Short Communication

The Receptor-Like Kinase SOL2 Mediates CLE Signaling in *Arabidopsis*

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  Arabidopsis sol2 mutants showed CLV3 peptide resistance. Twenty-six synthetic CLE peptides were examined in the *clv1*, *clv2* and *sol2* mutants. *sol2* showed different levels of resistance to the various peptides, and the spectrum of peptide resistance was quite similar to that of *clv2*. *SOL2* encoded a receptor-like kinase protein which is identical to CORYNE (CRN). GeneChip analysis revealed that the expression of several genes was altered in the *sol2* root tip. Here, we suggest that SOL2, together with CLV2, plays an important role in the regulation of root meristem development through the CLE signaling pathway.

Keywords: *Arabidopsis thaliana* — CLE peptides — CLV — Meristem — Receptor kinase — SOL2.

Abbreviations: CLE, CLAVATA3/ESR-related; CLV, CLAVATA; GUS, β-glucuronidase; LRR, leucine-rich repeat; NB, nucleotide-binding site; RAM, root apical meristem; RLK, receptor-like kinase; RT–PCR, reverse transcription–PCR; SAM, shoot apical meristem.

  In multicellular organisms, intercellular communication is a fundamental mechanism for coordinating growth and differentiation. In the plant shoot apical meristem (SAM), the CLAVATA (CLV) signaling pathway plays an important role in regulating stem cell fate. CLV3 is a peptide ligand of *Arabidopsis thaliana* that interacts with the receptor complex of CLV1 and CLV2 to restrict the stem cell population in the SAM in a non-cell-autonomous manner (Fletcher et al. 1999). CLV1 is a leucine-rich repeat receptor-like kinase (LRR-RLK), and CLV2 is an LRR protein without a kinase domain (Clark et al. 1997, Jeong et al. 1999). CLV3 belongs to the 32 member *CLV3/ESR* (*CLE*) gene family in *Arabidopsis*. *CLE* genes encode small proteins with a conserved 14 amino acid motif (CLE motif) at or near the C-terminus (Sharma et al. 2003, Kondo et al. 2006).

  It has been suggested that there is functional redundancy among the CLE proteins in the regulation of meristem homeostasis (Fiers et al. 2004, Kinoshita et al. 2007). Similarly, several lines of evidence suggest that multiple receptors for CLE peptides function redundantly (Diévart et al. 2003).

  The *suppressor of overexpression of LLP1-1* and *-2* (*sol1*, *sol2*) mutants have been isolated, which suppress a short root phenotype of transgenic plants constitutively overexpressing the *CLE19* gene (Casamitjana-Martínez et al. 2003). We examined the CLV3 peptide resistance of the *sol2* mutant. In the absence of CLV3 peptide, the *sol2* mutant roots were slightly shorter than the wild type (Figs. 1A, B and 2A). We could not detect any difference regarding root apical meristem (RAM) size between the *sol2* and wild-type plants grown on agar medium without peptide (Fig. 1I, K). The application of 1 μM CLV3 peptide severely inhibited root growth in the wild type; however, the *sol2* mutant was more resistant to the peptide treatment (Fig. 1A–D, I-L). These results suggested that the synthetic CLV3 peptide induces RAM consumption, as has been demonstrated previously (Fiers et al. 2005, Kinoshita et al. 2007), and functions in a SOL2-dependent signaling pathway. Carpel number is used as an indicator of the *clv* phenotype (Ni and Clark 2006). The carpel number per flower was significantly increased in the *sol2* mutant (Fig. 1E–H), as has been reported previously (Casamitjana-Martínez et al. 2003). The presence of extra carpels in the *sol2* flowers, which is the typical phenotype of the *clv* mutants, also indicates that SOL2 functions in the same CLV signaling pathway.

