A Peptide Encoding Gene *MdCLE8* Regulates Lateral Root Development in Apple

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Abstract

Nitrogen is not only an essential nutrient for plant, but also an important signal molecule to integrate and regulate gene expression, metabolism and growth. Plant peptides are considered as a new hormone, and play an important regulatory role in plant growth and development. However, there are few researches on the co-regulation network between nitrogen and peptide hormones in plant. Here we identified an apple *MdCLE8* gene, which encodes a putative peptide, induced by nitrogen deficiency in apple. Ectopic expression of *MdCLE8* inhibited lateral root formation in *Arabidopsis* under nitrogen deficiency. Similarly, overexpression of *MdCLE8* inhibited lateral root development in apple adventitious roots, and this inhibition was amplified under nitrogen deficiency treatment. Further studies showed that *MdCLE8* may inhibit the expression of several key genes during lateral root emergence stage in *Arabidopsis*, thereby inhibiting the emergence of lateral root from root cortex cells. Collectively, our study not only broadened the gene regulatory network under the influence of nitrogen in apple, but also expanded the function of CLE peptide hormones in apple.

Key Message

The apple peptide encoding gene MdCLE8 is induced by nitrogen deficiency signaling and negatively regulates lateral root formation in plants.

Introduction

Nitrogen (N) is not only one of the most important nutrients for plants, but also a signal substance to affect plant growth and development (Vidal et al. 2010, 2013). Root system architecture (RSA) plays an important role in nutrient and water absorption, and exhibits considerable developmental plasticity to changing environmental conditions (Malamy 2005; Osmont et al. 2007). Lateral root (LR) accounts for most of the total root system and displays higher plasticity to external nutrient utilization than primary root (Giehl et al. 2014). In *Arabidopsis*, nitrogen is an important environmental factor affecting lateral root development: Local nitrate treatment can not only stimulate the initiation of lateral root (Vidal et al. 2013), but also significantly promote lateral root elongation (Zhang and Forde 1998; Remans et al. 2006a); Mild nitrogen deficiency can promote the growth of lateral root, while prolonged nitrogen deficiency or excessive nitrogen supply is not conducive to lateral root growth (Forde and Lorenzo 2002; Remans et al. 2006b; Ruffel et al. 2011; Gruber et al. 2013).

The postembryonic and multistage nature of lateral root development makes it subject to more complex gene network regulation: In *Arabidopsis*, lateral roots originate from founder cells formed from xylem pole pericycle cells, and then undergo cell division and expansion to develop lateral root primordium (LRP) (Malamy and Benfey 1997; Casimiro et al. 2001; Dubrovsky et al. 2001). Lateral root primordium must pass through three cell layers: endodermis (EN), cortical layer (CO), and epidermal layer (EP) successively before it can appear on the surface of primary root (Péret et al. 2009a, b; Vilches-Barro and Maizel 2015). In this process, the cell layer provides the greatest resistance, which may affect the number of lateral root.

To reduce this resistance, cells covering the lateral root primordium undergo cell wall remodeling (CWR) to promote cell separation and thus expand the growth pathway of lateral root primordium, and a process requires proper spatiotemporal expression of cell wall remodeling genes (Lewis et al. 2013). CWR genes are expressed not only in the floral abscission zones, but also in regions where cell separation occurs, including dehiscence zones of the siliques and the cells overlaying emerging lateral root primordium (González-Carranza et al. 2007; Cai and Lashbrook 2008; Ogawa et al. 2009; Lewis et al. 2013). During lateral root emergence, auxin induces the expression of *LAX3* in cortical cells directly covering lateral root primordium, which is also where some CWR genes are specifically expressed. *LAX3* encodes a high affinity auxin influx carrier and functions in lateral root emergence by targeting the auxin-inducible expression of CWR genes (Swarup et al. 2008; Kumpf et al. 2013). In addition, peptide signaling also plays a key role in lateral root emergence by regulating the expression of CWR genes. INFLORESCENCE DEFICIENT IN ABSCISSION (*IDA*) encodes a small protein ligand that co-regulates floral organ abscission with its receptor HAE/HSL2 (Sung et al. 2008; Santiago et al. 2016). Moreover, *IDA* also has an overlapping expression region with CWR genes in the root (Kumpf et al. 2013). IDA-HAE/HLS2 module and its downstream MKK4/MKK5–MPK3/MPK6 module jointly regulate the expression of CWR genes and thus play a key role in the lateral root emergence process (Kumpf et al. 2013; Zhu et al. 2019).

Peptides are typically processed from a small protein precursor, of which several hundred are expected in *Arabidopsis* (Hanada et al. 2007, 2013; Murphy et al. 2012). Recent studies have shown that peptides play an important role in plant growth and development (Czyzewicz et al. 2013; Fletcher 2020). The CLAVATA3/EMBRYO SURROUNDING REGION-related (*CLE*) gene encodes a large peptide family (Goad et al. 2017). Among 32 *CLE* genes reported in *Arabidopsis* (Cock and McCormick 2001), *CLV3* and *CLE40* are the two best characterized members, which play key roles in stem cell population maintenance in shoot apical meristem and root apical meristem, respectively (Fletcher 1999; Hobe et al. 2003). The *CLE* gene is also widely present in other species (Goad et al. 2017). We previously identified 25 *MdCLE* genes in the apple genome, and they have very similar structural characteristics to *CLE* genes reported in other species (Zhang et al. 2021), but their functions in apple are unknown.

Here, we identified and analyzed the function of the *MdCLE8* gene, which is adjacent to CLE1/3/4/7 of *Arabidopsis* in the evolutionary tree and encodes highly similar CLE motif (Fig. S1 and S2). In this study, we found that the expression of *MdCLE8* was significantly induced by nitrogen deficiency in apple. Overexpression of *MdCLE8* inhibited lateral root development in apple adventitious roots, and this inhibition was further enhanced by nitrogen deficiency treatment. Further studies showed that *MdCLE8* may inhibit lateral roots formation by affecting the expression of related genes during lateral root emergence.

