Master's Thesis of Science in Agriculture

The Tomato *naked* Mutant Affects Trichome Development and Plant Growth

토마토 모상체 발달과 식물 생장에 관여하는

naked 돌연변이에 대한 연구

August 2019

Won-Ki Hong

Department of International Agricultural Technology

Graduate School of International Agricultural Technology

Seoul National University

The Tomato *naked* Mutant Affects Trichome Development and Plant Growth

A thesis
submitted in partial fulfillment of the requirements to the faculty
of Graduate School of International Agricultural Technology
for the Degree of Master of Science in Agriculture

By
Won-Ki Hong

Supervised by
Prof. Jin-Ho Kang

Major of International Agricultural Technology
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University

June 2019

Approved as a qualified thesis
for the Degree of Master of Science in Agriculture
by the committee members

Chairman	**Choonkyun Jung, Ph.D.**	
Member	**Jin-Ho Kang, Ph.D.**	
Member	**Jang-Kyun Seo, Ph.D.**	

Abstract

The Tomato *naked* Mutant Affects Trichome Development and Plant Growth

Won-Ki Hong

Department of International Agricultural Technology

The Graduate School of International Agricultural Technology

Seoul National University

Trichomes are hair-like structure derived from plant epidermis that acts as a plant defense against biotic and abiotic stresses. Trichomes exist in the wide range of plant species and are classified as either glandular or non-glandular types. Glandular trichomes function in chemical defense against herbivore, and non-glandular trichomes function as physiological barriers for biotic and environmental stresses. In this study, we characterized a monogenic recessive tomato mutant called *naked*. To analyze the morphology of trichomes on the *naked* mutant in detail, we observed trichomes with a dissecting microscope (DMS) and a scanning electron microscopy (SEM). Compared with wild-type plants which have four types of glandular (type I, IV, VI, VII) and three types of non-glandular (type II, III, V) trichomes on the all aerial tissue, the *naked* mutant does not have all types of trichomes on young stems. In addition, the *naked* mutant has shorter stems and fewer branches compared with wild-type plants. Genetic mapping experiments positioned the *Naked* locus within a 1.9 cM interval on chromosome 9. The physical distance of the mapping region is

about 250 kb and this region contains 31 hypothetical genes. Based on candidate gene approaches, RT-PCR and genomic DNA PCR, we identified that 3' region of *Solyc09g075140* (monoacylglycerol lipase, MAGL) gene was deleted in the *naked* mutant, suggesting that *Solyc09g075140* is *Naked* gene. Now we are doing complementation experiment with *naked* mutant by expressing the wild-type *SlMAGL* gene from its native promoter.

Keyword: map-based cloning, monoacylglycerol lipase, *naked*, tomato, trichome

Student Number: 2017-25003

Contents

List of Figures

List of Tables

List of Abbreviations

bp	base pair
cv	cultivar
BLAST	Basic Local Alignment Search Tool
ITAG	International Tomato Annotation Group
RNA	Ribonucleic Acid
mRNA	messenger RNA
gDNA	Genomic Deoxyribonucleic Acid
cDNA	Complementary DNA
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcription PCR
qRT-PCR	Quantitative Real-time PCR
DEGs	Differential expression genes
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
35S	Cauliflower mosaic virus 35S promoter
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
sgRNA	single-guide RNA
MYB	Myeloblastosis
bHLH	basic helix-loop-helix
MAGL	monoacylglycerol lipase

Introduction

The epidermis of a plant is varied and multifunctional tissue. The epidermal cells directly interact with the surrounding environment and adapt to the environment. To adapt effectively to the surroundings the epidermal cells differentiate into various types of cells such as stomata guard cells, pavement cells, and trichomes.

Trichomes differentiate from epidermal pavement cells, having uni- or multicellular structures and functions as a physical and chemical deterrent to herbivores. Trichomes have been implicated in several physiological functions against various biotic and abiotic stress conditions. For example, trichomes protect plants from infections caused by various insects and pathogens (Shepherd ., 2005; Kang *et al*., 2016), prevent water loss in drought conditions (Ju *et al*., 2012), and may provide protection from UV-B radiation (Yan *et al*., 2012). Moreover, due to its relatively simple cell structure, trichomes serves *et al*as an excellent model for studying gene regulatory networks that control the fate of plant cells.

In addition, these structures are often distinguished as being non-glandular (simple hairs) or glandular. Generally, non-glandular trichomes play a role in physical defense and glandular trichomes oversee physical defense and chemical defense (Werker *et al*., 2000). Glandular trichomes include various structures and shapes, but they can be identified through their capacity to synthesize, store, or secrete large quantities of specialized metabolites,

including alkaloids, polysaccharides, terpenoids polyphenols, and organic acids (Yan *et al*., 2016). These compounds can protect plants from pests and pathogens and have high commercial value (Wagner *et al*., 1991). Thus, the glandular trichomes are a suitable model for metabolic engineering and breeding, as it is capable of producing chemicals of high commercial value (Xiao *et al.*, 2016). However, almost nothing is known about the molecular mechanisms based on the development of the glandular trichome.

The model plant Arabidopsis has only one type of unicellular structure and a non-glandular trichome composed of three branches. The trichome initiation pathway in Arabidopsis has been well studied and over 40 other genes were known to control the developmental processes (Pattanaik *et al*., 2014; Perazza *et al*., 1999).

The *GLABROUS 1* (*GL1*) gene encodes an R2R3 MYB family featuring two MYB repeat (Oppenheimer *et al*., 1991) and essential for trichome initiation and subsequent outgrowth (Marks and Feldmann *et al*., 1989). *TRANSPARENT TESTA GLABRA 1* (*TTG1*) gene plays a crucial role in trichome formation in Arabidopsis (Koornneef *et al*., 1981), which encodes a protein containing four conserved WD repeats (Walker *et al*., 1999). Genetic studies show that *GL1* and *TTG1* regulate the same pathway in the trichome initiation (Larkin *et al*., 1999).

Another two bHLH regulators, *GLABRA 3* (*GL3*) and *ENHANCER OF GLABRA 3* (*EGL3*), functions in a redundant manner to specify trichome cell fate in Arabidopsis (Bernhardt *et al*., 2003). The WD Repeats/bHLH/MYB complexes consisting of these four genes triggers trichome formation in

Arabidopsis by enhancing the expression of three downstream genes, *GLABRA 2* (*GL2*), *ENHANCER OF GLABRA 2* (*EGL2*), and *TRANSPARENT TESTA GLABRA 2* (*TTG2*) (Ishida *et al*., 2007; Zhao *et al*., 2008; Qi *et al*., 2011). Simultaneously, this complex promotes the expression of several single-repeat R3 MYB genes, *CAPRICE* (*CPC*), *TRIPTYCHON* (*TRY*), *ENHANCER OF TRY AND CPC 1* (*ETC1*), *ETC2*, *ETC3*, *TRICHOMELESS 1* (*TCL1*), and *TRICHOMELES 2* (*TCL2*), which functions as a negative regulator by competing with GL1 for binding to the bHLH factors when they move to adjacent cells (Chang *et al*., 2018).

In contrast to Arabidopsis, cultivated tomato (*Solanum lycopersicum* L.) and its wild relatives have at least seven distinct types of trichomes that differ with respect to size, cell number, and the presence of glandular secreting cells (Simmons and Gurr *et al*., 2005). The *Woolly* (*Wo*) gene, encoding an HD-ZIP IV transcription factor, mainly regulates type I trichome development through heterodimer formation with the B-type cyclin SlCycB2 in tomato (Yang *et al*., 2011). Ectopic expression of *Wo* in *Nicotiana tabacum* (tobacco) and *Solanum tuberosum* (potato) induces to increased trichome density, implying that trichomes in the Solanaceae family may share a common regulatory pathway (Yang *et al*., 2015). It has been pointed out that the initiation of multicellular trichomes may be controlled by a different pathway to unicellular trichomes (Payne *et al*., 1999). However, the actual molecular mechanism that forms the basis of multicellular trichome formation in tomato has not yet been identified.

naked is a radiation mutation in tomato (*Solanum lycopersicum*), which is responsible for plant growth and trichome development on young stems. In this study, we analyzed the *naked* mutants absent trichomes on young stems. We

report the results of a map-based identification of the best candidate gene which may be responsible for trichome development in tomato. We identified a deletion in *Solyc09g075140*, which may cause the trichome absent on the young stem. Such a truncation of Solyc09g075140 protein most probably to regulate the trichome initiation pathway and results in the absent of trichome in the *naked* mutant.

Materials and Methods

1. Plant materials and growth conditions

Tomato (*Solanum lycopersicum*) cv M82 (LA3475) was used as the wild-type (WT) for all experiments. Seeds for WT and wild tomato species seeds *S. pimpinellifolium* (LA1589) were obtained from C.M. Rick Tomato Genetics Resource Center (University of California, Davis, CA, USA). Tomato seeds were sown on half-strength Murashige and Skoog (MS) and Gamborg's B5 medium (#M0231, Duchefa, Haarlem, Netherlands) containing 2 % sucrose, 0.05 % MES, and 0.75 % agar and placed in a growth room at 25 °C under a 16-h light/8-h dark cycle with the light intensity of 50 μE m^{-2} s^{-1}. After 6- to 7-days, seedlings were transferred to Jiffy peat pots (Hummert International, USA) and grown in a greenhouse under natural light. 3- to 4-week-old plants were sampled for morphological analysis.

2. Morphology analysis

A dissecting microscope (DMS) CH-M205A (Leica, Germany) was used to analyze tomato trichomes on leaves, young stems, old stems, and hypocotyls. The images were analyzed with Leica Application Software (LAS v4.8) and assembled with Photoshop Imaging Suite. Scanning electron microscopy (SEM) TM3030plus (Hitachi High-technologies Corporation, Japan), and equipped with DEBEN Coolstage (Deben, UK) was used to freeze and fix

examine epidermal tissues of tomato. Images were captured using 15 kV to minimize surface charging of the trichomes. The images were analyzed with TM3030 plus application software (ver. 01-05-02) and assembled with Photoshop Imaging Suite. All measurements were performed on wild-type (WT) plants and *naked* mutants grew the same growth room at 25 °C under a 16-h light/8-h dark cycle with the light intensity of 50 μE m^{-2} s^{-1}.

3. Map-based cloning of *Naked*

Fine mapping of *Naked* was performed with F_2 population derived from a cross between *naked* mutant and *S. pimpinellifolium* (LA1589) and was predicted based on ITAG 3.2 gene models offered by the Sol Genomics Network (https://solgenomics.net/jbrowse_solgenomics/). A population of 91 F_2 plants was scored for does not have any trichome on the young stem compared to WT. The recombination frequency was identified in the mapping population by designed primer using putative Single nucleotide polymorphisms (SNPs) containing contigs that showed no significant homology to the *naked* mutant and LA1589. Recombination frequency in tomato genome with recurrent crossover events was measured using allele-specific digital PCR (Integrated Fluidic Circuit, Fluidigm, USA). And the Cleaved Amplified Polymorphic Sequences (CAPS), insertion/deletion (InDel) markers in Table 1, were used to narrow down the genomic region of 211 F_2 plants. Genomic DNA extraction was as described previously (Kang *et al*., 2010).

4. RT-PCR and genomic DNA PCR

Total RNA extracted from the young stems in about 2 cm length of right below the apical bud of *naked* mutants and WT plants for cDNA synthesis with Invitrogen™ TRIzol™ Reagent (#15596018, Thermo Fisher Scientific, USA) according to manufacturer's protocol. cDNA was synthesized with 2 µg of total RNA using RevertAid Reverse Transcriptase (#EP0442, Thermo Fisher Scientific, USA) and Oligo (dT) 18 Primer (#SO131, Thermo Fisher Scientific, USA) according to manufacturer's protocol. The PCR reaction was performed with Solg™ 2X Taq PCR Smart mix 2 (#SEF02, Solgent, Korea) in a 20 µL using a T100™ Thermal Cycler (#186-1096, Bio-Rad, USA) with the following cycling program: 2 min at 95 °C, 30 or 35 cycles of 20 s at 95 °C, 40 s at 60 °C, 30 s - 3 min at 72 °C, and one cycle of 5 min at 72 °C. Amplified DNA products were separated on a 1 - 2% agarose gel. Primers used to amplify each gene are listed in Table 2.

5. Total RNA extraction and qRT-PCR for expression pattern analysis

The individual core of stem right below the shoot apical meristem tissues of *naked* mutants and WT plants samples were collected by snap-freezing in liquid nitrogen. Total RNA extraction and cDNA synthesis were performed as mentioned above. Quantitative Real-time PCR analysis was performed using 2X Real-Time PCR Smart mix (25 mM $MgCl_2$) and 20X EvaGreen™ (#SRH71, Solgent, Korea) with an AriaMX Real-time PCR system (#G8830A, Agilent Technologies, USA). *ACT* (*Solyc03g078400*) was used as an internal

standard. Data are shown as the mean ± standard deviation (SD) of three biological and three technical replicates.

6. Vector construction

The coding sequence of *Solyc09g075140* was amplified from WT plant cDNA using PrimeSTAR® GXL DNA Polymerase (#R050A, TaKaRa, Japan) with 5' – GTCTCTAGAATGGGTGTTGAATATCATGAGGTATT – 3' and 5' – CGCCTCGAGCTACATAGATGAATGATGATGCATTC – 3' primer set. pBI-121 binary vector, which has a 35S promoter, and PCR product were digested with *Xba*I and *Xho*I restriction enzyme and purified using a LaboPass™ Gel and PCR Clean-up Kit (#CMA0112, Cosmogenetech, Korea). Purified PCR products were inserted into the pBI-121 vector. The promoter of *Solyc09g075140* was amplified from WT plant gDNA using PrimeSTAR® GXL DNA Polymerase (#R050A, TaKaRa, Japan) with 5' – GCGCCTGCAGGAAAGGGAGAAGCGTGTATGAAGT – 3' and 5' – GCATCTAGAATATGATGAGGAGAAGTGTATTCG – 3' primer set. pBI-121-*Solyc09g075140* and PCR product were digested with *Sbf*I and *Xba*I restriction enzyme and purified. Purified PCR products were inserted into the pBI-121-*Solyc09g075140.* To generate *Solyc09g075140* knockout vector, single-guide RNA (sgRNA) candidates for *Solyc09g075140* were designed by using CRISPR RGEN Tools (http://rgenome.ibs.re.kr). pHAtC binary vector was received from Dr. Sang-Gyu Kim (Institute for Basic Science, Korea) and pHAtC-*Solyc09g075140*-sgRNA vectors were constructed as described previously (Kim *et al*., 2016). The sequence information of sgRNAs are listed

in Table 2. The final constructs were introduced into *Agrobacterium tumefaciens* strain LBA4404 and used to transform WT cotyledon explants.

7. Phylogenetic analysis and conserved sequence analysis

The homologs of Solyc09g075140 protein in tomato and Arabidopsis were obtained from BLAST searches using the non-redundant protein sequences database at the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov). Phylogenetic tree of *Solyc09g075140* made in MEGA7 software using Neighbor-Joining method based on an amino acid sequence in tomato and Arabidopsis. The reliability of the tree was tested by bootstrap resampling (1,000 replications). The amino acid sequences of *Solyc09g075140* protein homologs are aligned by using the Multiple Sequence Alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo/).

8. RNA extraction, library preparation, and sequencing

Total RNA was extracted from the young stems in about 2 cm length of right below the apical bud of *naked* mutants and WT plants using the RNA Isolation Kit (Qiagen, Germany), according to the manufacturer's instructions. To check the RNA quality, all the RNA samples were examined for concentration and purity using an Agilent Bioanalyzer 2100 (Agilent Technologies, USA). RNA-Seq. paired end libraries were prepared using the Illumina TruSeq RNA Sample Preparation Kit v2 (#RS-122-2001, Illumina, USA). And the library was quantified using the KAPA library quantification kit (#KK4854, Kapa

Biosystems, USA) following the manufacturer's instructions. Each library was loaded on the HiSeq^{TM} 2000 System (#SY-401-1001, Illumina, USA), and we performed for high-throughput sequencing to ensure that each sample met the desired average sequencing depth.

9. Reads mapping to the reference genome

To collect high-quality trimmed data, we filtered the sequencing data by phred score (DynamicTrim, $Q \geq 20$) and minimum length (LengthSort, short read length ≥ 25 bp) using the SolexaQA software. The paired-end clean reads were aligned to the reference genome using TopHat v2.1.1. The number of mapped reads for each mRNA was counted and then normalized using the DESeq library in R package (Anders *et al*., 2010). The filtered reads were mapped to 35,768 reference transcripts from *Solanum lycopersicum* (cv Heinz 1706) using the SGN SL3.2 gene models.

10. Differential expression genes (DEGs) analysis

Differentially expressed genes (DEGs) analysis were performed using the DESeq library in R package. DESeq library provides statistical routines for determining DEGs using a model based on a binomial test. Genes with an adjusted *p*-value determined to be ≤ 0.01 (false discovery rate, FDR ≤ 0.01) using DESeq library and that had a fold change value ≥ 2 ($|\text{Log}_2 \text{ fold change}| \geq 1$) between wild-type and *naked* mutants were considered to be differentially expressed.

