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A CLE-SUNN module regulates strigolactone content and fungal colonization in arbuscular mycorrhiza

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During arbuscular mycorrhizal symbiosis, colonization of the root is modulated in response to the physiological status of the plant, with regulation occurring locally and systemically. Here, we identify differentially expressed genes encoding CLAVATA3/ESR-related (CLE) peptides that negatively regulate colonization levels by modulating root strigolactone content. CLE function requires a receptor-like kinase, SUNN; thus, a CLE-SUNN-strigolactone feedback loop is one avenue through which the plant modulates colonization levels.

Most land plants have the capacity to form mutualistic associations with arbuscular mycorrhizal (AM) fungi, which increase phosphate (Pi) acquisition and thus provide substantial benefit to plants growing in phosphorous-limited environments. Both initiation of this symbiosis and its continued development¹ are influenced by carotenoid-derived plant hormones, namely, strigolactones. Strigolactones act as pre-symbiotic signals for AM fungi, and they promote fungal energy metabolism and hyphal branching, as well as the exudation of fungal chitin oligomers, which in turn activate the plant common symbiosis signalling pathway^{2–5}. The common symbiosis signalling pathway, so-called because in legumes it is also required for symbiosis with rhizobia⁶, is essential for symbiosis and ultimately activates cellular programmes for accommodation of the fungus. Physiological studies indicate that symbiotic development is regulated systemically in response to the phosphorous status of the plant⁶: a high phosphorous status suppresses initiation of symbiosis and also suppresses further development of pre-established associations⁷. Strigolactone biosynthesis and export are promoted by Pi starvation and suppressed if the phosphorous status is elevated^{8,9}; therefore, these hormones provide one means of regulating symbiotic development in response to plant phosphorous status. However, hormones alone are not sufficient to explain all aspects of Pi-mediated regulation of AM symbiosis⁷.

An additional factor influencing symbiotic development is colonization itself; several reports indicate that colonization by AM fungi negatively regulates further symbiotic development independent of the plant's phosphorous status^{10,11}. These systemic suppressive effects, originally detected in split-root experiments, are dependent on *NARK*, which encodes a leucine-rich receptor-like kinase (LRR-RLK) related to *Arabidopsis thaliana* *CLAVATA1* (*AtCLV1*)¹². Mutants in *CLV1* orthologues in other legumes including *Medicago truncatula* *sunn*, *Pisum sativum* *sym29* and *Lotus japonicus* *har1*, as well as mutants in the tomato receptor *SlCLAVATA2* (*SlCLV2*), also show slightly increased fungal colonization levels relative to

controls^{13–15}. This suggests a potential role for *SUNN*/*SYM29*/*HAR1*/*NARK* and *CLV2* in quantitative regulation of fungal colonization. *SUNN*/*SYM29*/*HAR1*/*NARK* were initially characterized as negative regulators of nodulation (in rhizobia–legume symbiosis) where they function in concert with nodulation-induced or nitrate-induced CLE peptides in a process called autoregulation of nodulation¹⁵, which also involves a microRNA and cytokinin signalling^{16,17}.

CLE peptides are small, secreted peptides characterized initially in *Arabidopsis* as non-cell-autonomous regulators of shoot meristem development¹⁸. Large CLE gene families have been discovered in many plant species^{19,20}, and considerable functional diversity, spanning a range of developmental and physiological roles, has been uncovered²¹. Although transcriptional responses of genes encoding CLEs to Pi and AM symbiosis have been noted^{22–24}, functional roles for these CLEs have not been reported previously.