Materials And Methods

2.1. Plant materials, growth conditions and treatments

Tissue cultured ‘M26’ apple plants were subcultured on Murashige and Skoog (MS) medium (MS + 3% sucrose + 0.5 mg/L 6-BA + 0.2 mg/L NAA + 0.2 mg/L GA, pH5.8) under a long-day conditions (16 h light/8 h dark) at 24 ˚C for 30 d.

The Columbia ecotype (Col-0) *Arabidopsis* seeds were cultured on 1/2 MS medium for 4 days, then transferred to new 1/2 MS medium or N-deficient 1/2 MS medium (Nitrogen concentration was 0.1 mM, KNO_3 was replaced by KCl and nitrogen was supplemented by NH_4NO_3) for further growth for 7 days.

To determine the expressions of *MdCLE* genes in response to different nitrogen concentrations, one-month-old ‘Pingyitiancha’ (*Malus × hupehensis*) apple seedlings were pretreated with 1/2 medium for 3 d, then they were treated with 0 mM, 0.1 mM, 1 mM and 10 mM nitrogen medium (KNO_3 was replaced by KCl and nitrogen was supplemented by NH_4NO_3) for the indicated times, respectively. The seedlings were growning at 24˚C with a 16 h light/8 h dark photoperiod.

2.2. Vector construction and plant transformation

The open reading frame (ORF) of *MdCLE8* was fused to pRI-GFP to generate 35S::MdCLE8-GFP. Then 35S::MdCLE8-GFP and empty expression vector were introduced into *Agrobacterium rhizogenes* strain K599 and transformed into ‘M26’ apple stem segments to induce hairy roots (Zhou et al. 2019), and the empty expression vector (CK) was used as a control. GFP fluorescence of transgenic adventitious roots was identified by fluorescence microscopy.

35S::MdCLE8-GFP vector was transformed into *Arabidopsis* by the *Agrobacterium*-mediated floral dip method (Clough and Bent 1998).

2.3. Gene expression analysis

Total RNA was extracted from apple seedlings, ‘M26’ transgenic adventitious roots, and *Arabidopsis* using RNA Plant Plus reagent (Tiangen, Beijing, China) according to the instructions, and reverse transcription assay was performed by using the PrimeScript cDNA Synthesis Kit (Takara, Liaoning, China). RT-qPCR was performed with the UltraSYBR mixture (Takara, Liaoning, China) by an ABI7500 RT-PCR system (An et al. 2020). Primers used for RT-qPCR are listed in supplementary Table S1, and 18S rRNA was used as an internal control.

2.4. GUS staining

After germinating and growing for 4 days on 1/2 MS medium, DR5-GUS and DR5-GUS/MdCLE8-OE transgenic *Arabidopsis* seeds were transferred to N-deficient 1/2 MS medium for 3 days. 7-day-old transgenic *Arabidopsis* seedlings were stained using a GUS solution (Clough and Bent 1998), then the total number of lateral root primordium was counted under the type microscope.

2.5. Determination of polygalacturonase and pectinase activities

Polygalacturonase and pectinase activity detection kits were purchased from Comin Biotechnology (Suzhou, China).

2.6. Root measurement

For *Arabidopsis*, the primary root length and the lateral root number were analyzed by Digimizer software. For apple seedlings, the phenotypes of apple transgenic adventitious roots were scanned with a root scanner (Perfection V850 Pro Photo, Epson), and the lateral root length and number were measured with Digimizer software.

2.7. Statistical analysis

SPSS v17.0 software was used for statistical analysis. Statistical analysis was performed using a Student's t-test, where ns $P > 0.05$, *$P < 0.05$ and **$P < 0.01$. Different letters indicate significant difference ($P < 0.05$) as obtained by one-way ANOVA test.

2.8. Accession Numbers

MdCLE1 (MDP0000312687), *MdCLE3* (MDP0000223926), *MdCLE4* (MDP0000825799), *MdCLE5* (MDP0000226320), *MdCLE6* (MDP0000262449), *MdCLE8* (MDP0000240955), *MdCLE10* (MDP0000149789), *MdCLE21* (MDP0000224389), *MdCLE23*(MDP0000119279), *MdLAX-like1* (MDP0000885425), *MdLAX-like2* (MDP0000020317), *MdLAX-like3* (MDP0000155113), *MdLAX-like4* (MDP0000089124), *MdLAX-like5* (MDP0000080407), *LAX3* (AT1G77690), *PGLR* (AT5G14650), *PGAZAT* (AT2G41850), *XTR6*/*XTH23* (AT4G25810), *EXP17* (AT4G01630). The *MdXTH* gene family information as described by Atkinson et al. (Atkinson et al. 2009). All primers used are listed in Table S1.

Results

1. *MdCLE8* is a nitrogen-responsive gene that encodes a putative peptide in apple

Nitrogen is one of the essential elements for plant growth and development. In order to investigate the relationship between nitrogen and MdCLE peptide hormones in apple, we quantitatively detected the expression levels of *MdCLE* genes in subfamily III under different nitrogen concentrations (Fig. S1). With the decrease of nitrogen concentration in nutrient solution, the expression levels of *MdCLE8* and *MdCLE23* in apple seedling were significantly induced, while the other detected that *MdCLEs* remained basically unchanged (Fig. 1). These results suggested that *MdCLE8* and *MdCLE23* might be functional genes under the influence of nitrogen in apple.

To investigate further, we cloned the *MdCLE8* gene. By sequence alignment, we found that MdCLE8 protein had a conserved CLE motif at the C terminal similar to that of AtCLE1/3/4/7 (Fig S2). Our previous study showed that the external application of *MdCLE8* synthetic peptide (MdCLE8p) had no significant effect on the normal growth of *Arabidopsis*, showing similar activity to AtCLE3p [36,41].