11. Gene Ontology (GO) and KEGG enrichment analysis

Gene Ontology (GO) term annotation including all three GO categories (biological process, cellular component, and molecular function) of a subset of DEGs ($p < 0.05$) was conducted using the Blast2GO with default parameters. In addition, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) to identify the biological mechanisms and metabolic pathways associated with the DEGs corresponding their enzyme commission numbers.

Results

1. *naked* mutant affect plant growth and trichome development on stems

The overall plant growth of *naked* mutant was smaller than that of its wild-type parent (cv M82, WT). For example, the length of main stem of *naked* mutant was extremely significantly reduced to 38% of that of WT plants in the 3-week-old plants (unpaired *t*-test: **** $p < 0.0001$, Fig. 1A and 1B). Branch number was also extremely significantly reduced to 30% in *naked* mutant compared to WT plants (unpaired *t*-test: **** $p < 0.0001$, Fig. 1C and 1D). In addition, fruit morphology was different. The *naked* mutant had smaller fruits with less gloss on the surface compared with WT plants (Fig. 1E).

The most noticeable phenotype of *naked* mutant was trichome development. The dissecting microscope (DMS) was used to compare the morphology of trichomes on the leaves, stems, and hypocotyls of the *naked* mutant to WT plants. Compared to WT in which type I trichomes were aligned perpendicular to the epidermal surface, *naked* mutant had normal type I trichomes on leaves and hypocotyls but no type I trichomes on young stems or smaller type I trichomes on old stems (Fig. 2) The identity of other types of trichomes as well as type I trichomes was confirmed low-temperature scanning electron microscopy (Cryo-SEM). This analysis showed that the *naked* mutant has all types of trichomes including type I on leaves, old stems, and hypocotyls although the length of type I trichomes on old stems and hypocotyls of *naked* mutant is smaller than WT plants (Fig. 3). However, the effect of the *naked*

mutant on trichome morphology in young stems was not specific to type I trichomes but rather extended to other types of trichomes as well. The young stems of *naked* mutant did not have all types of trichomes (Fig. 3). Also, the length of type I trichome, an additional defect related to trichome, was observed in the *naked* mutant (Fig. 4). The length of WT and *naked* type I trichomes on the leaves was 10.1 ± 1.4 mm and 7.0 ± 2.1 mm, respectively (mean ± SD; n = 35 type I trichomes on each of three leaflets; unpaired t-test: **** $p < 0.0001$). In the WT of young stems, the type I trichomes were 35.4 ± 4.1 mm, while the young stems of *naked* were impossible to measure because all trichome was absent (mean ± SD; n = 35 type I trichomes of three WT stems; unpaired t-test: **** $p < 0.0001$). The length of type I trichomes on *naked* old stems (16.1 ± 3.4 mm) was also short in comparison with the WT (27.8 ± 3.4 mm; n = 35 type I trichomes on each of three stems; unpaired t-test: **** $p < 0.0001$), as was the length of type I trichomes on *naked* hypocotyls (34.9 ± 4.6 mm and 19.6 ± 3.2 mm; n = 35 type I trichomes on each of three hypocotyls; unpaired t-test: **** $p < 0.0001$).

2. Genetic mapping of *Naked*

F_1 plants derived from a cross between *naked* and its wild-type parent showed normal trichome phenotypes, indicating that the mutation is recessive. An F_2 population obtained by selfing the F_1 plants was scored at the seedling stage (3-week-old plants) for the trichome phenotype (no trichome on young stems). Among 80 F_2 plants, 18 plants exhibited no trichomes, whereas the remaining F_2 plants appeared normal trichomes on stems. This ratio (3.4:1) is

in good agreement with that predicted for a single recessive mutation (χ^2 = 0.267; P = 0.606). To identify the *Naked* gene, we conducted the map-based cloning (Fig. 5). We used a 211 F_2 population derived from a cross between *naked* mutant and *S. pimpinellifolium* (LA1589) to locate the map position of *Naked* to ~250 kb region flanked by markers Solyc09g075010 and Solyc09g075360 on chromosome 9 (Fig. 5). The ~250 kb region is predicted to contain 31 hypothetical genes (ITAG 3.2 gene models; https://solgenomics.net/jbrowse_solgenomics/) (Fig. 5, Table 2).

3. *Naked* likely encodes the tomato homolog of monoacylglycerol lipase

Among the hypothetical genes, 13 genes related to the development of trichomes or epidermal cells in the previous studies were selected and conducted RT-PCR to predict *Naked* candidate genes (Fig. 6). RT-PCR results showed that the expression level of *Solyc09g075130*, *Solyc09g075140*, *Solyc09g075170*, and *Solyc09g075260* genes were less in *naked* stems compared to WT stems (Fig. 6). We conducted RNA-Seq. using RNA derived from WT and *naked* stems to study which genes are regulated by *Naked* gene and to predict *Naked* gene. Among the 31 hypothetical genes, four genes were differentially expressed between WT and *naked* plants and two genes (*Solyc09g075140* and *Solyc09g075260*) were down-regulated in *naked* compared to WT plants (Table 3). Sequencing of fragments amplified from genomic DNA PCR of *Solyc09g075260* containing ~3 kb promoter and whole structural region from WT and *naked* plants did not reveal nucleotide polymorphisms at this locus (Fig. 7). RT-PCR of *Solyc09g075140* gene

showed that full-length and 3-termins region were not amplified in *naked* mutant (Fig. 8A and 8B). Genomic DNA PCR of this gene also showed that full-length and 3-termins region were not amplified in *naked* mutant (Fig. 8C). Further genomic PCR with a primer set (F and R) covering 3' structural gene and 3' intergenic region showed that *naked* mutant produced a smaller fragment compared to WT plants (Fig. 8D). The sequencing of these fragments confirmed that ~2.l kb including translation termination sequence is deleted in *naked* mutant (Fig. 9). This deletion generates a premature stop codon that truncates the C-terminal 224 amino acids of the protein (Fig. 10), suggesting that *Solyc09g075140* is *Naked* gene.

4. Expression pattern of *Solyc09g075140*

To analyze the tissue-specific expression pattern of *Solyc09g075140* gene, we measured mRNA levels of leaves, floral buds, roots, epidermal stems, and peeled stems in wild-type (cv Ailsa craig) plants by qRT-PCR analysis (Fig. 11). The *Solyc09g075140* mRNA was constitutively expressed in all analyzed tissues, with higher levels detected in leaves and floral buds, and the lowest expression in roots.

5. Phylogenetic and conserved sequence analysis

The homologs of *Solyc09g075140* gene in Arabidopsis and tomato are used to construct the phylogenetic tree (Fig. 12). *Solyc09g075140* and 8 tomato genes are annotated as α/β-hydrolases superfamily protein, Arabidopsis 10

genes are annotated as monoacylglycerol lipase (MAGL). MAGL, a member of the serine hydrolase family, shares fold of the α/β-hydrolase which consisting of a central β-sheet surrounded by a variable number of α-helices (Ollis *et al*., 2009). In Arabidopsis, *MAGL8* known to associated with the surface of oil bodies in germinating seeds and leaves accumulating oil bodies (Kim *et al*., 2016). While the function of tomato genes are not studied yet. Alignment of the amino acid sequences from the Arabidopsis MAGL genes and mouse MAGL gene showed that α/β-hydrolase domain and catalytic triad (Ser, Asp, and His residues) corresponding to the active site of MAGL was conserved in *Solyc09g075140* (Karlsson *et al*., 1997) (Fig. 13). Also, α/β-hydrolase is related to the cuticle layer formation of the epidermis (Kurdyukov *et al*., 2006). The cuticle layer consists of cutin, which biosynthesis genes affect the development of trichomes in Arabidopsis (Kurdyukov *et al*., 2006) and *Artemisia annua* (Shi *et al*., 2017). So, we predicted the Solyc09g075140 protein is may function in cuticle synthesis. Furthermore, cutin synthase homologs in the cutin synthesis pathway of Arabidopsis were down-regulated in *naked* (Fig. 14, Table 4). This result suggests that *naked* has no trichome due to an abnormal cuticle layer of the epidermis. These findings support the inference that *Solyc09g075140* is the gene responsible for *naked* phenotypes and will confirm through a plant transformation (Fig. 15).

6. Identification of DEGs and functional classification

RNA-Seq. data were used for identification of DEGs in young stem tissue of WT and *naked* mutants. DEGs were identified using the following filters:

adjusted *p*-value < 0.01, and |Log_2 fold change| ≥ 1. From the 35,768 genes, which mapped by the RNA-Seq. data (Table 5), 797 DEGs were identified (Fig. 16).

To overview functions of DEGs, the 2,285 annotated DEGs were assigned to at least one Gene Ontology (GO) category that belonged to three major terms: cellular component, molecular function, and biological process. GO term enrichment analysis results varied from GO classification and expression change of DEGs. As to biological process, the up-regulated DEGs in *naked* significantly enriched in biological process, response to stimulus and oxidation-reduction process, and the down-regulated DEGs in *naked* significantly enriched in oxidoreductase activity, carbohydrate metabolic process, lipid metabolic process, and oxoacid metabolic process. For cellular component, the up-regulated DEGs in *naked* were no enriched in this category, and the down-regulated DEGs in *naked* significantly enriched in cell wall, external encapsulating structure, and cell periphery. About molecular function, the up-regulated DEGs in *naked* significantly enriched in catalytic activity and oxidoreductase activity, and the down-regulated DEGs in *naked* significantly enriched in catalytic activity, oxidoreductase activity, cofactor binding, iron ion binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, heme binding, and tetrapyrrole binding. More detailed GO enrichment analysis results are shown in Figs. 17 and 18.

Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of down-regulated genes in *naked* were mainly involved in metabolism and included biosynthesis of other secondary metabolites (163

genes), lipid metabolism (31 genes), metabolism of terpenoids and polyketides (40 genes) (Fig. 19).

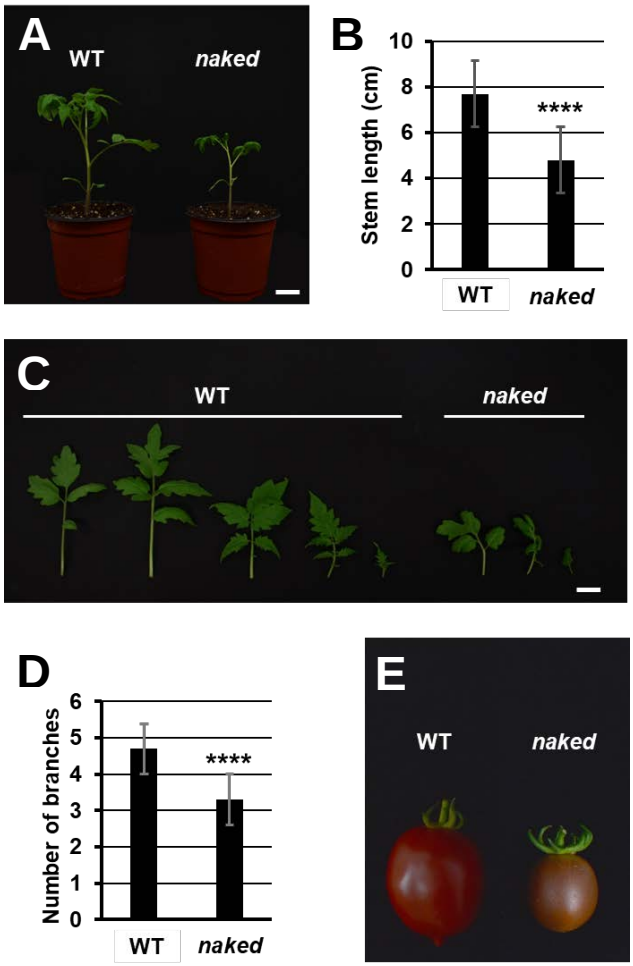


Figure 1. Phenotypic appearance of wild-type (WT) and *naked* plants. (A) Three-week-old WT and *naked* plants. Scale bar represent 2 cm. **(B)** Stem length of three-week-old WT and *naked* plants. Each mean represents data from 36 replicates. Asterisks denote extremely significant differences between WT and *naked* plants (unpaired *t*-test: **** $p < 0.0001$). **(C)** Detached branches from three-week-old WT and *naked* plants. **(D)** Branch number of three-week-old WT and *naked* plants. Each mean represents data from 36 replicates. Asterisks denote extremely significant differences between WT and *naked* plants (unpaired *t*-test: **** $p < 0.0001$). **(E)** Ripened fruit of WT and *naked* plants.

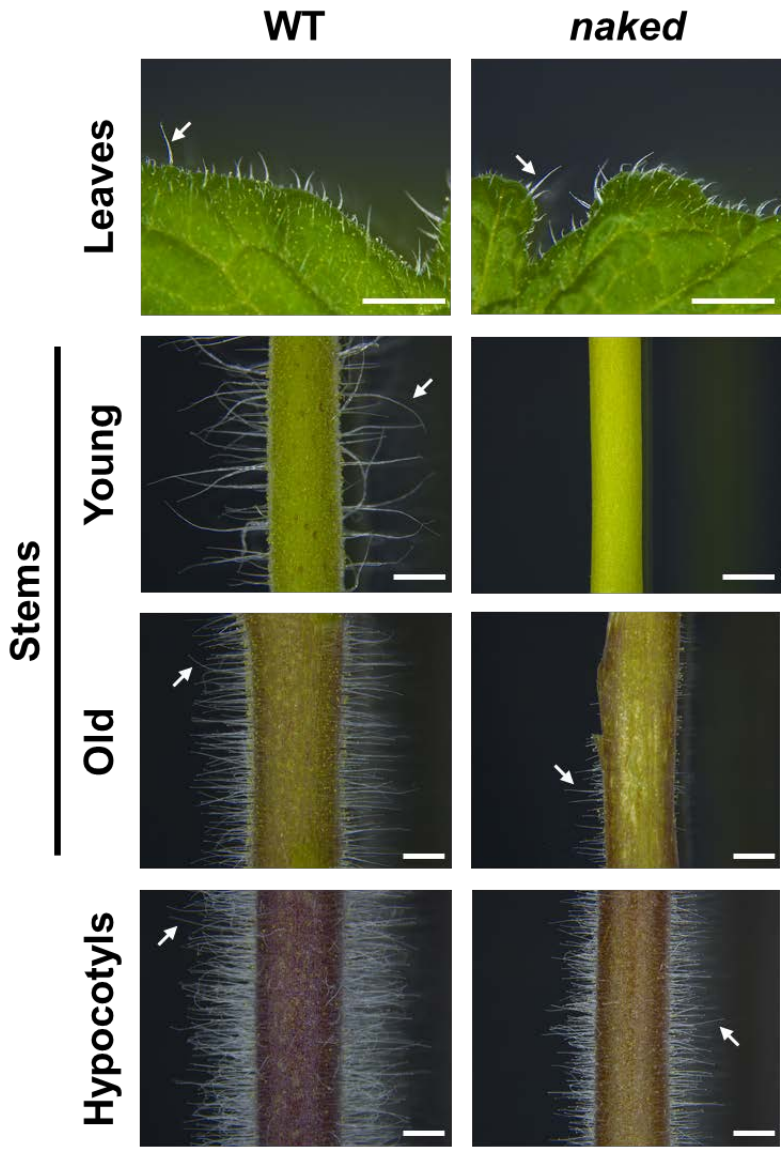


Figure 2. Light micrographs of trichomes on the leaf, young stem, old stem, and hypocotyl of wild-type (WT) and *naked* plants. Photographs show the adaxial leaf surface (1st row), young stem (2nd row), old stem (3rd row), and hypocotyl (4th row) of each genotype. All photos were taken from 3-week-old plants. The arrows indicate type I trichomes. All scale bars represent 2 mm.

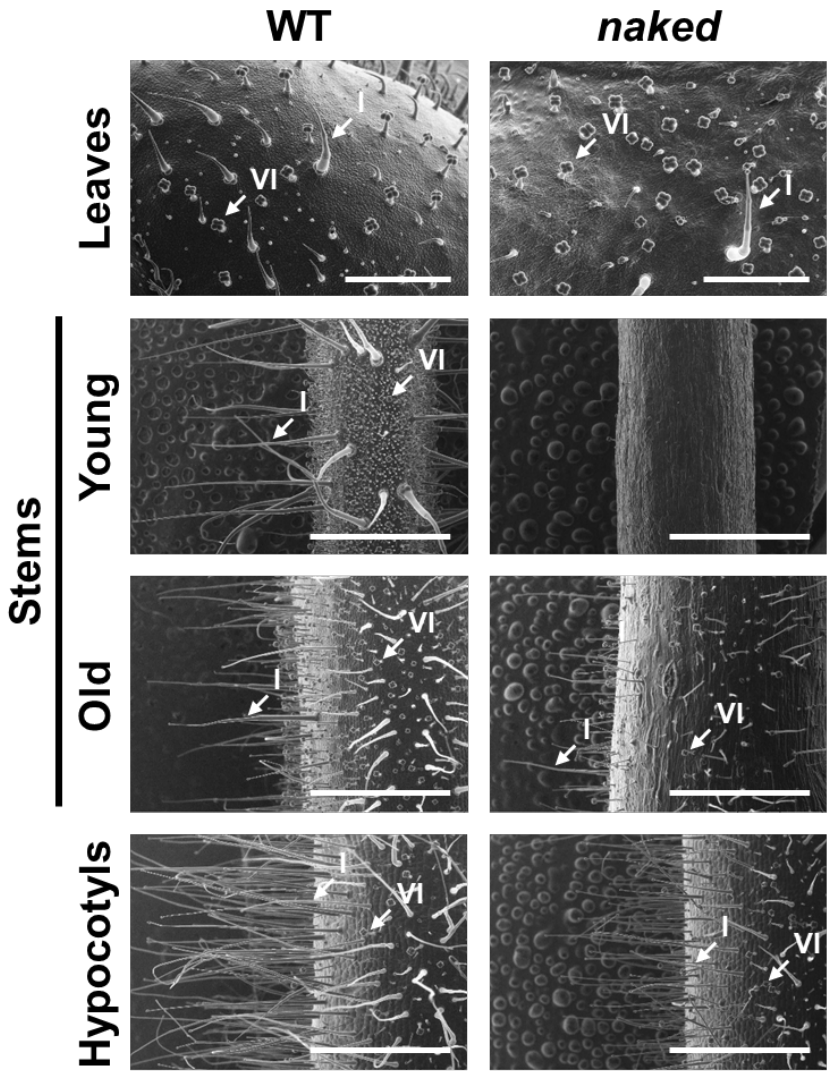


Figure 3. SEM micrographs of trichomes on the leaf, young stem, old stem, and hypocotyl of wild-type (WT) and *naked* plants. Photographs show the adaxial leaf surface (1st row), young stem (2nd row), old stem (3rd row), and hypocotyl (4th row) of each genotype. All photos were taken from 3-week-old plants. Type I and VI trichomes are indicated by arrows. Scale bars represent 500 μm in the leaves, and 2 mm in the young stems, old stems, and hypocotyls.

