciences, USA* **105**: 15 196–15 201.

Gillaspy G, Ben-David H, Gruissem W. 1993. Fruits: a developmental perspective. *Plant Cell* **5**: 1439–1451.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489–500.

Goldwasser Y, Yoneyama K, Xie X, Yoneyama K. 2008. Production of strigolactones by *Arabidopsis thaliana* responsible for *Orobanche aegyptiaca* seed germination. *Plant Growth Regulation* **55**: 21–28.

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C *et al.* 2008. Strigolactone inhibition of shoot branching. *Nature* **455**: 189–194.

Helliwell CA, Wesley SV, Wielopolska AJ, Waterhouse PM. 2002. High-throughput vectors for efficient gene silencing in plants. *Functional Plant Biology* **29**: 1217–1225.

Hewitt EJ. 1966. *Sand and water culture methods used in the study of plant nutrition.* Farnham Royal, UK: Commonwealth Agricultural Bureaux.

Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station* **347**: 1–32.

Kapulnik Y, Delaux P-M, Resnick N, Mayzlish-Gati E, Wininger S, Bhattacharya C, Séjalon-Delmas N, Combier J-P, Bécard G, Belausov E *et al.* 2011. Strigolactones affect lateral root formation and root-hair elongation in Arabidopsis. *Planta* **233**: 209–216.

Klerk G-Jd, Krieken Wvd, Jong JCd. 1999. Review: the formation of adventitious roots: new concepts, new possibilities. *In Vitro Cellular & Developmental Biology. Plant* **35**: 189–199.

Klironomos JN, Hart MM. 2002. Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza* **12**: 181–184.

Kohlen W, Charnikhova T, Liu Q, Bours R, Domagalska MA, Beguerie S, Verstappen FWA, Leyser HMO, Bouwmeester HJ, Ruyter-Spira C. 2011a. Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host Arabidopsis. *Plant Physiology* **155**: 974–987.

Kohlen W, Ruyter-Spira C, Bouwmeester HJ. 2011b. Strigolactones: a new musician in the orchestra of plant hormones. *Botany* **89**: 827–840.

Koltai H, Dor E, Hershenhorn J, Joel D, Weininger S, Lekalla S, Shealtiel H, Bhattacharya C, Eliahu E, Resnick N *et al.* 2010a. Strigolactones' effect on root growth and root-hair elongation may be mediated by auxin-efflux carriers. *Journal of Plant Growth Regulation* **29**: 129–136.

Koltai H, LekKala SP, Bhattacharya C, Mayzlish-Gati E, Resnick N, Wininger S, Dor E, Yoneyama K, Yoneyama K, Hershenhorn J *et al.* 2010b. A tomato strigolactone-impaired mutant displays aberrant shoot morphology and plant interactions. *Journal of Experimental Botany* **61**: 1739–1749.

Kretzschmar T, Kohlen W, Sasse J, Borghi L, Schlegel M, Bachelier JB, Reinhardt D, Bours R, Bouwmeester HJ, Martinoia E. 2012. A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. *Nature* **483**: 341–344.

Kroschel J. 2001. *A technical manual for parasitic weed research and extension.* Dordrecht, The Netherlands: Kluwer Academic Publishers.

Ledger SE, Janssen BJ, Karunairetnam S, Wang T, Snowden KC. 2010. Modified CAROTENOID CLEAVAGE DIOXYGENASE8 expression correlates with altered branching in kiwifruit (*Actinidia chinensis*). *New Phytologist* **188**: 803–813.

Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J *et al.* 2009. DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. *Plant Cell* **21**: 1512–1525.

Liu W, Kohlen W, Lillo A, Op den Camp R, Ivanov S, Hartog M, Limpens E, Jamil M, Smaczniak C, Kaufmann K *et al.* 2011. Strigolactone biosynthesis in Medicago truncatula and rice requires the symbiotic GRAS-type transcription factors NSP1 and NSP2. *Plant Cell* **23**: 3853–3865.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* **25**: 402–408.

López-Ráez JA, Charnikhova T, Fernández I, Bouwmeester H, Pozo MJ. 2011a. Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato. *Journal of Plant Physiology* **168**: 294–297.

López-Ráez JA, Charnikhova T, Gómez-Roldán V, Matusova R, Kohlen W, Vos RD, Verstappen FWA, Puech-Pages V, Bécard G, Mulder P *et al.* 2008a. Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. *New Phytologist* **178**: 863–874.

López-Ráez JA, Charnikhova T, Mulder P, Kohlen W, Bino R, Levin I, Bouwmeester HJ. 2008b. Susceptibility of the tomato mutant high pigment-2dg (hp-2dg) to *Orobanche spp.* infection. *Journal of Agricultural and Food Chemistry* **56**: 6326–6332.

López-Ráez JA, Kohlen W, Charnikhova T, Mulder P, Undas AK, Sergeant MJ, Verstappen F, Bugg TDH, Thompson AJ, Ruyter-Spira C *et al.* 2010. Does abscisic acid affect strigolactone biosynthesis? *New Phytologist* **187**: 343–354.

López-Ráez JA, Matusova R, Cardoso C, Jamil M, Charnikhova T, Kohlen W, Ruyter-Spira C, Verstappen F, Bouwmeester H. 2009. Strigolactones: ecological significance and use as a target for parasitic plant control. *Pest Management Science* **65**: 471–477.

