

SUPPLEMENTAL INFORMATION

Supplemental Table S1. *MtCLE* identification.

Identified *MtCLE* genes with their corresponding names in the general nomenclature and number of the bacterial artificial clone or of the expressed sequence tag. General nomenclature according to Cock and McCormick (2001) and Oelkers et al. (2008), who numbered each CLE member independently of the species origin and prefixed the numbers with "CLE".

<i>M. truncatula</i> nomenclature	General nomenclature	BAC clone, EST number
<i>MtCLE1</i>	CLE74	AW586793, BQ139113
<i>MtCLE2</i>	CLE67	AC137080.13, AC135319.28, AC147499.5, BI311733
<i>MtCLE3</i>	CLE69	BQ157494
<i>MtCLE4</i>	CLE71	AW329414
<i>MtCLE5</i>	CLE70	BM812815, BE999212
<i>MtCLE6</i>	CLE64	BF650504, BE941833, CX518468
<i>MtCLE7</i>		AC157502.1
<i>MtCLE8</i>	CLE73	AC151458.10, CX530629, CX530352
<i>MtCLE9</i>		AC155894.1
<i>MtCLE10</i>		AC157779.7
<i>MtCLE11</i>	CLE66	BQ122845
<i>MtCLE12</i>	CLE65	AC144893.15, AL381238, AL381237
<i>MtCLE13</i>	CLE39	AC144893.15, AL380420, AL380419
<i>MtCLE14</i>		AC155894.1
<i>MtCLE15</i>		AC151522.2
<i>MtCLE16</i>	CLE35	AC146785.12
<i>MtCLE17</i>	CLE72	AC123570.7, AC147960.8, AC147961.3, AY379776.1
<i>MtCLE18</i>	CLE36	AC146570.4
<i>MtCLE19</i>		AC150244.2
<i>MtCLE20</i>	CLE37	AC158501.3
<i>MtCLE21</i>	CLE38	CT009655_15.4
<i>MtCLE22</i>	CLE68	AC151522_11.4
<i>MtCLE23</i>		CU024869_31.4
<i>MtCLE24</i>		CR955009_22.5
<i>MtCLE25</i>		CU013514_50.4

Supplemental Table S2. Comparison of the CLE peptide sequence of *MtCLE4*, *MtCLE12*, and *MtCLE13* with the CLE peptide sequences from Arabidopsis *CLE* genes and of *LjCLE1*, *LjCLE2*, and *LjCLE3*.

CLE peptide sequences homologous to *MtCLE12* and *MtCLE13* are framed in blue and those to *MtCLE4* in red.

<i>MtCLE12</i>	R	L	S P G G P N H I H N
<i>MtCLE13</i>	R	L	S P A G P D P Q H N
<u><i>LjCLE1</i></u>	R	L	S P G G P D P Q H N
<u><i>LjCLE2</i></u>	R	L	S P G G P D P Q H N
<i>AtCLE7</i>	R	F	S P G G P D P Q H H
<i>AtCLE5/6</i>	R	V	S P G G P D P Q H H
<i>AtCLE2</i>	R	L	S P G G P D P Q H H
<i>AtCLE1/3/4</i>	R	L	S P G G P D P R H H
<i>AtCLE46</i>	H	K	H P S G P N P T G N
<i>AtCLE41/44</i>	H	E	V P S G P N P I S N
<i>AtCLE42</i>	H	G	V P S G P N P I S N
<u><i>LjCLE3</i></u>	R	V	V P T G P N P L H N
<i>MtCLE4</i>	R	G	V P S G A N P L H N
<i>AtCLE9/10</i>	R	L	V P S G P N P L H N
<i>AtCLE11</i>	R	V	V P S G P N P L H H
<i>AtCLE12</i>	R	R	V P S G P N P L H H
<i>AtCLE13</i>	R	L	V P S G P N P L H H
<i>AtCLE14</i>	R	L	V P K G P N P L H N
<i>AtCLE16</i>	R	L	V H T G P N P L H N
<i>AtCLE17</i>	R	V	V H T G P N P L H N
<i>AtCLE18</i>	R	Q	I P T G P D P L H N
<i>AtCLE19</i>	R	V	I P T G P N P L H N
<i>AtCLE20</i>	R	K	V K T G S N P L H N
<i>AtCLE21</i>	R	S	I P T G P N P L H N
<i>AtCLE22</i>	R	R	V F T G P N P L H N
<i>AtCLE25</i>	R	K	V P N G P D P I H N
<i>AtCLE26</i>	R	K	V P R G P D P I H N
<i>AtCLE27</i>	R	I	V P S C P D P L H N
<i>AtCLE40</i>	R	Q	V P T G S D P L H H
<i>AtCLE45</i>	R	R	V R R G S D P I H N
<i>AtCLV3</i>	R	T	V P S G P D P L H H

