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A carlactonoic acid methyltransferase that contributes to the inhibition of shoot branching in *Arabidopsis*

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Strigolactones (SLs) are plant hormones that regulate shoot branching and diverse developmental processes. They are biosynthesized from carotenoid molecules via a key biosynthetic precursor called carlactone (CL) and its carboxylated analog, carlactonoic acid (CLA). We have previously identified the methyl esterified derivative of CLA, methyl carlactonoate (MeCLA), as an endogenous SL-like molecule in *Arabidopsis*. Neither CL nor CLA could interact with the receptor protein, *Arabidopsis* DWARF14 (AtD14), in vitro, while MeCLA could, suggesting that the methylation step of CLA is critical to convert a biologically inactive precursor to a bioactive compound in the shoot branching inhibition pathway. Here, we show that a member of the SABATH protein family (At4g36470) efficiently catalyzes methyl esterification of CLA using *S*-adenosyl-L-methionine (SAM) as a methyl donor. We named this enzyme CLAMT for CLA methyltransferase. The *Arabidopsis* loss-of-function *clamt* mutant accumulated CLA and had substantially reduced MeCLA content compared with wild type (WT), showing that CLAMT is the main enzyme that catalyzes CLA methylation in *Arabidopsis*. The *clamt* mutant displayed an increased branching phenotype, yet the branch number was less than that of severe SL biosynthetic mutants. Exogenously applied MeCLA, but not CLA, restored the branching phenotype of the *clamt* mutant. In addition, grafting experiments using the *clamt* and other SL biosynthetic mutants suggest that CL and CLA are transmissible from root to shoot. Taken together, our results demonstrate a significant role of CLAMT in the shoot branching inhibition pathway in *Arabidopsis*.

plant hormones | biosynthesis | terpenoid | strigolactone

Significance

Strigolactones (SLs) are a group of apocarotenoid hormones, which regulates shoot branching and other diverse developmental processes in plants. The major bioactive form(s) of SLs as endogenous hormones has not yet been clarified. Here, we identify an *Arabidopsis* methyltransferase, CLAMT, responsible for the conversion of an inactive precursor to a biologically active SL that can interact with the SL receptor in vitro. Reverse genetic analysis showed that this enzyme plays an essential role in inhibiting shoot branching. This mutant also contributed to specifying the SL-related metabolites that could move from root to shoot in grafting experiments. Our work has identified a key enzyme necessary for the production of the bioactive form(s) of SLs.

The apocarotenoid strigolactones (SLs) act as plant hormones that regulate shoot branching (1, 2). They also have multiple hormonal roles in diverse developmental processes in plants (3–5). In addition, SLs serve as rhizosphere signals for symbiotic and parasitic interactions with arbuscular mycorrhizal fungi and root parasitic plants, respectively (6, 7). In 2012, a key biosynthetic intermediate called carlactone (CL) was identified through in vitro functional analysis of three enzymes, DWARF27 (D27), CAROTENOID CLEAVAGE DIOXYGENASE7 (CCD7), and CCD8 (8) (Fig. 1). Later, CL was identified as an endogenous metabolite both in *Arabidopsis* and rice. In addition, CL was converted to SLs such as 4-deoxyorobanchol (4DO) and orobanchol in vivo by feeding experiments using a stable isotope-labeled CL (9). A cytochrome P450, MORE AXILLARY GROWTH1 (MAX1), in *Arabidopsis* catalyzes a three-step oxidation of the C-19 of CL to yield a carboxylated analog, carlactonoic acid (CLA) (10) (Fig. 1), while one of the five MAX1 homologs in rice, Os01g0700900 (Os900), is a multifunctional enzyme catalyzing the conversion of CL into a four ring–type SL, 4DO (11). On the other hand, another MAX1 homolog, Os01g0701400 (Os1400) catalyzes hydroxylation of 4DO to produce orobanchol (11), showing that MAX1 homologs have functional diversity (Fig. 1).

Previously, we identified an endogenous SL-like compound in *Arabidopsis*, which we first named SL-LIKE1 (9). SL-LIKE1 was later demonstrated to be the methyl esterified analog of CLA, methyl carlactonoate (MeCLA) (10) (Fig. 1). SLs are perceived by an α/β-fold hydrolase receptor, DWARF14 (D14), and they are hydrolytically degraded by D14 after signal transmission (12). Interestingly, we found that neither CL nor CLA could physically interact with *Arabidopsis* DWARF14 (AtD14), while MeCLA could (10). These results suggest that MeCLA is a biologically active hormone in the shoot branching inhibition pathway in *Arabidopsis*, and that the methylation of CLA is a critical step to convert a biologically inactive precursor to an active hormone molecule. Recently, an SL biosynthetic enzyme, LATERAL BRANCHING OXIDOREDUCTASE (LBO), which belongs to the 2-oxoglutarate-dependent dioxygenase family, was identified in *Arabidopsis* (13). LBO catalyzes the formation of a MeCLA+16 Da reaction product from MeCLA. The *Arabidopsis lbo* knockout mutant