2. Ectopic expression of *MdCLE8* inhibits lateral root formation in *Arabidopsis* under nitrogen deficiency

To investigate the function of *MdCLE8* gene, we constructed *MdCLE8* overexpression vector, and transformed it into wild type *Arabidopsis* (MdCLE8-OE1, MdCLE8-OE2, and MdCLE8-OE3). Subsequently, we treated transgenic *Arabidopsis* lines with 1/2 MS and N-deficiency 1/2 MS medium, and used the wild type (WT) as a control. The results showed that there was no difference between WT and MdCLE8-OE transgenic *Arabidopsis* growing on 1/2 MS medium, and the primary root length, lateral root number and density statistics all indicate this (Fig. 2A and 2C-D). In contrast, the growth of MdCLE8-OE transgenic *Arabidopsis* was significantly inhibited in N-deficient 1/2 MS medium. Compared with the WT, the primary root length of MdCLE8-OE transgenic *Arabidopsis* was slightly reduced, and the lateral root number and density were significantly reduced (Fig. 2B and 2C-D). The above results revealed that overexpression *MdCLE8* inhibited lateral root growth and development under nitrogen deficiency condition.

3. *MdCLE8* inhibits lateral root formation in apple adventitious roots under nitrogen deficiency

To explore the function of *MdCLE8* on the growth of apple root system, the 35::MdCLE8-GFP vector and empty expression vector pRI-GFP (CK) were transformed into ‘M26’ apple shoot base cells by *Agrobacterium rhizogenes*-mediated genetic transformation (Fig. S3A). The false positive adventitious roots without GFP fluorescence were removed under fluorescence microscope (Fig. S3B), and apple seedlings were transplanted into vermiculite without any nutrition. Finally, the apple seedlings were watered with 1/2 MS or N-deficiency 1/2 MS for 30 days, respectively.

Our results showed that prolonged nitrogen deficiency increased the empty expression vector transgenic adventitious roots length, but significantly decreased the lateral root tips number and density (Fig. 3A, 3C and 3E-G), this finding indicating that prolonged nitrogen deficiency will seriously affect the growth and development of apple lateral root. Under the 1/2 MS condition, the total lateral root tips number and density of 35S::MdCLE8-GFP transgenic adventitious roots were significantly decreased (Fig. 3A-B and 3E-G). Moreover, nitrogen deficiency intensified the effect of 35S::MdCLE8-GFP on the growth of apple adventitious roots (Fig. 3C-G). The root length of MdCLE8-OE transgenic adventitious roots decreased significantly, the lateral root number decreased from 45.64–79.58%, and the lateral root density decreased from 44.37–68.71%. Taken together, our results indicate that *MdCLE8* inhibited lateral root formation in apple, and this inhibition was enhanced by nitrogen deficiency treatment.

4. *MdCLE8* is involved spatiotemporal regulation of key genes during lateral root emergence

The above experimental results revealed that the overexpression of *MdCLE8* gene inhibited lateral root formation in *Arabidopsis* under nitrogen deficiency. An unsolved question is what the underlying mechanism is. To answer this question, we obtained a hybrid *Arabidopsis* material of DR5-GUS and MdCLE8-OE (DR5-GUS/MdCLE8-OE). The development of lateral root primordia of DR5-GU5/MdCLE8-OE was observed by GUS staining (Fig. S4A), and DR5-GUS was used as control. The results showed that the total lateral root primordia number and density of DR5-GUS/MdCLE8-OE on N-deficiency 1/2 MS medium for 7 days were not significantly different from control (Fig. S4B-D), suggesting that *MdCLE8* does not affect lateral root primordia formation in *Arabidopsis*.

We hypothesized that the inhibition of MdCLE8-OE on lateral root development was caused by affecting lateral root emergence stage. Therefore, we examined the expression levels of key genes in *Arabidopsis* that affect the lateral root emergence, including auxin influx vector gene *LAX3* (Swarup et al. 2008), cell wall remodeling gene *PGAZAT*, *PGLR*, *XTR6* and *EXP17* (Laskowski et al. 2006; González-Carranza et al. 2007; Swarup et al. 2008; Kumpf et al. 2013). The results showed that overexpression of *MdCLE8* inhibited the expression of *LAX3*, *PGAZAT*, *PGLR* and *XTR6* genes in *Arabidopsis* under nitrogen deficiency conditions (Fig. 4A). At the same time, we detected the expression levels of *MdLAX3-like* and *MdXTH* genes, as well as polygalacturonase (PG) and pectinase activity in apple. The results showed that overexpression of *MdCLE8* inhibited the expression of some *MdLAX-like* and *MdXTH* genes (Fig. 4B-C), and decreased the activities of PG and pectinase (Fig. 4D-E), and this inhibition was particularly obvious under nitrogen deficiency conditions. In conclusion, *MdCLE8* may inhibit the formation of lateral root by affecting lateral root emergence stage.

Discussions

Root system is an important organ for plants to obtain water and nutrients from the soil, and the growth and development of the aboveground parts of plants as well as the yield and quality of fruits are highly dependent on the underground parts (Takatsuka and Umeda 2014). Studies in a variety of plants have shown that the root architecture of plants is highly plastic under the influence of the environment (Malamy 2005; Osmont et al. 2007). Plant peptides are considered as a new type of plant hormones, which play important regulatory roles in many aspects of plant growth and development (Motomitsu et al. 2015; Gancheva et al. 2019). In our previous study, we identified 25 *MdCLE* genes in apple, and by applying their presumed synthetic peptides (MdCLEp) to *Arabidopsis*, we found that most of the MdCLEp showed strong effects on root growth and development (Zhang et al.). In fact, *CLE* genes and their synthetic peptides found in other plant species also showed inhibitory effects on plant root growth (Kinoshita et al. 2007; Whitford et al. 2008; Tian et al. 2019), suggesting that CLE peptide plays an important role in the regulation of root system architecture. Here, we found a low-nitrogen induced expression of *MdCLE8* gene in apple, which encodes a putative plant peptide and plays an important regulatory role in the growth and development of apple lateral roots.