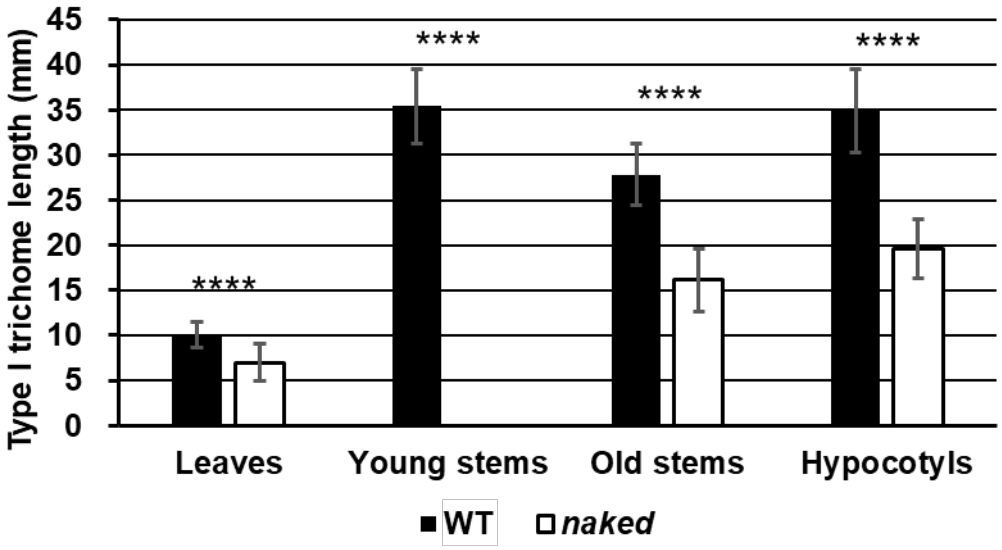


Figure 4. The length of type I trichomes on the leaves, young stems, old stems, and hypocotyls of wild-type (WT) and *naked* plants. All the plants were three-week-old. Type I trichome length is defined as the distance from the base of the cell to the tip. Bars represent the mean values (± SD) of the leaf, young stem, old stem, and hypocotyl samples from different three plants. Asterisks denote extremely significant differences between WT and *naked* plants (unpaired *t*-test: **** $p < 0.0001$).

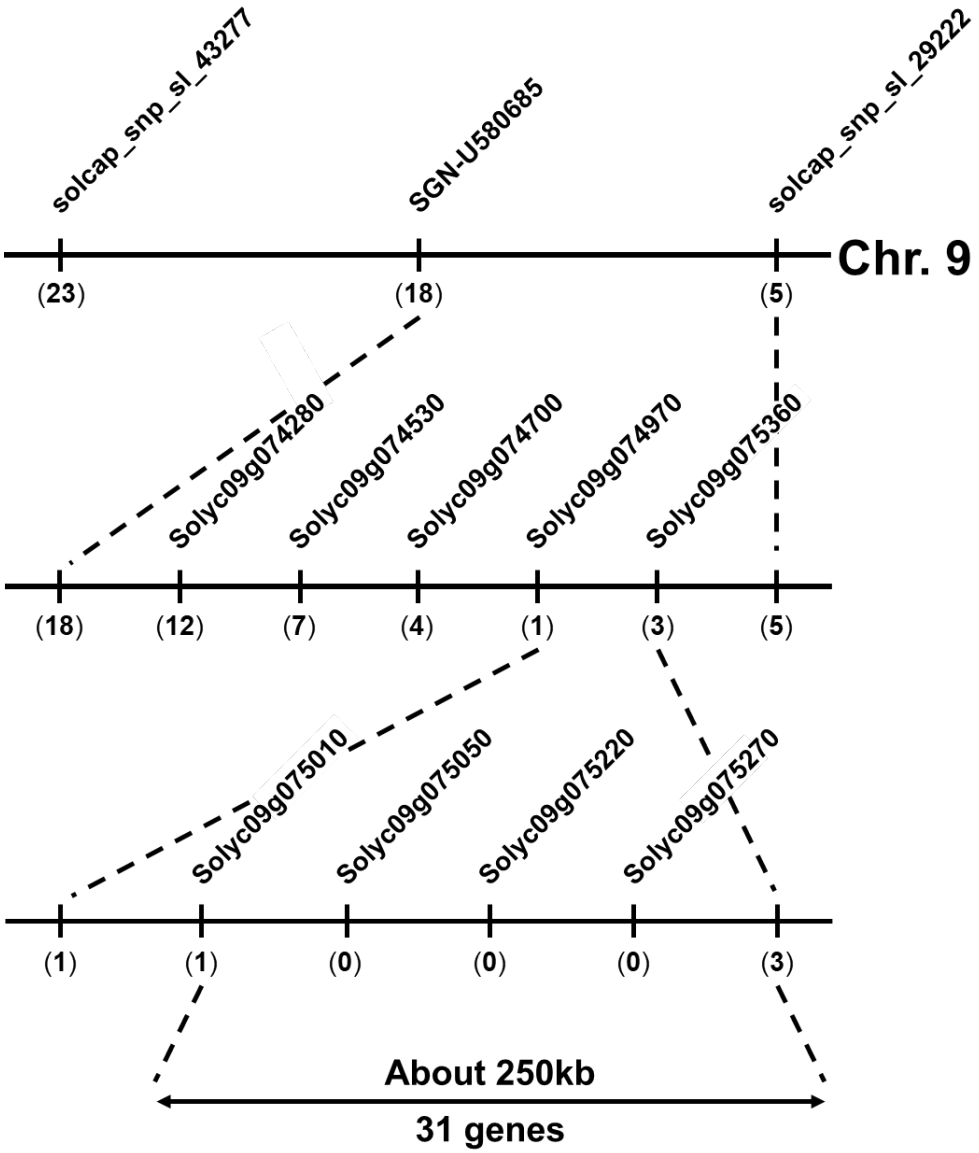


Figure 5. Fine genetic mapping of *Naked* gene. Genetic mapping of *Naked* gene delimited the target gene to an interval between marker Solyc09g075010 and Solyc09g075360 on tomato chromosome 9. Numbers in parentheses indicate the number of recombination events identified between markers and the target gene.

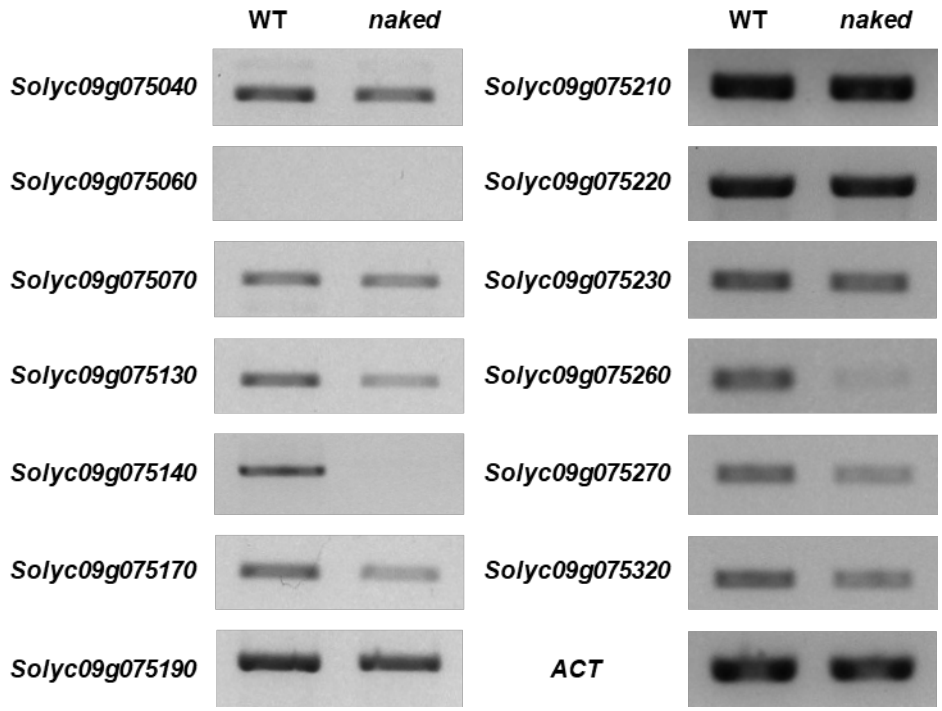


Figure 6. RT-PCR for candidate genes in the mapping region from wild-type (WT) and *naked* stems. *Actin* (*ACT*) mRNA was used as a loading control.

A

CGGCGCTGAATGACTTAAATTTCATTTCTTTAGAAAAAAATGGTGAAAAGTATTAAATGTAATAAAAATAATGTGC
CTAGAGATGCAAGCTTTGGTCAAATTTTGTAATGTAGCTTAGAAAACAAGACAAAAATATATTAATGACAACCCTT
CTTTCAGTCCCAACAGTTCTCATCACACTCCTAATTTTTCTCACCTACTTCCCCATAGAAGCTAAAAAAGAAATAG
CAGTTCATAGTGGTTAAGAATAATAATTTAAAGAATGTGTCATTTTTTTTTTAATTATTTGGTAATTAAGACTTAAT
ATATTCTATTAATTTAAATTTGTATGCATCTCATCATTACCTATTAAGGAATAATGTTCCCTTTTTTTTTTCAAGA
ACATCTTTCTAACTATATTCCAAACTTCAATTATATCCTTAGAGTATTCAGTAGAAAACATTTTTAAGTATTTAAA
ATGGAAAATTTTCATCCTTCTATTTGAATTTATTGGACAACGATTAAATTTCTCAAAAATTGAGCCATTTCTTCGT
AATTATATGTTATTCTGTATACATCAAGAATATTATCGTGAACTTTTTCAGTAATTAAGTTCAGCATTATGAAAAA
GAAATTCATTTGACAGTTTTATCATTTATTTTTTCATGTAATTTGATATTAAATATATTTTAGTTGAAATATATTT
CTTCTCAGTTGATATGCTAGCTAGAAGCAGCGCTTATTGAAGATTTTTTATTCTAATACTATAGAATGAAACTCAA
ATACGAGACATAATCAATATTTTAATCACTTCTATATTAAATTGACTGTAACATTTATTGATCTAAATACAATCTA
GCTTTGTAAAAGGTAAATATATTTAAGTCCAGCTTCTTTCAGTTACTAATGTTAAAAAGAGCAGGTCGTGTATTAT
CACACCTTTCAAATATTTTTTTATTATAGTAAGCAGTAGTGTCTAAGATTTTAAATATTTTTTTAGGAACTTCTTA
CACTAGGTAATGAAATTAGACAGAAAAACTTTGTTGATAATCACGTGAACTATTCATGTTTTGTAAGATCCCTTA
ATTTTTTTGAAAATATCTAATAAAAGGATGAAAATTATTCACTTTTAAATACTTGAATAATGTTTCCTGATAAAAT
TAAACTTTGAGGATATAGTTAAATTTGAGGTATAATTTAACAATATTTTTTAAAAAAAACTCTTCGAATCTCATAT
TTCAATTCACACCACGAAAAATCAAAAACTTCATATTCAAAACCAAAATTTAATTTTTTTTATTAAAGATTAAACG
GTCACATTCATTGTGTCATTTCAAACTTATTTGGGCATCACCATTGAAGGTGTTGTAATAGGGTAAAAATAAGCAT
TACATACTCTATATAAATTCATATGTAATACACCTATTTATGTTATTGGTAACTACGATTGGTGTATTTTTTATAT
TTGCAAGGAATGAGTTTAAACATTTTATTTTATTTTTAAAGAATTTTCTATGAAATTAAAAATATTATGATATTTA
AAAATTTATTAGTTTCTATAAGAATCTTATAATTAAATTATGGGAGTAGATAATTGTTGTAAATCGCTCAAGTTGA
TCTTTTCACCAAACAATATGTCAAAGAAGAGGACACGTGCTGACCCGTAGAGTTCTCACGTGCAGCGTGTGGGTTC
TTGGGTTTGCCCACGTGAAGTGTGAGGAAGTAGAAAAAATAAATTTTATCTTTTCTTCTAAGGTATTTTGATTTTG
CGTAAATTTAAGAAAGAGATTTTTAAAATTTATGATGTAAAAAAATATTACATAATTGTGGATTTTATTAAGAATA
AAATAGCTACTTTATTTTTAAATTGTTATTCATTCCGTTTATTATAATTTGATAAGTACACTAAAATAATTGTCCT
AACTAATTGTCAATTTAAATAATTAACAAATAATTAGTTATGTTTTTTCAATTTTGTCCTTAATAATTACATAGCT
ACTTAAATAGTTATCTAAACGAATTGTTGATTTTAAACAATCAATAAACTGTTAGTGATTTTATAATACTAATTTC
AAAAAGTAATCATTTTAATGTTAAACGACTACTTTAATGTGAAAAAAGTTGTTTAGACCATTTTAATTATGATTAG
TAGTAAGGTTATATTAATAAAATTGCACCTTATATTAATTTTTTTTTCAAGGACATGATTTTATCATACAATCAAT
AGTGAATGAAGGGAATACTTAATATAGAAATGTGTCAATCTTTTTGAGATCGACTAAAAAGAAAAATAAAGAGAAA
ATGTCAAAAATCACATACTGTTACGACAAATGTACTATTTGTCCCTTATAAGTTTATGACTACATAAAATGCTATA
TACCCAATTTACACTTATAACTTGATATTTATCATCTTAAATGTGTGTGGAGTGTTATTTTTATATAAAGTTATAT
AAATTGTGTTTCTAATTGTATAAAGCGAGAGAAAATTATATAAACACATTCAAATACATGTATTTTTATCATATAC
GCTTATAATTATTACAATAAAAGTACTCCCCTGTCCAATCTCTCTCTTTCTCGTATTATACAAATTCAAATTGTAT
ATAATTTATCTATTTCTCGTTTTATACAATTCGATTCAGTTGTATATTCCCTACCAAAGTCTCTTTTGTCTTTCTC
TCTTTCTCATTTTATACAAATTCAAATCATATAATCGTTCTATACACTTATAATTATACACTTCGTTCTATGCAAT
TCTCTAGCCAAGTCTCTTTATCTTTCTCTTTTTATACACTTTCTTTTATACAATTCACTTAAATTGTATATGTACA
GCAAATTATACATATATATGTTTGCTATACAACGCAATTATGCAAACTTTGTTATAGTATACAAATATAAATTTTA
TATTCGCTATATGTGAAAGTTGCACTTACAGAAAAACTTAAACTGATATAATAATATAAGTGTTGTACATTAATCT
GATGCACGAATACATTAATCGTTAAGTAAGATACAT**TATATTTTACACATGATACACTAATATAATATAACTTTTAT**
ACATGATAAGCTAATTTAATGCTCGAGATACATTGCAACAAGATGATAAAATAATTCATCGGCAATTACTTATTTTGA
TATAGCAATATTATTTTTATCTTTGATTAGAAAATAATTAATTAATTCTAAAATAACTTATTTCCGTTGTCTTCCCCA
TTTACCCAACGTTTTTCATAGGGCTACACCATAGCTACTCAATAGTTCAAGTTTCAAATTCAAGCTCTTCAATATTTA
AGATTAATATCTCAATTTCAAATTAAAATAAATAAGAGTAATATCTCAACTTCAAGTTAATTTAATTTAACTACTAAC
CAGAT

B

ATGCCTTCTGTAGCTTTTCCCTTATGCATTTTCCTCCTCCTACTCTTTGCAGCTTCTCCGGTCATCGGCGATGAGG
ATTCTCAGTCCCCGACGGCATACGAGATTCTACAGGAATACGATATTCCGGCAGGGATACTTCCGAAGGGCGTAAC
AAGGTACGAACTCGACAAAACAACGGGCAATTTCGCCGTTTACTTTAACAAATCGTGCAGTTTCAGCATAAGCGGC
TATGATCTCAAGTATATGAGTAAAATTACTGGTAAAATCTCTAAGGATCGGCTTGCGAACTTGAAAGGCGTACAGG
TGAAACTGCTTTTTTTTTTTGTCAATATTGTGGGAGTTACTCGTGACGGCGATGATCTCGGTTTCTCCGTCGGTGT
TTCTTCTGTTGATTTTGCAATTGAGTACTTCTATGAACCGCCAGAGTGTGGATGTGGATTTGATTGTGTCAATTCT
GGAGAAAACGGTACTGGTGAGTTCAATTTAAAGCAGCTTATCTCTTCCACTTGAATTGTTAACTAAAATTTTCAGA
GATTGGCTGTAAATTCAGTTGATTTGGGATGGAATTTGGAATTAGGGCAACTTTGTATTTCAGAATCCCACGGGAC
TGGAAGCATATGCTTTTGATGCTCCTTTAGAATGAATATGTATTTTTTTTCATATATCTCCGTGGACTCTTATGGA
AATTGAAATAAATAAAATTTTCTTTTATTCGCTAC

Figure 7. Genomic DNA sequence of *Solyc09g075260* in wild-type and *naked* plants. (A) Sequence of 3 kb promoter region. 5' UTR region is in bold. **(B)** Sequence of structural region. Translation initiation and termination sequence are underlined.