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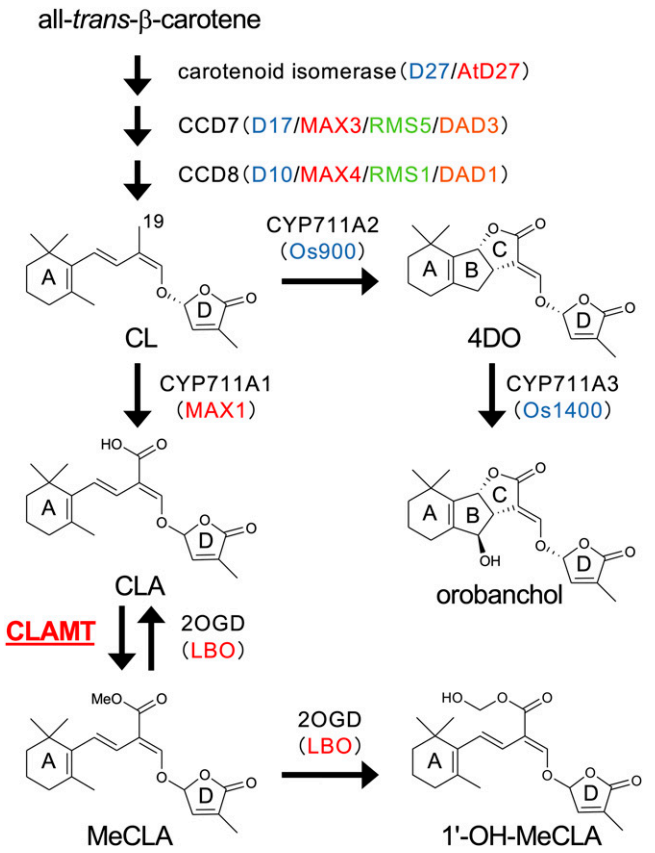

Fig. 1. Proposed biosynthetic pathway of SLs. Blue, red, green, and orange letters indicate genes of rice, *Arabidopsis*, pea, and petunia, respectively. 2OGD, 2-oxoglutarate-dependent dioxygenase; CCD, carotenoid cleavage dioxygenase.

exhibits a weaker shoot branching phenotype than the several SL biosynthetic mutants such as *max4* (defective in CCD8) and accumulates MeCLA. These results suggest that the pathway downstream of MeCLA has a more significant role than does MeCLA in the shoot branching inhibition in *Arabidopsis*. More recently, the LBO reaction product was identified to be hydroxymethyl carlactonoate (1′-OH-MeCLA), while LBO also catalyzed demethylation of MeCLA to produce CLA (14) (Fig. 1).

In order to further understand the role of the MeCLA pathway in *Arabidopsis*, the identification of the elusive CLA methyltransferase is indispensable. Among the methyltransferase protein families, the SABATH family has been demonstrated to include enzymes that methylate phytohormones, such as gibberellin (15), jasmonate (16), auxin (17), and salicylic acid (18). Thus, we hypothesized that CLA methyltransferase(s) also belongs to the SABATH protein family. Among 24 SABATH members in *Arabidopsis*, the gene product of *At4g36470*, which we named CLA methyltransferase (CLAMT), indeed efficiently catalyzed CLA methylation. Our finding is consistent with a recent report that At4g36470 catalyzed the methylation of (11*R*)-CLA (19). In addition, the *clamt* knockout mutant highly accumulated CLA, whereas MeCLA was significantly reduced in the mutant. Notably, the *clamt* mutant exhibited an increased shoot branching phenotype. Taken together, we successfully identified a CLA methyltransferase in *Arabidopsis*, which has a critical role in producing biologically active substances in the shoot branching regulation pathway.