  To examine the sensitivity of *sol2* against the various peptides, we tested whether 26 synthetic 12 amino acid peptides derived from the CLE motif could trigger RAM consumption. *Arabidopsis* seeds were germinated on vertical plates with medium containing each peptide (1 μM), and the root length was measured at 10 d after germination. Treatment with CLE41/44 or CLE46 did not affect root elongation, and CLE1/3/4, CLE2, CLE5/6, CLE7 and

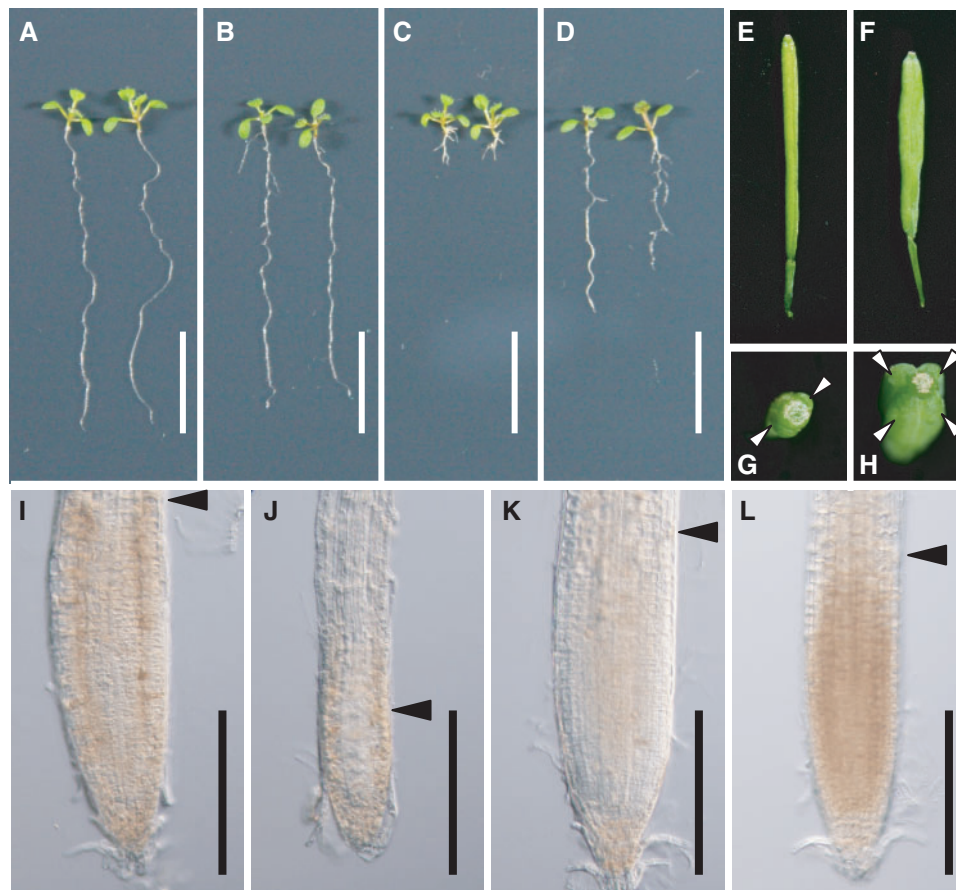*Corresponding author: E-mail, sawa@biol.s.u-tokyo.ac.jp; Fax, +81-3-5841-4464.

Fig. 1 Root and shoot phenotypes of the *sol2* mutant. (A–D) Ten-day-old seedlings of the wild type, Utr (A), and the *sol2* mutant (B) grown on MS agar plates, and of the wild type (C) and the *sol2* mutant (D) grown on MS agar plates containing 1 µM CLV3 peptide. Scale bars: 10 mm. (E, G) Carpels of the wild type. The wild type has two carpels. (F, H) Carpels of the *sol2* mutant. This sample has four carpels. The arrowhead shows the point of the carpels. (I–L) Nomarski images showing the root meristem boundary (arrowhead) of a 10-day-old wild-type root (I, J) and a *sol2* mutant root (K, L) treated without (I, K) and with (J, L) 1 µM CLV3 peptide. Scale bars: 200 µm.

