GLABROUS INFLORESCENCE STEMS regulates trichome branching by genetically interacting with *SIM* in *Arabidopsis*[*]

Li-li SUN[1], Zhong-jing ZHOU[1], Li-jun AN[1], Yan AN[1], Yong-qin ZHAO[1],

Xiao-fang MENG[1], Clare STEELE-KING[2], Yin-bo GAN[†‡1]

(*[1]Department of Agronomy, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China*)

(*[2]Centre for Novel Agricultural Products, Department of Biology, University of York, York, YO10 5DD, UK*)

[†]E-mail: ygan@zju.edu.cn

Received Dec. 17, 2012; Revision accepted Jan. 15, 2013; Crosschecked June 17, 2013

Abstract: *Arabidopsis* trichomes are large branched single cells that protrude from the epidermis. The first morphological indication of trichome development is an increase in nuclear content resulting from an initial cycle of endoreduplication. Our previous study has shown that the C2H2 zinc finger protein GLABROUS INFLORESCENCE STEMS (GIS) is required for trichome initiation in the inflorescence organ and for trichome branching in response to gibberellic acid signaling, although *GIS* gene does not play a direct role in regulating trichome cell division. Here, we describe a novel role of *GIS*, controlling trichome cell division indirectly by interacting genetically with a key endoreduplication regulator SIAMESE (*SIM*). Our molecular and genetic studies have shown that *GIS* might indireclty control cell division and trichome branching by acting downstream of *SIM*. A loss of function mutation of *SIM* signficantly reduced the expression of *GIS*. Futhermore, the overexpression of *GIS* rescued the trichome cluster cell phenotypes of *sim* mutant. The gain or loss of function of *GIS* had no significant effect on the expression of *SIM*. These results suggest that *GIS* may play an indirect role in regulating trichome cell division by genetically interacting with *SIM*.

Key words: *Arabidopsis thaliana*, GLABROUS INFLORESCENCE STEMS (GIS), Endoreduplication, *SIAMESE* (*SIM*), Trichome branching, Genetic interaction

doi:10.1631/jzus.B1200349   **Document code:** A   **CLC number:** S5-3

1 Introduction

Trichomes are present on most aerial organs of the plant including leaves, stems, and flower in *Arabidopsis*, and exist as a parrying barrier to protect plants from biotic and abiotic stress (Johnson, 1975; Marks, 1997; Rodney and Mark, 1997). Trichome cells provide an excellent model system to study the interplay of different development processes and

cellular functions (Hülskamp *et al.*, 1994). Trichomes are initiated in the epidermis of developing organs, and their distribution is well regulated; in wild type (WT) leaves, the clustering of trichomes is rare (Hülskamp *et al.*, 1994; Larkin *et al.*, 2003). Cells destined to become trichomes stop mitotic division and enter into an endoreduplication cycle. The trichome cells undergo four endoreduplication cycles, resulting in protrusion from the leaf surface, which is followed by two successive branching events (Hülskamp *et al.*, 1994; 1999; Schnittger *et al.*, 2002a; Larkin *et al.*, 2003; Bramsiepe *et al.*, 2010). Ultimately, the mature trichome develops into a three-branched structure with a DNA content of 32C. The process of trichome development is complex and involves many different genes that regulate their

