Doctoral Dissertation (Censored)

博士論文（要約）

Studies on shoot regeneration from the epidermis of cultured stem segments of *Torenia fournieri*

(トレニア茎断片培養系における表皮起源シュート再生の研究)

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Abstract

Shoot regeneration involves reprogramming of somatic cells and *de novo* organization of shoot apical meristems (SAMs). In the best-studied model system of shoot regeneration using Arabidopsis, regeneration occurs mediated by auxin-responsive pluripotent callus formation from pericycle or pericycle-like tissues according to the lateral root development pathway. In contrast, shoot regeneration can be induced directly from fully differentiated epidermal cells of stem explants of *Torenia fournieri* (Torenia) without intervening callus formation in culture with cytokinin, yet its molecular mechanisms remain unaddressed. Here I characterized this direct shoot regeneration by cytological observation and transcriptome analyses. The results showed that the gene expression profile rapidly changes upon culture to acquire a mixed signature of multiple organs/tissues, possibly associated with epidermal reprogramming. Comparison of transcriptomes between three different callus-inducing cultures (callus induction by auxin, callus induction by wounding, and protoplast culture) of Arabidopsis and the Torenia stem culture identified genes upregulated in all the four culture systems as candidates of common factors of cell reprogramming. These initial changes proceeded independently of cytokinin, followed by cytokinin-dependent, transcriptional activations of nucleolar development and cell cycle. Later, SAM regulatory genes became highly expressed, leading to SAM organization in the foci of proliferating cells in the epidermal layer. My findings revealed three distinct phases with different transcriptomic and regulatory features during direct shoot regeneration from the epidermis in Torenia, which provides a basis for further investigation of shoot regeneration in this unique culture system.

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List of abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
AIR	*AUXIN-INDUCED IN ROOT CULTURES*
alf	*aberrant lateral root formation*
BA	N^6- benzyl adenine
CDKA	*CYCLIN DEPENDENT KINASE A*
CUC	*CUP-SHAPED COTYLEDON*
CYCB	*CYCLIN B*
CYCD	CYCLIN D
DAPI	4',6-diamidino-2-phenylindole
FDR	false discovery rate
GAPC	*GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE C SUBUNIT*
GO	gene ontology
HDA	*HISTONE DEACETYLASE*
IAA	indole-3-acetic acid
LBD	LATERAL ORGAN BOUNDARIES DOMAIN
LRR	*LEUCINE-RICH REPEAT*
MES	2-(-*N*-morpholino)ethanesylfonic acid
MS	Murashige and Skoog
NAA	1-Naphthaleneacetic acid
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDIL	*PROTEIN DISULFIDE ISOMERASE-LIKE*
PLT	PLETHORA

PMT	*POLYOL/MONOSACCHARIDE TRANSPORTER*
RLK	*RECEPTOR-LIKE KINASE*
RT-qPCR	reverse transcription-quantitativePCR
SAM	shoot apical meristem
slr	*solitary root*
STM	*SHOOT MERISTEMLESS*
TOLS	*TARGET OF LBD SIXTEEN*
TPM	transcripts per kilobase million
WIND	*WOUND-INDUCED DEDIFFERENTIATION*
WUS	*WUSCHEL*
XYL	*ALPHA-XYLOSIDASE*

Chapter 1. Introduction

Plant development is often featured by its high plasticity in contrast to the limited plasticity of animal development. The plastic nature of plant development can be typically seen in regeneration phenomena such as organ regeneration and somatic embryogenesis, through which many plants are able to recreate most parts of or even entire plant body (Birnbaum and Alvarado, 2008; Ikeuchi et al., 2016). The regeneration processes generally involve some kinds of cell reprogramming and *de novo* organization of meristems that contain stem cells. For a representative example, shoot regeneration from mature tissues relies on reprogramming from the original, differentiated state and the subsequent generation of the shoot apical meristem (SAM) of an adventitious bud.

Since Skoog and Miller (1957) discovered that callus, shoots, and roots can be artificially induced and manipulated by application of the phytohormones auxin and cytokinin in tissue culture, tissue culture has become one of main tools to study plant organogenesis including shoot regeneration. For the efficient induction of shoot regeneration, two-step culture systems, consisting of callus induction by auxin-rich culture in the first step and adventitious shoot induction by cytokinin-rich culture in the second step, have been developed in many plants and widely used (e.g., Nishi et al., 1968; Christianson and Warnick, 1983; Koornneef et al., 1987; Coleman and Ernst, 1990).

A similar two-step culture system was also established in the model plant *Arabidopsis thaliana* (Arabidopsis) (Valvekens et al., 1988; Akama et al., 1992), which has facilitated molecular biological analysis of shoot regeneration. In this culture system, if skipping the first step for callus induction, explants can form no or few adventitious buds, which implies that explant cells undergo reprogramming to acquire competence for shoot regeneration in the first step culture.