Supplemental Table S3. Primers used in the analysis.

Gene	Sense Primer	Anti-sense Primer
Gateway cloning		
<i>ORF-MtCLE4</i>	ATGCTAACAAAGTGAAATGAGCG	CTACCGATTATGCAGAGGATTAGC
<i>ORF-MtCLE12</i>	ATGGAGAATTCAAATAAAGTGCCAAT	TTAGTTATGTATATGGTTTGGTCCAC
<i>ORF-MtCLE13</i>	ATGGGCCGGTATACAACC	CTACTTCTTGGTGGTGATCT
<i>promoter-MtCLE12</i>	TGTAATGTGATGGTGGACTT	CATGCAAAGAACAAGAAGTTGG
<i>promoter-MtCLE13</i>	TCATTCCCTAGTAGAACGGCG	GCCCATGTGTGATTTTAATC
qRT-PCR amplification		
<i>MtCLE1</i>	TGGTGGGGTTTGTGTTGTTTATGG	GTCTTGTAGTTGTTTGTCTCTGTTCC
<i>MtCLE2</i>	ATGAATGTGAATGTTCTCTCTAGGC	GGTTGTCTATATTTCCCTGCTCTCC
<i>MtCLE3</i>	GGTTCAAGCAACAAGAATGAAACAC	AGGATCTGGGCAGCTTGGTAC
<i>MtCLE4</i>	AATTTCACAAGTTCTGCTTCATCGC	TGGCACACCTCTCTTGTCTTCC
<i>MtCLE5</i>	AATGCAAGCTACCACGTCTCG	GCAGAGGATTTGGTCCAGAAGG
<i>MtCLE6</i>	TCTATCTCACCAACCAACAACAACC	TCCAAATCTTTTTCCAGCTCCATCC
<i>MtCLE7</i>	AACTGTGTTTTGTTATTCTTCTTC	AGATGATGATGATTTGAATGAACC
<i>MtCLE8</i>	TTCTCAAAGTAGGAAAATGTTAGC	CGATGATGAAGACGACTATGG
<i>MtCLE9</i>	GCTCGCTATGTTTCCCTTAC	AGAGATGGACTTAGATTATTCTGC
<i>MtCLE10</i>	GCACATGGAAGAAAGAACAAGAGTC	CCCCTGGAGTAGATGTATGTGAAAC
<i>MtCLE11</i>	TGTTAGCTTCTCAACCATAACATTGC	TGAAGGTGAAGGTGCCAAATCC
<i>MtCLE12</i>	CAACGTCTCTTGCATGAGTTAATGG	ACCTGGTGAAAGCCTATCTCCTG
<i>MtCLE13</i>	CCGAAGCCTTCTACAGAACTACG	TCTTGGTGGTGATCTTCCATTATGC
<i>MtCLE14</i>	ACACCACCACAATTCCTTAC	ATATGATGAACATTCTCAGATAGC
<i>MtCLE15</i>	CAGTGGTTGGAGAGTTGAGAAAAGG	TCAAGGGTTTTTCAGGCTTAATAGGG
<i>MtCLE16</i>	AACACTCAAACAACCAACAACAACATC	ACTCTTCTTCTCTCAGCACCAAATC
<i>MtCLE17</i>	TCTCAAGCCCTTCTTCTACTTC	TCCTGTAGGCACTTTGCGTTTC
<i>MtCLE18</i>	TTCACATTCTACTATGGCTATCTC	ATGATGACTTGCTTGTATGATTTG
<i>MtCLE19</i>	TTGATACTTTTCTTTTCTTGTGTTGG	TTGAAACTGACATCACTTCT
<i>MtCLE20</i>	TGTTACCACTTCATCACGGCATC	TGTGGCTCAACAACCTGAAGACTC
<i>MtCLE21</i>	GCTAAAGGAGGTTCAAGACAG	GTAAGGATTAGGACCAGTGTAG
<i>MtCLE22</i>	TCATCCCTACCTCTGTCAATTATAC	ATCCTTGCTTACCAACCTTCC
<i>MtCLE23</i>	CTTCTTACACCTACATCTTACAC	TTACCTCTGCTGCTGATTGG
<i>MtCLE24</i>	AAGAAGAGTTTCGGCTAATGTC	CTGTTGTGAATAGGATCTGACC
<i>MtCLE25</i>	CACTCAAACATGATGATGAACAAG	TGAATAGGATCTGGTCCATTAGG
<i>40S Ribos</i>	GCCATTGTCCAAGTTTGATGCTG	TTTTCTACCAACTTCAAAACACCG
<i>GFP</i>	ACGGCATCAAGGTGAACTTC	GTGCTCAGGTAGTGGTTGTC
<i>GUS</i>	CTACACCACGCCGAACAC	CACCACCTGCCAGTCAAC

