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Note

Effect of the CLE14 polypeptide on *GLABRA2* homolog gene expression in rice and tomato roots

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Abstract The CLAVATA3/ESR (CLE) plant polypeptides act as peptide hormones in various physiological and developmental aspects in a diverse array of land plants. One of the *CLE* family of genes, *CLE14*, is reported to induce root hair formation in *Arabidopsis thaliana* roots. Previously, we demonstrated that the application of synthetic CLE14 polypeptide treatment induced excess root hairs, and reduced the expression level of the non-hair cell fate determinant gene, *GLABRA2* (*GL2*) in *Arabidopsis* roots. In this study, we investigated the function of synthetic CLE14 polypeptide in rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*) roots. We measured the expression levels of the *OsGL2* and *SlGL2* genes, i.e., homologs of the *Arabidopsis GL2* gene, in rice and tomato seedlings, respectively. Although CLE14 polypeptide treatment induced excess root hair formation in rice roots, substantial root hair induction was not observed in tomato roots. However, the CLE14 polypeptide treatment significantly inhibited the expression of the *GL2* homolog genes of rice (*OsGL2*) and tomato (*SlGL2*). Our findings thus indicated that CLE14 can inhibit the *GL2* gene expression in both rice and tomato plants, similar to the effect seen in *Arabidopsis*.

Key words: CLE14, *OsGL2*, rice, *SlGL2*, tomato.

The *CLE* family of genes acts as 12 to 13 amino acid polypeptide hormones that regulate cellular activity in the shoot apical meristem, root apical meristem, and vascular tissues in *Arabidopsis* (Cock and McCormick 2001; Ito et al. 2006; Kondo et al. 2006; Ohyama et al. 2008, 2009). Previously, one of the *CLE* genes, *CLE14*, was reportedly expressed in the root tips of *Arabidopsis*, and overexpression of the *CLE14* gene triggered the early differentiation of root hair cells (Meng and Feldman 2010; Meng et al. 2010). The *GL2* gene encodes a homeodomain leucine-zipper protein (Rerie et al. 1994). The *GL2* gene is thought to be a decisive factor, acting farthest downstream in the root hair/non-hair cell regulatory cascade in *Arabidopsis* (Masucci et al. 1996). Previously, we demonstrated that the exogenous application of synthetic CLE14 polypeptide induced excessive and ectopic root hair formation by inhibiting the *GL2* gene expression in *Arabidopsis* roots (Hayashi et al. 2018). For agricultural use, it is necessary to verify the activity of exogenous CLE14 application (as opposed to endogenous overexpression) on crop roots. Therefore, in this study, we further investigated the effect of synthetic CLE14 polypeptide treatment on crops by analyzing the transcriptional changes of *GL2* homolog genes in rice and tomato roots. The rice *OsGL2* gene was identified as a homolog of the *Arabidopsis GL2* gene by BLAST search of the rice proteome (Zheng et al. 2016). Unlike in *Arabidopsis*, elevated expression of *OsGL2* was reported in R3 MYB transgenic rice plants (Zheng et al. 2016). The tomato *SlGL2* gene was identified as a homolog of the *Arabidopsis GL2* gene by BLAST search using the SOL Genomics Network database (Lashbrooke et al. 2015). *SlGL2* was reportedly down-regulated in the *SlMIXTA-RNAi* tomato lines (Lashbrooke et al. 2015).

Rice (*Oryza sativa* L. Japonica Group cv. Hinohikari, and Indica Group cv. Kasalath) seeds were surface-sterilized and sown on 1.5% agar plates containing 1/2 MS and then incubated at 22°C under constant white light (50–100 μmol m^{-2} s^{-1}). Tomato (*Solanum lycopersicum* L. 'Micro-Tom') seeds were grown on 1.5% agar plates, as previously described (Tominaga-Wada et al. 2013). Synthetic CLE14 peptides were obtained from Eurofins Genomics (Tokyo, Japan) and were used as previously described (Hayashi et al. 2018). Real-time