With the two-step culture system of Arabidopsis, transcriptome analysis was performed for gene expression profiling of callus formation and adventitious SAM formation (Che et al., 2002, 2006, 2007; Liu et al., 2010; Sugimoto et al., 2010; Xu et al., 2012), and spatial and temporal expression

patterns and the function of genes encoding major SAM regulatory transcription factors and phytohormone signaling factors were investigated in the process leading to SAM formation (Gordon et al., 2007; Cheng et al., 2013; Zhang et al., 2017). Through forward genetics and functional screening of cDNA library using the two-step shoot induction culture of Arabidopsis, several factors involved in shoot regeneration process were identified (Yasutani et al., 1994; Ozawa et al., 1998; Banno et al., 2001; Tamaki et al., 2009). Recently, studies of the two-step shoot regeneration have been expanded, incorporating various new lines of research such as functional analysis of epigenetic regulation (He et al., 2012; Lee and Seo, 2018; Ishihara et al., 2019) and genome wide association analysis of natural variations (Lardon et al., 2020), which has accumulated increasing pieces of information.

Molecular studies using the two-step induction culture have also revealed the mechanism how the expression of key regulators of SAM is induced during adventitious SAM formation. The expression of *SHOOT MERISTEMLESS* (*STM*), a homeodomain transcriptional factor involved in meristem maintenance throughout the SAM (Long et al., 1996), is induced by two NAC transcription factors, *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2* (Daimon et al., 2003). Another homeodomain transcriptional factor, *WUSCHEL* (*WUS*), which is expressed in the center of the SAM, also plays an essential role in meristem maintenance (Schoof et al., 2000). The expression of *WUS* is upregulated in the downstream of cytokinin as a direct target of type-B ARRs (Meng et al., 2017; Zhang et al., 2017). This upregulation of *WUS* is considered to be a critical event of adventitious SAM formation because a loss of function mutation of *WUS* severely disrupts shoot regeneration (Gordon et al., 2007).

One of the most important outcomes of research concerning the two-step culture of Arabidopsis over the last decade is the understanding that callus formed in the first step is not a fully undifferentiated cell mass but a disorganized root meristem-like tissue originating from pericycle or pericycle-like tissues via the pathway of lateral root formation (Che et al., 2007; Atta et al., 2009;

Sugimoto et al., 2010; Kareem et al., 2015; Shang et al., 2016). Mutants incapable of lateral root formation, *aberrant lateral root formation 4* (*alf4*) and *solitary root* (*slr*) are also found to be defective in callus formation (Sugimoto et al., 2010; Shang et al., 2016). Furthermore, it was also shown that transcription factors regulating lateral root formation, such as LATERAL ORGAN BOUNDARIES DOMAINs (LBDs) and PLETHORAs (PLTs), participate not only in callus formation but also in acquisition of shoot regeneration competence (Fan et al., 2012; Kareem et al., 2015; Kim et al., 2018; Liu et al., 2018). These findings suggest that the root development pathway offers a mechanism of cell reprogramming during auxin-induced callus formation. It is noted here that this reprogramming starts not from fully differentiated cells but from pericycle (or pericycle-like tissue) cells that are generally considered to remain partially meristematic (De Smet et al., 2006; Atta et al., 2009).

Arabidopsis plants can occasionally form regenerative callus at wounded sites without hormone application. The molecular basis of this wound-induced callus formation has been also studied well for cell reprogramming, resulting in the identification of several key regulatory factors including the AP2/ERF transcription factor WOUND-INDUCED DEDIFFERENTIATION 1 (WIND1) (Iwase et al., 2011, 2015, 2017). Another good material for studying cell reprogramming is mesophyll protoplasts, which are reactivated from the quiescent state to enter the cell cycle and form regenerative callus in culture (Zelcer and Galun, 1976). Transcriptome analysis of these processes revealed dynamic transcriptional changes possibly associated with cell reprogramming during wound-induced callus formation and protoplast culture (Chupeau et al., 2013; Ikeuchi et al., 2017).

In other plants, there are more diverse paths of shoot regeneration. In not a few cases, a preparatory callus formation step is dispensable for shoot regeneration (Hicks, 1980). Moreover, the origin of regenerated shoots is not restricted to pericycle or pericycle-like cells in some cases. For example, adventitious buds directly arise from epidermal and subepidermal cells when thin cell layers prepared from internodes of *Nicotiana tabacum* are cultured in the presence of auxin and

cytokinin (Creemers-Molenaar et al., 1994). Shoot regeneration from the epidermis without callus induction step was also described in *Begonia rex* (Chlyah and Van, 1975) and *Nautilocalyx lynchei* (Van, 1973).

In 1973, Chlyah reported a notable system for the induction of this type of shoot regeneration with tissue culture of *Torenia fournieri* (Torenia). In this culture, adventitious bud SAMs formed directly on the surface of stem segments without intervening callus growth phase (Chlyah, 1973, 1974a, 1974b). Importantly, histological analysis demonstrated that these adventitious bud SAMs originated exclusively from epidermal cells (Chlyah, 1974a). The contribution of the epidermal and inner tissues to shoot regeneration was further investigated using stem segments split into the epidermal layer and the remaining inner tissue block. When the epidermal and inner tissue explants were cultured separately, either of them did not form adventitious buds. However, when these explants were cultured in contact with each other, only the epidermal explants formed adventitious buds (Chlyah, 1974c). These results indicated an essential supportive role of inner tissues in shoot regeneration from the epidermis.