López-Ráez JA, Pozo MJ, García-Garrido JM. 2011b. Strigolactones: a cry for help in the rhizosphere. *Botany* **89**: 513–522.

Mapelli S, Frova C, Torti G, Soressi GP. 1978. Relationship between set, development and activities of growth regulators in tomato fruits. *Plant and Cell Physiology* **19**: 1281–1288.

Mashiguchi K, Sasaki E, Shimada Y, Nagae M, Ueno K, Nakano T, Yoneyama K, Suzuki Y, Asami T. 2009. Feedback-regulation of strigolactone biosynthetic genes and strigolactone-regulated genes in Arabidopsis. *Bioscience, Biotechnology, and Biochemistry* **73**: 2460–2465.

Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ. 2005. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche spp.* are derived from the carotenoid pathway. *Plant Physiology* **139**: 920–934.

Napoli C. 1996. Highly branched phenotype of the petunia *dad1-1* mutant is reversed by grafting. *Plant Physiology* **111**: 27–37.

Pattison RJ, Catalá C. 2012. Evaluating auxin distribution in tomato (*Solanum lycopersicum*) through an analysis of the PIN and AUX/LAX gene families. *Plant Journal* **70**: 585–598.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158–161, IN116–IN118.

Rani K, Zwanenburg B, Sugimoto Y, Yoneyama K, Bouwmeester HJ. 2008. Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signalling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. *Plant Physiology and Biochemistry* **46**: 617–626.

Rasmussen A, Mason M, De Cuyper C, Brewer PB, Herold S, Agusti J, Geelen DNV, Greb T, Goormachtig S, Beeckman T *et al.* 2012. Strigolactones suppress adventitious rooting in Arabidopsis and pea. *Plant Physiology* **158**: 1976–1987.

van Roekel J, Damm B, Melchers L, Hoekema A. 1993. Factors influencing transformation frequency of tomato (*Lycopersicon esculentum*). *Plant Cell Reports* **12**: 644–647.

Rotenberg D, Thompson TS, German TL, Willis DK. 2006. Methods for effective real-time RT-PCR analysis of virus-induced gene silencing. *Journal of Virological Methods* **138**: 49–59.

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

Sergeeva LI, de Bruijn SM, Koot-Gronsveld EAM, Navratil O, Vreugdenhil D. 2000. Tuber morphology and starch accumulation are independent phenomena: evidence from ipt-transgenic potato lines. *Physiologia Plantarum* **108**: 435–443.

Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG, Klee HJ. 2005. The Decreased apical dominance 1/petunia hybrida carotenoid cleavage dioxygenase8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. *Plant Cell* **17**: 746–759.

Sorefan K, Booker J, Haurogné K, Goussot M, Bainbridge K, Foo E, Chatfield S, Ward S, Beveridge C, Rameau C *et al.* 2003. *MAX4* and *RMS1* are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Genes & Development* **17**: 1469–1474.

Spinsanti G, Panti C, Lazzeri E, Marsili L, Casini S, Frati F, Fossi CM. 2006. Selection of reference genes for quantitative RT-PCR studies in striped dolphin (*Stenella coeruleoalba*) skin biopsies. *BMC Molecular Biology* **7**: 1–11.

Stirnberg P, van de Sande K, Leyser HMO. 2002. MAX1 and MAX2 control shoot lateral branching in Arabidopsis. *Development* **129**: 1131–1141.

Tsuchiya Y, Vidaurre D, Toh S, Hanada A, Nambara E, Kamiya Y, Yamaguchi S, McCourt P. 2010. A small-molecule screen identifies new functions for the plant hormone strigolactone. *Nature Chemical Biology* **6**: 741–749.

Turnbull CGN, Booker JP, Leyser HMO. 2002. Micrografting techniques for testing long-distance signalling in Arabidopsis. *Plant Journal* **32**: 255–262.

Umehara M, Hanada A, Magome H, Takeda-Kamiya N, Yamaguchi S. 2010. Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. *Plant and Cell Physiology* **51**: 1118–1126.

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

Vogel JT, Walter MH, Giavalisco P, Lytovchenko A, Kohlen W, Charnikhova T, Simkin AJ, Goulet C, Strack D, Bouwmeester HJ *et al.* 2010. SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *Plant Journal* **61**: 300–311.

Walter M, Floss D, Strack D. 2010. Apocarotenoids: hormones, mycorrhizal metabolites and aroma volatiles. *Planta* **232**: 1–17.

Xie X, Yoneyama K, Yoneyama K. 2010. The strigolactone story. *Annual Review of Phytopathology* **48**: 93–117.

Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H. 2007. Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. *Planta* **225**: 1031–1038.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Relative *SlCCD7* expression.

Fig. S2 MRM-LC-MS/MS analysis of tomato xylem sap.

Fig. S3 Analysis of sucrose in xylem sap.

Fig. S4 Strigolactone analysis in tomato root and hypocotyl tissue under limiting phosphate conditions.

Fig. S5 Distribution of lateral branches.

Fig. S6 Average seed size.

Fig. S7 Tomato seed germination.

Fig. S8 Strigolactone analysis in tomato root exudates under sufficient and limiting phosphate conditions.

Table S1 *SlCCD8* complete nucleotide sequence

Table S2 SlCCD8 complete amino acid sequence

Table S3 Sequence homology of *SlCCD8*

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