We surveyed transcript levels of 47 *M. truncatula* CLE genes¹⁹ in roots colonized with the AM fungus *Rhizophagus irregularis* relative to mock-inoculated control roots. *MtCLE16*, for which AM-induced gene expression has been reported previously²⁴, *MtCLE45* and *MtCLE53* showed the largest increases in expression (Fig. 1a). A recent clustering analysis assigned 1,628 plant CLE genes to 12 groups with potentially shared functions²⁰; *MtCLE53* is a member of group 3 with CLE genes from non-legumes and legumes involved in nutrient signalling and nodulation. *MtCLE16* and *MtCLE45* were assigned to group 1, a highly heterogeneous cluster containing many CLE genes with developmental roles²⁰. Focusing on CLE genes with potential roles in symbiosis, we further evaluated the expression of the ten *MtCLE* genes assigned to group 3 in *M. truncatula* roots colonized with the AM fungus *Glomus versiforme*. Again, *MtCLE53* showed a significant transcriptional increase in colonized roots, while the other nine genes showed either no change or a small decrease (Fig. 1b and Supplementary Fig. 1). In addition, the expression of *MtCLE32* and *MtCLE33*, but not *MtCLE53*, was significantly induced in roots grown under high (2 mM) Pi conditions compared to roots grown without Pi application (Fig. 1c and Supplementary Fig. 1). We selected the AM-induced *MtCLE53* and the Pi-induced *MtCLE33* for further analysis because they show the largest transcriptional increases in response to these stimuli and have not been analysed previously.

Using promoter- β -*GLUCURONIDASE* (*GUS*) reporter gene fusions, we evaluated the spatial expression patterns of *MtCLE33* and *MtCLE53*. The *MtCLE33* promoter (*pMtCLE33*) was active in the root vascular tissue, with the strongest expression in the pericycle and xylem parenchyma (Supplementary Fig. 2) and with no

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apparent difference between colonized and non-colonized roots. By contrast, MtCLE53 promoter-GUS (pMtCLE53::GUS) activity was detected in the vascular tissue, and, consistent with the gene expression data, the intensity of vascular GUS staining increased near colonized regions of the root (Fig. 1d and Supplementary Fig. 2). This local increase in GUS staining intensity was seen in 77.19% of fungal infection units but was only rarely observed in roots expressing 35S::GUS (7.69%; see Supplementary Fig. 2). A local increase in vascular pMtCLE53::GUS expression near colonized cortex cells was also observed in the *pt4-2* mutant (78.95% of infections showed locally increased vascular GUS staining intensity, $n=38$ across 4 root samples; see Supplementary Fig. 2), where symbiotic Pi transfer to the root cell, and, consequently, maintenance of the symbiosis, is impaired²⁵. Thus, transcriptional induction of MtCLE53 is triggered in the vascular tissue in the local vicinity of colonized cells, and the fact that this occurs also in the *pt4-2* mutant is consistent with the finding that MtCLE53 expression is induced in response to colonization rather than a secondary effect of local increases in Pi levels.

To gain insight into the roles of these CLEs, we evaluated the AM symbiosis phenotypes of composite *M. truncatula* plants ectopically overexpressing MtCLE33 or MtCLE53 in the roots. In both cases, the transgenic roots showed normal growth, but colonization levels were reduced significantly relative to roots expressing a 35S::GUS control construct (Fig. 1e); fungal entry into the root (which here comprises both primary and secondary infections) was reduced by an average of 1.9-fold and 1.5-fold in roots expressing 35S::MtCLE33 or 35S::MtCLE53, respectively (Fig. 1f), while infection unit length was not affected (Fig. 1g). Reduced colonization was not observed when MtCLE33 or MtCLE53 were expressed ectopically in the root cortex (Supplementary Fig. 3), suggesting that the high vascular expression conferred by the 35S promoter is probably required for this effect. In addition, roots expressing 35S::MtCLE10, a gene that is not differentially regulated in colonized roots, or 35S::MtCLE13, which is induced by nodulation²⁶ but not by AM symbiosis (Fig. 1a), did not show reduced fungal colonization levels (Supplementary Fig. 3). Overall, this indicates that MtCLE33 and MtCLE53 overexpression negatively regulates AM symbiosis and, furthermore, that the effect is specific. This is interesting considering that the CLE signalling domains encoded by these genes are very similar (Supplementary Fig. 1). To determine whether reduction in MtCLE53 expression shows the converse effect, we downregulated MtCLE53 by RNA interference. Roots in which MtCLE53 expression was reduced to 1–12% compared to the average expression in

control roots showed a 2.4-fold and 3.3-fold increase in transcript levels of the genes encoding the AM symbiosis markers MtPT4 and *G. versiforme* α -tubulin, respectively (Supplementary Fig. 3), consistent with increased colonization, although microscopy analyses were not sensitive enough to detect this change.