From 1970s to 1990s, several physiological studies were carried out with the Torenia culture system. In the experiments to examine the effects of phytohormones, it was shown that cytokinins, such as N^6- benzyladenine (BA), zeatin, and *N*-phenyl-*N*′-(4-pyridyl)urea, drastically promote shoot regeneration while auxins, such as indole-3-acetic acid and α-naphthaleneacetic acid, are only weakly promotive (Kamada and Harada, 1979; Tanimoto and Harada, 1982, 1984). The effects of abiotic stresses were also examined, and wound stress was found to be another promoting factor of shoot regeneration because additional wounding to stem segments elevated the number of adventitious buds (Takeuchi et al., 1985). The early histological studies and these following physiological studies established the basis of the Torenia stem culture system. However, this culture system has never been used for molecular biological studies of shoot regeneration.

The Torenia stem culture system has three distinct features of shoot regeneration in contrast to the Arabidopsis two-step culture system: First, the entire process of shoot regeneration is triggered simply by one-step culture; second, drastic reprogramming should occur during transformation of fully differentiated epidermal cells into meristem cells; and third, the initial process of *de novo* organization of SAMs takes place in a two-dimensional field of the epidermis. Because of these features, the Torenia stem culture can serve as a unique and advantageous experimental system for studying cell reprogramming and SAM organization during shoot regeneration. In the present study, I performed cytological and transcriptomic characterization of shoot regeneration with the Torenia culture system to uncover hidden aspects of shoot regeneration with this unique system. I also compared the transcriptome data of Torenia with those reported for Arabidopsis to gain information of core reprogramming mechanisms common to various types of regeneration-related events. The results obtained depict global and temporal changes in the gene expression profile that are likely to associate with each elementary process of shoot regeneration in the Torenia stem culture, which provides a basis for further investigation of the relevant molecular mechanisms. Comparison of transcriptome data between three different callus-inducing cultures of Arabidopsis and the Torenia stem culture identified candidates of factors universally involved in the molecular network of cell reprogramming.

Chapter 2. Results

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Chapter 3. Discussion

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Chapter 4. Materials and Methods

Plant Material and Growth Conditions

All experiments were carried out using a genetically homogeneous inbred line of *Torenia fournieri* Lind. that had been developed through 11 generations of self-pollination. Surface-sterilized and stratified seeds were sown on basal medium, which was half-strength Murashige and Skoog (MS) medium containing 2% (w/v) of sucrose, buffered with 0.05% (w/v) of 2-morpholinoethanesulfonic acid at pH 5.7, and solidified with 0.25% (w/v) of gellan gum, and plants were aseptically grown at 22°C under continuous light (60–100 $\mu mol \cdot s^{-1} \cdot m^{-2}$).

Tissue Culture

Stems were excised from the internodes between the cotyledons and the first pair of true leaves of 4-week-old plants. Each internode stem of a quadrangular prism shape with wider and narrower lateral faces was sliced longitudinally along the midline of the narrower side into two sections and then cut into 1.5-mm-long segments. The stem segments were placed on the basal medium described above or the basal medium supplemented with 1 mg/L of BA such that the sliced surface was in contact with the medium. Subsequent culture was conducted at 22°C under continuous light (50–70 $\mu mol \cdot s^{-1} \cdot m^{-2}$) or in the dark.

Flow cytometric Analysis of Nuclear DNA Content

To isolate nuclei, samples were chopped in CyStain UV Precise P Nuclei Extraction Buffer (sysmex) in petri dishes placed on ice and filtered through 20-μm CellTrics filter (sysmex) after 1-minute incubation on ice. Isolated nuclei were stained with CyStain UV Precise P Staining Buffer (sysmex) and then the DNA content of each nucleus was quantified with SyFlow SL (Partec).

Microscopic Analysis

For RNAselect and DAPI staining, stripped epidermis of stem explants was fixed in methanol at -20°C for at least 10 minutes. The fixed samples were washed in phosphate buffered saline at pH 7.2 (PBS) and then stained in PBS solution containing 25% (v/v) CyStain UV Precise P Staining Buffer (sysmex), 1 μM CYTO RNAselect Green Fluorescent Cell Stain (Invitrogen), and 0.1% (w/v) TritonX-100 for 30 minutes at room temperature while being protected from light. The stained samples were washed in PBS before observation.

For detection of nascent cell walls, epidermis of stem explants was stained with aniline blue according to the protocol described in Schenk and Schikora (2015) with minor modifications. Stripped epidermis was fixed in a 1:3 mixture of acetic acid and ethanol for at least 24 hours at room temperature. After washing in 150 mM K_2HPO_4 for 30 minutes, the samples were stained in 1% (w/v) aniline blue solution containing 150 mM K_2HPO_4 for 2.5 hours at room temperature while being protected from light. The stained samples were washed in 150 mM K_2HPO_4 before observation. All nascent cell walls detected on the epidermal layer were counted.

The epidermis samples stained with DAPI, RNAselect, or aniline blue were observed under the Olympus BX50F4 microscope.

Serial observation of the surface of cultured explants was performed with a metallurgical microscope (WRAYMER, BM-3400TL).

Transcriptome Analysis

All transcriptome analyses were carried out in three biological replicates. Collected samples were immediately frozen with liquid nitrogen and stored at −80°C until use. Total RNA was isolated from the frozen samples with Direct-zol RNA MiniPrep Kit (Zymo research).