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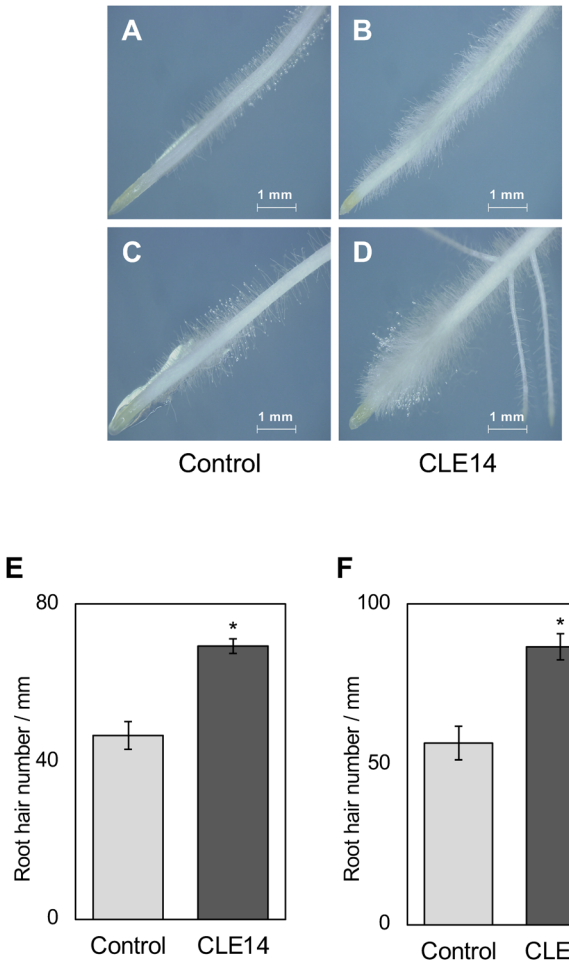

Figure 1. Root phenotypes of CLE14-treated rice seedlings. 10-day-old Hinohikari and Kasalath seedlings grown in control medium (1/2 MS) (A and C, respectively), or in 1/2 MS medium containing 0.5 μM of the CLE14 polypeptide (B and D, respectively). Number of root hairs of Hinohikari (E) and Kasalath (F) seedlings grown in 1/2 MS or in 1/2 MS medium containing 0.5 μM of the CLE14 polypeptide. The number of root hairs per millimeter was determined from 10-day-old seedlings (n=5). Error bars indicate the standard deviations. Asterisks indicate significant differences between the control and CLE14-treated plants (Student's t-test, p<0.05). Scale bars: 1 mm.

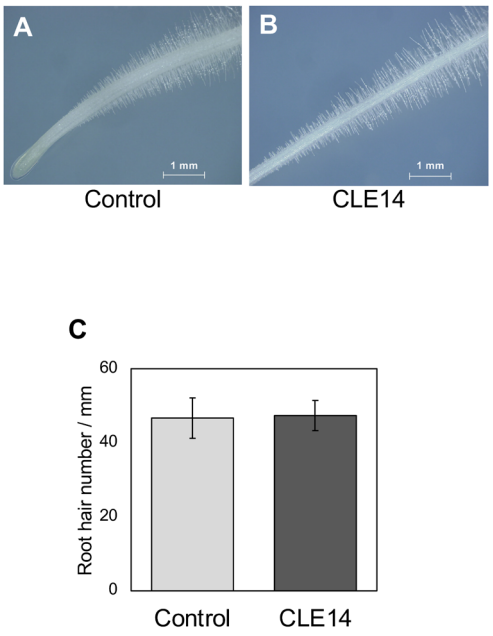

Figure 2. Root phenotypes of CLE14-treated tomato seedlings. 10-day-old tomato seedlings grown in control medium (1/2 MS) (A), or in 1/2 MS medium containing 0.5 μM of the CLE14 polypeptide (B). Root hair number of tomato seedlings grown in 1/2 MS or in 1/2 MS medium containing 0.5 μM of the CLE14 polypeptide (C). The number of root hairs per millimeter was determined from 10-day-old seedlings (n=5). Error bars indicate the standard deviations. Scale bars: 1 mm.

PCR was performed as previously described (Hayashi et al. 2018) to analyze the mRNA levels of the transcripts encoding *OsGL2* in rice and *SlGL2* in tomato. *OsUBQ* and *LeAction* were used as the endogenous controls to normalize the expression levels of *OsGL2* or *SlGL2*, respectively. The primers were: *OsGL2-qF* and *OsGL2-qR* for *OsGL2* (Zheng et al. 2016), *UBQ5-qPCRF* and *UBQ5-qPCRR* for *OsUBQ* (Guo et al. 2015), *SlGL2RT-F* and *SlGL2RT-R* for *SlGL2* (Lashbrooke et al. 2015), and *LeAction-F* and *LeAction-R* for *LeAction* (Girardi et al. 2006).

The findings of the present study revealed that exogenous application of synthetic CLE14 polypeptide clearly induced root hair formation in rice roots (Figure 1A–D). Compared with the control plants of both the Japonica cultivar Hinohikari and Indica cultivar Kasalath, the roots of 10-day-old rice seedlings showed significant increases in root hair density when treated with the CLE14 polypeptide (Figure 1E, 1F).