Results

Biochemical Screening of CLAMT in the *Arabidopsis* SABATH Protein Family. Because of its role in methylation of plant hormones (15–18), we hypothesized that CLA methyltransferase(s) is a SABATH protein family member. The *Arabidopsis* genome contains 24 genes belonging to the *SABATH* family. We examined the enzyme activities of 13 SABATH proteins that were randomly selected from each clade of the phylogenetic tree or that were expressed in axillary buds in the preliminary RT-PCR analysis (*SI Appendix*, Fig. S1). Each of these was cloned into a protein expression vector, pET47b, and expressed in *Escherichia coli* as a His-tag fusion protein. The methylation activity against CLA was tested using the soluble protein fraction of each transformant, and we found that the cell lysate expressing At4g36470 efficiently catalyzed CLA methylation using SAM as a methyl donor (Fig. 2 and *SI Appendix*, Fig. S2). To further validate the biochemical function of At4g36470, we reconstituted the *Arabidopsis* SL biosynthetic pathway in *Nicotiana benthamiana*. Production of MeCLA was observed in *N. benthamiana* leaves coexpressing At4g36470 and the CLA biosynthetic enzymes (AtD27, MAX3/CCD7, MAX4/CCD8, and MAX1) (*SI Appendix*, Fig. S3). Phylogenetic analysis of all 24 SABATH family proteins in *Arabidopsis* suggests that there are no closely related homologs of *At4g36470* (*SI Appendix*, Fig. S1). We named At4g36470 CLA methyltransferase (CLAMT) and further analyzed its physiological function using loss-of-function mutants.

Characterization of the *Arabidopsis clamt* Knockout Mutants and Their Phenotypic Analysis. We identified two independent alleles of the *Arabidopsis clamt* mutant in the Institute of Physical and Chemical Research (Japan) RIKEN *Arabidopsis* transposon-tagged collection in the Nossen (No-0) background (line RATM11-1868-1, *clamt-1* and RATM53-2997-1, *clamt-2*) (*SI Appendix*, Fig. S4*A*) (20). In both of these alleles, the *CLAMT* transcript levels were significantly reduced compared with wild-type (WT) No-0 (*SI Appendix*, Fig. S4*B*). In order to investigate the in vivo function of CLAMT, we measured the endogenous levels of CLA and MeCLA in these mutants. We found that CLA highly accumulated in both mutants, whereas the MeCLA level was drastically reduced compared with WT (Fig. 3*A*), demonstrating that CLAMT serves as the main enzyme that catalyzes CLA methylation in vivo. However, considering the presence of detectable levels of MeCLA in the *clamt* mutants, another methyltransferase(s) may contribute to the CLA methylation step in vivo. Both *clamt-1* and *clamt-2* had increased axillary branching compared with No-0 WT (Fig. 3*B*). To evaluate whether that is due to reduced production of the SL branching hormone, we performed a shoot branching inhibition assay with these *clamt* mutants using a number of different SLs. Treatment with GR24 or MeCLA rescued the shoot branching phenotypes of both mutants, whereas CLA was not effective, confirming that CLA itself is not biologically active as a shoot branching inhibitor (Fig. 3*C*). In addition, these results illustrate a critical role of CLA methylation to produce the biologically active hormone(s) in *Arabidopsis*, supporting our in vitro experimental results using the receptor protein, AtD14 (10).

In order to compare the branching phenotype of the *clamt* mutant with other SL biosynthetic mutants, we generated the *clamt-1* mutant in the Col-0 background by backcrossing with Col-0. After backcrossing seven times with Col-0, the phenotype of the *clamt-1* (Col-0) was compared with the *max1-4*,