spacing, density, and morphology (Hülskamp *et al.*, 1994; Szymanski and Marks, 1998; Hülskamp *et al.*, 1999; Larkin *et al.*, 2003; Bramsiepe *et al.*, 2010). *SIAMESE* (*SIM*) is a plant-specific cyclin-dependent kinase (CDK) inhibitor, and acts as a key regulator required for endoreduplication during trichome development (Walker *et al.*, 2000; Churchman *et al.*, 2006). *SIM* is considered to function as a repressor of cellular division in the endoreduplication cell cycle, because loss-of-function mutations of *SIM* display multicellular trichomes, in contrast to the unicellular trichome of WT plants (Walker *et al.*, 2000; Churchman *et al.*, 2006). The SIM protein has a motif similar to inhibitor/interactor of cyclin-dependent kinase/Kip-related proteins (ICK/KRP) proteins, and can interact with D-type cyclins (CYCDs) and A-type CDK (CDKA) (Churchman *et al.*, 2006), which are typically considered to function at the G1/synthesis transition (Schnittger *et al.*, 2002a). The overexpression of *CYCD* in trichomes produces multicellular trichomes and the *sim* mutant phenotype is rescued when CDK inhibitor ICK1/KRP1 is expressed in the trichomes (Weinl *et al.*, 2005). These results suggest that *SIM* acts within the CYCD-CDKA complexes to repress mitotic cycles. In addition, overexpression of the B-type cyclin (*CYCB*), which regulates the G2/M transition (Lilly and Duronio, 2005), results in a phenotype similar to that of the *sim* mutant (Schnittger *et al.*, 2002b). The detection of the *CYCB* transcript in the *sim* mutant, but not WT trichomes, indicates that *SIM* may inhibit *CYCB* expression (Schnittger *et al.*, 2002b). Furthermore, SIM has been shown to cooperate with CCS52/Fizzy-Related (FZR) family protein, which functions as an activator of anaphase-promoting complex, to establish endoreduplication in the trichome (Cebolla *et al.*, 1999; Kasili *et al.*, 2010).

Mutations that affect the number of endoreduplication cycles also lead to trichomes with supernumerary or reduced numbers of branches (Hülskamp *et al.*, 1994). Generally, DNA content correlates well with trichome branching, such that mutants with a reduced DNA content have fewer branches, and mutants with increased ploidy levels have more branches (Hülskamp *et al.*, 1994; Folkers *et al.*, 1997; Bramsiepe *et al.*, 2010). *GLABRA3* (*GL3*) and *TRIPTYCHON* (*TRY*) regulate not only trichome initiation, but also endoreduplication cycles

(Hülskamp *et al.*, 1994; Szymanski and Marks, 1998; Payne *et al.*, 2000). *GL3*, which encodes a helix-loop-helix transcription factor, is thought to facilitate the rapid growth of the trichome cell; mutation of the *gl3* locus results in an average DNA content of 16C, reduced trichome branching, and reduced trichome size (Hülskamp *et al.*, 1994). Further evidence indicates that *GL3* acts upstream of FURCA4 (*FRC4*), which positively regulates the trichome branching (Luo and Oppenheimer, 1999; Plett *et al.*, 2009). *TRY*, which encodes a small single R3-MYB-repeat protein (Szymanski and Marks, 1998), regulates lateral suppression during the process of trichome fate determination. Trichomes of the *try* mutant have increased branching and an average DNA content of 64C, which suggests an additional endoreduplication cycle has occurred (Szymanski and Marks, 1998; Rao and Venkatachalam, 1999; Schellmann *et al.*, 2002).

Genes which influence the branch number without affecting endoreduplication have also been identified. The STICHEL (*STI*) gene, which encodes a novel protein containing a domain similar to eubacterial DNA-polymerase III γ subunits (Bertram *et al.*, 1998; Ilgenfritz *et al.*, 2003), is considered to be one of the most important contributors for trichome branching. All trichomes are unbranched in the *sti* mutant, but endoreduplication levels were not affected in both *sti* mutants and *STI* overexpressors (Ilgenfritz *et al.*, 2003). BRANCHLESS TRICHOMES (*BLT*) gene, which encodes a protein of hitherto unknown function, was also reported to play a key role in trichome branch cell endoreduplication (Kasili *et al.*, 2010). *blt* mutant was found to enhance the multicellular trichome phenotype of mutants in the SIAMESE (*SIM*) gene and *BLT* interacts both genetically and physically with *STI* to regulate trichome branch, although *blt* mutants show normal trichome DNA content (Kasili *et al.*, 2010).