Unlike the results seen in rice, the CLE14 treatment did not have a remarkable effect on tomato root hair formation (Figure 2A, 2B), whereby the numbers of root hairs between the control and CLE14-treated tomato seedlings did not differ significantly (Figure 2C).

To investigate the effect of CLE treatment on the expression of the rice *OsGL2* gene, i.e., a homolog of the *Arabidopsis GL2* gene, we performed real-time PCR analyses. Consistent with the findings of *Arabidopsis*, we detected a significantly lower accumulation of *OsGL2* transcripts in the Hinohikari rice roots treated with the CLE14 polypeptide compared with that of the control plants (Figure 3A). These results suggested that CLE14 is functional in rice roots as well as in *Arabidopsis* roots, where it inhibits the expression of the *GL2* homolog gene *OsGL2* and promotes root hair formation.

Unlike the results seen in *Arabidopsis* and rice, CLE14 did not induce root hair formation in tomato seedlings (Figure 2). However, consistent with the results from *Arabidopsis* and rice, a significantly lower accumulation level of the *GL2* homolog gene (*SlGL2*) transcripts was detected in the CLE14 polypeptide-treated tomato roots compared with those of the control plants (Figure 3B). The level of *SlGL2* gene expression decreased to less than half of that of the control (Figure 3B). These results

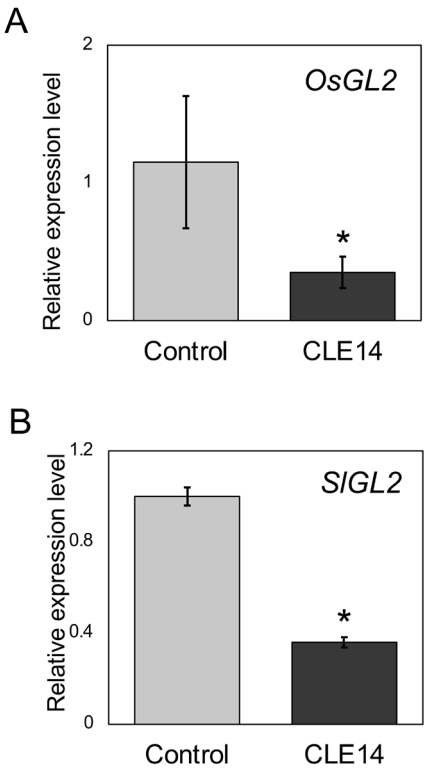

Figure 3. Expression analysis of the *GL2* homolog genes in rice and tomato roots treated with or without CLE14 polypeptide. (A) Real-time reverse transcription PCR analysis of *OsGL2* gene expression in the roots of 10-day-old Hinohikari seedlings grown in the different media. (B) Real-time reverse transcription PCR analysis of *SlGL2* gene expression in the roots of 10-day-old tomato seedlings grown in the different media. The experiments were repeated three times. Error bars indicate the standard deviations. Asterisks indicate significant differences between the control and CLE14-treated plants (Student's *t*-test, $p<0.05$).

suggested that regardless of the root hair phenotype, CLE14 polypeptide can suppress the *GL2* homolog gene expression in rice, tomato, and *Arabidopsis* seedlings.

Therefore, it is strongly suggested that the cascade of CLE14-*GL2* gene regulation is common among rice, tomato, and *Arabidopsis* plants. However, the CLE14 treatment was not found to increase tomato root hair formation. We proposed some possible explanations for this finding: 1) the CLE14 concentration or incubation time was not sufficient for tomato root hair induction. 2) Tomato, rice, and *Arabidopsis* have different types of root hair formation, i.e., type 1, type 2, and type 3, respectively (Dolan 1996), and the type 1 root hair formation of tomato plants, may be governed by different control systems from those of *Arabidopsis* and rice. Type 1 tomato root hair formation is usually random, however, the root hair induction system may have been abandoned with the CLE14 treatment. In addition, *SlGL2* may have evolutionarily lost the root hair formation function. 3) The function of *SlGL2* may differ from that of *GL2* or *OsGL2*, whereby downstream genes targeted by *SlGL2* under CLE14 treatment may differ from that of *GL2* or *OsGL2*.

As most CLE peptides function by interacting with receptor-like kinases in *Arabidopsis* (Gutierrez-Alanis et al. 2017), CLE14 is expected to also function in the same way in rice and tomato plants. Although further investigations of the effects of synthetic CLE14 polypeptide on crop plants are required, including the search for specific receptors, the present study offers new insights into the molecular basis of CLE peptide signaling in rice and tomato roots.

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Conflicts of interest

The authors declare no conflicts of interest.

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