CLE42 affected root growth only slightly in the wild types, Utr and L*er* (Fig. 2A, B). These five peptides did not have obvious effects in the wild type Col (Kinoshita et al. 2006), indicating that peptide sensitivity may be slightly different in each *Arabidopsis* wild-type accession. The other 19 peptides inhibited root growth and reduced the size of the RAM in wild-type plants (Figs. 1A, C, I, J, and 2A, B). Next, we used two *clv1* alleles, *clv1-4* and *clv1-6*, and the *clv2-1* and *sol2* mutants to compare root elongation. The strong allele of *clv1-4* and the weak allele of *clv1-6* tended to have shorter roots in the absence of externally added peptides (Supplementary Figs. S1, S2). This result may suggest that CLV1 has a positive role in root elongation in the absence of CLE peptides. The inhibition of root elongation by CLE peptides was not dramatically affected by the *clv1* mutations (Supplementary Figs. S1, S2), suggesting that CLV1 is not involved in CLE signaling in roots or that a gene(s) with redundant functions to

CLV1 operates in the roots. The *sol2* and *clv2* roots were also slightly shorter than those of the wild type. These mutant roots were partially resistant to the CLE peptides that inhibit wild-type root elongation (Fig. 2A, B). Interestingly, the *sol2* mutants showed various degrees of resistance to different synthetic peptides, and the level of the resistance to each peptide was quite similar to that of the *clv2* mutant (Fig. 2A, B). This result indicated the cooperative roles of CLV2 and SOL2 in mediating CLE signaling in roots.

To investigate the molecular basis of the *sol2* mutation, we attempted to isolate the affected gene by map-based cloning. For this purpose, the increased carpel number and CLV3 peptide-resistant root phenotypes were used as indicators to map the *SOL2* locus to chromosome 5 between markers RCI1B (14/704 recombinant/chromosome tested) and nga151 (1/704). Using the corresponding wild-type sequence, we identified a G→A transition at 220 bp
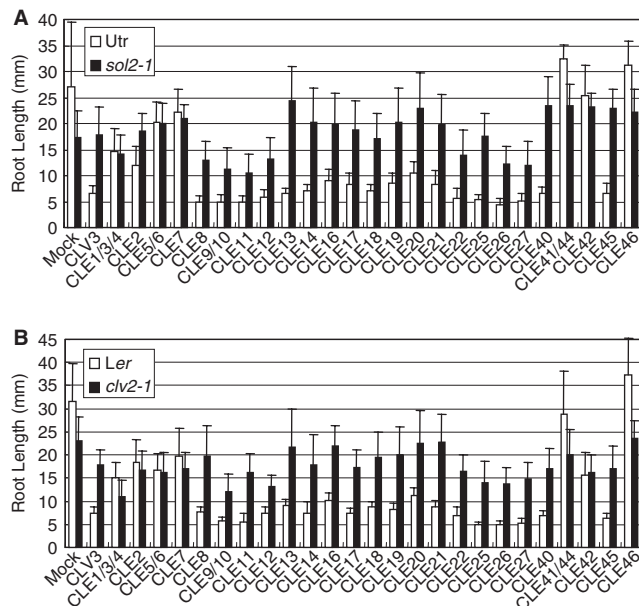
Fig. 2 CLE peptides affect root elongation in *Arabidopsis clv2* and *sol2* mutants. (A) Root length of the wild type Utr (parental strain of the *sol2*) and *sol2* mutant was measured 10 d after germination. (B) Root length of the wild type L*er* and *clv2*. Mean values and standard deviations were calculated from at least seven samples grown on agar medium containing various CLE peptides (1 μM).

Fig. 3 Structure and expression of SOL2. (A) Structure of the receptor-like kinase, At5g13290. SP, signal peptide; TM, transmembrane domain; KD, kinase domain. The single amino acid substitution (G→R) is indicated. (B) Complementation of the *sol2* mutant. Transgenic hairy roots of the *sol2* mutant, harboring the *SOL2* (pGWB-SOL2g) genome region, were sensitive to 1 μM CLV3 peptide. As a control, transgenic hairy roots of the *sol2* mutant expressing the GUS gene (pGWB-GUS) were resistant to CLV3 peptide. The white arrowhead indicates the root tip region (left). The RAM region of transgenic *sol2* hairy roots expressing *SOL2* showed RAM consumption (right). The black arrowhead indicates the root meristem boundary. (C) Expression analysis of the *SOL2* gene using total RNA from roots, rosette leaves, open flowers, stems, inflorescences, siliques and cauline leaves. *SOL2* expression was normalized against that of the *TUA4* gene.

downstream from the ATG start codon of *At5g13290* causing a single amino acid substitution (G→R) at codon 74 in a putative RLK (Fig. 3A). *At5g13290* encodes a 401 amino acid RLK protein that contains a predicted N-terminal signal peptide, a transmembrane domain and a kinase domain. The *sol2* mutation was found in the transmembrane domain (Fig. 3A).