Previously, we reported the discovery and identification of four *Arabidopsis* C2H2 zinc finger protein genes, *GIS*, *GIS2*, *ZFP5*, and *ZFP8*, which through hormone signalling, stimulate trichome initiation in inflorescence organs and cauline leaves (Gan *et al.*, 2006; 2007). As stated above, in general, a correlation exists between the trichome branch number and the DNA content (Hülskamp *et al.*, 1994; Folkers *et al.*, 1997; Bramsiepe *et al.*, 2010); however, *GIS* functions to suppress trichome branching, but it

not required for endoreduplication (An *et al.*, 2012). Thus, the aim of this study is to investigate further the role of *GIS* in the regulation of trichome cell division, by exploring the genetic interaction between *GIS* and *SIM*.

2 Materials and methods

2.1 Plant materials and growth condition

Arabidopsis thaliana ecotype Columbia *Col-0* was used for all experiments. Seeds were surface-sterilized and sown on Murashige and Skoog (MS) medium containing 4.4 g/L MS powder (Sigma), 1% sucrose (Sigma), and 0.5% agar. Seeds were imbibed for 2 d at 4 °C, before being transferred to long day conditions (16 h light/8 h dark) at 22 °C. Seedlings of a similar size were transferred to soil 7–8 d after germination on MS media (Gan *et al.*, 2006). Trichome branching phenotypes were analyzed at 35 d after sowing, when the plants had bolted. The numbers of trichome branches on the adaxial surface of the 3rd, 4th, and 8th rosette leaves were recorded. At least 16 plants per genotype were used for the trichome branch counting analysis. All experiments were duplicated. The *sim* mutant was a gift from Prof. Martin HÜLSKAMP (University of Cologne, Germany).

2.2 Construction of double mutant

The *gis sim* double mutant was constructed by crossing *gis* and *sim* homozygous mutants. F2 populations were screened for homozygous double mutants on selective MS medium containing sulfadiazine (50 mg/L) for *gis* mutant, and then verified by genomic polymerase chain reaction (PCR). The homozygous double mutant was also self-crossed to ensure that the homozygous double mutant *gis sim* did not exhibit a trichome segregation phenotype.

2.3 RNA extraction and real-time reverse transcription PCR (RT-PCR)

RNA was extracted using the TRIzol™ reagent (Invitrogen) according to the manufacturer's instructions. All RNA was isolated from pooled samples of at least eight 21-d old plants. The primers used for quantitative RT-PCR analysis were as follows: *GIS* (GISQF1: 5′-TTCATGAACGTCGAATCCTTCTC-3′; GISQR1: 5′-ACGAATGGGTTTAGGGTTCTTATC

T-3′), *SIM* (SIMQF: 5′-CCATCTTGAATTTCCCAC CAGCCATC-3′; SIMQR: 5′-GCAGCCGCCGCC GTCATCATCTC-3′), *UBQ10* (UBQ10F1: 5′-GGT TCGTACCTTTGTCCAAGCA-3′; UBQ10R1: 5′- CCTTCGTTAAACCAAGCTCAGTATC-3′). cDNA synthesis and RT-PCR were conducted as described by Gan *et al.* (2010). Optimization experiments were performed to establish the optimal concentration of primers. Melt curve analysis and gel electrophoresis of the PCR products were used to confirm the absence of non-specific amplification products. Transcripts from an ubiquitin gene (*UBQ10*) were detected and used as an endogenous control. Relative expression levels were calculated by subtracting the C_t (threshold cycle) values for *UBQ10* from those of the target gene (to give ΔC_t) and then calculating $2^{-\Delta C_t}$ (Gan *et al.*, 2005).

2.4 Molecular cloning

The cloning of the *35S:GIS* construct has been previously reported (Gan *et al.*, 2006). In brief, the *35S:GIS* construct was introduced into *Agrobacterium tumefaciens* strain GV3101. *Agrobacterium*-mediated transformation of all *Arabidopsis* genotypes was performed using the floral dip method (Clough and Bent, 1998), and transgenic seeds were selected using hygromycin (Gan *et al.*, 2006).

2.5 Statistical analyses

All presented data were tested for significance by means of analysis of variance using Statistix program Version 3.5 (Analytical Software, USA). A Student's *t*-test was calculated at the probability of either 5% ($P<0.05$) or 1% ($P<0.01$) as previously described (Bao *et al.*, 2011; Gan *et al.*, 2011).