Our previous research has shown that 1 μM MdCLE8p had no significant effect on the root system of *Arabidopsis* cultured in 1/2 MS medium (Zhang et al.). Similarly, in this study, we found that the ectopic overexpression of *MdCLE8* did not affect the growth of *Arabidopsis* in 1/2 MS medium, but significantly inhibited lateral root formation in N-deficiency 1/2 MS medium (Fig. 2). Furthermore, we also overexpressed the *MdCLE8* gene in apple adventitious roots. We found that *MdCLE8* gene also inhibited apple lateral roots formation, and this inhibition was further enhanced by nitrogen deficiency treatment (Fig. 3), suggesting that *MdCLE8* gene and nitrogen deficiency signal co-regulate the development of apple lateral roots.

Lateral root formation in plants is a complex physiological process. To put it simply, lateral root primordia are first produced from xylem pole pericycle cells, and then further developed and grow until they

successfully drill out of the parent root epidermis to produce lateral roots (Malamy and Benfey 1997; Casimiro et al. 2001; Dubrovsky et al. 2001). The growth of the lateral root primordium needs to pass through three cell layers, and the substances in the cell layer such as pectin provide resistance to this process (Lewis et al. 2013). Our results further showed that the overexpression of *MdCLE8* strongly inhibited the expression of cell wall remodeling genes (Fig. 4), thus impeding the normal separation of the cell layer. Therefore, we suggest that the influence of *MdCLE8* on lateral root formation may be due to the growth and emergence of lateral root primordium rather than the origin of lateral root primordium.

Previous studies have shown that the CLE-CLV1 signaling pathway is a core module that regulates the expansion of the lateral root system of *Arabidopsis* under low nitrogen conditions (Araya et al. 2014b, a). The MdCLE8 protein in apple has a highly similar CLE motif to that of AtCLE1/3/4/7 in *Arabidopsis* (Fig S2), and *MdCLE8* and *AtCLE3* have similar regulatory functions for plant lateral root formation (Fig. 2), suggesting that *CLE* genes with similar CLE motif in different species are functionally conserved. However, more detailed anatomical analysis of apple root system, and the receptors that CLE peptides act on remain to be studied. Therefore, the specific mechanism by which *MdCLE8* regulates lateral root development in apple still needs to be further analyzed.

Declarations

Data availability statement

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

Chun-xiang You, Qiang Zhao, and Tian-en Zhang conceived and designed the experiments. Chun-xiang You and Qiang Zhao supervised the experiments. Tian-en Zhang, Xiu-ming Li and Yan Shi performed the experiments. All the authors read and approved the final manuscript.

Disclosure statement

The authors have no conflict of interest to declare.

References

1. An JP, Wang XF, Espley R V., et al (2020) An apple b-box protein MdBBX37 modulates anthocyanin biosynthesis and hypocotyl elongation synergistically with MdMYBs and MdHY5. Plant Cell Physiol 61:130–143.
2. Araya T, Miyamoto M, Wibowo J., et al (2014a) CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. Proc Natl Acad Sci U S A 111:2029–2034.
3. Araya T, Von Wirén N, Takahashi H (2014b) CLE peptides regulate lateral root development in response to nitrogen nutritional status of plants. Plant Signal Behav 9: 7, e29302.
4. Atkinson RG, Johnston SL, Yauk YK., et al (2009) Analysis of xyloglucan endotransglucosylase/hydrolase (XTH) gene families in kiwifruit and apple. Postharvest Biol Technol 51:149–157.
5. Cai S, Lashbrook CC (2008) Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: Enhanced retention of floral organs in transgenic plants overexpressing arabidopsis Zinc Finger Protein2. Plant Physiol 146:1305–1321.
6. Casimiro I, Marchant A, Bhalerao RP., et al (2001) Auxin transport promotes arabidopsis lateral root initiation. Plant Cell 13:843–852.
7. Clough SJ, Bent AF (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16:735–743.
8. Cock JM, McCormick S (2001) A Large Family of Genes That Share Homology with CLAVATA3. Plant Physiol 126:939–942.
9. Czyzewicz N, Yue K, Beeckman T, De Smet I (2013) Message in a bottle: Small signalling peptide outputs during growth and development. J Exp Bot 64:5281–5296.
10. Dubrovsky JG, Rost TL, Colón-Carmona A, Doerner P (2001) Early primordium morphogenesis during lateral root initiation in Arabidopsis thaliana. Planta 214:30–36.
11. Fletcher JC (2020) Recent Advances in Arabidopsis CLE Peptide Signaling. Trends Plant Sci 25:1005–1016.
12. Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. Science 283:1911–1914.
13. Forde B, Lorenzo H (2001) The nutritional control of root development. Plant and Soil 232:51–68.
14. Gancheva MS, Malovichko Y V, Poliushkevich LO., et al (2019) Plant Peptide Hormones. Russ J Plant Physiol 66:171–189.
15. Giehl RFH, Gruber BD, von Wirén N (2014) It's time to make changes: modulation of root system architecture by nutrient signals. J Exp Bot 65:769–778.
16. Goad DM, Zhu C, Kellogg EA (2017) Comprehensive identification and clustering of CLV3/ESR-related (CLE) genes in plants finds groups with potentially shared function. New Phytol 216:605–616.