To investigate downstream responses to MtCLE53 expression, we compared transcript profiles of mock-inoculated and *G. versiforme*-colonized 35S::MtCLE53 and 35S::GUS roots (Supplementary Tables 1 and 2 and Supplementary Figs. 4 and 5). Differentially expressed genes were identified, the majority of which were downregulated in 35S::MtCLE53 relative to 35S::GUS roots in both colonized and control treatments (Supplementary Table 1). These included MtDXS2-2, MtHMGR4 and MtD27 (Supplementary Fig. 6). MtDXS2-2 and MtHMGR4 are members of multigene families, and although the function of these specific genes is unknown, 1-deoxy-D-xyllose-5-phosphate synthase (DXS) proteins are the rate-limiting enzymes of the plastid-localized methylerythritol phosphate pathway and 3-hydroxy-3-methylglutaryl coenzyme A reductases (HMGRs) govern the cytosolic mevalonate pathway, respectively²⁷. The final product of both interconnected parallel pathways is dimethylallyl pyrophosphate/isopentenyl pyrophosphate, the precursor molecule for the biosynthesis of various secondary metabolites including strigolactones. MtD27 encodes a β -carotene isomerase, the first enzyme of the strigolactone biosynthesis pathway²⁸. MtCLE53 overexpression not only decreases MtD27 transcript levels in both colonized and mock-inoculated roots (Supplementary Fig. 6) but also negatively affects the expression of genes encoding enzymes that catalyse subsequent steps of strigolactone biosynthesis (MtCCD7, MtCCD8 and MtMAX1) and exudation (MtPDR1) (Fig. 2a). The effect on the latter was stronger in the mock-inoculated root samples, which lack additional transcriptional changes resulting from fungal colonization. Genes involved in strigolactone signalling or perception (MtD14, MtD14L, MtMAX2 and MtD53)²⁹ were unaffected (Fig. 2a). Downregulation of the strigolactone biosynthesis pathway in MtCLE53-overexpressing roots could explain the reduction in fungal colonization levels, as this is the phenotype displayed by strigolactone biosynthesis mutants^{8,30}. Furthermore, overexpression of MtCLE33, but not of MtCLE10 or MtCLE13, also resulted in a significant decrease in MtD27 gene expression (Fig. 2b and Supplementary Fig. 7). Consistent with MtD27 transcript abundance, strigolactone levels in non-colonized roots overexpressing MtCLE33 or MtCLE53 were lower than those of roots expressing the 35S::GUS control construct; we were unable to detect orobanchol

Fig. 1 | Expression of *M. truncatula* CLE genes and MtCLE overexpression phenotypes. **a**, Gene expression fold changes of 47 *M. truncatula* CLE genes in roots colonized with *R. irregularis* (R.i.) compared to mock-inoculated roots. Bar plots represent the mean expression fold change ($\Delta\Delta Ct$) and the error bars represent the standard deviation of the fold change calculated from $n=3$ independent biological samples per treatment. Significance levels (two-sided Student's *t*-test): * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ND, not detected. **b**, Expression of MtCLE33 and MtCLE53 in *M. truncatula* A17 wild-type roots colonized with *G. versiforme* (G.v.) compared to mock-inoculated control roots. $n=5$ independent biological samples per treatment. Pairwise comparisons were calculated separately for each gene. $P=0.095$ for MtCLE33 (not significant (NS)) and ** $P=0.008$ for MtCLE53, two-sided Mann-Whitney *U*-test. **c**, Expression of MtCLE33 and MtCLE53 in non-colonized roots grown under different Pi regimes. $n=3$ independent biological samples per treatment. The Kruskal-Wallis test was performed separately for each gene: MtCLE33: $P=0.027$; MtCLE53: $P=0.145$ (not significant). The different letters denote significant differences after Dunn's post-hoc test; the two independent analyses are represented with the letters a, b and a', respectively. Scatterplots in **b** and **c** show individual measurements as coloured points; the horizontal bar represents the mean. **d**, GUS staining of mycorrhizal roots transformed with pMtCLE53::GUS. GUS is detected in the vascular tissue (v) with stronger GUS intensity in the vicinity of fungal infections (visualized by wheat germ agglutinin (WGA)-Alexa Fluor-488 counterstaining and indicated by the dashed lines). Representative images are shown based on data from three independent experiments. Scale bars, 250 μm . **e**, Overexpression of MtCLE33 ($n=10$ independent biological samples) or MtCLE53 ($n=9$) leads to a decrease in colonized root length compared to roots expressing the control construct 35S::GUS ($n=10$). $P=4.70\times 10^{-4}$ (Kruskal-Wallis test). **f**, Overexpression of MtCLE33 ($n=23$ root pieces derived from 10 independent biological samples) or MtCLE53 ($n=19$ root pieces derived from four independent biological samples) leads to lower numbers of fungal entries per centimetre of colonized root than controls ($n=61$ root pieces derived from 10 independent biological samples). $P=8.60\times 10^{-4}$ (Kruskal-Wallis test). **g**, Infection unit length in roots transformed with 35S::MtCLE33 ($n=56$ infection units from eight independent biological samples), 35S::MtCLE53 ($n=45$ from five independent biological samples) or 35S::GUS ($n=39$ from five independent biological samples) does not differ significantly. $P=0.003$ (Kruskal-Wallis test). The box-and-whiskers plots in **e–g** show the lower and upper quartiles, and the minimum and maximum values. The bar in the box represents the median, and the coloured points represent individual measurements. The different letters denote significant differences ($P<0.05$) after Dunn's post-hoc test.