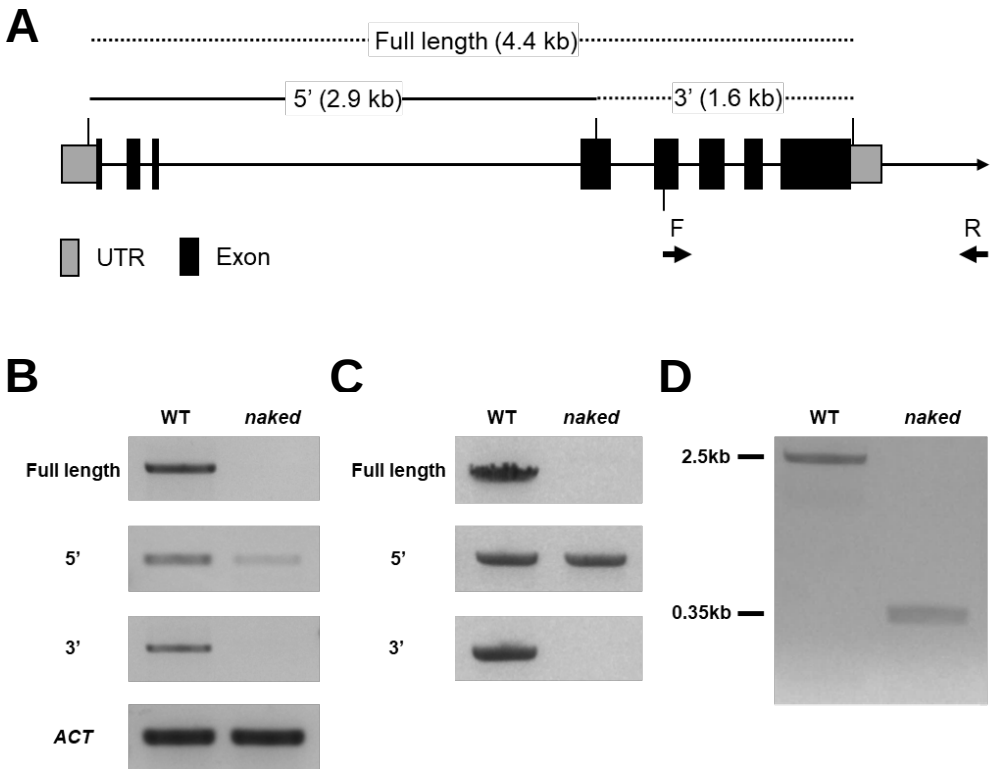


Figure 8. *Solyc09g075140* gene is mutated in *naked* mutant. (A) Genomic DNA structure of *Solyc09g075140*. **(B)** Agarose gel showing the results of RT-PCR amplification of *Solyc09g075140* cDNA using mRNA isolated from WT and *naked* stems. *Actin* (*ACT*) mRNA was used as a loading control. The amplified full length, 5', and 3' region is indicated in (A). **(C)** PCR amplification of *Solyc09g075140* genomic DNA from WT and *naked* plants. The amplified full length, 5', and 3' region is indicated in (A). **(D)** PCR amplification of *Solyc09g075140* genomic DNA containing the mutated region from WT and *naked* plants. F and R primer set indicated in (A) is used.

```
WT       AACGGTTTCTGTATGGGGAGTCGATGGGAGGGGCCGTGGCTCTTTTAACACACAAGAAGG  360
naked    AACGGTTTCTGTATGGGGAGTCGATGGGAGGGGCCGTGGCTCTTTTAACACACAAGAAGG  360
         ************************************************************

WT       ATCCTTCCTTTTGGCATGGTGCTCTTCTGGTTGCACCTATGTGTAAGGTAGGCCTATTGA  420
naked    ATCCTTCGTT----------------------------------------------------  370
         *******  **

WT       GATTTTTAGTCTCAACTCATTTACTGTAATATATATGCTTTAGTGCTTGCATTTAGTATT  480
naked    ------------------------------------------------------------

WT       GAGGGACCATTGGAAGTCGGTTATGGTGTTGCTAATGCATTGCAAAATCACCAGATATCT  540
naked    ------------------------------------------------------------

WT       GAGAAGGTGAAGCCACATCCTGTGGTTATAAGCTTACTAACTAAAGTGGAGGATGTCATA  600
naked    ------------------------------------------------------------

WT       CCAAGATGGAAGATAGTCCCTACGAAGGATGTCATTGATTCGGCCTTCAAGGACCCCGCT  660
naked    ------------------------------------------------------------

WT       AAAAGGGAAGAGGTGAGGCTAATTGTTAAGTTAATATGCAACATTTGCTAGCTTCCAAGT  720
naked    ------------------------------------------------------------

WT       ATTAATGATGTGCGGCATCCAATTCTTGTCCTCCATCAAAACATGATGTCCATTTCTTGT  780
naked    ------------------------------------------------------------

WT       CATTTTGCAAGTGTAGGTACGCGAGAACAAGTTAATTTATCAGGCAAAGCCAAGACTAAA  840
naked    ------------------------------------------------------------

WT       GACAGCTTTGGAAATGCTAAGAACCAGCATGCACCTTGAGGAAAGTTTGCACGAGGTAAC  900
naked    ------------------------------------------------------------

WT       TATTACCTAACCGTCTTCCATGTAAACAATAATTGAATCATTCCGACAAATTGGAAACTG  960
naked    ------------------------------------------------------------

WT       GAAATGATGTGTAGTTGAAAATGTTTCGACAGGTCACTGTACCATTTTTAGTGTTACATG 1020
naked    ------------------------------------------------------------

WT       GGGAAGCAGACATAGTAACTGATCCAGAAATAAGTAAGGCTTTATACGAGCAAGCGAGTA 1080
naked    ------------------------------------------------------------

WT       GCAAAGACAAGACTATAAAACTCTATCCAGGAATGTGGCATGGTTTGACATATGGTGAGC 1140
naked    ------------------------------------------------------------

WT       CAGAAGAAAACATTGAAATCGTATTTTCAGATATCATCTCGTGGCTTGACAAGCGAAATG 1200
naked    ------------------------------------------------------------

WT       GAGAGAATACTGGTGATGCTAGTTTAATCGAGAGATCAGTTTGTCGAGCTACATCTACTC 1260
naked    ------------------------------------------------------------

WT       CTCCATATGAGATGCACACAGTTTCTTCACCTGCAACAATGAAAGAAACAAAACCACATA 1320
naked    ------------------------------------------------------------

WT       GAACGCGTCCCCAAGCTAATTATCTGTGTGGATTGAAAGGACGTCGAATGCATCATCATT 1380
naked    ------------------------------------------------------------

WT       CATCTATGTAGAAACTTGATCGATAGATATGGCGTGTTGCATCTCCTTCATTTGTTCATT 1440
naked    ------------------------------------------------------------

WT       CGCGTTACGGTGGCATAAATGTTACTACTATTATTTATCAAGGAAGTGGAAAATAACATG 1500
naked    ------------------------------------------------------------
```

Figure 9. Comparison of *Solyc09g075140* gDNA sequence between wild-type (WT) and *naked* plants. Translation termination sequence is underlined in WT. In the *naked* mutants, *Solyc09g075140* gene is deleted about 2.1 kb containing translation termination sequence.

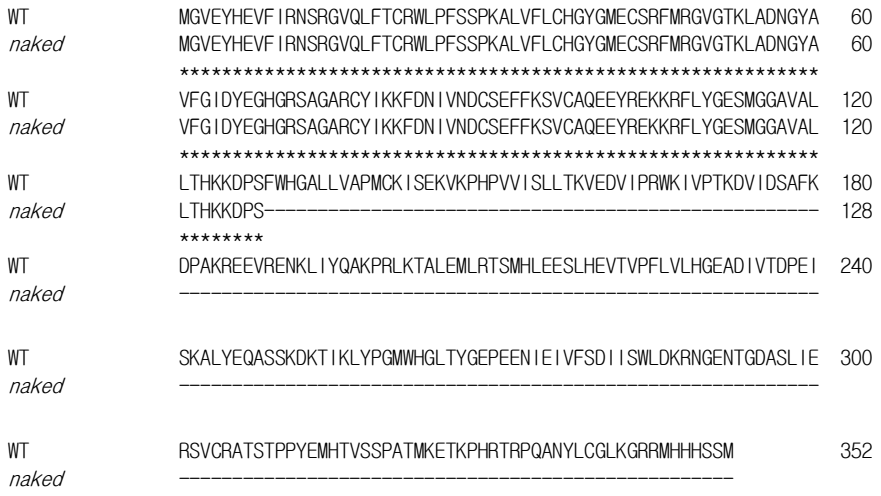


Figure 10. Alignment of Solyc09g075140 amino acid sequence from wild-type (WT) and *naked* mutant.

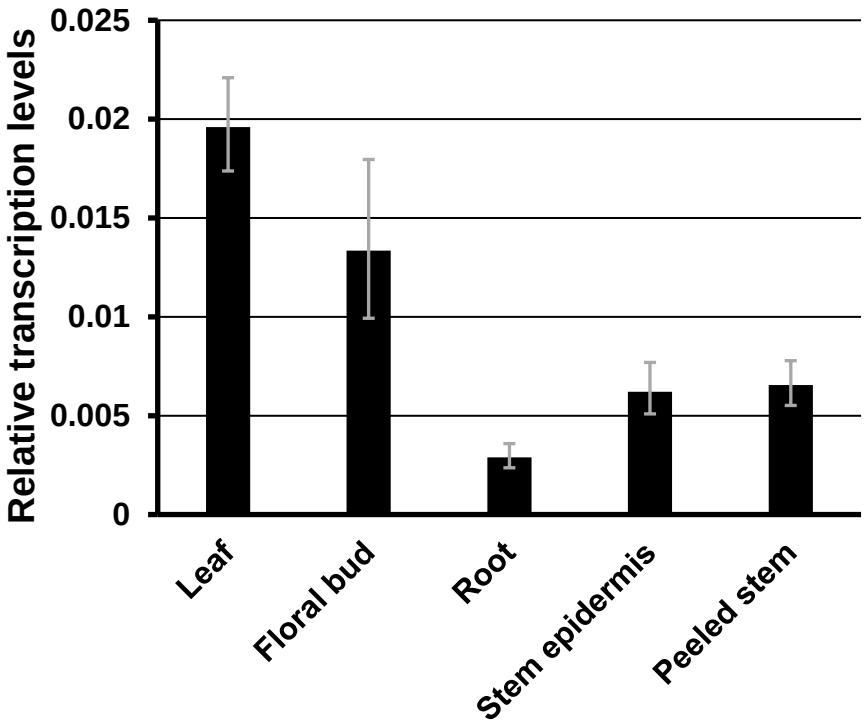


Figure 11. Expression pattern analysis of *Solyc09g075140*. Expression of *Solyc09g075140* was analyzed by qRT-PCR. Total RNA was extracted from different tissues in 4-week-old wild-type (cv Ailsa Craig) plants and used to synthesize cDNA. Values were normalized to actin. All results are expressed as the means ± SD of three biological and technical repeats.

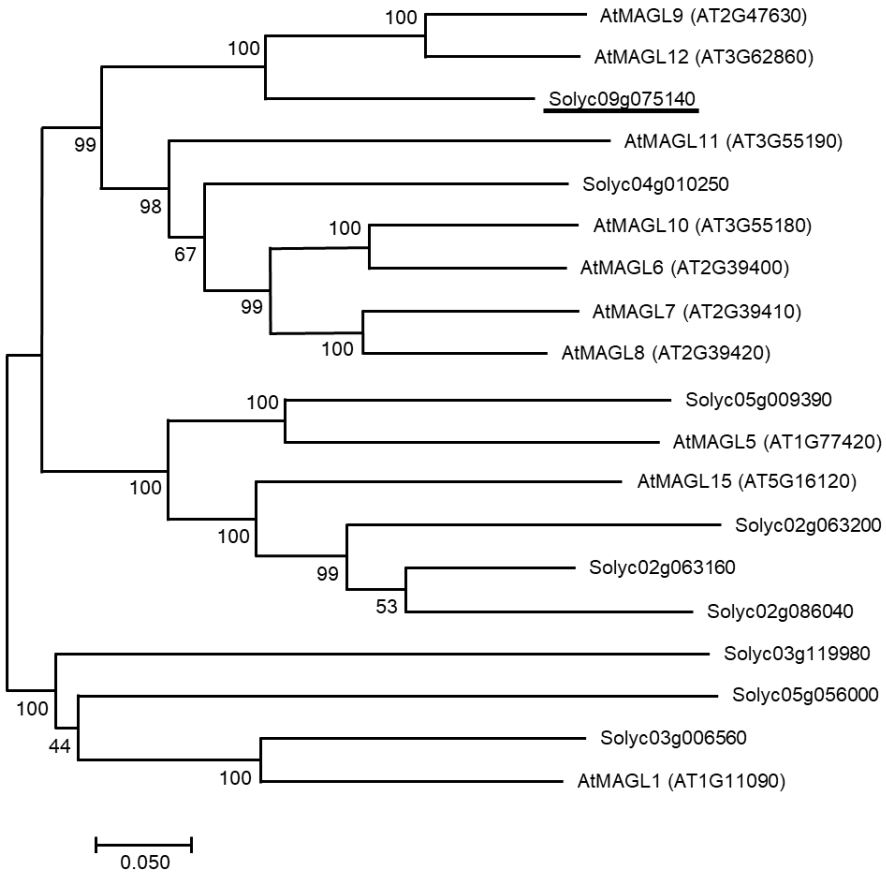
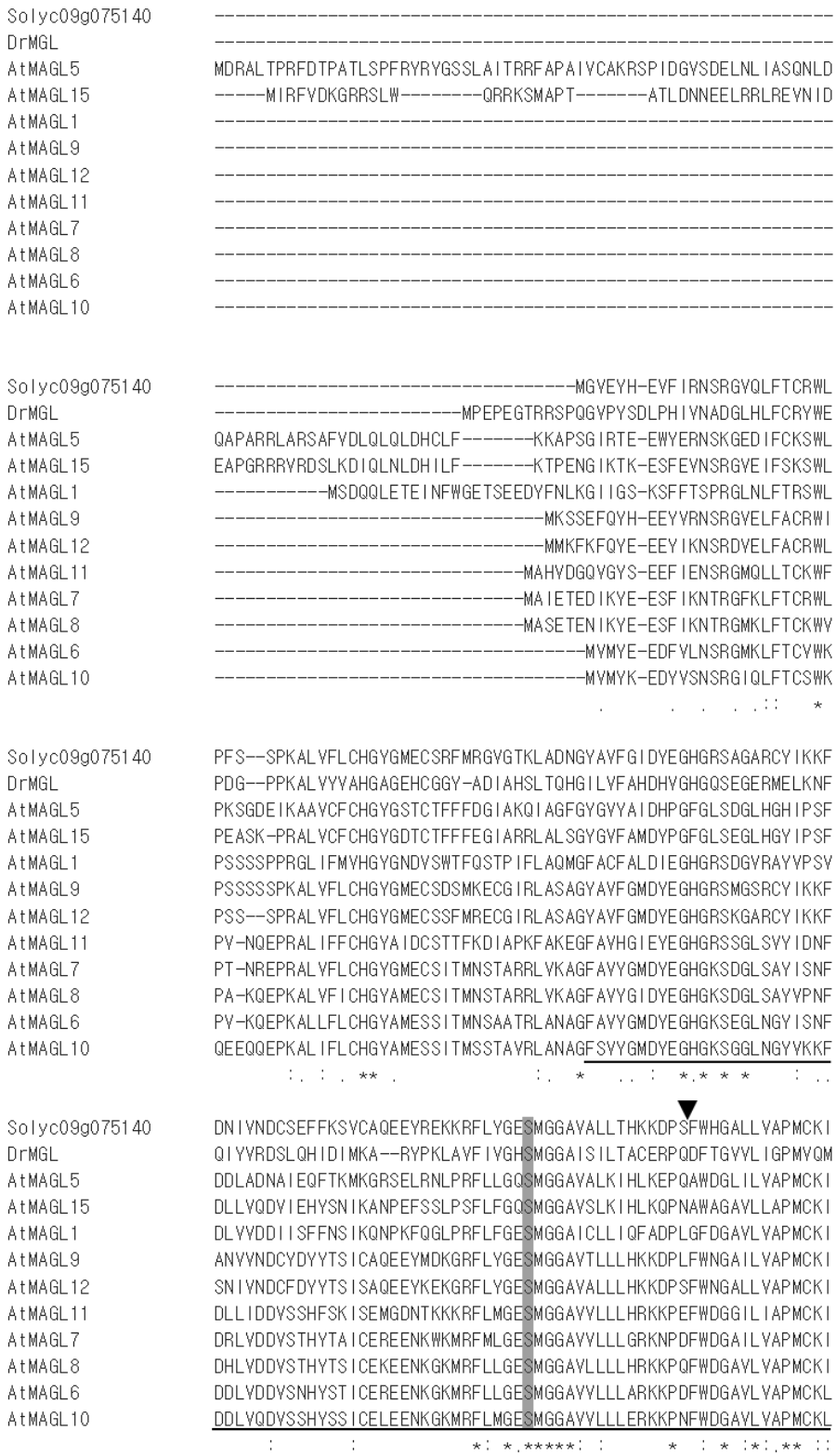