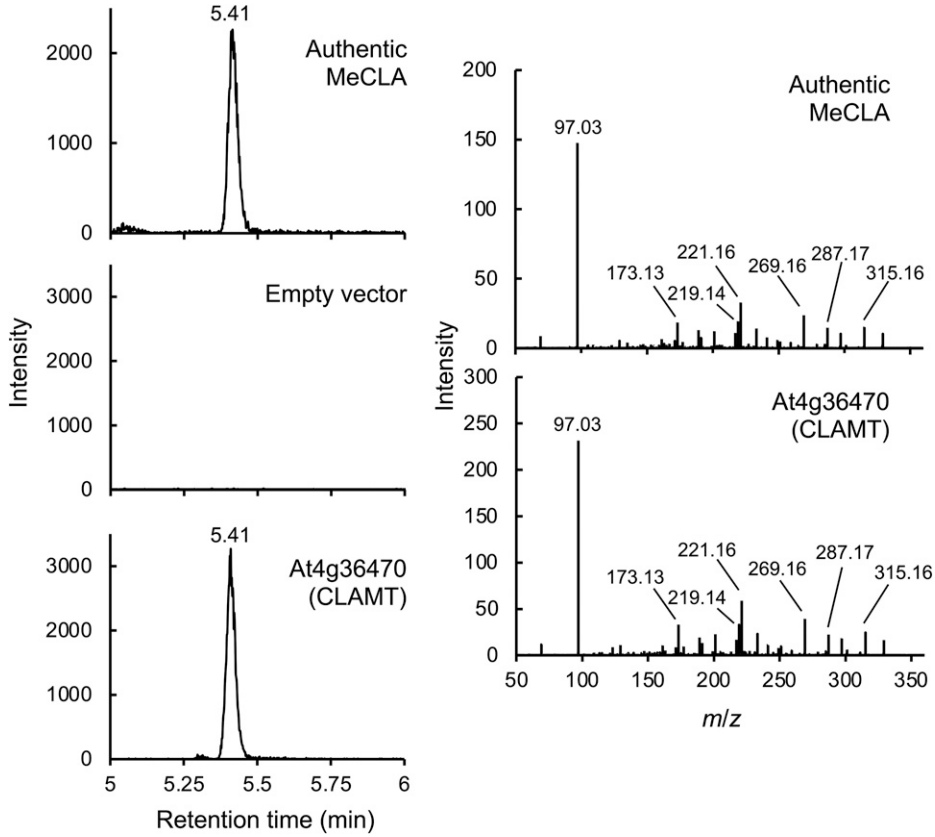

Fig. 2. Conversion of CLA to MeCLA by CLAMT. Enzymatic reaction was performed using CLA as a substrate in the presence of SAM. The reaction product of the cell lysate expressing the empty vector or At4g36470 (CLAMT) was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Sciex X500R quadrupole time-of-flight). The MS/MS chromatogram for MeCLA (*m/z* 347.18 > 97.03) (*Left*) and MS/MS scan spectra of fragment ions between *m/z* 50 and 380 from a precursor ion with *m/z* 347.18 (*Right*) are shown.

max3-11 (*ccd7*), and *max4-8* (*ccd8*) mutants, all of which were in the Col-0 background. *clamt-1* had fewer branches than the other SL biosynthetic mutants, but still significantly more than WT Col-0 (*SI Appendix*, Fig. S5 *A* and *B*). Notably, the *clamt* mutant exhibited a shoot branching phenotype similar to that of the *lbo* mutant (*SI Appendix*, Fig. S5 *C* and *D*). These results further support the idea that the methylation of CLA has an important role in generating the biologically active shoot branching inhibiting hormone.

Grafting Experiments Using the *clamt* Mutant. In previous reports, a series of grafting experiments using SL mutants revealed the relationship between the biosynthetic enzymes and the root-to-shoot translocation of the corresponding biosynthetic precursors. For example, the phenotype of the *max4* mutant was restored by grafting onto the *max1* mutant rootstock, suggesting that the substrate of MAX1 is able to translocate long distances from root to shoot (21). In addition, this result demonstrated that MAX1, a cytochrome P450, is functioning downstream of MAX4/CCD8—oxidizing CL, the product of CCD8—which was indeed experimentally verified later (10). Moreover, these results suggest that CL may be able to translocate from root to shoot, although the experimental evidence for this has not yet been provided.

We therefore grafted the *max4* mutant shoot onto the *max1* mutant root to evaluate the translocation of CL from root to shoot. We analyzed CL in the shoot (*max4*) part of the grafted plants, in which the branching phenotype was completely restored as was reported (*SI Appendix*, Fig. S6*A*) (21). In this grafted plant, CL was successfully detected in the shoot extracts (Fig. 4*A* and *SI Appendix*, Fig. S6*B*). As we reported previously, the *max1* mutant accumulated an extremely high level of CL. Thus, it would be possible that the translocation of CL occurs only when the *max1* mutant is used as the rootstock. We therefore grafted the *max4* mutant onto the WT rootstock, and even in this grafting combination CL was detected in the shoot part (Fig. 4*A* and *SI Appendix*, Fig. S6*C*), although the CL level in the shoot was lower than for the *max4*/*max1* (shoot/root) grafting combination. These results provide direct evidence for the root-to-shoot translocation of the SL biosynthetic precursor, CL.

Next, in order to assess the translocation ability of the MAX1 product, CLA, we performed grafting experiments using the *clamt-1* and the *max1-4* mutants. Because the background of each mutant was different (No-0 for *clamt-1*, Col-0 for *max1-4*), we prepared a control plant; namely, the shoot of the *max1-4* mutant was grafted onto the WT No-0 rootstock, which resulted in a favorable restoration of the *max1-4* branching phenotype. In a subsequent experiment, the *max1-4* phenotype was also restored by grafting onto the *clamt-1* mutant (Fig. 4*B* and *SI Appendix*, Fig. S7*A*). This result suggests that CLA is transmissible from root to shoot, and it might be converted into a bioactive substance for shoot branching inhibition. These results also confirm that CLAMT functions downstream of MAX1. Next, to examine the translocation ability of the CLAMT product such as MeCLA, we performed grafting experiments using the *clamt-1* mutant and WT plants. The branching phenotype of *clamt-1* was not complemented by grafting onto a WT rootstock in both the Col-0 and the No-0 backgrounds, indicating that the products downstream of