17. González-Carranza ZH, Elliott KA, Roberts JA (2007) Expression of polygalacturonases and evidence to support their role during cell separation processes in Arabidopsis thaliana. J Exp Bot 58:3719–3730.
18. Gruber BD, Giehl RFH, Friedel S, von Wirén N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. Plant Physiol 163:161–179.
19. Hanada K, Higuchi-Takeuchi M, Okamoto M, et al (2013) Small open reading frames associated with morphogenesis are hidden in plant genomes. Proc Natl Acad Sci U S A 110:2395–2400.
20. Hanada K, Zhang X, Borevitz JO, et al (2007) A large number of novel coding small open reading frames in the intergenic regions of the Arabidopsis thaliana genome are transcribed and/or under purifying selection. Genome Res 17:632–640.
21. Hobe M, Müller R, Grünewald M, et al (2003) Loss of CLE40, a protein functionally equivalent to the stem cell restricting signal CLV3, enhances root waving in Arabidopsis. Dev Genes Evol 213:371–381.
22. Kinoshita A, Nakamura Y, Sasaki E, et al (2007) Gain-of-function phenotypes of chemically synthetic CLAVATA3/ESR-Related (CLE) peptides in Arabidopsis thaliana and Oryza sativa. Plant Cell Physiol 48:1821–1825.
23. Kumpf RP, Shi CL, Larrieu A, et al (2013) Floral organ abscission peptide IDA and its HAE/HSL2 receptors control cell separation during lateral root emergence. Proc Natl Acad Sci U S A 110:5235–5240.
24. Laskowski M, Biller S, Stanley K, et al (2006) Expression profiling of auxin-treated Arabidopsis roots: Toward a molecular analysis of lateral root emergence. Plant Cell Physiol 47:788–792.
25. Lewis DR, Olex AL, Lundy SR, et al (2013) A kinetic analysis of the auxin transcriptome reveals cell wall remodeling proteins that modulate lateral root development in Arabidopsis. Plant Cell 25:3329–3346.
26. Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. Plant, Cell Environ 28:67–77.
27. Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of Arabidopsis thaliana. Development 124:33–44
28. Motomitsu A, Sawa S, Ishida T (2015) Plant peptide hormone signalling. Essays Biochem 58:115–131.
29. Murphy E, Smith S, De Smet I (2012) Small Signaling Peptides in Arabidopsis Development: How Cells Communicate Over a Short Distance. Plant Cell 24:3198–3217.
30. Ogawa M, Kay P, Wilson S, Swain SM (2009) Arabidopsis Dehiscence Zone Polygalacturonase1 (ADPG1), ADPG2, and Quartet2 are polygalacturonases required for cell separation during reproductive development in Arabidopsis. Plant Cell 21:216–233.
31. Osmont KS, Sibout R, Hardtke CS (2007) Hidden branches: developments in root system architecture. Annu Rev Plant Biol 58:93–113.

32. Péret B, De Rybel B, Casimiro I, et al (2009a) Arabidopsis lateral root development: an emerging story. Trends Plant Sci 14:399–408.
33. Péret B, Larrieu A, Bennett MJ (2009b) Lateral root emergence: a difficult birth. J Exp Bot 60:3637–3643.
34. Remans T, Nacry P, Pervent M, et al (2006a) The Arabidopsis NRT1 . 1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc Natl Acad Sci U S A 103: 19206-19211.
35. Remans T, Nacry P, Pervent M, et al (2006b) A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. Plant Physiol 140:909–921.
36. Ruffel S, Krouk G, Ristova D, et al (2011) Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. Proc Natl Acad Sci U S A 108:18524–18529.
37. Santiago J, Brandt B, Wildhagen M, et al (2016) Mechanistic insight into a peptide hormone signaling complex mediating floral organ abscission. Elife 5: e15075.
38. Sung KC, Larue CT, Chevalier D, et al (2008) Regulation of floral organ abscission in Arabidopsis thaliana. Proc Natl Acad Sci U S A 105:15629–15634.
39. Swarup K, Benková E, Swarup R, et al (2008) The auxin influx carrier LAX3 promotes lateral root emergence. Nat Cell Biol 10:946–954.
40. Takatsuka H, Umeda M (2014) Hormonal control of cell division and elongation along differentiation trajectories in roots. J Exp Bot 65:2633–2643.
41. Tian D, Liu Y, Tian L, et al (2019) Involvement of Populus CLEL peptides in root development. Tree Physiol 39:1907–1921.
42. Vidal EA, Moyano TC, Riveras E, et al (2013) Systems approaches map regulatory networks downstream of the auxin receptor AFB3 in the nitrate response of Arabidopsis thaliana roots. Proc Natl Acad Sci U S A 110:12840–12845.
43. Vidal EA, Tamayo KP, Gutierrez RA (2010) Gene networks for nitrogen sensing , signaling , and response in Arabidopsis thaliana. Syst Biol Med 2:683-693.
44. Vilches-Barro A, Maizel A (2015) Talking through walls: Mechanisms of lateral root emergence in Arabidopsis thaliana. Curr Opin Plant Biol 23:31–38.
45. Whitford R, Fernandez A, De Groodt R, et al (2008) Plant CLE peptides from two distinct functional classes synergistically induce division of vascular cells. Proc Natl Acad Sci U S A 105:18625–18630.
46. Zhang H, Forde BG (1998) An Arabidopsis MADS Box Gene That Controls Nutrient-Induced Changes in Root Architecture. Science 279:407–410.
47. Zhang TE, Li XM, Zhao Q, et al (2021) Genome-wide identification and functional characterization of the MdCLE peptide family in apple (*Malus × domestica*). Hortic Plant J (Accept).

48. Zhou LJ, Zhang CL, Zhang RF, et al (2019) The SUMO E3 ligase MdSIZ1 targets MdbHLH104 to regulate plasma membrane H $^{+}$ -ATPase activity and iron homeostasis. Plant Physiol 179:88–106.
49. Zhu Q, Shao Y, Ge S, et al (2019) A MAPK cascade downstream of IDA–HAE/HSL2 ligand–receptor pair in lateral root emergence. Nat Plants 5:414–423.