3 Results

3.1 Knockout of *SIM* affects the expression of *GIS*

The expression of *GIS* in the *sim* mutant was determined, which was significantly reduced in *sim* mutant plants in comparison to that in WT plants (Fig. 1a). However, the expression of *SIM* in *GIS* loss of function mutants was not significantly different from the WT control (Fig. 1b). The expression of *SIM* in *35S:GIS* transgenic plants was also measured and results show that the *SIM* transcript was not
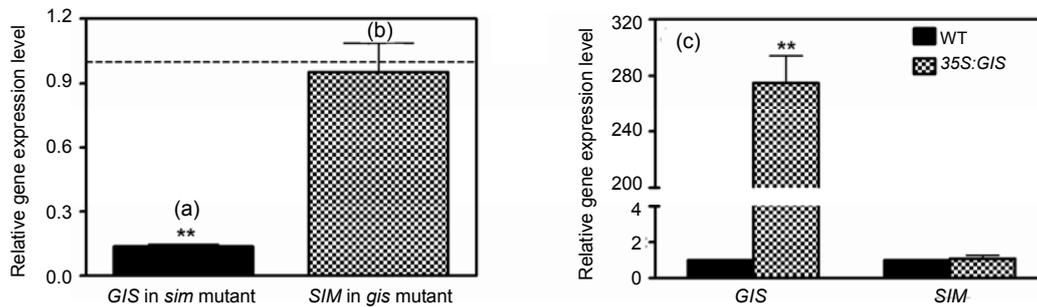
Fig. 1 Gene expression patterns of *GIS* and *SIM*

(a) *GIS* relative expression level in the *sim* mutant in comparison to that of the wild type; (b) *SIM* relative expression level in *gis* mutant in comparison to that of the wild type; (c) Expression levels of *GIS* and *SIM* in *35S:GIS* transgenic plants in comparison to those of the wild type. The relative gene expression value (mean±standard deviation (SD), *n*=3) was calculated by using *UBQ10* as the housekeeping gene against the wild type. The *t*-test was calculated at either 5% (* $P<0.05$) or at 1% (** $P<0.01$) probability
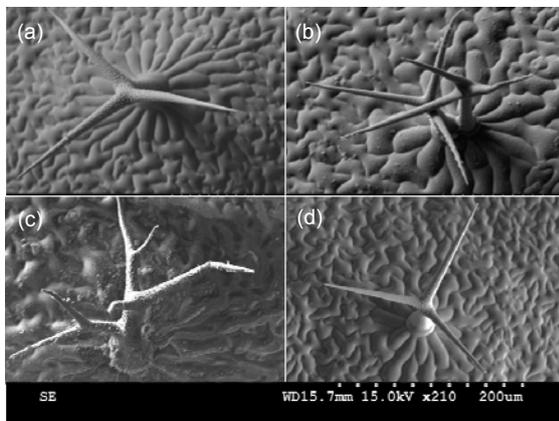

Fig. 2 Frozen scanning electron microscope images of genetic interactions between *GIS* and *SIM*

Trichome branching phenotypes were observed on the 8th rosette leaves of different genotypes. (a) In wild type, most of the trichomes are three-branched; (b) *sim* mutants frequently produce cluster trichomes; (c) Trichomes possess five-branched in *gis* background; (d) In trichome branching phenotype of *35S:GIS-sim* transgenic plants, no cluster trichomes could be found

significantly affected (Fig. 1c). These results suggest that *GIS* acts downstream of *SIM* to regulate trichome branching.