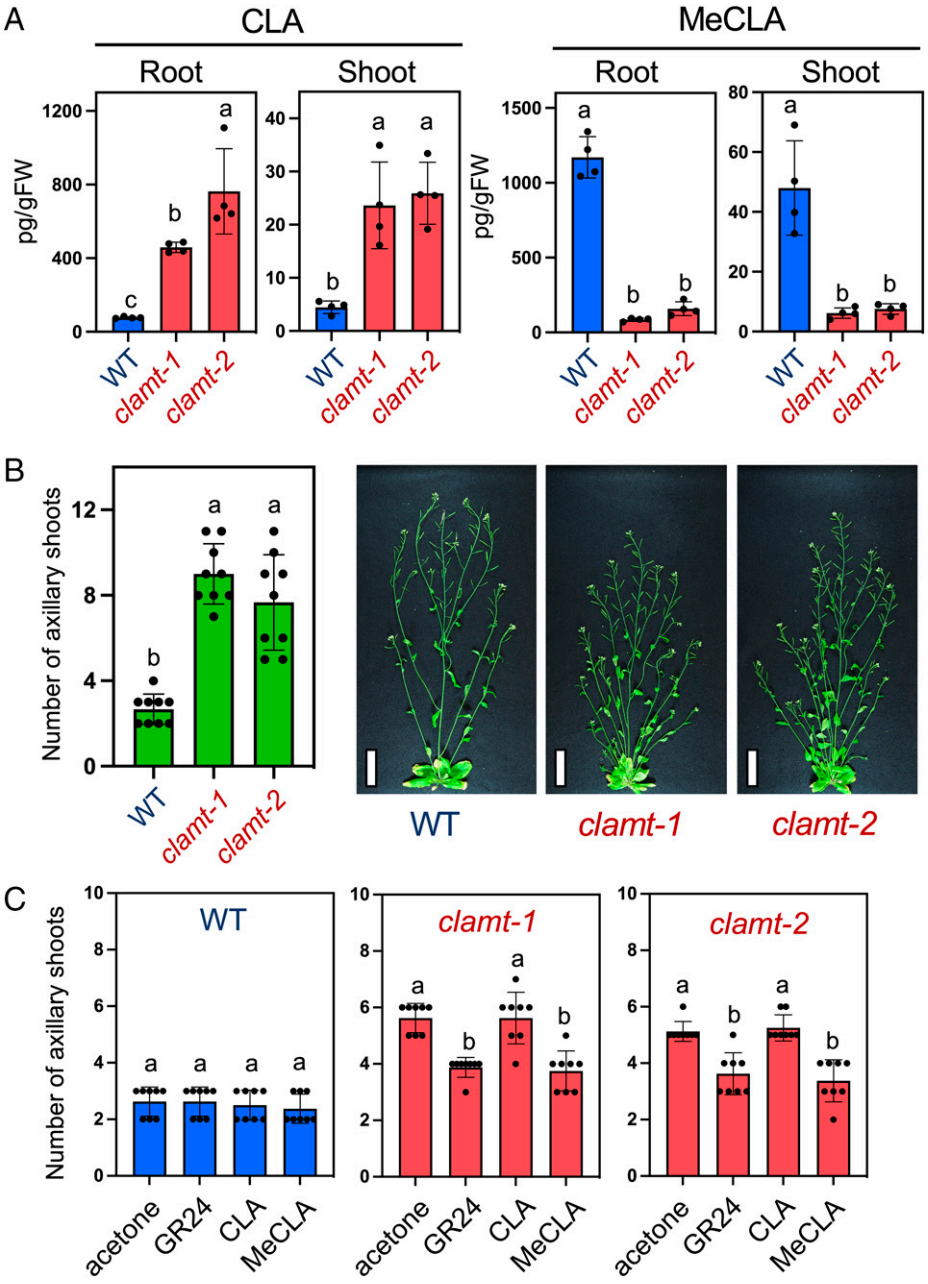

Fig. 3. Analysis of the *Arabidopsis clamt* mutants in the No-0 background. (*A*) Quantitative analysis of endogenous CLA and MeCLA levels in *Arabidopsis* WT (No-0) and the *clamt* mutants. Data are the means ± SD ($n = 4$). (*B*) Shoot branching phenotype of *Arabidopsis* WT (No-0) and the *clamt* mutants. The number of axillary shoots (>5 mm) per plant of 44-d-old plants is shown as the mean ± SD ($n = 9$). *Right* panels show the pictures of the aboveground part of these plants. (*C*) Effect of SLs (10 μM) on axillary bud outgrowth of *Arabidopsis*. A solution (10 μL) containing each compound was applied to axillary buds every other day for 29 d. The number of axillary shoots (>5 mm) per 46-d-old plant is shown as the mean ± SD ($n = 8$). Different letters indicate significant differences at $P < 0.05$, Tukey's honestly significant difference (HSD).