in wild-type roots overexpressing either *MtCLE33* or *MtCLE53* (Fig. 2c), but it was detected in four out of five biological replicate samples of the *GUS* control roots. In addition, the medicol content, a major strigolactone produced by *M. truncatula*³¹, was reduced significantly in non-colonized roots expressing 35S::*MtCLE33* and 35S::*MtCLE53* relative to 35S::*GUS* control roots (Fig. 2d and Supplementary Fig. 7).

To test the hypothesis that during AM symbiosis strigolactones act downstream of *MtCLE* signalling, we applied the strigolactone analogue *rac*-GR24 (ref. ³²) and its strigolactone-specific stereoisomer GR24^{5DS} (ref. ³³) individually to composite plants with roots overexpressing *MtCLE33* or *MtCLE53*. This resulted in colonization levels that were not significantly different from those of roots expressing the 35S::*GUS* control construct (Fig. 2e and Supplementary Fig. 7).

Hence, these data support the hypothesis that the negative regulatory effect of these *CLE* genes on fungal colonization is mediated by the downregulation of strigolactone biosynthesis, which leads to lower colonization levels.

Genes whose expression increased in 35S::MtCLE53 roots relative to 35S::GUS roots were also identified in our transcriptome data (Supplementary Table 1), and the LRR-RLK *SUNN* was upregulated significantly in both control and colonized treatments (Supplementary Fig. 8). *SUNN* expression also increased in response to overexpression of Pi-induced MtCLE33 and nodulation-induced MtCLE13 (Fig. 3a and Supplementary Fig. 7). Previous studies have indicated that overexpression of *CLE* genes can result in the increased transcription of their cognate receptors and also that *SUNN* and MtCLE13 act in the same pathway^{26,34}. Consequently, our transcript data led us to hypothesize that MtCLE53 and MtCLE33 could be perceived by *SUNN*. In addition to *SUNN*, overexpression of MtCLE53 also resulted in differential expression of several other genes encoding LRR-RLKs (Supplementary Fig. 9), which could potentially constitute additional players in the MtCLE53 signalling pathway; however, we focused on the well-characterized LRR-RLK *SUNN*.

Previous reports indicate that *sun*n mutants, as well as mutants in soybean and *L. japonicus* orthologues, display slight increases in root colonization by AM fungi^{12–14}. We evaluated colonization in two additional *sun*n alleles, *sun*n-1, a missense allele, and *sun*n-4, a null allele³⁵, which likewise showed small increases in colonization that were statistically significant only when grown under a high Pi fertilization regime but not under low Pi conditions (Fig. 3b and Supplementary Fig. 10). We then transformed the MtCLE overexpression constructs into *sun*n roots. The negative effects of MtCLE33 or MtCLE53 overexpression on colonization levels observed in the wild-type A17 did not occur in *sun*n-4 and *sun*n-1 roots, where colonization levels of MtCLE-overexpressing roots were not significantly different from those of the 35S::GUS control (Fig. 3b and Supplementary Fig. 10). This indicates that *SUNN* is required for the MtCLE-mediated reduction in colonization. Furthermore, MtCLE-induced downregulation of MtD27 expression was not observed in *sun*n-4 roots (Fig. 2b). Consistent with the transcript data, orobanchol and medicaol levels did not differ significantly in non-colonized *sun*n-4 MtCLE-overexpressing roots relative to the *sun*n-4 35S::GUS controls (Fig. 2c,d). In *sun*n-4 roots overexpressing MtCLE genes, medicaol levels remained approximately