For RNA-seq analysis of a set of samples consisting of stem explants cultured for 0, 2, 4, 6, and 8 days, libraries were prepared total RNA with mRNA-seq Kit with KAPA mRNA Capture Beads (KAPA), NEBNext Multiplex Oligos for Illumina Index Primers Set 1-4 (NEB), and Agincourt AMPure XP (Beckman Coulter) according to the manufacturers' protocols. The libraries were sequenced with Nextseq500 (Illmina). Raw reads containing adapter sequences were trimmed using bcl2fastq (Illumina), and nucleotides with low-quality (QV < 25) were masked by N with using the original script. Reads shorter than 50 bp were discarded, and the remaining reads were mapped to the Torenia cDNA database (http://dandelion.liveholonics.com/torenia/), which had been constructed from mRNAs of leaves and roots of young seedlings and floral organs of flowering plants, using Bowtie with the following parameters: "--all --best –strata" (Langmead et al., 2009). Reads were counted by transcript models.

For RNA-seq analysis of a set of samples consisting of various parts of 4-week-old plants (shoot apices, the first and second pairs of true leaves, the first internode stems, and whole roots) and stem explants at the early stage of culture (explants cultured for 0, 3, 6, 12, 24, and 48 hours), libraries were prepared with mRNA HyperPrep Kit (KAPA) and Multiplex Oligos for Illumina Index Primers Set 1-4 (NEB) according to the manufacturers' protocols. The libraries were sequenced with Novaseq6000 (Illmina). Reads were mapped to the Torenia cDNA database (http://dandelion.liveholonics.com/torenia/) using Bowtie2 (Langmead and Salzberg, 2012) and expression level of each transcript was quantified with Salmon (Patro et al., 2017).

Differential expression analysis was performed with edgeR (Robinson et al., 2009; McCarthy et al., 2012) and *limma* (Ritchie et al., 2015) packages of R. K-means clustering analysis was performed on Multiple Experiment Viewer platform (Saeed et al., 2003). Assignment of Gene Ontology (GO) annotation to transcript sequences of Torenia was conducted with Blast2GO (Conesa and Götz, 2008) based on the results of homology search against the Arabidopsis subset and Viridiplantae subset of the NCBI non-redundant database and also on the protein domains identified

by InterPro domain search also performed with Blast2GO. GO enrichment analysis was also carried out on Blast2GO by Fisher's exact test with cutoff at FDR < 0.05.

For comparison of transcriptome data between Arabidopsis and Torenia, RNA-seq data and microarray data of Arabidopsis were obtained from the public resource. The Arabidopsis RNA-seq data were processed as described above. The microarray data was normalized by a variant of MAS5.0 with robust radius-minimax estimators (Kohl and Deigner, 2010). Then differentially expressed genes were identified with the rank products method with a cut off at FDR < 0.05 unsing the Rank Prod R package (Del Carratore et al., 2017).

Identification of Torenia Orthologs to Arabidpsis Genes

Orthologs of Torenia to the SAM regulator genes of Arabidopsis were identified by homology search against the amino-acid sequence database deduced from the Torenia cDNA database (http://dandelion.liveholonics.com/torenia/) with the full-length amino-acid sequences of Arabidopsis SAM regulators as queries followed by phylogenetic tree construction. In other cases, Torenia orthologs to a set of Arabidopsis genes of interest were identified using OrthoFinder (Emms and Kelly, 2015, 2019).

RT-qPCR Analysis

Total RNA was isolated with Direct-zol RNA MiniPrep Kit (Zymo research). From each RNA preparation, potentially remaining genomic DNA was eliminated and the first-strand cDNA was synthesized using PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa). Then qPCR was performed with gene-specific primers (Table 4) using TB Green Premix Ex Taq II (Tli RNaseH Plus) (TaKaRa) on Step One Real Time PCR System (Applied Biosystems). The data were normalized with $\Delta\Delta C_t$ method using the ubiquitin gene *TfUBQ10*, a Torenia homolog of Arabidopsis *UBQ10*, as an internal control.

The sequences of genes used in this study are available at the Torenia cDNA database (http://dandelion.liveholonics.com/torenia/) under the following accession numbers: *TfSTM1* (TfB072761), *TfSTM2* (TfB084476, TfB084487), *TfSTM3* (TfB080768), *TfCUC1/2a* (TfB082036), *TfCUC1/2b* (TfB082143), *TfWUS1* (TfB099710), *TfWUS2* (TfB094340), and *TfUBQ10* (TfB084374).

References

Akama, K., Shiraishi, H., Ohta, S., Nakamura, K., Okada, K., and Shimura, Y. (1992). Efficient transformation of *Arabidopsis thaliana*: comparison of the efficiencies with various organs, plant ecotypes and *Agrobacterium* strains. Plant Cell Rep. **12**: 7–11.

Atta, R., Laurens, L., Boucheron-Dubuisson, E., Guivarc'h, A., Carnero, E., Giraudat-Pautot, V., Rech, P., and Chriqui, D. (2009). Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown *in vitro*. Plant J. **57**: 626–644.

Banno, H., Ikeda, Y., Niu, Q.W., and Chua, N.H. (2001). Overexpression of Arabidopsis ESR1 induces initiation of Shoot Regeneration. Plant Cell **13**: 2609–2618.