Figures

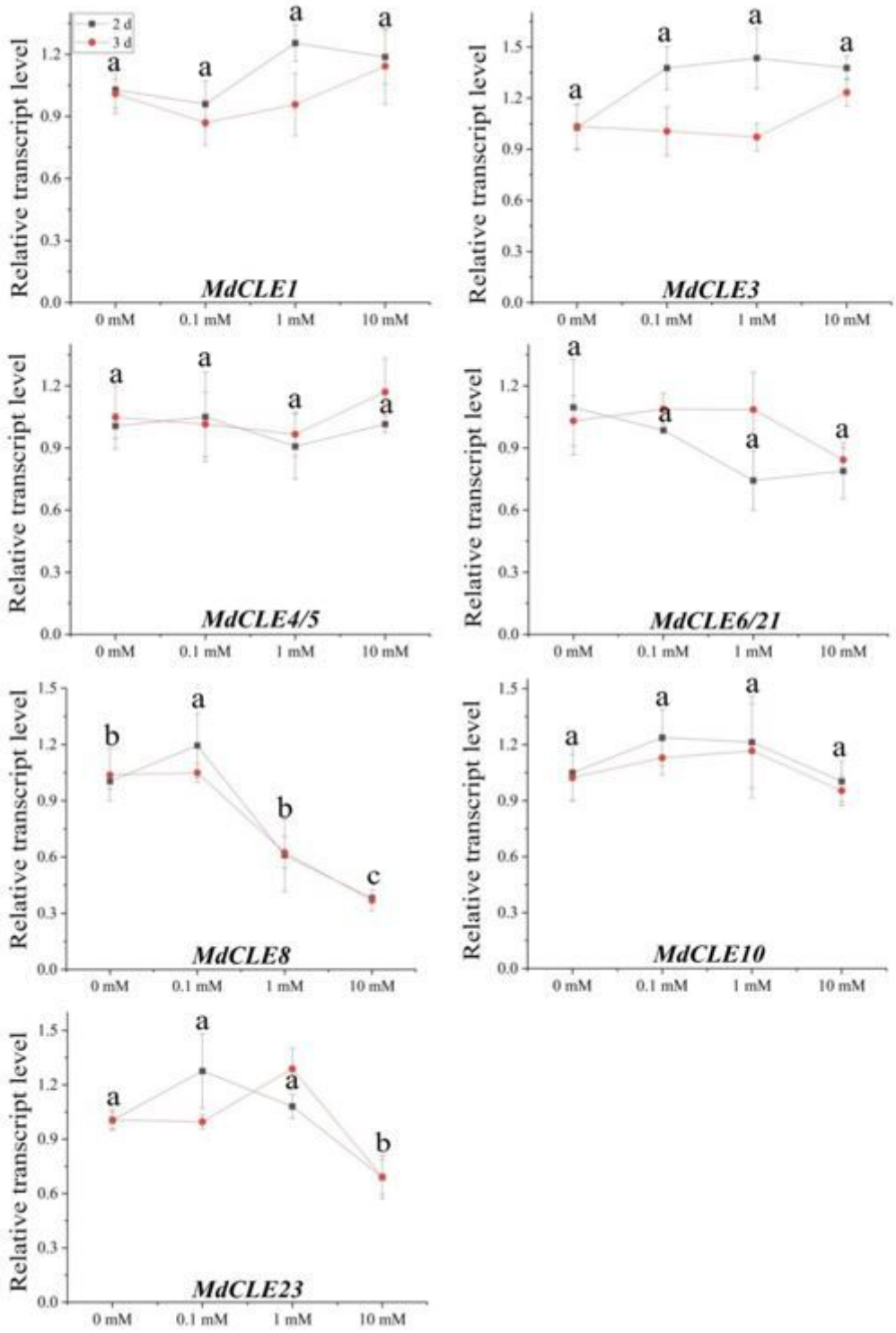

Figure 1

Expression patterns analysis of MdCLE genes under different nitrogen concentrations (0 mM, 0.1 mM, 1 mM, 10 mM). Different letters indicate significant differences ($P < 0.05$) as obtained by one-way ANOVA test. Data are shown as mean SD.