Figure 12. Phylogenetic tree of Solyc09g075140 and its homologs in other species. Phylogenetic tree of Solyc09g075140 made in MEGA7 software using Neighbor-Joining method based on amino acid sequence in tomato and Arabidopsis. *S. lycopersicum*, Solyc09g075140, *A. thaliana*, AtMAGL9 (NP_566106.1), *A. thaliana*, AtMAGL12 (NP_191845.1), *S. lycopersicum*, Solyc04g010250, *A. thaliana*, AtMAGL11 (NP_191079.1), *A. thaliana*, AtMAGL10 (NP_191078.1), *A. thaliana*, AtMAGL6 (NP_565903), *A. thaliana*, AtMAGL7 (NP_850316.1), *A. thaliana*, AtMAGL8 (NP_181474.2)), *S. lycopersicum*, Solyc05g009390, *A. thaliana*, AtMAGL5 (NP_177867.1) , *S. lycopersicum*, Solyc02g063200, *A. thaliana*, AtMAGL15 (NP_001119234), *S. lycopersicum*, Solyc02g063160, *S. lycopersicum*, Solyc02g086040, *S. lycopersicum*, Solyc03g119980, *S. lycopersicum*, Solyc05g056000, *S. lycopersicum*, Solyc03g006560, *A. thaliana*, AtMAGL1 (NP_172576.1). Numbers next to branch points are the percentage of replicate trees in which the associated taxa clustered together. The scale bar indicates the branch length corresponding to 0.050 amino acid substitutions per site.


Solyc09g075140 ---------- 0
DrMGL ---------- 0
AtMAGL5 MDRALTPRFDTPATLSPFRYRYGSSLAITRRFAPAIVCAKRSPIDGVSDELNLIASQNLD 60
AtMAGL15 -----MIRFVDKGRRSLW--------QRRKSMAPT--------ATLDNNEELRRLREVNID 40
AtMAGL1 ---------- 0
AtMAGL9 ---------- 0
AtMAGL12 ---------- 0
AtMAGL11 ---------- 0
AtMAGL7 ---------- 0
AtMAGL8 ---------- 0
AtMAGL6 ---------- 0
AtMAGL10 ---------- 0
Solyc09g075140 ----------MGVEYH-EVFIRNSRGVQLFTCRWL 24
DrMGL ----------MPEPEGTRRSPQGVPYSDLPHIVNADGLHLFCRYWE 36
AtMAGL5 QAPARRLARSAFYDLQLQLDHCLF--------KKAPSGIRTE-EWYERNSKGEDIFCKSWL 112
AtMAGL15 EAPGRRRYRDSLKDIQLNLDHILF--------KTPENGIKTK-ESFEVNSRGVEIFSKSWL 92
AtMAGL1 ----------MSDQQLETEINFWGETSEEDYFNLKGIIGS-KSFFTSPRGLNLFTRSWL 48
AtMAGL9 ----------MKSSEFQYH-EEYVRNSRGVELFACRWI 27
AtMAGL12 ----------MMKFKFQYE-EEYIKNSRDVELFACRWL 27
AtMAGL11 ----------MAHYDGQVGYS-EEFIENSRGMQLLTCKWF 29
AtMAGL7 ----------MAIETEDIKYE-ESFIKNTRGFKLFTCRWL 29
AtMAGL8 ----------MASETENIKYE-ESFIKNTRGMKLFTCKWY 29
AtMAGL6 ----------MVMYE-EDFYLNSRGMKLFTCVWK 23
AtMAGL10 ----------MVMYK-EDYVSNSRGIQLFTCSWK 23
PFS--SPKALVFLCHGYGMECSRFMRGVGTKLADNGYAVFGIDYEGHGRSAGARCYIKKF 82
PDG--PPKALVYVAHGAGEHCGGY-ADIAHSLTQHGILVFAHDHVGHGQSEGERMELKNF 93
PKSGDEIKAAVCFCHGYGSTCTFFFDGIAKQIAGFGYGVYAIDHPGFGLSDGLHGHIPSF 172
PEASK-PRALVCFCHGYGDTCTFFFEGIARRLALSGYGVFAMDYPGFGLSEGLHGYIPSF 151
PSSSSPPRGLIFMVHGYGNDVSWTFQSTPIFLAQMGFACFALDIEGHGRSDGVRAYVPSV 108
PSSSSSPKALVFLCHGYGMECSDSMKECGIRLASAGYAVFGMDYEGHGRSMGSRCYIKKF 87
PSS--SPRALVFLCHGYGMECSSFMRECGIRLASAGYAVFGMDYEGHGRSKGARCYIKKF 85
PV-NQEPRALIFFCHGYAIDCSTTFKDIAPKFAKEGFAVHGIEYEGHGRSSGLSVYIDNF 88
PT-NREPRALVFLCHGYGMECSITMNSTARRLVKAGFAVYGMDYEGHGKSDGLSAYISNF 88
PA-KQEPKALVFICHGYAMECSITMNSTARRLVKAGFAVYGIDYEGHGKSDGLSAYVPNF 88
PV-KQEPKALLFLCHGYAMESSITMNSAATRLANAGFAVYGMDYEGHGKSEGLNGYISNF 82
QEEQQEPKALIFLCHGYAMESSITMSSTAVRLANAGFSVYGMDYEGHGKSGGLNGYVKKF 83
DNIVNDCSEFFKSVCAQEEYREKKRFLYGESMGGAVALLTHKKDPSFWHGALLVAPMCKI 142
QIYVRDSLQHIDIMKA--RYPKLAVFIVGHSMGGAISILTACERPQDFTGVVLIGPMVQM 151
DDLADNAIEQFTKMKGRSELRNLPRFLLGQSMGGAVALKIHLKEPQAWDGLILVAPMCKI 232
DLLVQDVIEHYSNIKANPEFSSLPSFLFGQSMGGAVSLKIHLKQPNAWAGAVLLAPMCKI 211
DLVYDDIISFFNSIKQNPKFQGLPRFLFGESMGGAICLLIQFADPLGFDGAVLVAPMCKI 168
ANVVNDCYDYYTSICAQEEYMDKGRFLYGESMGGAVTLLLHKKDPLFWNGAILVAPMCKI 147
SNIVNDCFDYYTSISAQEEYKEKGRFLYGESMGGAVALLLHKKDPSFWNGALLVAPMCKI 145
DLLIDDVSSHFSKISEMGDNTKKKRFLMGESMGGAVVLLLHRKKPEFWDGGILIAPMCKI 148
DRLVDDVSTHYTAICEREENKWKMRFMLGESMGGAVVLLLGRKNPDFWDGAILVAPMCKI 148
DHLVDDVSTHYTSICEKEENKGKMRFLLGESMGGAVLLLLHRKKPQFWDGAVLVAPMCKI 148
DDLVDDVSNHYSTICEREENKGKMRFLLGESMGGAVVLLLARKKPDFWDGAVLVAPMCKL 142
DDLVQDVSSHYSSICELEENKGKMRFLMGESMGGAVVLLLERKKPNFWDGAVLVAPMCKL 143

```
Solyc09g075140      SEKVKPHPVYISLLTKVEDVIPRWK-IVPTKDVIDSAFKDPAKREEVRENKLIYQAKPRL  201
DrMGL               SAESATPFKV-FMAKVLNRLAPKLT-LGPID--PKFVSRDPKQVEAYEKDELNYHGGLRV  207
AtMAGL5             SEDVKPPPLYLKTLILMSTLFPKAK-LFPKRDLSDFFFRDLSKRKLCEYDVICYDDQTRL  291
AtMAGL15            ADDLVPPPVLKQILIGLANVLPKHK-LVPQKDLAEAGFRDIRKRDMTPYNMICYSGKPRL  270
AtMAGL1             SDKVRPKWPVDQFLIMISRFLPTWA-IVPTEDLLEKSIKVEEKKPIAKRNPMRYNEKPRL  227
AtMAGL9             SEKVKPHPIVINLLTRVEEIIPKWK-IVPTKDVIDAAFKDLVKREEVRNNKLIYQDKPRL  206
AtMAGL12            SEKVKPHPVVINLLTRVEDIIPKWK-IVPTKDVIDAAFKDPYKREEIRNNKLIYQDKPRL  204
AtMAGL11            AEEMKPSRMVISMINMVTNLIPSWKSIIHGPDILNSAIKLPEKRHEIRTNPNCYNGWPRM  208
AtMAGL7             AEEMKPSPFVISILTKLISIIPKWK-IIPSQDIIEISYKEPEIRKQVRENPLCSKGRPRL  207
AtMAGL8             AEEMKPSPLVISILAKLSGVIPSWK-IIPGQDIIETAFKQPEIRKQVRENPYCYKGRPRL  207
AtMAGL6             ADEIKPHPVYISILIKLAKFIPTWK-IVPGNDIIDIAIKEPHIRNQVRENKYCYKGRPRL  201
AtMAGL10            AEDIKPHPMVISFLTKLTRFIPTWK-IVPSNDIIDVAFKETHIRKQVRDNEYCYKGRPRL  202
                    : .     :     : .*   :       .  :         :        *:

Solyc09g075140      KTALEMLRTSMHLEESLHEVTVPFLVLHGEADIVTDPEISKALYEQASSKDKTIKLYPGM  261
DrMGL               SFGMQMLDATSRIERELPDIRWPFYILHGDADKLCDIRGSRLLYNEAKSTDKKLKVYEEA  267
AtMAGL5             KTAVELLNATRDIEMQVDKVSLPLLILHGDTDKVTDPTVSKFLHKHAVSQDKTLKLYPGG  351
AtMAGL15            RTAVEMLRTTQDIEKQLQEVSLPILILHGEADTVTDPSVSRELYEKAKSPDKKIVLYENA  330
AtMAGL1             GTVMELLRVTDYLGKKLKDVSIPFIIVHGSADAVTDPEVSRELYEHAKSKDKTLKIYDGM  287
AtMAGL9             KTALEMLRTSMNLEDTLHEITMPFFVLHGEADTVTDPEVSKALYEKASTRDKTLKLYPGM  266
AtMAGL12            KTALEMLRTSMDLEDTLHEITLPFFVLHGEADIVTDPEISKALFEKASTRDKTIKLYPGM  264
AtMAGL11            KTMSELFRISLDLENRLNEVTMPFIVLHGEDDKVTDKGGSKLLYEVALSNDKTLKLYPEM  268
AtMAGL7             KTAYELLRISNDLEKRLQEVSLPFLVLHGDDDKVTDKAVSQELYKVALSADKTLKLYPGM  267
AtMAGL8             KTAYELLRVSTDLEKRLNEVSLPFIVLHGEDDKVTDKAVSRQLYEVASSSDKTFKLYPGM  267
AtMAGL6             NTAYQLLLVSLDLEKNLHQVSIPFIVLHGEDDKVTDKSISKMLYEVASSSDKTFKLYPKM  261
AtMAGL10            KTAHQLLMVSLDLEKNLDQVSMPFIVLHGEDDKVTDKNVSKLLYEVASSSDKTFKLYPNM  262
                        :::   :   :    : .:   *: ::**. * : *   *: *.: * : **.: :*

Solyc09g075140      WHGLTYGEPEENIEIVFSDIISWLDKRNGENTGDASLIERSVCRATSTPPYEMHTVSSPA  321
DrMGL               YHALHH-DLPETIESVLKEVSTWILERYPAPQTS----------------------------  300
AtMAGL5             YHCILEGDTDENIFTVINDIVAWLDARYDPK-------------------------------  382
AtMAGL15            YHSLLEGEPDDMILRVLSDIISWLNDHSLQAEGSSVTTM-----------------------  369
AtMAGL1             MHSMLFGEPDDNIEIVRKDIVSWLNDRCGGDKTKTQV-------------------------  324
AtMAGL9             WHALTSGEPDCNVDLVFADIINWLDLRTADPASLTV---TPIRVGNTTS---VQRVTTVN  320
AtMAGL12            WHGLTSGEPDANVDLVFADIVNWLDARTGDSASLTV---TPYHDFTS------NVQKVYD  315
AtMAGL11            WHSLLFGEPPENSEIVFNDIVQWMQTRITTLQVKANNHEAKTSNLIT-----SDSV----  319
AtMAGL7             WHGLLTGETPENIEIVFADVISWLEKRSDYGNDRFESELKQRNDRLN-----FKK-----  317
AtMAGL8             WHGLLYGETPENIETVFADIIGWLDKKVADESGGFESELKRKNDGIP-----LKG-----  317
AtMAGL6             WHALLYGETNENSEIVFGDIINWLEDRATDSNGGLESQLKHKHDGFL-----KHK-----  311
AtMAGL10            WHGLLYGESPENLEIVFSDIISWLKERASVTNQKLETELKHYDDGFS-----MQK-----  312
                     * :   :       *  ::  *:  :

Solyc09g075140      TMKETKPHRTRPQANYLCGLKGRR--MHHHSSM        352
DrMGL               ---------------------------------        300
AtMAGL5             ---------------------------------        382
AtMAGL15            ---------------------------------        369
AtMAGL1             ---------------------------------        324
AtMAGL9             GVSNGHRRPKRPFFNLLCGLNRGR--LVPRSTV        351
AtMAGL12            GVSNGQGKSKRPQASLLCGLNGGGRRLVHRSSM        348
AtMAGL11            ---------------------------------        319
AtMAGL7             ---------------------------------        317
AtMAGL8             ---------------------------------        317
AtMAGL6             ---------------------------------        311
AtMAGL10            ---------------------------------        312
```

Figure 13. Protein sequence alignment of Solyc09g075140 and MAGLs. The predicted amino acid sequence alignments of *Solyc09g075140*, DrMGL (NP_956591.1), and 10 AtMAGLs by Clustal Omega. The position of premature stop codon in Solyc09g075140 of *naked* mutant is marked by a black triangle. The α/β-hydrolase domain is underlined and the catalytic triad (Ser, Asp, and His) residues are highlighted in grey color. AtMAGL1 (NP_172576.1), AtMAGL5 (NP_177867.1), AtMAGL6 (NP_565903), AtMAGL7 (NP_850316.1), AtMAGL8 (NP_181474.2), AtMAGL9 (NP_566106.1), AtMAGL10 (NP_191078.1), AtMAGL11 (NP_191079.1), AtMAGL12 (NP_191845.1), AtMAGL15 (NP_001119234). Species prefixes are as follows: Solyc, *Solanum lycopersicum*; Dr, *Danio rario*; At, *Arabidopsis thaliana*.