3.2 *GIS* functions downstream of *SIM* to regulate trichome branching

In the WT plants, most of the trichomes on rosette leaves are three-branched (Fig. 2a; Tables 1–4). However, in the *gis* mutant background, five-branched trichomes were observed on the 8th rosette leaves (Fig. 2c; Tables 1 and 2), which confirms that *GIS* acts to suppress trichome branching (An *et al.*, 2012). The *sim* mutant exhibited abundant trichome

clustering on the rosette leaves (Fig. 2b; Tables 1 and 3). These clusters are considered to be an abnormal trichome phenotype (Hülskamp *et al.*, 1994; Larkin *et al.*, 2003). To assess the genetic interaction between *GIS* and *SIM* further, *GIS* was overexpressed in a *sim* background. The abnormal branching phenotype of the *sim* mutant on the rosette leaves was rescued by this *GIS* overexpression (Fig. 2d; Tables 1 and 3). In *35S:GIS-sim* transgenic plants, we did not observe any trichome clusters on the 3rd, 4th, and 8th rosette leaves (Tables 1–3). Furthermore, the results show that the percentages of one- and two-branched trichomes in *35S:GIS-sim* plants were significantly increased while those of the three-branched trichomes on the 3rd and 4th rosette leaves in *35S:GIS-sim* plants were significantly decreased in comparison to those of the WT and *sim* mutant (Table 4). In the case of the 8th rosette leaves, the percentage of one-branched trichomes was also significantly increased, while the percentage of four-branched trichomes was significantly decreased (Table 2). These results are consistent with our previous findings that the overexpression of *GIS* suppresses trichome branching (An *et al.*, 2012). These results indicate a genetic interaction between *GIS* and *SIM* to control the trichome branching.

To further study the genetic relationship between *GIS* and *SIM*, the clustering and branching of trichomes in the *sim*, *gis*, and *gis sim* double mutants were analyzed. As shown in Tables 1 and 3, the clustering of trichomes was not found in the *gis sim* double mutant on either of 3rd, 4th, and 8th rosette leaves. The trichome branching pattern of *gis sim* plants most closely resembled that of *gis* single

Table 1 Frequency of trichome branching phenotypes observed at the 8th rosette leaves of different genotypes

Line	Number of branches[a]					
	1	2	3	4	5	Cluster
Wild type (*Col*)	0±0	4.0±1.3	22.2±6.2	1.5±0.8	0±0	0±0
gis	0±0	2.4±1.5[*]	20.7±4.0	6.5±1.5[**]	0.2±0.4[**]	0±0
sim	1.0±0.5[**]	2.7±1.1[**]	26.4±5.7[*]	1.5±0.5	0±0	1.7±1.9[**]
35S:GIS-sim	1.1±1.3[**]	4.8±1.8	32.4±7.5[**]	0±0	0±0	0±0
gis sim	0±0	2.4±1.5[*]	22.2±5.7	5.4±1.3[**]	0±0	0±0

[a] Trichomes were counted on the adaxial surface of the 8th rosette leaves of 16 plants. Values indicate mean±standard deviation (SD) (n=16). All significant test analyses were done using the corresponding wild type data as the control. The experiment was repeated at least once with similar results. t-test was calculated at the probability of either 5% ([*] $P<0.05$) or 1% ([**] $P<0.01$)

Table 2 Percentage of each trichome branching phenotype on the 8th rosette leaves of different genotypes

Line	Percentage of trichome branches[a]					
	1	2	3	4	5	Cluster
Wild type (*Col*)	0±0	14.8±3.3	81.6±7.2	3.6±1.8	0±0	0±0
gis	0±0	8.2±2.4[**]	69.2±3.0[**]	21.9±2.5[**]	0.7±0.4[**]	0±0
sim	2.9±0.5[**]	8.0±1.1[**]	79.4±6.7	4.5±2.3	0±0	5.2±2.0[**]
35S:GIS-sim	2.9±1.8[**]	12.5±2.8	84.6±5.1	0±0[**]	0±0	0±0
gis sim	0±0	7.9±5.5[**]	74.2±7.4[*]	17.9±5.9[**]	0±0	0±0

[a] Trichomes were counted on the 8th rosette leaves adaxial of 16 plants. Values indicate mean±standard deviation (SD) (n=16). All significant test analyses were done using the corresponding wild type data as the control. The experiment was repeated at least once with similar results. t-test was calculated at the probability of either 5% ([*] $P<0.05$) or 1% ([**] $P<0.01$)

Table 3 Frequency of trichome branching phenotypes observed at the 3rd and 4th rosette leaves of different genotypes