To confirm that *At5g13290* was the causative gene, we performed a complementation test using the hairy root transformation system. A genomic DNA fragment spanning the *At5g13290* locus was cloned into a binary vector and transformed into *Agrobacterium rhizogenes*. This *Agrobacterium* strain was then used to generate the *sol2* mutant plants with transgenic roots. After the emergence of hairy roots, plants were transferred to MS agar plates with and without the CLV3 peptide, and incubated for 7 d. The transgenic hairy roots of *sol2* mutants carrying the wild-type *SOL2* genome region showed a short root phenotype and RAM consumption when grown on agar plates with the CLV3 peptide, and we concluded that the *At5g13290* gene is the causative gene of the *sol2* mutant (Fig. 3B). As a control, we confirmed that the transgenic *sol2* hairy roots with the β-glucuronidase (GUS) gene showed resistance to the CLV3 peptide (Fig. 3B).

To investigate the expression pattern of *SOL2*, a quantitative reverse transcription–PCR (RT–PCR) analysis was conducted using total RNA isolated from the roots,
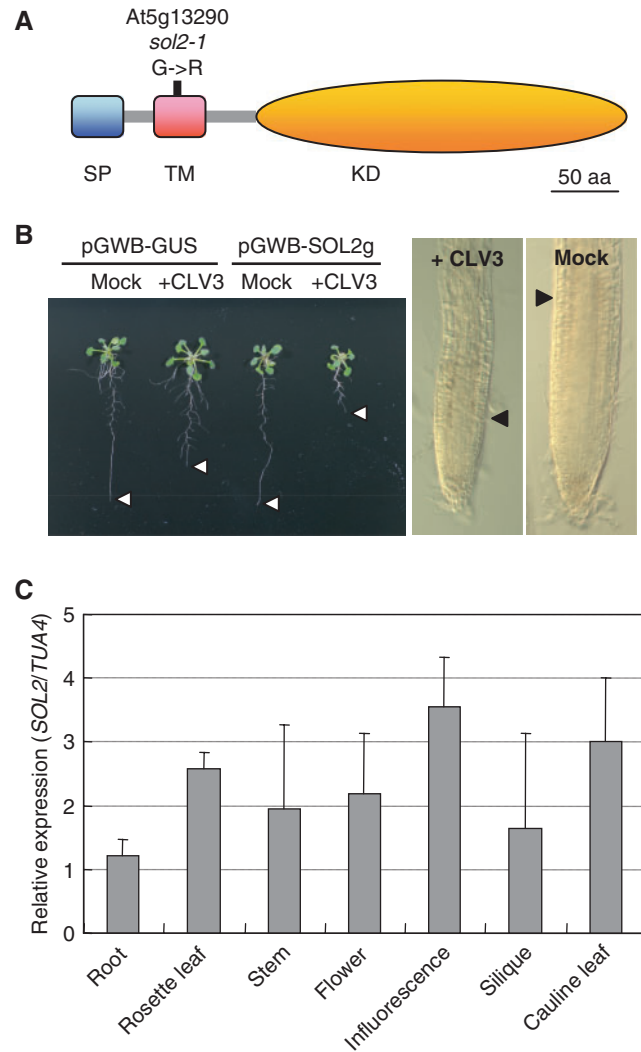