CLAMT are not translocated from root to shoot (Fig. 4*C* and *SI Appendix*, Fig. S7 *B–D*). By contrast, the branching phenotype of the *lbo* scion was partially rescued by a WT rootstock as observed in a previous study (13), and more clearly by a *d14* rootstock, in which SL biosynthesis is activated (*SI Appendix*, Fig. S8*A*). Furthermore, a WT rootstock moderately rescued the branching phenotype of the *clamt lbo* double mutant, which displayed an additive branching phenotype compared with the *clamt* and *lbo* single mutants (*SI Appendix*, Fig. S8 *B* and *C*), supporting the idea that the LBO product can move upward from the root. However, the insensitivity of the *clamt* scion to a WT rootstock is inconsistent with the hypothesis above. This point will be discussed below.

Expression of the *CLAMT* Gene. To analyze the expression pattern of *CLAMT* in *Arabidopsis*, we generated transgenic lines expressing *CLAMTpro*::*GUS*, in which β-glucuronidase (GUS) expresses under the regulation of the *CLAMT* promoter. *CLAMTpro*::*GUS* showed the strongest expression in the roots (Fig. 5*A*). This pattern correlated well with the higher levels of endogenous MeCLA in roots (Fig. 3*A*). *CLAMTpro*::*GUS* was also expressed in the vascular tissue, the flowers, and the basal part of cauline branches and the siliques (Fig. 5 *B–E*). Similar expression patterns have been reported for other SL biosynthetic genes although not entirely overlapping (13, 21, 22). The expression of *CLAMTpro*::*GUS* was particularly strong in the nodal part of young axillary buds, much more so than in

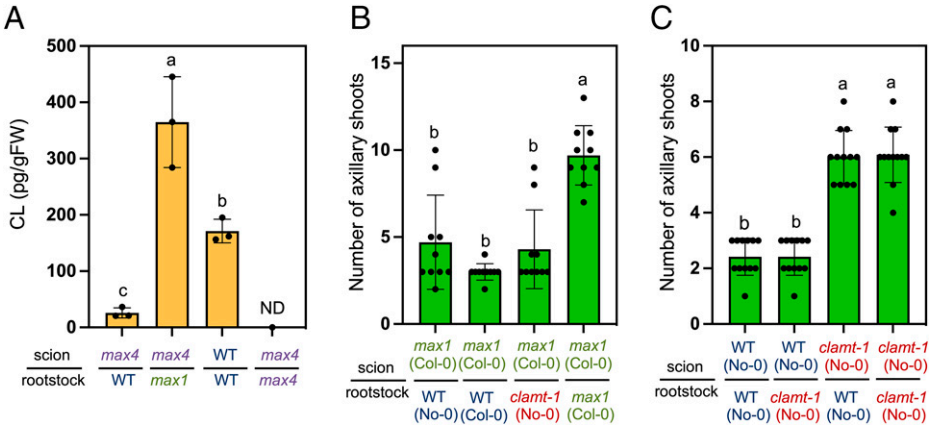

Fig. 4. Analysis of the root-to-shoot translocation of endogenous SL biosynthetic intermediates in the grafted plants. (*A*) Quantitative analysis of endogenous levels of CL in the shoot of the grafted plants. Data are the means ± SD (n = 3). (*B*) Shoot branching phenotype of 63-d-old grafted plants using WT (No-0 or Col-0), the *max1-4* (Col-0), and the *clamt-1* (No-0) mutants. The number of axillary shoots (>5 mm) per plant is shown as the mean ± SD (n = 10). (*C*) Shoot branching phenotype of 77-d-old grafted plants using WT (No-0) and *clamt-1* (No-0) mutants. The number of axillary shoots (>5 mm) per plant is shown as the mean ± SD (n = 12). Different letters indicate significant differences at $P < 0.05$, Tukey's HSD.

the older ones (*SI Appendix*, Fig. S9), suggesting that *CLAMT* functions locally to suppress bud outgrowth.