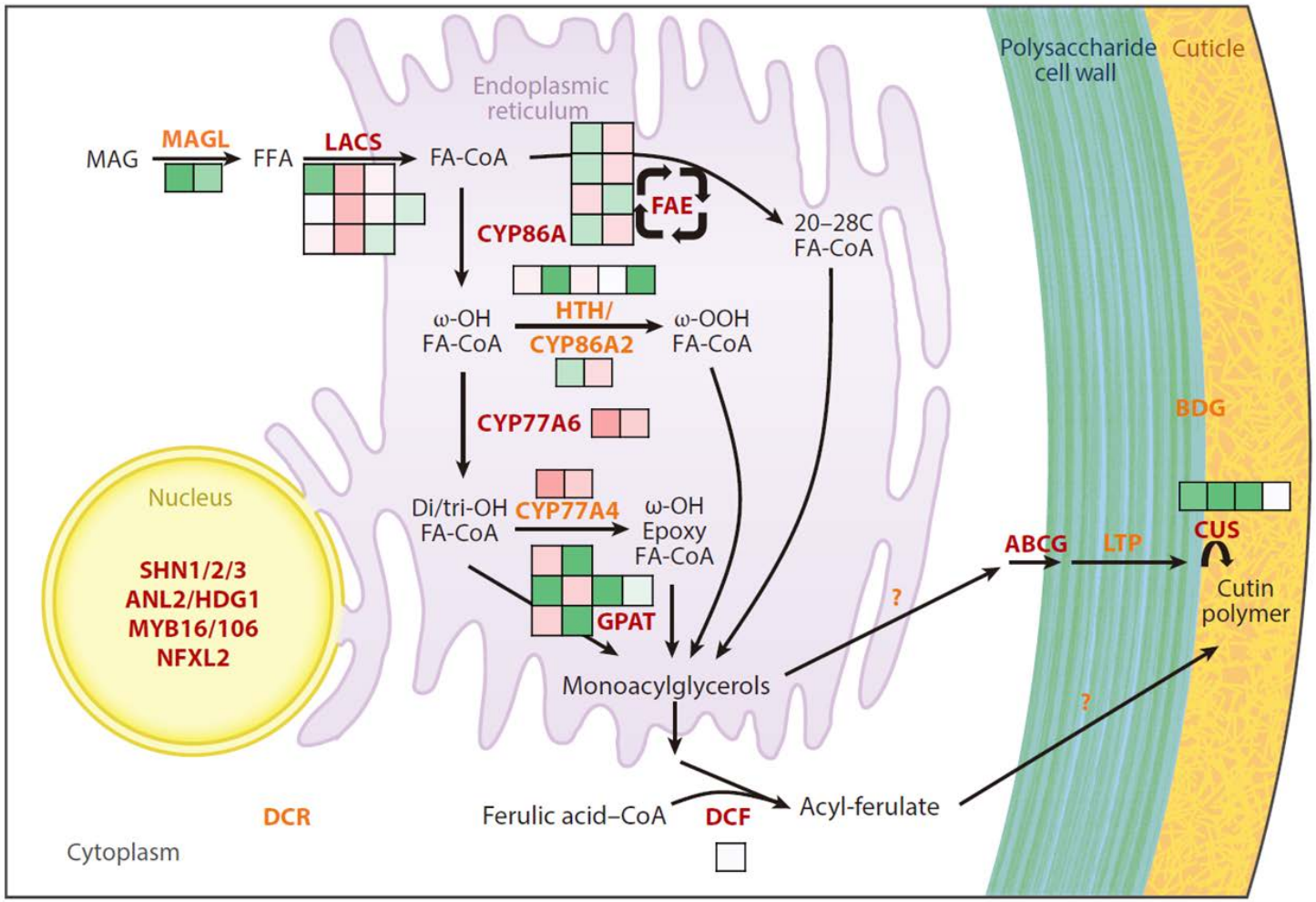

Endoplasmic reticulum
Polysaccharide cell wall
Cuticle
MAG
MAGL
FFA
LACS
FA-CoA
FAE
20–28C FA-CoA
CYP86A
ω-OH FA-CoA
HTH/ CYP86A2
ω-OOH FA-CoA
CYP77A6
Di/tri-OH FA-CoA
CYP77A4
ω-OH Epoxy FA-CoA
GPAT
Monoacylglycerols
?
ABCG
LTP
BDG
CUS
Cutin polymer
?
Nucleus
SHN1/2/3
ANL2/HDG1
MYB16/106
NFXL2
DCR
Ferulic acid–CoA
DCF
Acyl-ferulate
Cytoplasm

Figure 14. The expression level of biosynthetic and regulatory factors that control cutin synthesis and their localization in epidermal cells. Names shown in red denote proteins, or protein complexes, with a demonstrated function; names shown in orange denote proteins with an unknown or speculative function. Red squares represent genes or proteins that were significantly up-regulated in *naked*; green squares represent genes or proteins that were significantly down-regulated in *naked*. Abbreviations: C, carbon; CoA, coenzyme A; FA, fatty acid; FAE, fatty acid elongase complex; FAS, fatty acid synthase complex; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase. For steps involving multiple paralogs, only the gene subfamily name is given; for the names and IDs of each gene, in Table 4.

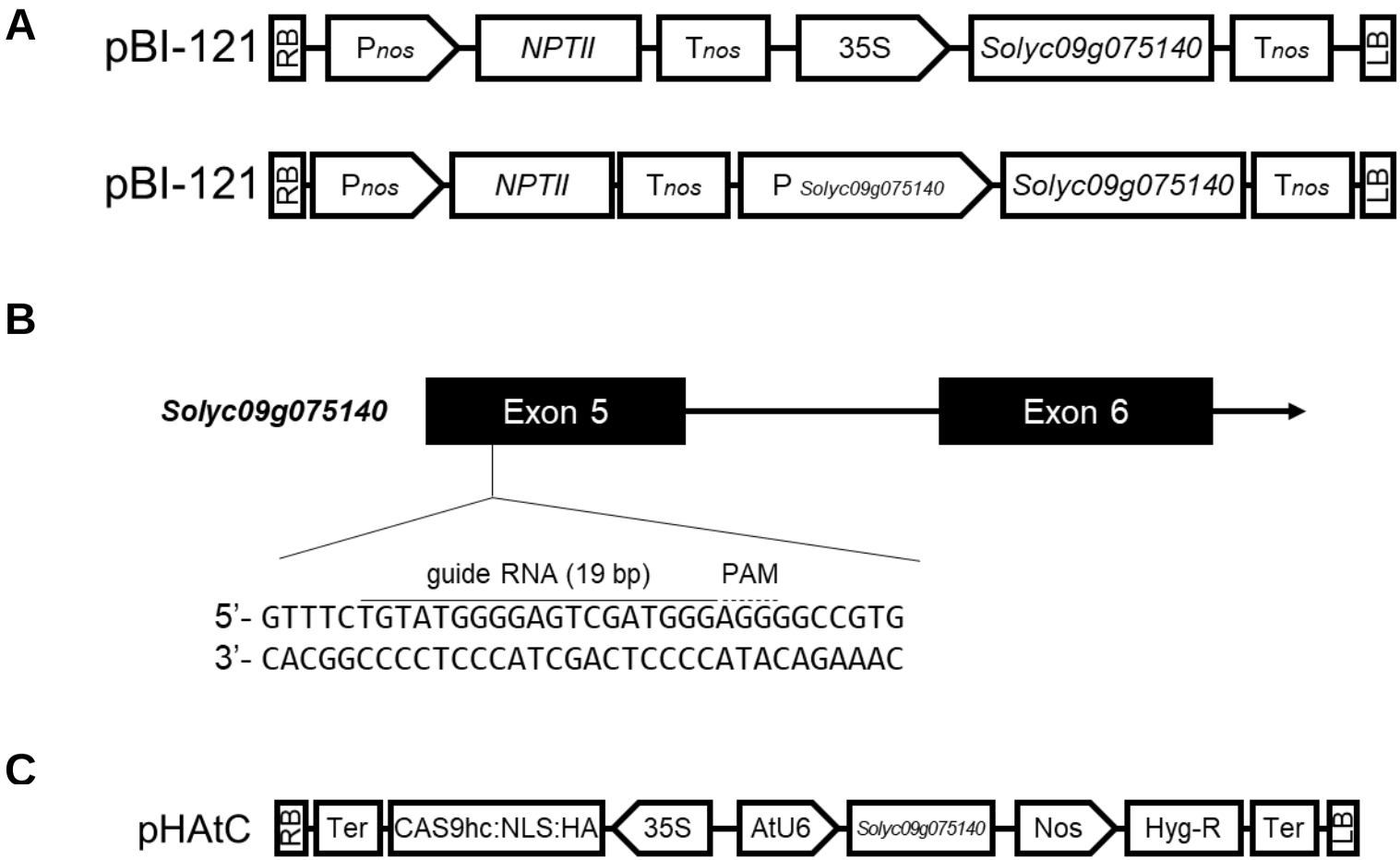

A
pBI-121
RB
Pnos
NPTII
Tnos
35S
Solyc09g075140
Tnos
LB
pBI-121
RB
Pnos
NPTII
Tnos
P Solyc09g075140
Solyc09g075140
Tnos
LB
B
Solyc09g075140
Exon 5
Exon 6
guide RNA (19 bp)
PAM
5'- GTTTCTGTATGGGGAGTCGATGGGAGGGGCCGTG
3'- CACGGCCCCTCCCATCGACTCCCCATACAGAAAC
C
pHAtC
RB
Ter
CAS9hc:NLS:HA
35S
AtU6
Solyc09g075140
Nos
Hyg-R
Ter
LB

Figure 15. Schematic information of binary vectors for plant transformation. (A) Vectors for overexpression of *Solyc09g075140* with 35S promoter and the native promoter. **(B)** and **(C)** Guide RNA sequence of *Solyc09g075140* used for knock-out (B) and schematic diagram of CRISPR-Cas9 vector containing *Solyc09g075140* guide RNA (C).

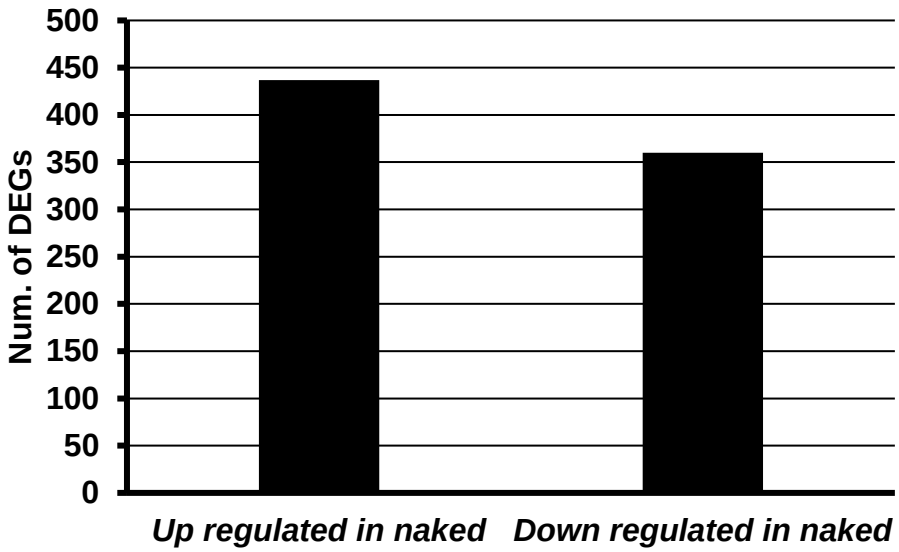


Figure 16. Number of identified DEGs in *naked*.

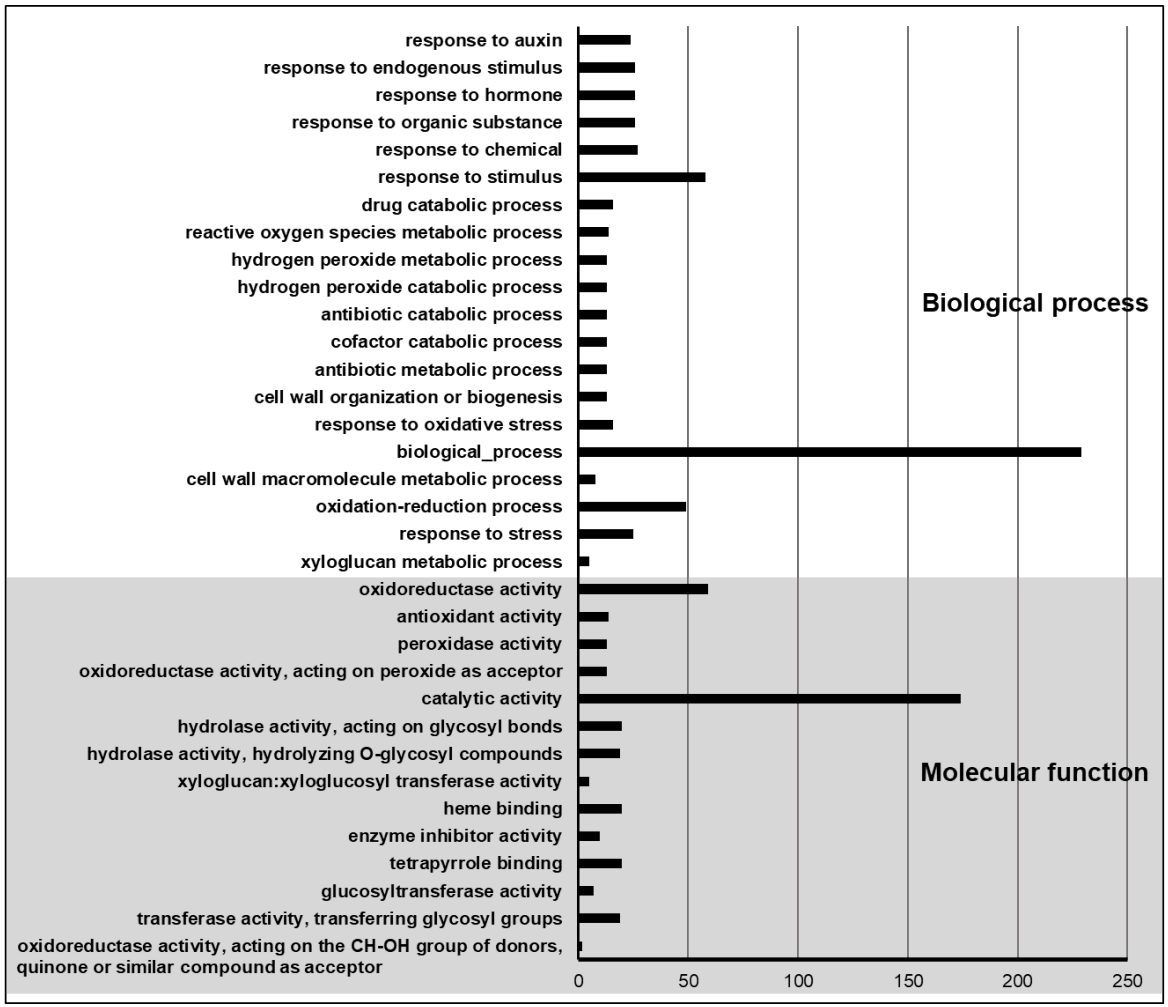

response to auxin
response to endogenous stimulus
response to hormone
response to organic substance
response to chemical
response to stimulus
drug catabolic process
reactive oxygen species metabolic process
hydrogen peroxide metabolic process
hydrogen peroxide catabolic process
antibiotic catabolic process
cofactor catabolic process
antibiotic metabolic process
cell wall organization or biogenesis
response to oxidative stress
biological_process
cell wall macromolecule metabolic process
oxidation-reduction process
response to stress
xyloglucan metabolic process
oxidoreductase activity
antioxidant activity
peroxidase activity
oxidoreductase activity, acting on peroxide as acceptor
catalytic activity
hydrolase activity, acting on glycosyl bonds
hydrolase activity, hydrolyzing O-glycosyl compounds
xyloglucan:xyloglucosyl transferase activity
heme binding
enzyme inhibitor activity
tetrapyrrole binding
glucosyltransferase activity
transferase activity, transferring glycosyl groups
oxidoreductase activity, acting on the CH-OH group of donors, quinone or similar compound as acceptor
Biological process
Molecular function
0
50
100
150
200
250

Figure 17. Functional analysis and classification of DEGs with Gene GO term (up-regulation). The top 20 Gene ontology (GO) terms of up-regulated DEGs in *naked*. The DEGs were summarized in 2 main GO categories, biological process and molecular function. The X-axis shows the number of DEGs while the Y-axis represents the GO terms.