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      10      20      30      40      50      60      70      80
HtCLE01 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE25 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE05 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE10 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE08 NSSYHNKLISSVACLAPOQTFKTPSSKTSIFIKLFFQKNSLSLHAIISISFTFTIARTIKLQVLSFIDGTFPTID
HtCLE18 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE17 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE23 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE16 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE03 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE07 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE15 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE22 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE04 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE21 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE11 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE24 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE02 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE06 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE09 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE14 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE13 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE12 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE20 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE19 .....|.....|.....|.....|.....|.....|.....|.....|

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      90     100     110     120     130     140     150     160
HtCLE01 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE25 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE05 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE10 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE08 HRFPGFCMISISTGKTLTG--NRPVVSQSKRLATGDFPTFINDHRSLEHR--RQDHYRSHYRHAAPNE
HtCLE18 HQYMLKY--SKYKQITDASERSSPSRERKVLAKRPFSPFF--RHLRP--QCFAPQD
HtCLE17 PRVYLRIT--RFSRPS--TSSRHLRPIFFPH--SP
HtCLE23 PTVYTRT--RFSRPS--TQQAIFR--WAM--SP
HtCLE16 NCRLESQT--RPSRPSRNTQHQQLLYCDSFRKNTESL--CINLQRIDHRLBNQSP
HtCLE03 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE07 IAP--CEAAA--LTGNKALPDR--FRGSS--FRSSSRF--HRCM
HtCLE15 ETSAGONTAY--ACSYANGESLKEPQNRRLSGLW/SLE--GSS--
HtCLE22 ETS--GCSAY--ACVDANGESLKKDG--RWR/GQO--GSSS
HtCLE04 NSGFCIAG--GLNFTS--SASSR--KQ
HtCLE21 NTSFSAQETA--RSONFRKSRKSPWF--QARGSR--QW
HtCLE11 QPTIACGLR--TKD--LWQISEMLKGDAREGLS--KDL
HtCLE24 QPKVSGLES--LELALRNGKDHV/LQORTLKAPEKELLATKSSA--MNRKSV
HtCLE02 SIVKFLBDIKH--EDTSRFSFSCRLFGALLSGLDHTDFLTISTHETKR--TLLVPRWVWLSRDLK
HtCLE06 NLSRQPTTM--PMPSSRSTKSTKSRSTSTSTSTTM--LHPK
HtCLE09 NAKTAAKTVSFTPTQNDPQKPTCKRCKEDRQMSLPSLPSFRKASISLPTGLASIQRYSLQPNWIVQVYRE
HtCLE14 RSRGGDPTDRL--SLQSDVHRIRLSGGDPRRESLQSDVHRIRLSGGDPRRWFLSDVHRIRLS
HtCLE13 PSLQAPS--LQDPLIDNIDRSLQLRL--KRI
HtCLE12 FRSYRARR--LRGSHDSQALLRSLRVDRLR--LII
HtCLE20 KAEFTVLLIT--FLVCGKQSSPVEPQKESQWRQ--RQ
HtCLE19 SFTFSRKRRC--QFQLKFLDEHQLDTRVWVWEDQ--ETVAL