Previous reports have suggested the presence of a negative feedback regulation for some SL biosynthetic genes by endogenous or exogenously applied SL (1, 23). In order to assess the SL-dependent regulation of the *CLAMT* gene expression, we performed qRT-PCR analysis using whole seedlings or roots of 14-d-old WT and SL-related mutants, where *MAX3* (24), *MAX4* (22, 23), and *CLAMTpro*::*GUS* (Fig. 5*A*) are strongly expressed. *CLAMT* expression levels were not altered in any of the SL mutants, suggesting that *CLAMT* expression is not regulated by endogenous SL levels (*SI Appendix*, Fig. S10 *A* and *B*). This observation is supported by the fact that *CLAMT* is not included in SL-responsive genes in a recent transcriptome analysis (25). In contrast, the expression of *MAX3* and *MAX4* was slightly elevated in the *clamt* mutant compared with that in WT, but was weaker than that in other SL-related mutants in whole seedlings (*SI Appendix*, Fig. S10*A*). In roots, the up-regulation of *MAX4* in the *clamt* mutant was significant, as observed in other SL-related mutants (*SI Appendix*, Fig. S10*B*). These results support the idea that CLAMT is involved in producing bioactive SLs that regulate the expression of upstream biosynthetic genes in *Arabidopsis*.

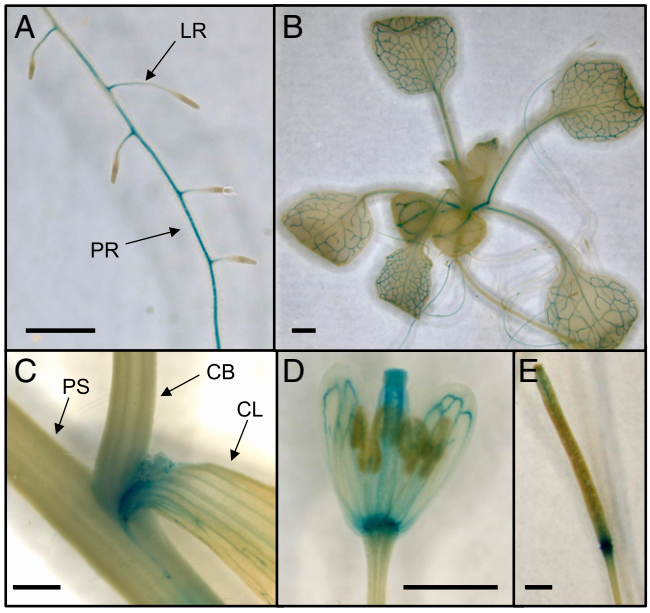

Fig. 5. The *GUS* expression patterns in transgenic lines carrying *CLAMTpro*::*GUS*. The 14-d-old (*A* and *B*) and 40-d-old (*C*–*E*) plants were stained with an X-gluc-containing solution. *A*, root; *B*, aerial tissue; *C*, basal part of the cauline branch; *D*, flower; and *E*, silique. GUS staining was performed for 1 h (*A*), 3 h (*B*), and 20 h (*C*–*E*). (Scale bar, 1 mm.) LR, lateral root; PR, primary root; CB, cauline branch; CL, cauline leaf; and PS, primary shoot.

Discussion

We have previously identified an endogenous SL-like molecule in *Arabidopsis* (9), which was later shown to be MeCLA (10). Neither CL nor CLA physically interacts with the receptor protein, AtD14, while MeCLA does, strongly suggesting that the methylation of CLA is critical for converting a biologically inactive biosynthetic precursor into a bioactive substance in the shoot branching inhibition pathway in *Arabidopsis*. Here, we report the characterization of CLAMT that efficiently methylates CLA. The *Arabidopsis* mutants that are defective in *CLAMT* showed an increased level of CLA, while the level of MeCLA was significantly reduced. In addition, these *clamt* mutants exhibited increased branching phenotypes, demonstrating an essential role of CLAMT in producing biologically active molecules that inhibit shoot branching. On the other hand, MeCLA was still detectable in the *clamt* mutants, suggesting that there might be another methyltransferase(s) that can catalyze CLA methylation, although the contribution of such an enzyme(s) should be much smaller than that of CLAMT. Moreover, we found that the branching phenotype of the *clamt* mutant is weaker than that of other SL biosynthetic mutants, such as *max1*, *max3*, and *max4*. There are multiple possible reasons to explain this weaker branching phenotype of the *clamt-1* mutant. First, the *clamt-1* mutant is still producing detectable amounts of MeCLA as mentioned above, and this remaining MeCLA or its downstream metabolites may still weakly inhibit shoot branching in the *clamt-1* mutant. Second, some SLs other than the products in the MeCLA pathway might inhibit shoot branching in the *clamt-1* mutant. In fact, the presence of orobanchol and some other four ring–type SLs in *Arabidopsis* were reported (26), although we were unable to detect these. It is also important to clarify whether CLAMT is involved in the production of MeCLA derivatives (4-OH-MeCLA and 16-OH-MeCLA) that were recently identified in *Arabidopsis* (14).