Birnbaum, K.D. and Alvarado, A.S. (2008). Slicing across kingdoms: Regeneration in plants and animals. Cell **132**: 697–710.

Brenner, W.G., Romanov, G.A., Köllmer, I., Bürkle, L., and Schmülling, T. (2005). Immediate-early and delayed cytokinin response genes of Arabidopsis thaliana identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. Plant J. **44**: 314–333.

Buganim, Y., Faddah, D.A., Cheng, A.W., Itskovich, E., Markoulaki, S., Ganz, K., Klemm, S.L., Van Oudenaarden, A., and Jaenisch, R. (2012). Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. Cell **150**: 1209–1222.

Del Carratore, F., Jankevics, A., Eisinga, R., Heskes, T., Hong, F., and Breitling, R. (2017). RankProd 2.0: a refactored bioconductor package for detecting differentially expressed features in molecular profiling datasets. Bioinformatics **33**: 2774–2775.

Che, P., Gingerich, D.J., Lall, S., and Howell, S.H. (2002). Global and hormone-induced gene expression changes during shoot development in Arabidopsis. Plant Cell **14**: 2771–2785.

Che, P., Lall, S., and Howell, S.H. (2007). Developmental steps in acquiring competence for shoot

development in *Arabidopsis* tissue culture. Planta **226**: 1183–1194.

Che, P., Lall, S., Nettleton, D., and Howell, S.H. (2006). Gene expression programs during shoot, root, and callus development in Arabidopsis tissue culture. Plant Physiol. **141**: 620–637.

Cheng, Z.J., Wang, L., Sun, W., Zhang, Y., Zhou, C., Su, Y.H., Li, W., Sun, T.T., Zhao, X.Y., Li, X.G., Cheng, Y., Zhao, Y., Xie, Q., and Zhang, X.S. (2013). Pattern of auxin and cytokinin responses for shoot meristem induction results from the regulation of cytokinin biosynthesis by AUXIN RESPONSE FACTOR3. Plant Physiol. **161**: 240–251.

Chlyah, A. and Van, M.T.T. (1975). Differential reactivity in epidermal cells of Begonia rex excised and grown in vitro. Physiol. Plant. **35**: 16–20.

Chlyah, H. (1973). Directed neoformation of organ fragments of Torenia fournieri (Lind.) cultivated *in vitro*. Biol. Plant. **15**: 80–87.

Chlyah, H. (1974a). Etude histologique de la néoformation de méristèmes caulinaires et radiculaires à partir de segments d'entre-noeuds de Torenia fournieri cultivés in vitro. Can. J. Bot. **52**: 473–476.

Chlyah, H. (1974b). Formation and propagation of cell division-centers in the epidermal layer of internodal segments of *Torenia fournieri* grown *in vitro*. Simultaneous surface observations of all the epidermal cells. Can. J. Bot. **52**: 867–872.

Chlyah, H. (1974c). Inter-tissue correlations in organ fragments. Plant Physiol. **54**: 341–348.

Chlyah, H., Van, M.T.T., and Demarly, Y. (1975). Distribution pattern of cell division centers on the epidermis of stem segments of *Torenia fournieri* during *de novo* bud formation. Plant Physiol. **56**: 28–33.

Christianson, M.L. and Warnick, D.A. (1983). Competence and determination in the process of in vitro shoot organogenesis. Dev. Biol. **95**: 288–293.

Chupeau, M.C., Granier, F., Pichon, O., Renou, J.P., Gaudin, V., and Chupeaua, Y. (2013). Characterization of the early events leading to totipotency in an *Arabidopsis* protoplast liquid culture by temporal transcript profiling. Plant Cell **25**: 2444–2463.

Coleman, G.D. and Ernst, S.G. (1990). Shoot induction competence and callus determination in *Populus deltoides*. Plant Sci. **71**: 83–92.

Conesa, A. and Götz, S. (2008). Blast2GO: A comprehensive suite for functional analysis in plant genomics. Int. J. Plant Genomics **2008**: 619832.

Creemers-Molenaar, J., Hakkert, J.C., Van Staveren, M.J., and Gilissen, L.J.W. (1994). Histology of the morphogenic response in thin cell layer explants from vegetative tobacco plants. Ann. Bot. **73**: 547–555.

Daimon, Y., Takabe, K., and Tasaka, M. (2003). The *CUP-SHAPED COTYLEDON* genes promote adventitious shoot formation on calli. Plant Cell Physiol. **44**: 113–121.

Efroni, I. (2018). A conceptual framework for cell identity transitions in plants. Plant Cell Physiol. **59**: 691–701.

Efroni, I., Mello, A., Nawy, T., Ip, P.L., Rahni, R., Delrose, N., Powers, A., Satija, R., and Birnbaum, K.D. (2016). Root regeneration triggers an embryo-like sequence guided by hormonal interactions. Cell **165**: 1721–1733.

Emms, D.M. and Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. Genome Biol. **20**: 238.

Emms, D.M. and Kelly, S. (2015). OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol. **16**: 157.

Fan, M., Xu, C., Xu, K., and Hu, Y. (2012). LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in Arabidopsis regeneration. Cell Res. **22**: 1169–1180.

Feldman, L.J. and Torrey, J.G. (1977). Nuclear changes associated with cellular dedifferentiation in pea root cortical cells cultured *in vitro*. J. Cell Sci. **28**: 87–105.