threefold higher than the wild-type roots overexpressing MtCLE genes, which correlated with colonization levels that we observed previously (Fig. 3b).

Non-colonized *sun*n-4 and wild-type roots each expressing the 35S::GUS control construct do not differ in MtD27 expression or strigolactone content (Fig. 2b–d). This is consistent with a previous study that reported comparable strigolactone levels in a non-colonized pea *nark*-mutant allele and the wild-type control³⁶. Following inoculation, the native MtCLE53 should be active and, therefore, we might have expected the wild-type 35S::GUS-transformed roots to show lower colonization levels than the *sun*n-4 35S::GUS roots. However, as observed in comparisons of *sun*n and wild type (native roots or transformed with 35S::GUS), the changes in colonization are small³⁷ (Fig. 3b and Supplementary Fig. 10). In this case, it is possible that colonization levels did not show a statistically significant difference because the endogenous MtCLE53 expression had not yet reached a sufficiently high level to result in a measurable difference in colonization.

AM symbiosis occurs widely across the plant kingdom, and therefore we looked for evidence of a similar CLE–receptor module in monocots. A *Brachypodium distachyon* mutant in a LRR-RLK orthologous to *sun*n (*fon1-1*; Supplementary Fig. 11) showed higher colonization levels than wild-type segregants, and BdCLE genes that show transcriptional increases in response to AM fungal colonization or to Pi were also identified (Supplementary Figs. 12–14). These findings provide initial evidence that similar inducible CLE genes and an orthologous receptor quantitatively modulating AM colonization exist also in monocots.

Previous studies indicated that strigolactones influence colonization^{2–4,8} and that root strigolactone production decreases following colonization³⁸. Taken together with our data, we propose a model in which colonization of roots by AM fungi gradually leads to increased expression of MtCLE53, which acts via *SUNN* to reduce strigolactone biosynthetic gene expression and strigolactone content in the roots, and consequently reduces the promotion of further colonization (Fig. 3c). This effect is mimicked—although with higher intensity and thus heightened effects relative to the native situation—by ectopically overexpressing MtCLE53. The resulting negative-feedback loop could provide a mechanism to fine-tune colonization levels in response to colonization, a so-called autoregulatory effect as proposed previously^{10,11}. The colonization-induced expression of pMtCLE53::GUS in the vascular tissue in the