rosette leaves, open flowers, stems, inflorescences, siliques and cauline leaves. The expression of *SOL2* was observed in all the tissues tested (Fig. 3C), and the highest expression was detected in the inflorescence, suggesting that it probably functions in various developmental processes.
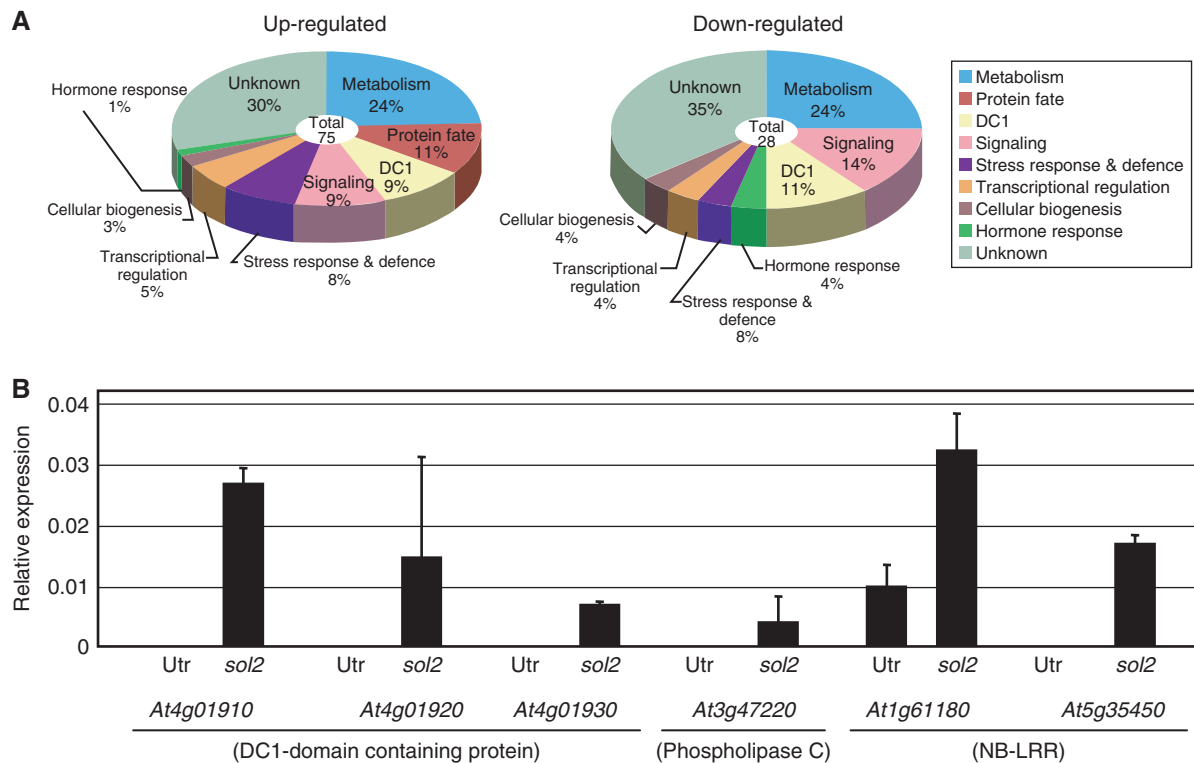
Fig. 4 (A) Identification of genes down- or up-regulated in the *sol2* mutant root tip using GeneChip analysis. Samples were grown on MS agar plates without CLV3 peptide for 4 d and the RAM region of 2 mm from the root tip was collected for this analysis. Gene expression profiles of the wild-type and the *sol2* mutant root tips were analyzed with GeneChip. The up- and down-regulated genes, in both the duplicated experiments, were categorized into nine groups by the common functions of their putative gene products. Lists of the genes are given in Supplementary Tables S1 and S2. (B) Quantitative RT–PCR of up-regulated genes in the *sol2* mutant root tip. Changes in gene expression of *At4g01910, At4g019420, At4g01930, At3g47220, At1g61180* and *At5g35450* were calculated relative to the abundance of the *TUA4* gene. Bars denote standard deviations (n = 3). For all samples, *P* <0.01 (*t*-test, Utr vs. *sol2*).