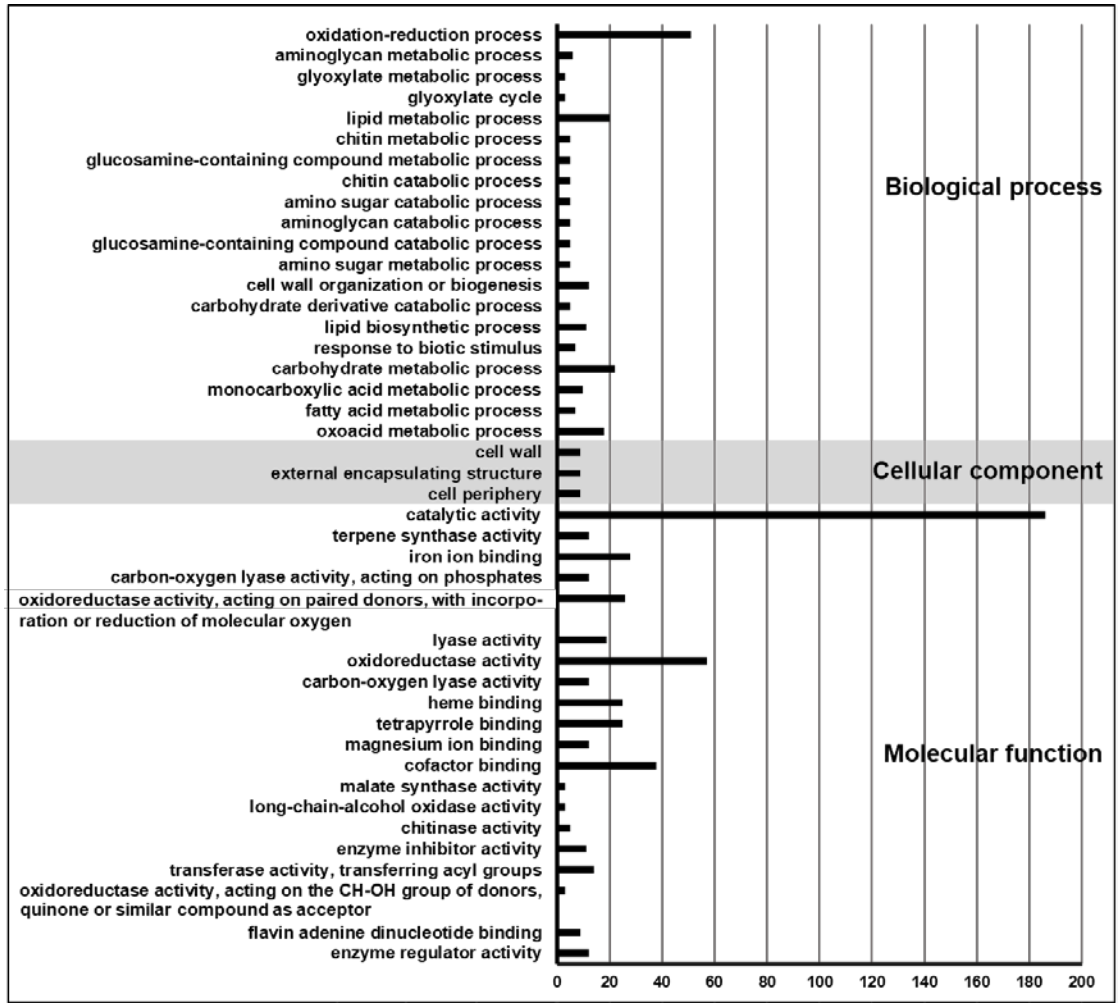

oxidation-reduction process
aminoglycan metabolic process
glyoxylate metabolic process
glyoxylate cycle
lipid metabolic process
chitin metabolic process
glucosamine-containing compound metabolic process
chitin catabolic process
amino sugar catabolic process
aminoglycan catabolic process
glucosamine-containing compound catabolic process
amino sugar metabolic process
cell wall organization or biogenesis
carbohydrate derivative catabolic process
lipid biosynthetic process
response to biotic stimulus
carbohydrate metabolic process
monocarboxylic acid metabolic process
fatty acid metabolic process
oxoacid metabolic process
cell wall
external encapsulating structure
cell periphery
catalytic activity
terpene synthase activity
iron ion binding
carbon-oxygen lyase activity, acting on phosphates
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen
lyase activity
oxidoreductase activity
carbon-oxygen lyase activity
heme binding
tetrapyrrole binding
magnesium ion binding
cofactor binding
malate synthase activity
long-chain-alcohol oxidase activity
chitinase activity
enzyme inhibitor activity
transferase activity, transferring acyl groups
oxidoreductase activity, acting on the CH-OH group of donors, quinone or similar compound as acceptor
flavin adenine dinucleotide binding
enzyme regulator activity
Biological process
Cellular component
Molecular function
0
20
40
60
80
100
120
140
160
180
200

Figure 18. Functional analysis and classification of DEGs with GO term (down-regulation). The top 20 Gene ontology (GO) terms of down-regulated DEGs in *naked*. The DEGs were summarized in 3 main GO categories, biological process, cellular component , and molecular function. The X-axis shows the number of DEGs while the Y-axis represents the GO terms.

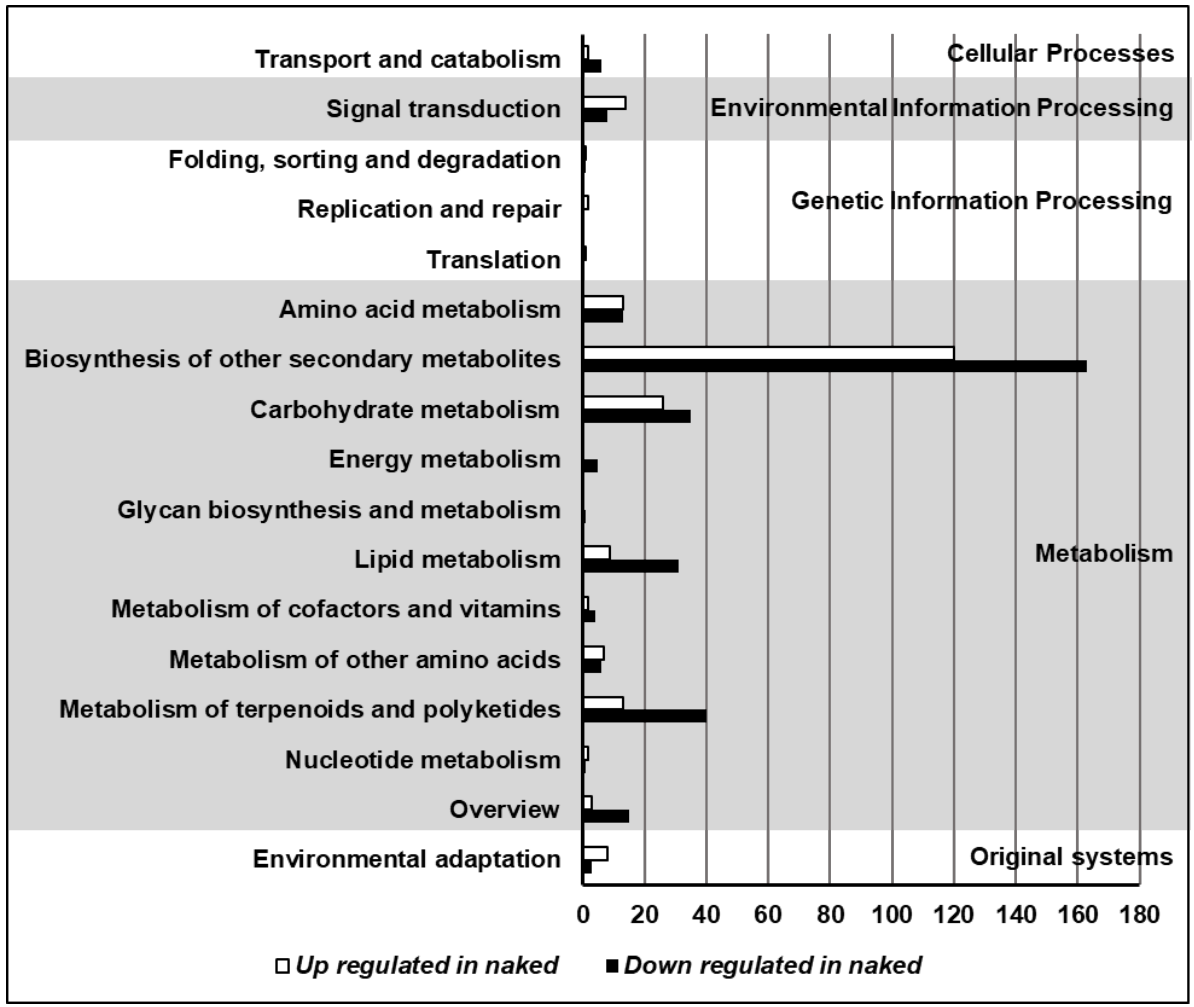

Transport and catabolism
Signal transduction
Folding, sorting and degradation
Replication and repair
Translation
Amino acid metabolism
Biosynthesis of other secondary metabolites
Carbohydrate metabolism
Energy metabolism
Glycan biosynthesis and metabolism
Lipid metabolism
Metabolism of cofactors and vitamins
Metabolism of other amino acids
Metabolism of terpenoids and polyketides
Nucleotide metabolism
Overview
Environmental adaptation
Cellular Processes
Environmental Information Processing
Genetic Information Processing
Metabolism
Original systems
0 20 40 60 80 100 120 140 160 180
Up regulated in naked
Down regulated in naked

Figure 19. Functional analysis and classification of DEGs with KEGG.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of significantly DEGs between the wild-type (WT) and *naked* mutants. The X-axis shows the number of DEGs while the Y-axis represents the sub classification.

Table 1. Description of PCR-based mapping markers.

Marker name	Map unit (Mb)	Primer sequence		PCR size M82/LA1589 (bp)	Mapping enzyme	Digest size M82/LA1589 (bp)
		Forward (5'-3')	Reverse (5'-3')			
Solyc09g074280	66.50	GAGACGATGAACTTGAAATGTCATG	TAAGAATCAAGCTGTTTCAATCTGC	387/387	*Bsr*GI	387/129+258
Solyc09g074530	66.78	AGCATTCAGTTCTTCACACATCG	ACGAATGGTGTAATATAATTAGGAGG	180/158	PCR	
Solyc09g074700	66.94	AATGCAGATCCCTTGGCGG	ACCGTGACCATTTGTGTGGC	325/325	*Hae*III	325/201+124
Solyc09g074970	67.20	TACTAGTGTATGATGTGTTAAGGG	ATGGTGTTAACAAAGTAGGCCTC	153/234	PCR	
Solyc09g075010	67.22	AAGTTCATTAGTCTGCCTCTCG	TACACCACATATTGTGAGAGTGG	213/243	PCR	
Solyc09g075050	67.30	ATGAACAGTGGCTATGTTGAGC	AAGCAAGTGAGATTAGACTGCAC	195/168	PCR	
Solyc09g075220	67.42	TACTACTTGGTTGCTCACTGAC	AGCTTCAAGAATATTCTCCAACTG	153/172	PCR	
Solyc09g075270	67.44	TCGAGCAAACCTTGAATATGGG	AATCCTACCTGATTCTGACGAC	413/413	*Eco*RV	413/283+130
Solyc09g075360	67.51	TTGATGTGATTGGTGGCTGTG	AACCCAAGTTCAGGATTCTACC	321/403	PCR	

Table 2. Description of PCR primers used in this study.

Gene	Purpose	Primer sequence	
		Forward (5'-3')	Reverse (5'-3')
Solyc03g078400	Control (Actin)	GGGATGGAGAAGTTTGGTGGTGG	CTTCGACCAAGGGATGGTGTAGC
Solyc09g075040		TTGATGGTTGCTCCCTGGTG	TCAGACGCCCATAAATGCCC
Solyc09g075070		TTCTCCACCAGATCAGTTTGAG	AGAATGAAGTACTTTATCTTCAATCTG
Solyc09g075130		TTGAACTAGAGCGCCGAACG	TCACAGAATCTCCCCGAAGG
Solyc09g075170		ATCTCAGGTCTTGCGAAACATG	TCTCCACACATCCTAGCTCC
Solyc09g075190		TGGATGTGATAGAATCTCAGTGG	AGAACAGATACGGATGGGCG
Solyc09g075210	RT-PCR	AGGTGCAGTTTCCGGTGTAG	AAGCCCGAAGATTAACACTAATAC
Solyc09g075220		TCCTTACAACTCTACCGCCG	ATTGTAAGTGGGAAAGCTAGCC
Solyc09g075230		TCCTACTCCTACTCCAACTCTC	ACGAGTAACTTCGACAATATTAATCC
Solyc09g075260		AATTGTTAACTAAAATTTTCAGAGATTGG	ATTTCCATAAGAGTCCACGGAG
Solyc09g075270		ATGGGGATTCTTCCAAAAGGAG	TCAACACAATTCAATCCACGTCC
Solyc09g075320		ACGCTAATCGATTCCTCCTCC	ACCATTATTACAACTTCATCTTCTCC
Solyc09g075140	5' region amplifying	TTACTTACCCATGAATGTTGAGTC	ACCTTCATAATCTATTCCAAACACC
	3' region amplifying	AAGCTGGCAGATAATGGATATGC	TGAAGGAGATGCAACACGCC
	3' downstream region amplifying	TAACACACAAGAAGGATCCTTCC ATCAGGCAAAGCCAAGACTAAAG	TTACTGTTCACGTTTATCTGATAGG
	qRT-PCR	TGATTCGGCCTTCAAGGACC	TATGTCTGCTTCCCCATGTAAC
	Cloning (Promoter)	GCGCCTGCAGGAAAGGGAGAAGCGTGTATGAAGT	GCATCTAGAATATGATGAGGAGAAGTGTATTCG
	Cloning (CDS)	GTCTCTAGAATGGGTGTTGAATATCATGAGGTATT	CGCCTCGAGCTACATAGATGAATGATGATGCATTC
	gRNA	GATTGTGTATGGGGAGTCGATGGG	AAACCCCATCGACTCCCCATACAC
Solyc09g075260	RT-PCR	AATTGTTAACTAAAATTTTCAGAGATTGG	ATTTCCATAAGAGTCCACGGAG
	Full length amplifying	ATACATGATAAGCTAATTTAATGCTCG	AACAATTCAAGTGGAAGAGATAAGC
	Promoter region amplifying	ATAATGTGCCTAGAGATGCAAGC	TCTTGTTGCAATGTATCTCGAGC

Table 3. List of genes in the mapping region between marker Solyc09g075010 and Solyc09g075360. *: DEG comparison obtained from RNA-seq. data between WT and *naked* stems.

Name	Description	*naked* vs WT *
Solyc09g075010	HSP20-like chaperones superfamily protein	0.07
Solyc09g075020	Multidrug resistance protein ABC transporter family protein	0.26
Solyc09g075030	Nucleolar 16	-0.04
Solyc09g075040	E3 ubiquitin-protein ligase	0.07
Solyc09g075050	Mannose-binding lectin superfamily protein	0.10
Solyc09g075060	Beta-glucosidase	0.39
Solyc09g075070	Beta-glucosidase	0.69
Solyc09g075080	Phytochrome A-associated F-box protein	0.27
Solyc09g075090	RNA-binding (RRM/RBD/RNP motifs) family protein	0.00
Solyc09g075100	Complex 1 family protein	0.08
Solyc09g075110	Vacuolar sorting protein 39	0.60
Solyc09g075120	UDP-glucuronic acid decarboxylase 1	-0.03
Solyc09g075130	Small nuclear ribonucleoprotein family protein	-0.36
Solyc09g075140	alpha/beta-Hydrolases superfamily protein; Lipase-like protein	-3.24
Solyc09g075150	60S ribosomal protein L22-2	0.19
Solyc09g075160	60S ribosomal protein L22-2	0.06
Solyc09g075170	Pentatricopeptide repeat (PPR) superfamily protein	-0.38
Solyc09g075180	cryptochrome DASH family protein	0.19
Solyc09g075190	Gamma-tubulin complex component	-0.11
Solyc09g075200	U1 small nuclear ribonucleoprotein	-0.03
Solyc09g075210	Late embryogenesis abundant protein Lea5	1.68
Solyc09g075220	LOW QUALITY:pectinesterase	0.47
Solyc09g075230	pectinesterase (Protein of unknown function, DUF538)	0.03
Solyc09g075260	Pectinesterase	-3.59
Solyc09g075270	transmembrane protein	-0.49
Solyc09g075280	exocyst complex component sec15B	0.06
Solyc09g075290	60S ribosomal protein L18	-0.02
Solyc09g075300	Epoxide hydrolase, putative	-0.30
Solyc09g075310	Unknown Protein	-
Solyc09g075320	RING/U-box superfamily protein	-0.69
Solyc09g075330	Pectinesterase	5.15
Solyc09g075350	Pectinesterase	-0.33
Solyc09g075360	4-beta-glucanase precursor	-0.04

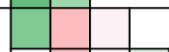
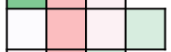
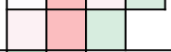
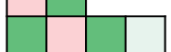
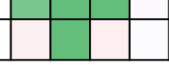
Gene family	Gene name	Gene ID (*At*)	Gene ID (*Sl*)	Function	*naked* vs WT*
Monoacylglycerol lipase	*MAGL12*	AT3G62860	**Solyc09g075140**, Solyc04g010250	Hydrolysis of monoacylglycerides	
Long-chain acyl-CoA synthetase	*LACS1*	AT2G47240	Solyc01g079240, Solyc08g008310, Solyc08g082280	Attachment of CoA to free fatty acids	
	LACS2	AT1G49430	Solyc01g109180, Solyc08g008310, Solyc08g082280, Solyc01g095745		
	LACS3	AT1G64400	Solyc08g082280, Solyc08g008310, Solyc01g095745		
Cytochrome P450	*CYP86A2*	AT4G00360	Solyc01g094750, Solyc08g081220	ω-Hydroxylase; diacid synthesis	
	CYP86A4	AT1G01600	Solyc01g094750, Solyc08g081220		
	CYP86A7	AT1G63710	Solyc08g081220, Solyc01g094750		
	CYP86A8	AT2G45970	Solyc01g094750, Solyc08g081220		
Cytochrome P450	*CYP77A6*	AT3G10570	Solyc05g055400, Solyc11g007540	Midchain hydroxylase	
Cytochrome P450	*CYP77A4*	AT5G04660	Solyc05g055400, Solyc11g007540	Epoxidase	
Glycerol-3-phosphateacyltransferase	*GPAT4*	AT1G01610	Solyc01g094700, Solyc09g014350	Synthesis of 2-monoacylglycerols	
	GPAT6	AT2G38110	Solyc09g014350, Solyc01g094700, Solyc10g084900, Solyc07g056320		
	GPAT8	AT4G00400	Solyc01g094700, Solyc09g014350		
BAHD feruloyl transferase	*DCF*	AT3G48720	Solyc03g097500	Transfer of ferulate from CoA to acyl group	
GDSL lipase (cutin synthase)	*CUS1*	AT3G04290	Solyc04g050730, Solyc04g050570, Solyc11g006250, Solyc06g083650	Polymerization of 2-monoacylglycerols monomers	
None	*HOTHEAD*	AT1G72970	Solyc06g062600, Solyc03g121600, Solyc08g080190, Solyc08g080200, Solyc06g035580	Diacid synthesis	

Table 4. Genes involved in cutin biosynthesis, transport, and regulation in Arabidopsis and tomato. Names shown in red denote proteins, or protein complexes, with a demonstrated function; names shown in orange denote proteins with an unknown or speculative function. Red squares represent genes or proteins that were significantly up-regulated in *naked*; green squares represent genes or proteins that were significantly down-regulated in *naked*. Species prefixes are as follows: *Sl*, *Solanum lycopersicum*; *At*, *Arabidopsis thaliana*. *: DEG comparison obtained from RNA-seq. data between WT and *naked* stems.