Gibson, S.W. and Todd, C.D. (2015). Arabidopsis AIR12 influences root development. Physiol. Mol. Biol. Plants **21**: 479–489.

Gordon, S.P., Heisler, M.G., Reddy, G.V., Ohno, C., Das, P., and Meyerowitz, E.M. (2007).

Pattern formation during *de novo* assembly of the *Arabidopsis* shoot meristem. Development **134**: 3539–3548.

Haga, N., Kobayashi, K., Suzuki, T., Maeo, K., Kubo, M., Ohtani, M., Mitsuda, N., Demura, T., Nakamura, K., Jurgens, G., and Ito, M. (2011). Mutations in *MYB3R1* and *MYB3R4* cause pleiotropic developmental defects and preferential down-regulation of multiple G2/M-specific genes in Arabidopsis. Plant Physiol. **157**: 706–717.

He, C., Chen, X., Huang, H., and Xu, L. (2012). Reprogramming of H3K27me3 Is Critical for Acquisition of Pluripotency from Cultured Arabidopsis Tissues. PLoS Genet. **8**: e1002911.

Hicks, G.S. (1980). Patterns of organ development in plant tissue culture and the problem of organ determination. Bot. Rev. **46**: 1–23.

Hu, M., Krause, D., Greaves, M., Sharkis, S., Dexter, M., Heyworth, C., and Enver, T. (1997). Multilineage gene expression precedes commitment in the hemopoietic system. Genes Dev. **11**: 774–785.

Ikeuchi, M., Iwase, A., Rymen, B., Lambolez, A., Kojima, M., Takebayashi, Y., Heyman, J., Watanabe, S., Seo, M., De Veylder, L., Sakakibara, H., and Sugimoto, K. (2017). Wounding triggers callus formation via dynamic hormonal and transcriptional changes. Plant Physiol. **175**: 1158–1174.

Ikeuchi, M., Ogawa, Y., Iwase, A., and Sugimoto, K. (2016). Plant regeneration: Cellular origins and molecular mechanisms. Development **143**: 1442–1451.

Ikeuchi, M., Sugimoto, K., and Iwase, A. (2013). Plant callus: Mechanisms of induction and repression. Plant Cell **25**: 3159–3173.

Ishihara, H., Sugimoto, K., Tarr, P.T., Temman, H., Kadokura, S., Inui, Y., Sakamoto, T., Sasaki, T., Aida, M., Suzuki, T., Inagaki, S., Morohashi, K., Seki, M., Kakutani, T., Meyerowitz, E.M., and Matsunaga, S. (2019). Primed histone demethylation regulates shoot regenerative competency. Nat. Commun. **10**: 1–15.

Iwase, A., Harashima, H., Ikeuchi, M., Rymen, B., Ohnuma, M., Komaki, S., Morohashi, K.,

Kurata, T., Nakata, M., Ohme-Takagi, M., Grotewold, E., and Sugimoto, K. (2017). WIND1 promotes shoot regeneration through transcriptional activation of *ENHANCER OF SHOOT REGENERATION1* in Arabidopsis. Plant Cell **29**: 54–69.

Iwase, A., Mita, K., Nonaka, S., Ikeuchi, M., Koizuka, C., Ohnuma, M., Ezura, H., Imamura, J., and Sugimoto, K. (2015). WIND1-based acquisition of regeneration competency in Arabidopsis and rapeseed. J. Plant Res. **128**: 389–397.

Iwase, A., Mitsuda, N., Koyama, T., Hiratsu, K., Kojima, M., Arai, T., Inoue, Y., Seki, M., Sakakibara, H., Sugimoto, K., and Ohme-Takagi, M. (2011). The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. Curr. Biol. **21**: 508–514.

Kamada, H. and Harada, H. (1979). Influence of several growth regulators and amino acids on *in vitro* organogenesis of *Torenia fournieri* Lind. J. Exp. Bot. **30**: 27–36.

Kareem, A., Durgaprasad, K., Sugimoto, K., Du, Y., Pulianmackal, A.J., Trivedi, Z.B., Abhayadev, P. V, Pinon, V., Meyerowitz, E.M., Scheres, B., and Prasad, K. (2015). *PLETHORA* genes control regeneration by a two- step mechanism. Curr. Biol. **25**: 1–14.

Kiba, T., Naitou, T., Koizumi, N., Yamashino, T., Sakakibara, H., and Mizuno, T. (2005). Combinatorial microarray analysis revealing *Arabidopsis* genes implicated in cytokinin responses through the His→Asp phosphorelay circuitry. Plant Cell Physiol. **46**: 339–355.

Kim, J., Yang, W., Forner, J., Lohmann, J.U., Noh, B., and Noh, Y. (2018). Epigenetic reprogramming by histone acetyltransferase HAG1/AtGCN5 is required for pluripotency acquisition in *Arabidopsis*. EMBO J. **37**: e98726.

Klepek, Y.S., Volke, M., Konrad, K.R., Wippel, K., Hoth, S., Hedrich, R., and Sauer, N. (2010). Arabidopsis thaliana POLYOL/MONOSACCHARIDE TRANSPORTERS 1 and 2: Fructose and xylitol/H+ symporters in pollen and young xylem cells. J. Exp. Bot. **61**: 537–550.

