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
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-qPCR for OsUBQ (Guo et al. 2015), SIGL2RT-F and SIGL2RT-R for SIGL2 (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 (SIGL2) transcripts was detected in the CLE14 polypeptide-treated tomato roots compared with those of the control plants (Figure 3B). The level of SIGL2 gene expression decreased to less than half of that of the control (Figure 3B). These results...
under CLE14 treatment may differ from that of GL2 or SlGL2, whereby downstream genes targeted by OsGL2. The function of GL2 may differ from that of SlGL2 or evolutionarily lost the root hair formation function. 3) May have SlGL2 with the CLE14 treatment. In addition, the root hair induction system may have been abandoned

1 tomato root hair formation is usually random, however, Arabidopsis control systems from those of formation of tomato plants, may be governed by different time was not sufficient for tomato root hair induction. 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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