Table 5. Summary of transcriptome sequencing

Sample name		Num. of raw reads	Num. of trimmed reads	Num. of mapped reads
WT	Sample 1	20,589,815	20,589,815	19,234,631
	Sample 2	22,505,430	22,505,430	22,770,619
	Sample 3	15,602,866	15,602,866	13,884,541
naked	Sample 1	22,930,335	22,930,335	21,463,867
	Sample 2	16,907,944	16,907,944	14,969,348
	Sample 3	22,836,773	22,836,773	21,305,891

Discussion

The plant epidermal surface provides a powerful protective barrier against various biotic and abiotic stress. Trichomes, the epidermal outgrowths covering most aerial plant tissues, are found in a very large number of plant species and are composed of uni- or multicellular structures. The genetic analysis of trichome development has been done extensively in the Rosids including Arabidopsis and cotton, which are composed of unicellular structure trichome. The homologs responsible for unicellular trichome development in Rosids were analyzed in Asterids to find multicellular structure development pathways, but the results showed no correlation. In tomato, the molecular mechanisms of trichome development are still far from being fully understood. The mutant analysis is an effective approach to explore the function of genes in trichome development. Thus, we used forward genetics to find genes involved in the multicellular trichome development pathway.

In this study, we used tomato mutant *naked,* which has a young stem trichome absent phenotype. A map-based cloning strategy was used to select a potential candidate gene responsible for the young stem trichome absent phenotype. Genetic mapping experiments narrowed down the physical distance of the mapping region about 250 kb. This region is predicted to contain 31 hypothetical genes. To predict the located of the genes, Sol Genomics Network (https://solgenomics.net/jbrowse_solgenomics/) was used for annotation of the sequence. Among the 31 hypothetical genes, protein families of the 13 genes have been identified to either regulate the development

of trichomes or epidermal cells in previous studies. For example, *Solyc09g075040* gene is predicted to encode an E3 ubiquitin ligase. Previous studies had suggested that the E3 ubiquitin ligase regulates branching of trichome in Arabidopsis (Downes *et al*., 2003). The *Solyc09g075060*, *Solyc09g075070* gene is predicted to encode a β-glucosidase protein which is involved with the densities of trichome in Arabidopsis. (Singh *et al*., 2016). The *Solyc09g075130* gene is predicted to encode a small nuclear ribonucleoprotein (snRNP), which is involved in branching and density in trichome (Swaraz *et al*., 2011). The *Solyc09g075140* gene is predicted to encode α/β-hydrolase, which is known to control the cuticle development of the epidermis (Kurdyukov *et al*., 2006).

Through RT-PCR and genomic DNA PCR results, we identified that 3' region of *Solyc09g075140* gene was deleted in the *naked* mutant. *Solyc09g075140* gene is expected to encode α/β-hydrolase and has been found to belong to monoacylglycerol lipase 9 (MAGL9) which is homologs to Arabidopsis. Generally, MAGL acts to breakdown monoacylglycerol into fatty acid and glycerol, but its function is not well known in plants. MAGLs have been identified to function in cytosol and plasma membranes in humans and rabbits (Gkini *et al*., 2009). Animal MAGL represents the specificity of MAG hydrolysis and can effectively hydrolyze substrates with various double bonds and acyl chain lengths (Labar *et al*., 2010).

We also revealed that *Solyc09g075140* gene could be involved in the biosynthesis pathways of cutin, a component of the cuticle layer of the epidermal cell in the cytosol (Fich *et al*., 2016). We found the genes related to cutin biosynthesis in tomato and compared them with RNA-Seq. data. We

confirmed that the cutin synthases were down-regulated in the *naked* mutant. Previous studies have shown that trichome development is repressed if the cutin synthesis gene is defective (Kurdyukov *et al*., 2006). It is expected that poor synthesis of the cutin affects the epidermal cells, ultimately causing the *naked* mutant phenotype. Considering these findings, the *Solyc09g075140* gene is highly similar to the genes encoding MAGL, which is considered responsible for the *Naked* gene. Based on our genetic analysis, we assume that the mutation in *Solyc09g075140* may be responsible for the absence of trichomes on the young stem in the *naked* mutant, although the complementation experiment of the *naked* mutant must be carried out to confirm this. We are currently executing this experiment with the *naked* mutant by expressing the wild-type *Solyc09g075140* gene through the 35S promoter or its native promoter.

In addition to the defects in trichome development, *naked* mutants also exhibit several developmental phenotypes, including stem length, branch number, and fruit color. It is thus possible that *Naked* serves a primary role in the plant developmental process, the perturbation of which alters trichome- and defense-related traits. According to RNA-Seq. data, genes related to lipid metabolism and secondary metabolism are predominantly down-regulated in the *naked* mutant. It is assumed that the function of MAGL is critical in lipid metabolism. It is also predicted that the absence of trichomes on the young stem in *naked* mutant contributes to the down-regulation to secondary metabolism-related genes.

In conclusion, the identification of the *Naked* genes will provide the basis for multicellular trichome development research. In addition, revealing the

pathway of the trichome development will provide a cornerstone for plant breeding in terms of metabolic engineering and enhancing plant stress tolerance.

References

A.M.Swaraz, Young-Doo Park, Yoonkang Hur. (2011) Knock-out mutations of Arabidopsis *SmD3-b* induce pleotropic phenotypes through altered transcript splicing. *Plant Science*. **180**(5): 661-671.

Aaron T. Simmons, Geoff M. Gurr. (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. *Agricultural and Forest Entomology*. **7**(4): 265-276.

Amanda R. Walker, Paul A. Davison, Agnese C. Bolognesi-Winfield, Celia M. James, N. Srinivasan, Tom L. Blundell, Jeffrey J. Esch, M. David Marks, John C. Gray. (1999) The *TRANSPARENT TESTA GLABRA1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. *Plant Cell*. **11**:1337-1350.

An Yan, Minjie Wu, Yongqin Zhao, Aidong Zhang, Bohan Liu, John Schiefelbein, Yinbo Gan. (2014) Involvement of C2H2 zinc finger proteins in the regulation of epidermal cell fate determination in Arabidopsis. *Journal of Integrative Plant Biology*. **56**(12): 1112–1117.

Brian P. Downes, Robert M. Stupar, Derek J. Gingerich, Richard D. Vierstra. (2003) The HECT ubiquitin-protein ligase (UPL) family in Arabidopsis: UPL3 has a specific role in trichome development. *The Plant Journal*. **35**(6): 729-742.

Changxian Yang, Hanxia Li, Junhong Zhang, Zhidan Luo, Pengjuan Gong, Chanjuan Zhang, Jinhua Li, Taotao Wang, Yuyang Zhang, Yong'en Lu, Zhibiao Ye. (2011) A regulatory gene induces trichome formation and embryo lethality in tomato. *PNAS*. **108**(29): 11839-11841.

Christine Bernhardt, Myeong Min Lee, Antonio Gonzalez, Fan Zhang, Alan Lloyd, John Schiefelbein. (2003) The bHLH genes *GLABRA3 (GL3)* and *ENHANCER OF GLABRA3 (EGL3)* specify epidermal cell fate in the Arabidopsis root. *Development*. **130**:6431-6439.

Daniel Perazza, Michel Herzog, Martin Hülskamp, Spencer Brown, Anne-Marie Dorne, Jean-Marc Bonneville. (1999) Trichome Cell Growth in *Arabidopsis thaliana* Can Be Derepressed by Mutations in at Least Five Genes. *Genetics*. **152**(1): 461-176.

David G. Oppenheimer, Patricia L. Herman, Shan Sivakumaran, Jeffrey Esch, M. David Marks. (1991) A *myb* gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. *Cell*. **67**(3): 483-493.

David L. Ollis, Paul D. Carr. (2009) α/βHydrolase Fold: An Update. *Protein & Peptide Letters*. **16**(10): 1137-1148.

Eleni Gkini, Dimitris Anagnostopoulos, Mary Mavri-Vavayianni, Athanasia Siafaka-Kapadai. (2009) Metabolism of 2-acylglycerol in rabbit and human platelets. Involvement of monoacylglycerol lipase and fatty acid amide hydrolase. *Platelets*. **20**(6): 376-385.

G. Labar, J. Wouters, D.M. Lambert. (2010) A review on the monoacylglycerol lipase: at the interface between fat and endocannabinoid signalling. *Current Medicinal Chemistry*. **17**(24): 2588-2607.

George J. Wagner. (1991) Secreting Glandular Trichomes: More than Just Hairs. *Plant physiology*. **96**(3): 675–679.

Hyeran Kim, Sang-Tae Kim, Jahee Ryu, Min Kyung Choi, Jiyeon Kweon, Beum-Chang Kang, Hyo-Min Ahn, Suji Bae, Jungeun Kim, Jin-Soo Kim, Sang-Gyu Kim. (2016) A simple, flexible and high-throughput cloning system for plant genome editing via CRISPR-Cas system. *Journal of Integrative Plant Biology*. 58(8): 705-712.

Jiang Chang, Ting Yu, Qihong Yang, Changxing Li, Cheng Xiong, Shenghua Gao, Qingmin Xie, Fangyan Zheng, Hanxia Li, Zhendong Tian, Changxian Yang, Zhibiao Ye. (2018) *Hair*, encoding a single C2H2 zinc-finger protein, regulates multicellular trichome formation in tomato. *The Plant Journal*. **96**(1): 90-102.

Jie Ju, Hao Bai, Yongmei Zheng, Tianyi Zhao, Ruochen Fang, Lei Jianga. (2012) A multi-structural and multi-functional integrated fog collection system in cactus. *Nature Communications*. **3**: 1247.

Jin-Ho Kang, Marcelo L. Campos, Starla Zemelis-Durfee, Jameel M. Al-Haddad, A. Daniel Jones, Frank W. Telewski, Federica Brandizzi, Gregg A. Howe. (2016) Molecular cloning of the tomato *Hairless* gene implicates actin dynamics in trichome-mediated defense and mechanical

properties of stem tissue. *Journal of Experimental Botany*. **67**(18): 5313–5324.

John C. Larkin, Jason D. Walker, Agnese C. Bolognesi-Winfield, John C. Gray, Amanda R. Walker. (1999) Allele-specific interactions between *ttg* and *gl1* during trichome development in *Arabidopsis thaliana. Genetics*. **151**(4): 1591-1604.

Ling Xiao, Hexin Tan, Lei Zhang. (2016) *Artemisia annua* glandular secretory trichomes: the biofactory of antimalarial agent artemisinin. *Science Bulletin*. **61**(1): 26-36.

M. David Marks, Kenneth A. Feldmann. (1989) Trichome Development in Arabidopsis thaliana. I. T-DNA Tagging of the GLABROUS1 Gene. *Plant Cell*. **1**(11): 1043-1050.

Maarten Koornneef. (1981) The complex syndrome of *ttg* mutants. *Arabidopsis Inf Serv*. **18**:45-51.

Marie Karlsson, Juan A. Contreras, Ulf Hellman, Hans Tornqvist, Cecilia Holm. (1997) cDNA Cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. *Journal of Biological Chemistry*. **272**: 27218-27223.

Mingzhe Zhao, Kengo Morohashi, Greg Hatlestad, Erich Grotewold, Alan Lloyd. (2008) The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. *Development.* **135**:1991-1999.

Nameirakpam Dolendro Singh, Shashi Kumar, Henry Daniell. (2016) Expression of beta-glucosidase increases trichome density. *Plant Biotechnology*. **14**:1034-1045.

Pu Shi, Xueqing Fu, Qian Shen, Meng Liu, Qifang Pan, Yueli Tang, Weimin Jiang, Zongyou Lv, Tingxiang Yan, Yanan Ma, Minghui Chen, Xiaolong Hao, Pin Liu, Ling Li, Xiaofen Sun, Kexuan Tang. (2017) The roles of *AaMIXTA1* in regulating the initiation of glandular trichomes and cuticle biosynthesis in *Artemisia annua*. *New phytologist*. **217**(1): 261-276.

Ryan W. Shepherd, W. Troy Bass, Robert L. Houtz, George J. Wagner. (2005) Phylloplanins of tobacco are defensive proteins deployed on aerial surfaces by short glandular trichomes. *Plant Cell*. **17**(6): 1851–1861.

Ryeo Jin Kim, Hae Jin Kim, Donghwan Shim, Mi Chung Suh. (2016) Molecular and biochemical characterizations of the monoacylglycerol lipase gene family of *Arabidopsis thaliana*. *The Plant Journal*. **85**(6): 758-771.

Simon Anders, Wolfgang Huber. (2010) Differential expression analysis for sequence count data. *Genome Biology*. **11**:R106.

Sitakanta Pattanaik, Barunava Patra, Sanjay Kumar Singh, Ling Yuan. (2014) An overview of the gene regulatory network controlling trichome development in the model plant, *Arabidopsis*. *Frontiers in Plant Science*. **5**: 259.

Tetsuya Ishida, Sayoko Hattori, Ryosuke Sano, Kayoko Inoue, Yumiko Shirano, Hiroaki Hayashi, Daisuke Shibata, Shusei Sato, Tomohiko Kato, Satoshi Tabata, Kiyotaka Okada, Takuji Wada. (2007)

Arabidopsis *TRANSPARENT TESTA GLABRA2* is directly regulated by R2R3 MYB transcription factors and is involved in regulation of *GLABRA2* transcription in epidermal differentiation. *Plant Cell*. **19**(8): 2531-2543.

Thomas Payne, John Clement, David Arnold, Alan Lloyd. (1999) Heterologous myb genes distinct from *GL1* enhance trichome production when overexpressed in *Nicotiana tabacum. Development*. **126**: 671-682.

Tiancong Qi, Susheng Song, Qingcuo Ren, Dewei Wu, Huang Huang, Yan Chen, Meng Fan, Wen Peng, Chunmei Ren, Daoxin Xie. (2011) The Jasmonate-ZIM-Domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate Jasmonate-mediated Anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. *Plant Cell*. **23**(5): 1795-1814.

Tingxiang Yan, Minghui Chen, Qian Shen, Ling Li, Xueqing Fu, Qifang Pan, Yueli Tang, Pu Shi, Zongyou Lv, Weimin Jiang, Ya-nan Ma, Xiaolong Hao, Xiaofen Sun, Kexuan Tang. (2016) HOMEODOMAIN PROTEIN 1 is required for jasmonate-mediated glandular trichome initiation in *Artemisia annua*. *New phytologist*. **213**(3): 1145-1155.

Werker E. (2000) Trichome diversity and development. Advances in botanical research incorporating advances. *Plant Pathology*. **31**: 1–35.

Abstract in Korean

토마토 모상체 발달과 식물 생장에 관여하는 *naked* 돌연변이에 대한 연구

홍원기

서울대학교 국제농업기술대학원 국제농업기술학과

지도교수 강진호

모상체는 식물 표피에서 유래한 털과 유사한 구조로 단일 또는 다세포로 구성되어 식물에서 생물학적 및 비생물적 스트레스에 대항하여 방어 작용을 한다. 모상체는 넓은 범위의 식물 종에서 존재하며 glandular 또는 non-glandular 로 나뉜다. Glandular 모상체는 초식 곤충에 대한 화학 방어 작용을 하며 non-glandular 모상체는 생물학적 및 환경적 스트레스에 대한 생리적 장벽으로 작용한다. 본 연구에서는 *naked* 라는 열성 단일인자 돌연변이 토마토를 이용하였다. *naked* 돌연변이의 모상체 형태를 상세히 분석하기 위하여 해부 현미경과 주사 전자 현미경으로 모상체를 관찰한 결과, 4 종류의 glandular 및 3 종류의 non-glandular 모상체가 있는 야생종에 비하여 *naked* 돌연변이는 어린 줄기에서 모상체가 전혀 존재하지 않는 것을 관찰하였다. 또한 *naked* 돌연변이는 야생종에 비하여 줄기가 짧고 가지가 적은 결과도 확인할 수

있었다. *naked* 돌연변이에서 모상체 발달에 관여하는 유전자를 동정하기 위하여 유전자 지도 기반 클로닝을 수행한 결과 토마토 9 번 염색체에서 1.9 cM 간격 내에 *Naked* 의 유전자좌를 배치시켰다. 후보 유전자 영역은 약 250 kb 이고 이 영역은 31 개의 후보 유전자를 포함한다. 후보 유전자 접근법, RT-PCR 및 genomic DNA PCR 결과를 바탕으로 *Solyc09g075140* (monoacylglycerol lipase, MAGL) 유전자의 3’ 영역이 *naked* 돌연변이체에서 결실되어 *Solyc09g075140* 이 *Naked* 유전자임을 확인하였다. 현재 야생종의 *SlMAGL* 유전자를 천연 프로모터에서 발현시켜 *naked* mutant 의 보완 실험을 진행하고 있다.

주요어: 모상체, 유전자 지도 기반 클로닝, MAGL, *naked*, 토마토
학번: 2017-25003