MeCLA was reported to be a potential substrate for LBO. In agreement with this biochemical property, the *Arabidopsis lbo* mutant accumulated a larger amount of endogenous MeCLA. In addition, the *lbo* mutant was insensitive to exogenously applied MeCLA (13). These results suggest that the downstream product(s) of LBO, but not MeCLA, functions as the active hormone in the shoot branching inhibition pathway. Very recently, the reaction product of LBO when MeCLA was used as a substrate was identified to be 1′-OH-MeCLA (14). However, the main product was not 1′-OH-MeCLA, but the demethylation product, CLA. The authors proposed a possibility that 1′-OH-MeCLA is nonenzymatically converted into CLA because of its instability. It would also be possible, however, that LBO mainly catalyzes the oxidative demethylation of MeCLA and that 1′-OH-MeCLA is a reaction intermediate of the demethylation reaction. Moreover, another possibility is that 1′-OH-MeCLA is further converted into another metabolite by an unknown enzyme(s), and this metabolite may act as the true active hormone in shoot branching inhibition (14). In either case, our results clearly demonstrate that the CLA methylation step plays an essential role in synthesizing the active hormone for shoot branching inhibition. Because the *clamt* mutants can still synthesize a measurable level of MeCLA (Fig. 3*A*), the more severe phenotype observed in the *clamt lbo* mutant may be explained if both MeCLA (or its metabolite that does not pass the LBO pathway) and 1′-OH-MeCLA (or its unknown metabolite) independently function as bioactive hormones in inhibiting shoot branching as previously discussed to explain the weak phenotype of the *lbo* mutant (13). A clear understanding of the biochemical function of LBO will be necessary to elucidate the whole picture of SL biosynthesis, including the characterization of the as-yet-unidentified active hormone structure in the shoot branching inhibition pathway.

Grafting experiments using the *clamt* mutant demonstrated that CLA, but not MeCLA or its downstream metabolite(s), can be translocated long distance from root to shoot (Fig. 4 *B* and *C*). In contrast, the branching phenotype of the *lbo* mutant could be restored by grafting onto a WT rootstock, suggesting that the LBO downstream product(s) is transmissible (13) (*SI Appendix*, Fig. S8*A*). These results appear to be contradictory. However, it might be possible that MeCLA, which was produced from root-derived CL and CLA, accumulated and inhibited shoot branching in the scion of the *lbo*/WT graft, but not in that of the *clamt*/WT graft. Alternatively, CLAMT and LBO may function partially independently for producing shoot branching inhibiting hormones because both the *clamt* and *lbo* mutants are weak and additive (*SI Appendix*, Fig. S8*B*). Further experiments, such as local quantification of MeCLA in the grafted plants and substrate specificity analysis of CLAMT toward hydroxylated CLA derivatives (14), will provide clues to evaluate these possibilities.

Interestingly, possible CLAMT and LBO orthologous genes are widely distributed in seed plants, including rice, in which CLA was identified as an endogenous product (10), suggesting that rice also produces MeCLA-type SLs. This is supported by the detection of as-yet-unidentified SLs, tentatively coined methoxy-5-deoxystrigol isomers, in rice (27). Indeed, a phylogenetic analysis of *SABATH* genes shows that there are two rice genes in the same clade as the *Arabidopsis CLAMT* (28). Canonical SLs such as 4DO and orobanchol have been identified in rice. Thus, if MeCLA, and its downstream SLs, exist in rice, rice would be an appropriate species to investigate the functional differences between canonical and noncanonical SLs.

In conclusion, we have successfully identified and characterized a CLA methyltransferase in *Arabidopsis*. This will significantly contribute to understanding how structurally diverse SL molecules regulate plant growth and development and act as rhizosphere signals for symbiotic and parasitic interactions.

Materials and Methods

Plant Materials and Growth Conditions. We used *Arabidopsis* ecotype Col-0 and No-0 as the WT and *max1-4* (9), *max3-11* (1), *max4-7* (1), and *atd14-2* (9) mutants. The *clamt* mutants (RATM11-1868-1, *clamt-1* and RATM53-2997-1, *clamt-2*) were obtained from the RIKEN BioResource Research Center. Genotyping was carried out by a PCR-based method using the primers listed in *SI Appendix*, Table S1. The *clamt-1* and *clamt-2* mutants were originally in the No-0 background. To compare their phenotypes with other SL biosynthetic mutants, all of which were in the Col-0 background, they were backcrossed with Col-0 seven times. The *lbo* (Col-0) mutant, which was generated by backcrossing *lbo-1* (Wassilewskija) with Col-0 six times, was kindly provided by Christine Beveridge, The University of Queensland, St. Lucia, QLD, Australia. The *clamt lbo* mutant was generated by crossing *clamt-1* (Col-0) and *lbo* (Col-0) mutants. Details of growth conditions and other experiments are described in *SI Appendix*, SI Materials and Methods.

Data Availability. All study data are included in the article and/or *SI Appendix*.

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