Kohl, M. and Deigner, H.P. (2010). Preprocessing of gene expression data by optimally robust estimators. BMC Bioinformatics **11**: 583.

Koornneef, M., Hanhart, C.J., and Martinelli, L. (1987). A genetic analysis of cell culture traits in

tomato. Theor. Appl. Genet. **74**: 633–641.

Langmead, B. and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat. Methods **9**: 357–359.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. **10**: R25.

Lardon, R., Wijnker, E., Keurentjes, J., and Geelen, D. (2020). The genetic framework of shoot regeneration in *Arabidopsis* comprises master regulators and conditional fine-tuning factors. Commun. Biol. **3**: 549.

Lee, K., Park, O.S., Jung, S.J., and Seo, P.J. (2016). Histone deacetylation-mediated cellular dedifferentiation in *Arabidopsis*. J. Plant Physiol. **191**: 95–100.

Lee, K. and Seo, P.J. (2018). Dynamic epigenetic changes during plant regeneration. Trends Plant Sci. **23**: 235–247.

Liu, H.L., Wang, G.C., Feng, Z., and Zhu, J. (2010). Screening of genes associated with dedifferentiation and effect of LBD29 on pericycle cells in *Arabidopsis thaliana*. Plant Growth Regul. **62**: 127–136.

Liu, J., Hu, X., Qin, P., Prasad, K., Hu, Y., and Xu, L. (2018). The *WOX11* - *LBD16* pathway promotes pluripotency acquisition in callus cells during de novo shoot regeneration in tissue culture. Plant Cell Physiol. **59**: 734–743.

Long, J.A., Moan, E.I., Medford, J.I., and Barton, M.K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. Nature **379**: 66–69.

McCarthy, D.J., Chen, Y., and Smyth, G.K. (2012). Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res. **40**: 4288–4297.

Meng, W.J., Cheng, Z.J., Sang, Y.L., Zhang, M.M., Rong, X.F., Wang, Z.W., Tang, Y.Y., and Zhang, X.S. (2017). Type-B ARABIDOPSIS RESPONSE REGULATORs specify the shoot stem cell niche by dual regulation of *WUSCHEL*. Plant Cell **29**: 1357–1372.

Moris, N., Pina, C., and Arias, A.M. (2016). Transition states and cell fate decisions in epigenetic

landscapes. Nat. Rev. Genet. **17**: 693–703.

Mozgová, I., Muñoz-Viana, R., and Hennig, L. (2017). PRC2 represses hormone-induced somatic embryogenesis in vegetative tissue of *Arabidopsis thaliana*. PLoS Genet. **13**: e1006562.

Neuteboom, L.W., Ng, J.M.Y., Kuyper, M., Clijdesdale, O.R., Hooykaas, P.J.J., and Van Der Zaal, B.J. (1999). Isolation and characterization of cDNA clones corresponding with mRNAs that accumulate during auxin-induced lateral root formation. Plant Mol. Biol. **39**: 273–287.

Nishi, T., Yamada, Y., and Takahashi, E. (1968). Organ redifferentiation and plant restoration in rice callus. Nature **219**: 508–509.

Omary, M., Gil-Yarom, N., Yahav, C., Steiner, E., and Efroni, I. (2020). A conserved superlocus regulates above- and belowground root initiation. bioRxiv: 2020.11.11.377937.

Orchard, C.B., Siciliano, I., Sorrell, D.A., Marchbank, A., Rogers, H.J., Francis, D., Herbert, R.J., Suchomelova, P., Lipavska, H., Azmi, A., and Onckelen, H. Van (2005). Tobacco BY-2 cells expressing fission yeast cdc25 bypass a G2/M block on the cell cycle. Plant J. **44**: 290–299.

Ozawa, S., Yasutani, I., Fukuda, H., Komamine, A., and Sugiyama, M. (1998). Organogenic responses in tissue culture of srd mutants of *Arabidopsis thaliana*. Development **125**: 135–142.

Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods **14**: 417–419.

Paul, E., Harikrishna, K., Fioroni, O., and Draper, J. (1989). Dedifferentiation of *Asparagus officinalis* L. mesophyll cells during initiation of cell cultures. Plant Sci. **65**: 111–117.

Pitorre, D., Llauro, C., Jobet, E., Guilleminot, J., Brizard, J.P., Delseny, M., and Lasserre, E. (2010). RLK7, a leucine-rich repeat receptor-like kinase, is required for proper germination speed and tolerance to oxidative stress in *arabidopsis thaliana*. Planta **232**: 1339–1353.

Riou-Khamlichi, C., Huntley, R., Jacqmard, A., and Murray, J.A.H. (1999). Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Science. **283**: 1541–1544.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). *limma*

powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. **43**: e47.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2009). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics **26**: 139–140.

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V., and Quackenbush, J. (2003). TM4: A free, open-source system for microarray data management and analysis. Biotechniques **34**: 374–378.

Schenk, S. and Schikora, A. (2015). Staining of callose depositions in root and leaf tissues. Bio-Protocol **5**: e1429.

Schneider, M., Knuesting, J., Birkholz, O., Heinisch, J.J., and Scheibe, R. (2018). Cytosolic GAPDH as a redox-dependent regulator of energy metabolism. BMC Plant Biol. **18**: 184.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F.X., Jürgens, G., and Laux, T. (2000). The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell **100**: 635–644.