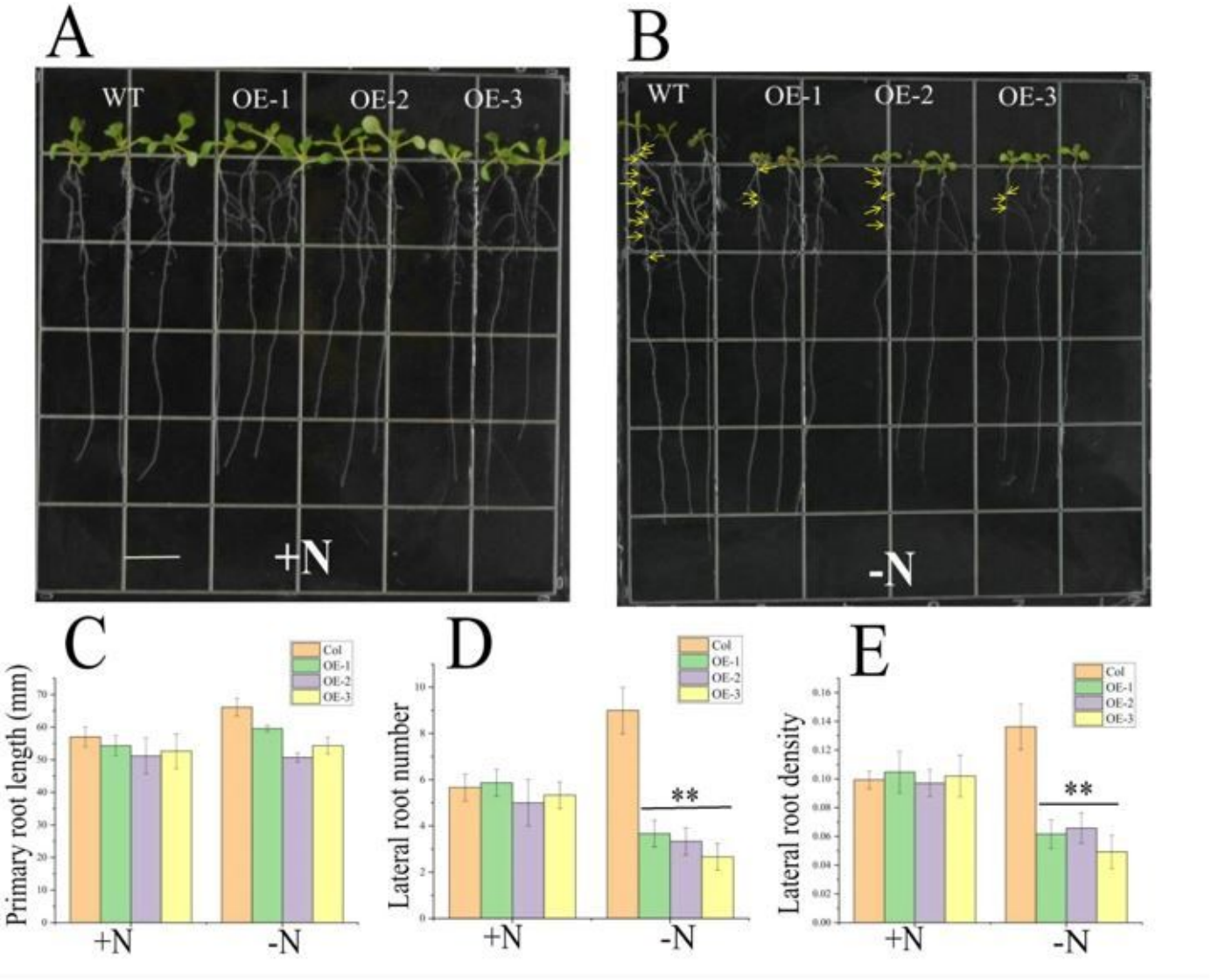

Figure 2

Ectopic expression of MdCLE8 inhibits Arabidopsis lateral root development. A-B. The phenotypes of wild type (WT) and MdCLE8 overexpressed Arabidopsis under 1/2 MS and N-deficiency 1/2 MS conditions, respectively. Bar = 10 mm. C-E. Primary root length, lateral root number and density of Arabidopsis in (A) and (B). Results shown are means ± SE, based on three independent biological replicates. Statistical significance was determined using Student's t-test: **, $P < 0.01$.