Fig. 2 | Overexpression of MtCLE33 and MtCLE53 in roots reduces strigolactone levels in a *SUNN*-dependent manner. **a**, Heatmap of *M. truncatula* homologues of gene families involved in strigolactone biosynthesis and transport (dark grey highlight), or strigolactone perception and signalling (light grey highlight). Gene families involved in strigolactone biosynthesis and transport are downregulated in non-colonized 35S::MtCLE53 roots, whereas genes involved in strigolactone perception and signalling are largely unaffected. The gene expression level fold change between mock-inoculated MtCLE53-overexpressing ($n=3$ independent biological samples) and control roots ($n=4$) is shown; *** $P<0.001$, Benjamini–Hochberg adjusted. Note, it has been shown previously that MtD27L is not a functional orthologue of *Oryza sativa* D27 (ref. 28). **b**, In the A17 wild-type background, but not in *sun*n-4 mutants, MtD27 transcript levels are reduced in non-colonized roots expressing 35S::MtCLE33 or 35S::MtCLE53 relative to control roots ($P=2.09\times10^{-4}$, as determined by analysis of variance (ANOVA); $n=6$ independent biological samples for A17 35S::GUS, $n=5$ for A17 35S::MtCLE33, $n=8$ for A17 35S::MtCLE53, $n=7$ for *sun*n-4 35S::GUS, $n=7$ for *sun*n-4 35S::MtCLE33, $n=8$ for *sun*n-4 35S::MtCLE53). **c**, Orobanchol content in non-colonized A17 and *sun*n-4 roots expressing 35S::MtCLE33 or 35S::MtCLE53. Out of five biological replicates each, no orobanchol was detected in A17 roots transformed with 35S::MtCLE33 or 35S::MtCLE53, whereas it was detected in four out of five control roots. In *sun*n-4 mutants, out of five replicates each, orobanchol was detected in four samples expressing 35S::GUS, three samples expressing 35S::MtCLE33 and five samples expressing 35S::MtCLE53. Missing values were probably caused by very low concentrations of orobanchol close to the detection limit and were omitted. ND, not detected. **d**, Medicaol content is decreased in non-colonized A17 roots overexpressing MtCLE33 or MtCLE53 compared to roots overexpressing the control construct, whereas such an effect was not detected in the *sun*n-4 background ($P=4.04\times10^{-6}$, as determined by ANOVA); $n=5$ independent biological samples per genotype and treatment. Measurements shown in graphs **b–d** were conducted on root samples from a single experiment for which plants were grown under a low Pi fertilization regime (20 μ M potassium phosphate). **e**, External application of the strigolactone analogue GR24^{50S} renders fungal colonization levels in MtCLE33- and MtCLE53-overexpressing roots indistinguishable from 35S::GUS roots ($P=0.001$ across all samples, as determined by ANOVA). $n=5$ independent biological samples for control 35S::MtCLE33, $n=7$ for control 35S::MtCLE53, $n=9$ for GR24 35S::GUS, $n=5$ for GR24 35S::MtCLE33, $n=7$ for GR24 35S::MtCLE53, $n=8$ control 35S::GUS. The box-and-whiskers plots in **b–e** show the lower and upper quartiles, and the minimum and maximum values. The bar in the box represents the median, and the coloured points represent individual measurements. The different letters denote significant differences ($P<0.05$) after Tukey's HSD post-hoc test.

immediate vicinity of the infected cells provides an explanation for previous observations that inhibition of colonization was directly correlated with the infection level¹¹. In *sunm* mutants, MtCLE53-mediated downregulation of strigolactone biosynthesis does not occur, and strigolactone levels are maintained. Over time, we predict that this would lead to increased colonization levels in *sunm* relative to wild type as observed for several *sunm* alleles, as well as *nark* and *har1* (refs. ^{12–14}). It is likely that SUNN acts in concert with other receptors. In addition, parallel signalling pathways, as

reported for CLAVATA signalling in meristems³⁹, could lead to redundancy, which might account for the relatively weak *sunm* AM symbiosis phenotype.

This study also shows that similar negative effects on strigolactone biosynthetic gene expression are obtained by overexpression of the Pi-induced MtCLE33, but not MtCLE10 or the nodulation-induced MtCLE13. Thus, it is possible that MtCLE33 also contributes to the fine-tuning of the symbiosis through modulation of strigolactone content.