The *sol2* mutant did not show RAM consumption in response to the CLV3 peptide treatment, indicating the importance of *SOL2* in RAM maintenance. GeneChip analysis was performed to compare the gene expression profiles of the wild-type and the *sol2* mutant root apex region, about 2 mm in length, when they were grown on agar plates without CLE peptides. Genes with an expression ratio (*sol2*/wild type) of <1/4 or >4 were selected. We identified 28 down-regulated genes and 75 up-regulated genes in the RAM region of the *sol2* mutant (Fig. 4A; Supplementary Tables S1, S2). Of the up-regulated genes, 24% encoded metabolic enzymes, including oxidoreductase, cytochrome P450 and dioxygenase. Furthermore, 9% of the up-regulated genes encoded various signaling-related proteins and proteins involved in protein fate decision and transcriptional regulation. Expression of peptidase and a set of genes involved in disease resistance/stress response was also up-regulated in the *sol2* mutant. Interestingly, 9% of the up-regulated genes and 11% of the down-regulated genes encoded DC1 domain-containing proteins. The DC1 domain is considered to be a type of Zn^{2+}-binding RING

finger domain, which probably acts as a transcription factor, although its biological function is largely unknown (Shinya et al. 2007). The *Arabidopsis* genome encodes 146 putative DC1 domain-containing proteins. Our GeneChip analysis detected 10 of them, suggesting that the DC1 domain-containing transcription factors might have specific roles in SOL2-mediated CLE signaling.

To confirm the results of the GeneChip analyses, we conducted a real-time RT–PCR experiment to monitor the transcript levels of six genes, *At4g01910* (DC1), *At4g01920* (DC1), *At4g01930* (DC1), *At3g47220* (phospholipase C), *At1g61180* (nucleotide-binding site–LRR; NB-LRR) and *At5g35450* (NB-LRR), in the wild type and *sol2* mutants without CLV3 peptide treatment. As a result, all of the transcripts accumulated to higher levels in the *sol2* mutant (Fig. 4B), indicating that these six genes may be involved in CLE signaling under the control of the SOL2 RLK in the RAM.

Here, we suggest that SOL2 functions in the CLE signaling pathway for plant meristem maintenance. From our root elongation assay, we found the *sol2* and *clv2*

mutants showed similar sensitivities to each CLE peptide, and the levels of sensitivities to various CLE peptides differed (Fig. 2A, B). This result may suggest the existence of a complex signaling network for meristem maintenance. However, we cannot exclude the possibility that the different sensitivity simply reflects the different binding affinities of CLE peptides for their receptors.

On the other hand, the spectrum of CLE sensitivity seems very similar between the *sol2* and *clv2* mutants, suggesting functional similarities between the *SOL2* and *CLV2* genes in meristem maintenance. Recently, Müller et al. found that the *coryne* (*crn*) mutant also showed a *clv*-like phenotype and resistance to the defect caused by CLV3 overexpression. *crn* has a mutation in the *At5g13290* gene (Müller et al. 2008), and the results support our idea that the *At5g13290* gene is involved in the CLE signaling pathway. The RLK has a cytoplasmic kinase domain and a short extracellular domain. In contrast, CLV2 has an extracellular LRR domain and a short intracellular domain. Together with our results showing that both *sol2* and *clv2* showed similar resistance to various CLE peptides, SOL2/CRN might be responsible for meristem maintenance synergistically working with CLV2, for example in the same receptor complex. The kinase domain of SOL2/CRN might complement the lack of an intracellular domain in CLV2.

The *clv1* and wild-type plants showed similar CLE peptide sensitivities, and CLV1 does not seem to function in RAM maintenance. However, we cannot deny the possibility that some other functionally redundant receptors complement the effects of the *clv1* mutation. It remains to be investigated whether CLV1 and SOL2/CRN have redundant functions in the RAM maintenance system.

Our GeneChip analysis of the *sol2* mutant showed that the expression levels of a number of genes are affected by the loss of the *SOL2/CRN* gene, giving rise to several remarkable observations. First, the gene expression of two peptidases is significantly up-regulated in the *sol2* plants compared with that in the wild type. Because meristem homeostasis is mediated by the CLV system, it is plausible that the loss of SOL2/CRN-mediated signaling up-regulates peptidase gene expression, which is possibly involved in the CLE protein maturation processes. Secondly, a set of genes involved in the disease resistance/stress response, including several genes encoding putative plant immune receptor types of NB-LRR proteins, is significantly up-regulated in *sol2* plants. SOL2/CRN may not only be responsible for the meristem maintenance, but may also play a role in disease resistance/stress responses.