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      170     180     190     200     210     220     230     240
HtCLE01 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE25 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE05 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE10 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE08 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE18 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE17 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE23 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE16 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE03 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE07 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE15 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE22 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE04 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE21 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE11 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE24 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE02 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE06 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE09 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE14 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE13 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE12 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE20 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE19 .....|.....|.....|.....|.....|.....|.....|.....|

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      250     260     270     280     290
HtCLE01 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE25 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE05 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE10 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE08 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE18 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE17 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE23 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE16 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE03 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE07 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE15 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE22 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE04 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE21 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE11 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE24 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE02 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE06 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE09 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE14 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE13 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE12 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE20 .....|.....|.....|.....|.....|.....|.....|.....|
HtCLE19 .....|.....|.....|.....|.....|.....|.....|.....|

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Figure S1. Sequence alignment of the *MtCLE* genes. In the *M. truncatula* genome, 25 *MtCLE* peptide genes were found. Alignment was created by ClustalW analysis. Conserved amino acid residues are indicated in color and the conserved 12 amino-acid-long CLE peptides are framed.

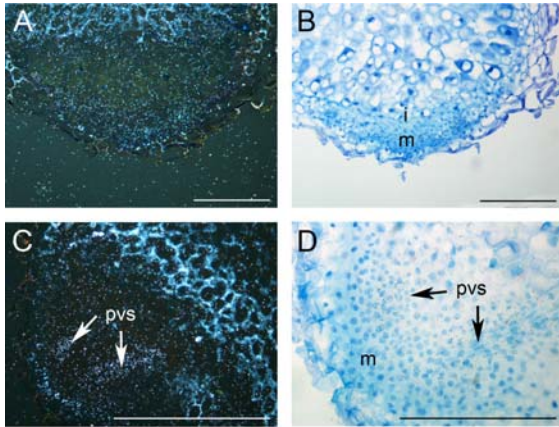


Figure S2. *MtCLE13* transcript accumulation in mature nodules by *in situ* hybridization. *MtCLE13* mRNA was localized by *in situ* hybridization with a *MtCLE13* antisense probe covering the complete open reading frame. Dark-field (A and C) and bright-field (B and D) microscopic images. Signal is seen as white and black dots in dark-field and bright-field micrographs, respectively. A and B, Detail of the meristematic zone of a mature nodule showing low signal in the meristem (m) and infection zone (i). C and D, Detail of a mature nodule in which the signal is localized in the provascular strands (pvs). Scale bars = 500 μ m.

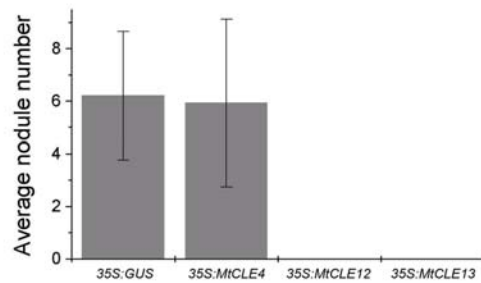


Figure S3. Average nodule number on roots ectopically expressing *GUS*, *MtCLE4*, *MtCLE12*, or *MtCLE13* at 11 dpi with a NF-overproducing *Sinorhizobium meliloti* strain. Average nodule number on plants with transgenic roots carrying the *35S:GUS*, *35S:MtCLE4*, *35S:MtCLE12* or *35S:MtCLE13* constructs at 11 dpi (n=6-17). Inoculation was done with a NF-overproducing *Sinorhizobium meliloti* strain (GMI6390:2011(pMH682)). Data and error bars represent mean \pm SD.