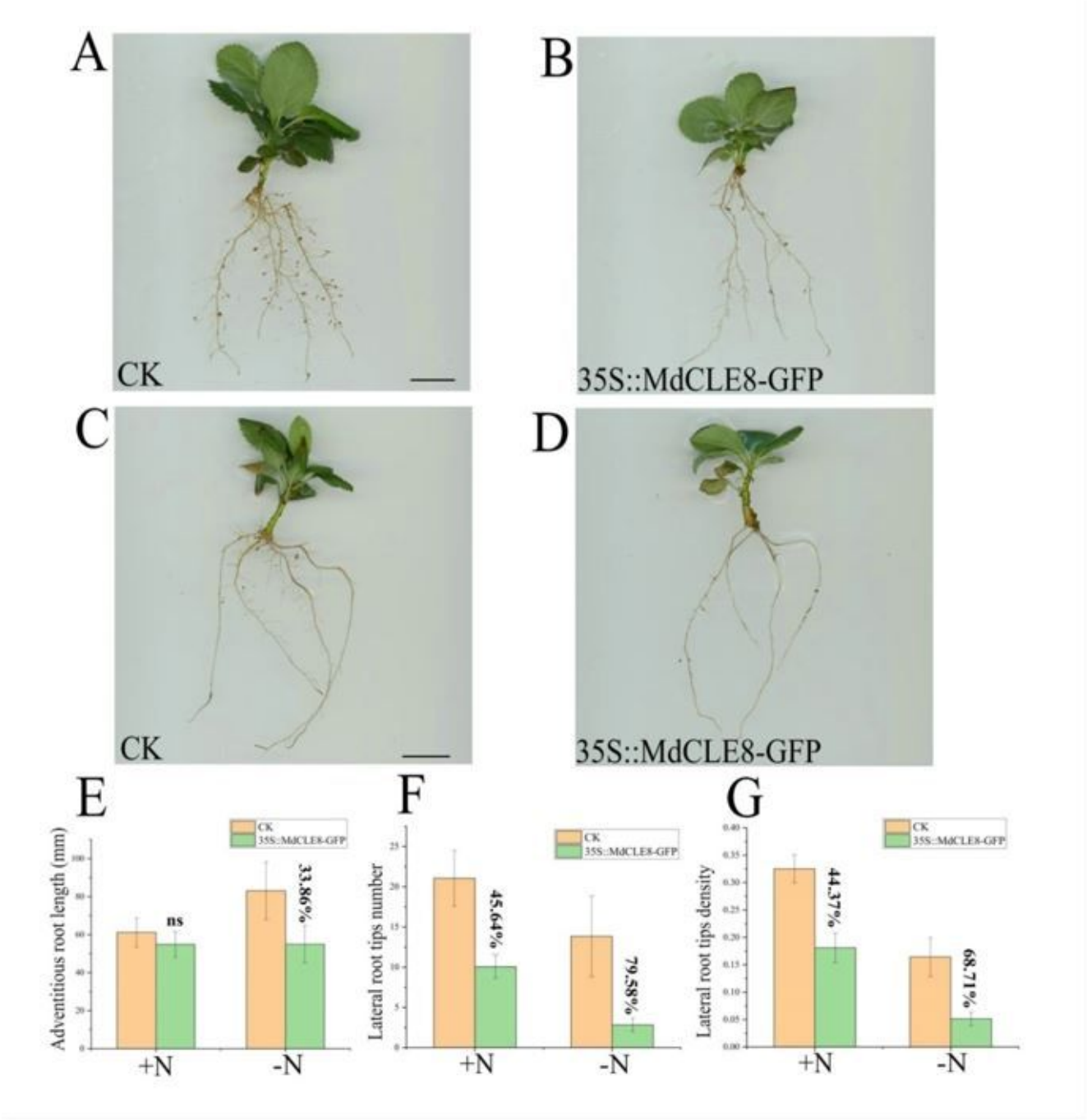

Figure 3

Overexpression of MdCLE8 inhibited lateral root development in apple adventitious roots. A-B. Phenotype of apple seedlings transgenic adventitious root with empty vector (CK) and 35S::MdCLE8-GFP grow for 30 d under 1/2 MS condition. Bar=10 mm. C-D. Phenotype of apple seedlings transgenic adventitious root with empty vector (CK) and 35S::MdCLE8-GFP grow for 30 d under N-deficient 1/2 MS condition. E-G. Transgenic adventitious root length, lateral root tips number and density in (A-D). Results shown are means ± SE, based on at least 5 independent transgenic lines. Statistical significance was determined using Student's t-test: ns, $P > 0.05$.

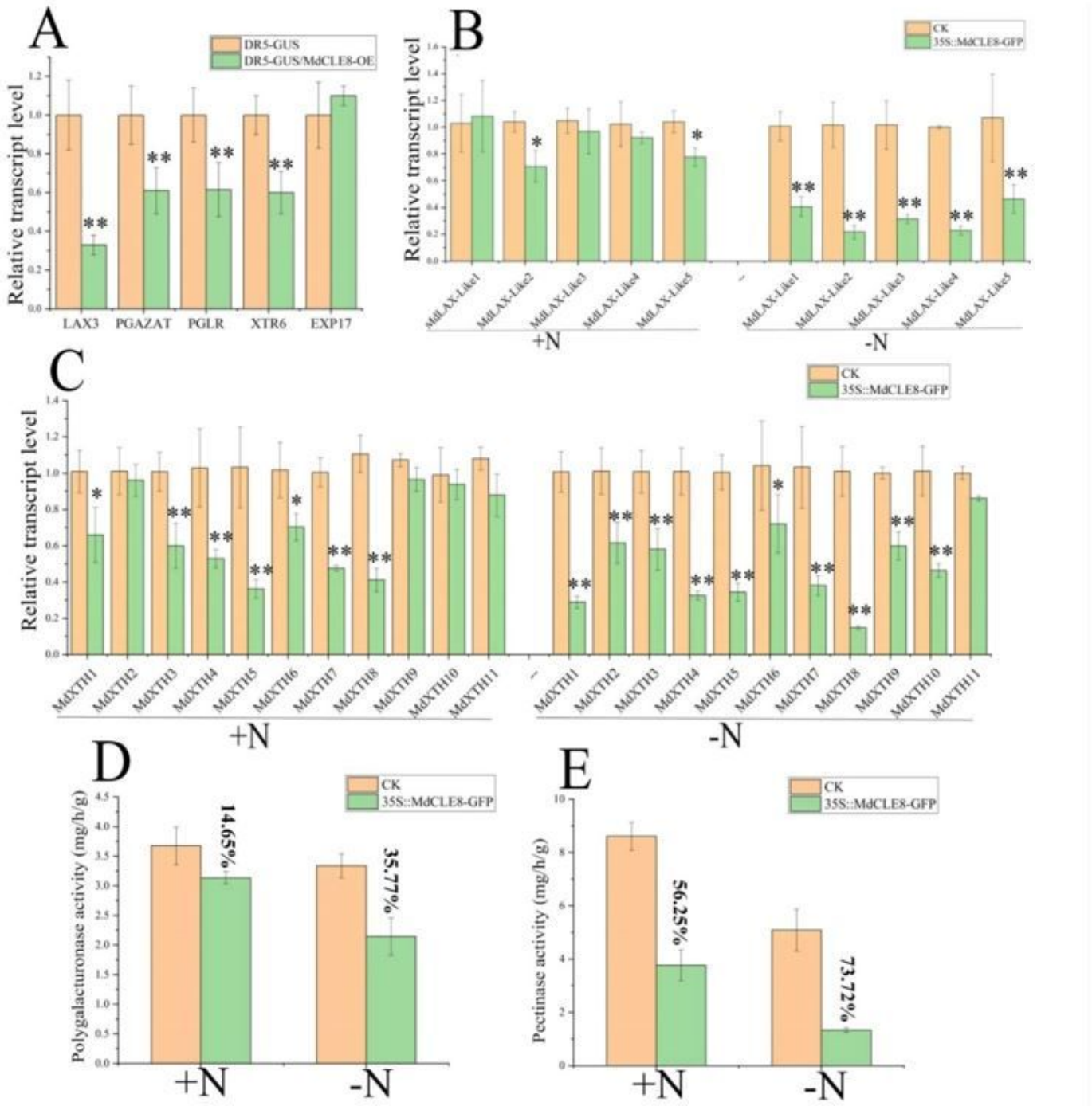

Figure 4

MdCLE8 affected the expression of related genes during lateral root emergence. A. Expression of key genes during lateral root emergence of DR5-GUS and DR5-GUS/MdCLE8-OE transgenic Arabidopsis. B-C. Quantitative analysis of MdLAX-like and MdXTH in transgenic adventitious roots of empty vector and 35S::MdCLE8-GFP. D-E. Detection of polygalacturonase and pectinase activities in transgenic adventitious roots of empty vector and 35S::MdCLE8-GFP.

Supplementary Files

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