In conclusion, our data support a role for SOL2/CRN in RAM homeostasis, possibly in conjunction with CLV2, to perceive CLE signaling molecules leading to the regulation of DC1 and NB-LRR transcript levels. A further analysis of SOL2/CRN downstream gene candidates provides new opportunities to broaden our understanding of the signaling components involved in plant meristem maintenance.

Materials and Methods

Plants (*A. thaliana*) were grown on soil, as described previously (Kinoshita et al. 2007). Wild-type *A. thaliana* (Utr and L*er*) and several mutants related to CLV signaling, *clv1-4* (L*er*), *clv1-6* (L*er*), *clv2-1* (L*er*) and *sol2-1* (Utr), were used in this study. *Arabidopsis* seeds germinated on MS plates were sterilized, as described previously (Kinoshita et al. 2007). CLE peptides were obtained and stored as described previously (Ito et al. 2006).

The *SOL2* locus was mapped by crossing the *sol2-1* mutant with L*er*. DNA was prepared from 352 *sol2* mutant F$_2$ generation plants (96 plants showed increased carpel number; 256 plants showed CLV3 peptide resistance). Using simple sequence length polymorphic markers and cleaved amplified polymorphic sequence markers, recombination events between *sol2* and the surrounding regions were identified.

Total RNA was extracted from about 2,000 main root tips (2 mm in length) of 4-day-old wild-type and *sol2* plants with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and purified with the RNeasy Micro Kit (Qiagen, Valencia, CA, USA), according to the manufacturers' instructions. GeneChip analyses were independently performed twice with the *Arabidopsis* ATH1 Genome Array (Affymetrix, Santa Clara, California, USA) as described in the GeneChip Expression Analysis Technical Manual. Comparative analysis of the wild type (as the baseline sample) and *sol2* (as the experimental sample) was performed with the GeneChip Operating Software (Affymetrix) with the standard parameters. After the elimination of 'Increased' genes with 'Absent' flags in the experimental sample and 'Decreased' genes with 'Absent' flags in the baseline sample, genes with a signal log ratio of >2 or <–2, in two experiments, were selected.

For a complementation test, pGWB-SOL2g vector carrying a 5 kb genomic DNA fragment of the *SOL2* region amplified using a primer set (5′-cgttaacactttccagagtaatattagt-3′ and 5′-acaatgatccggctcgcaaacatagctaag-3′), and a pGWB-GUS control vector carrying the GUS gene, were transformed into *A. rhizogenes* LBA1334. The *sol2* mutant plants were transformed by the hairy root system with several modifications (Kereszt et al. 2007). Plants were germinated and grown in MS medium in Petri dishes. At 7 d after germination, roots were cut and the aerial parts of plants were inoculated with the bacterial culture. After incubation for 7 d, seedlings were transferred to MS medium with and without 1 μM CLV3 peptide, which contained 200 μg ml^{-1} cefotaxime to eliminate the bacteria. At 7 d after transfer to MS medium, the transgenic hairy roots obtained were examined for sensitivity to the CLV3 peptide. Twenty plants were transformed, and 80% of plants were phenotypically rescued (Fig. 3B). The insertions of transgene in the longest roots were confirmed by PCR.

For the quantitative RT–PCR analysis, PCR and detection with a Taqman probe for *SOL2* and *alpha tubulin A4* (*TUA4*; *At1g04820*) were performed on the LightCycler (Roche Diagnostics, Indianapolis, IN, USA). To quantify the transcripts, the copy number of each gene was determined with a standard curve constructed using a diluted series of samples. Each reaction was repeated three times. For the quantitative RT–PCR analysis in Fig. 4, PCR was conducted three times by using the 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

Gene-specific primers were described in Supplementary Table S3. The copy numbers of the transcripts were normalized against the copy numbers of the *TUA4* transcripts, which are often used as internal controls (Sawa et al. 2002). RNA used for RT–PCR was biologically different from that for GeneChip analysis.

Supplementary data

Supplementary data are available at PCP Online.

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