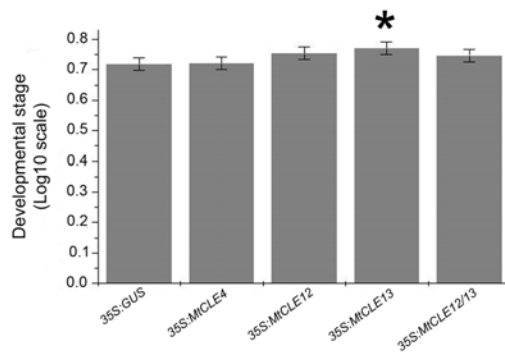


Figure S4. Log10 of the developmental stage of composite plants carrying roots ectopically expressing *GUS*, *MtCLE4*, *MtCLE12*, *MtCLE13*, or *MtCLE12* and *MtCLE13*.

Developmental stage was measured according to Bucciarelli et al., 2006. Data and error bars represent mean \pm SE. Asterisk marks a group statistically different from the control (35S:*GUS*).

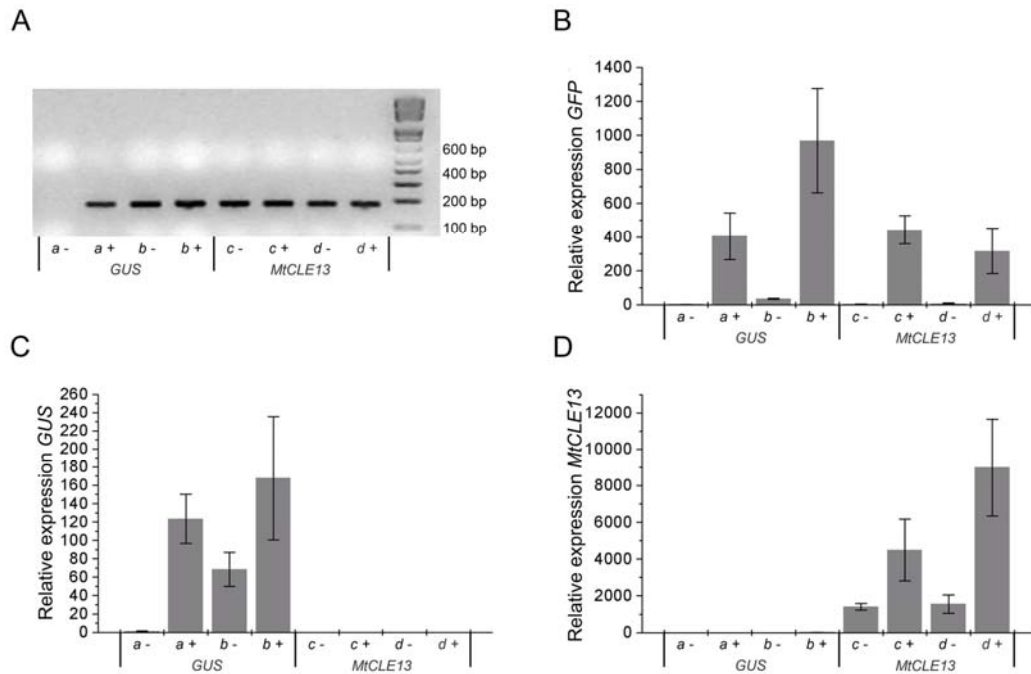


Figure S5. Detailed genotypic analysis of transgenic roots obtained by *A. rhizogenes* transformation.

Jemalong J5 plants were transformed with either *35S:GUS* (plants *a* and *b*) or *35S:MtCLE13* (plants *c* and *d*). Roots with (+) and without (-) GFP fluorescence were kept on the plants and harvested separately at 7 dpi. gDNA as well as cDNA were prepared from these samples. A, Gel electrophoresis of PCR products obtained with GFP-specific primers on the gDNA samples of these roots. B-D, Expression analysis of *GFP*, *GUS*, and *MtCLE13* by qRT-PCR on the cDNA samples of the roots.