Fig. 3 | Model for MtCLE33 and MtCLE53 function and their dependency on SUNN. **a**, Transcript levels of *SUNN* in mock-inoculated A17 wild-type and *sunn-4* roots. *SUNN* transcript levels increase in roots expressing 35S::MtCLE33 or 35S::MtCLE53 ($P = 1.5 \times 10^{-14}$, as determined by ANOVA). $n = 6$ independent biological samples for A17 35S::GUS, $n = 5$ for A17 35S::MtCLE33, $n = 8$ for A17 35S::MtCLE53, $n = 7$ for *sunn-4* 35S::GUS, $n = 7$ for *sunn-4* 35S::MtCLE33, $n = 8$ for *sunn-4* 35S::MtCLE53. **b**, The reduction in colonized root length observed in A17 roots overexpressing MtCLE33 or MtCLE53 is not observed when these genes are overexpressed in *sunn-4* roots ($P = 3 \times 10^{-10}$, as determined by ANOVA, across all samples). $n = 6$ independent biological samples for A17 35S::GUS, $n = 9$ for A17 35S::MtCLE33, $n = 9$ for A17 35S::MtCLE53, $n = 7$ for *sunn-4* 35S::GUS, $n = 11$ for *sunn-4* 35S::MtCLE33, $n = 10$ for *sunn-4* 35S::MtCLE53. Plants were grown under a low Pi fertilization regime (20 μ M potassium phosphate). The box-and-whiskers plots in **a** and **b** show the lower and upper quartiles, and the minimum and maximum values. The bar in the box represents the median, and the coloured points represent individual measurements. The different letters denote significant differences ($P < 0.05$) after Tukey's HSD post-hoc test. **c**, Schematic model for MtCLE-mediated regulation of AM symbiosis. MtCLE53 expression is induced in AM-colonized roots and triggers a negative feedback loop that negatively impacts colonization by reducing strigolactone levels. This effect is dependent on the LRR-RLK SUNN, but potentially also involves other, yet unknown, receptors. Strigolactones, whose production is regulated in part by Pi⁸, are positive regulators of AM symbiosis by directly influencing the fungus¹⁻⁴. Thus, following AM-dependent MtCLE53 induction, a reduced root strigolactone content provides one possible avenue for a negative, autoregulatory feedback loop conferred by this gene. Pi-induced MtCLE33 elicits similar responses when overexpressed; however, the integration of this gene into the depicted pathway deserves further study.

It is now emerging that CLE peptides function to integrate external cues with plant development or cellular responses and therefore enable plants to adapt to a changing environment²¹. Several CLEs act by modifying hormone biosynthesis or signalling⁴⁰. Our data concur with these observations and also indicate that CLE genes modulate the expression of strigolactone biosynthesis genes and consequently root strigolactone content; thus, we provide initial mechanistic insight into one way in which plants control fungal colonization.

Methods

Plant material, growth conditions and inoculation with AM fungi. *M.*

truncatula plants were grown in a growth chamber under a 16-h light (25°C)/8-h dark (22°C) regime at 40% relative humidity. *B. distachyon* plants were grown in a growth chamber under a 12-h light (24°C)/12-h dark (22°C) regime. Plants were inoculated with spores of the AM fungi *G. versiforme* or *R. irregularis*, and root colonization was visualized using WGA-Alexa Fluor-488. For details, see Supplementary Methods.

Statistical analyses and data representation. For all comparisons, we analysed data from independent biological replicates. Multiple comparisons were evaluated by analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test, or by Kruskal–Wallis tests followed by Dunn's post-hoc test. Pairwise differences were assessed by a two-tailed Student's *t*-test or Mann–Whitney *U*-test. For details and further testing, see Supplementary Methods.

UHPLC–MS/MS-based analysis of strigolactones in *M. truncatula* roots.

Strigolactones were identified and quantified using the Acquity UPLC System (Waters) coupled to a Xevo TQ-XS triple-quadrupole mass spectrometer (Waters

MS Technologies) with electrospray (ESI) interface. For further details on sample preparation and data analysis, see Supplementary Methods.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author on request. RNA sequencing raw reads are deposited in the NCBI Sequence Read Archive database (accession number [SRP198429](https://www.ncbi.nlm.nih.gov/sra/SRP198429)).

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Author contributions

L.M.M. and M.J.H. conceived the experiments and analysed the data. E.S. and J.F. provided genome-level identification of *CLE* genes and *sumn* mutants. K.F. and H.J.B. analysed and interpreted strigolactone levels. X.S. and Z.F. processed the RNA sequencing data and generated differential expression data. L.M.M. carried out all other experiments. L.M.M. and M.J.H. wrote the manuscript.

Competing interests

The authors declare no competing interests.

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Data exclusions	No data were excluded.
Replication	Each colonisation experiment was replicated at least two times with consistent results. In all instances where the experiments were replicated, the data were reproduced in the replicate experiments. Some experiments were performed only once. This includes CLE gene expression data for B. distachyon grown in different Pi conditions, the RNAseq experiment, and the strigolactone measurements ; however each of these experiments has a sufficient number of biological replicates. The reproducibility between independent biological samples indicates that these data can be replicated.
Randomization	Plants subjected to different treatments (e.g. colonisation vs. mock-inoculation, GR24 vs. acetone treatment) were randomly selected. After harvest, all biological replicates were analysed.
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