Shang, B., Xu, C., Zhang, X., Cao, H., Xin, W., and Hu, Y. (2016). Very-long-chain fatty acids restrict regeneration capacity by confining pericycle competence for callus formation in *Arabidopsis*. Proc. Natl. Acad. Sci. U. S. A. **113**: 5101–5106.

Shigeyama, T., Watanabe, A., Tokuchi, K., Toh, S., Sakurai, N., Shibuya, N., and Kawakami, N. (2016). α-Xylosidase plays essential roles in xyloglucan remodelling, maintenance of cell wall integrity, and seed germination in *Arabidopsis thaliana*. J. Exp. Bot. **67**: 5615–5629.

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. **11**: 118–130.

De Smet, I., Vanneste, S., Inzé, D., and Beeckman, T. (2006). Lateral root initiation or the birth of a new meristem. Plant Mol. Biol. **60**: 871–887.

Sugimoto, K., Jiao, Y., and Meyerowitz, E.M. (2010). *Arabidopsis* regeneration from multiple tissues occurs via a root development pathway. Dev. Cell **18**: 463–471.

Takeuchi, N., Tanimoto, S., and Harada, H. (1985). Effects of wounding on adventitious bud formation in Torenia stem segments cultured in vitro. J. Exp. Bot. **36**: 841–847.

Tamaki, H., Konishi, M., Daimon, Y., Aida, M., Tasaka, M., and Sugiyama, M. (2009). Identification of novel meristem factors involved in shoot regeneration through the analysis of temperature-sensitive mutants of Arabidopsis. Plant J. **57**: 1027–1039.

Tanimoto, S. and Harada, H. (1982). Effects of cytokinin and anticytokinin on the initial stage of adventitious bud differentiation in the epidermis of *Torenia* stem segments. Plant Cell Physiol. **23**: 1371–1376.

Tanimoto, S. and Harada, H. (1984). Roles of auxin and cytokinin in organogenesis in *Torenia* stem segments cultured *in vitro*. J. Plant Physiol. **115**: 11–18.

Toyokura, K., Goh, T., Shinohara, H., Shinoda, A., Kondo, Y., Okamoto, Y., Uehara, T., Fujimoto, K., Okushima, Y., Ikeyama, Y., Nakajima, K., Mimura, T., Tasaka, M., Matsubayashi, Y., and Fukaki, H. (2019). Lateral inhibition by a peptide hormone-receptor cascade during *Arabidopsis* lateral root founder cell formation. Dev. Cell **48**: 64–75.

Valvekens, D., Van Montagu, M., and Van Lijsebettens, M. (1988). Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana root explants by using kanamycin selection. Proc. Natl. Acad. Sci. U. S. A. **85**: 5536–5540.

Van, M.T.T. (1973). *In vitro* control of *de novo* flower, bud, root, and callus differentiation from excised epidermal tissues. Nature **246**: 44–45.

Vandepoele, K., Vlieghe, K., Florquin, K., Hennig, L., Beemster, G.T.S., Gruissem, W., Van De Peer, Y., Inzé, D., and De Veylder, L. (2005). Genome-wide identification of potential plant E2F target genes. Plant Physiol. **139**: 316–328.

Williams, L., Zhao, J., Morozova, N., Li, Y., Avivi, Y., and Grafi, G. (2003). Chromatin reorganization accompanying cellular dedifferentiation is associated with modifications of

histone H3, redistribution of HP1, and activation of E2F-target genes. Dev. Dyn. **228**: 113–120.

Williams, L.M. and Jordan, E.G. (1980). Nuclear and nucleolar size changes and nuclear pore frequency in cultured explants of jerusalem artichoke tubers (*Helianthus tuberosus* L.). J. Exp. Bot. **31**: 1613–1619.

Xu, K., Liu, J., Fan, M., Xin, W., Hu, Y., and Xu, C. (2012). A genome-wide transcriptome profiling reveals the early molecular events during callus initiation in *Arabidopsis* multiple organs. Genomics **100**: 116–124.

Yasutani, I., Ozawa, S., Nishida, T., Sugiyama, M., and Komamine, A. (1994). Isolation of temperature-sensitive mutants of *Arabidopsis thaliana* that are defective in the redifferentiation of shoots. Plant Physiol. **105**: 815–822.

Zelcer, A. and Galun, E. (1976). Culture of newly isolated tobacco protoplasts: Precursor incorporation into protein, RNA and DNA. Plant Sci. Lett. **7**: 331–336.

Zhang, K., Letham, D.S., and John, P.C.L. (1996). Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of $p34^{cdc2}$-like H1 histone kinase. Planta **200**: 2–12.

Zhang, T.Q., Lian, H., Zhou, C.M., Xu, L., Jiao, Y., and Wang, J.W. (2017). A two-step model for de novo activation of *WUSCHEL* during plant shoot regeneration. Plant Cell **29**: 1073–1087.

Zhang, Z., Liu, X., Li, R., Yuan, L., Dai, Y., and Wang, X. (2018). Identification and functional analysis of a protein disulfide isomerase (AtPDI1) in *Arabidopsis thaliana*. Front. Plant Sci. **9**: 913.

Figures and tables

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