Line	Number of branches[a]					
	1	2	3	4	5	Cluster
Wild type (*Col*)	2.2±1.2	9.2±3.4	59.4±11.7	0±0	0±0	0±0
gis	1.0+1.1	8.3±2.7	63.1±9.9	8.1±3.3[**]	0±0	0±0
sim	1.9±1.6	7.1±3.4	52.4±7.9[*]	2.0±1.8[**]	0±0	9.6±3.3[**]
35S:GIS-sim	14.6±5.2[**]	57.1±17.7[**]	94.3±26.7[**]	0±0	0±0	0±0
gis sim	0.9±1.5	8.4±2.5	60.1±9.1	4.7±1.9[**]	0±0	0±0

[a] Trichomes were counted on the adaxial surface of the 3rd and 4th rosette leaves of 16 plants. Values indicate mean±standard deviation (SD) (n=16). All significant test analyses were done using the corresponding wild type data as the control. The experiment was repeated at least once with similar results. t-test was calculated at the probability of either 5% ([*] $P<0.05$) or 1% ([**] $P<0.01$)

Table 4 Percentage of each trichome branching phenotype on the 3rd and 4th rosette leaves of different genotypes

Line	Percentage of trichome branches[a]					
	1	2	3	4	5	Cluster
Wild type (*Col*)	2.9±1.3	18.7±7.9	78.4±7.3	0±0	0±0	0±0
gis	1.3±0.8[*]	10.3±3.7[**]	78.4±3.8	10.0±3.0[**]	0±0	0±0
sim	2.6±1.2	9.7±1.8[**]	71.9±5.1[*]	2.7±1.4[**]	0±0	13.1±3.5[**]
35S:GIS-sim	8.8±2.0[**]	34.4±5.6[**]	56.8±5.2[**]	0±0	0±0	0±0
gis sim	1.2±2.7[*]	11.3±4.3[*]	81.2±3.7	6.3±3.8[**]	0±0	0±0

[a] Trichomes were counted on the adaxial surface of the 3rd and 4th rosette leaves of 16 plants. Values indicate mean±standard deviation (SD) (n=16). All significant test analyses were done using the corresponding wild type data as the control. The experiment was repeated at least once with similar results. t-test was calculated at the probability of either 5% ([*] $P<0.05$) or 1% ([**] $P<0.01$)

mutant phenotype (Tables 2 and 4). Taken together with results from gene expression data and genetic interaction analyses, we conclude that *GIS* acts downstream of *SIM* to control trichome branching and cell division in an indirect manner.

4 Discussion and conclusions

The development of eukaryotes requires coordinated cell division and growth to produce specific organs and tissues (Churchman *et al.*, 2006). The cell cycle is essential in the establishment of developmental procedures, because it determines the cell type and function, as well as the types and functions of the organs and tissues (Lilly and Duronio, 2005). In *Arabidopsis*, no differentiation of the epidermal cells of aerial organs is apparent until cells switch from mitotic division to endoreduplication, which initiates the differentiation into trichome cells, where endoreduplication levels also influence the degree of trichome branching (Hülskamp *et al.*, 1994; Ishida *et al.*, 2008; Bramsiepe *et al.*, 2010; Tominaga-Wada *et al.*, 2011). Reduced endoreduplication rates result in smaller trichomes with fewer branches, while an increased level of endoreduplication leads to larger trichomes with more branches (Hülskamp *et al.*, 1994; Folkers *et al.*, 1997; Bramsiepe *et al.*, 2010). These results indicate that the endoreduplication rate is regulated precisely and functions to regulate trichome development.

A role for the C2H2 zinc finger protein GIS in controlling trichome initiation and branching through gibberellic acid signaling has been established previously (Gan *et al.*, 2006; An *et al.*, 2012). This current study demonstrates that *GIS* acts downstream of the key endoreduplication regulator, *SIM*, to regulate trichome branching. Furthermore, *GIS* may regulate the endoreduplication pathway indirectly by genetically interacting with *SIM* (Fig. 2; Tables 1 and 2). Further research is needed to explore the mechanism and components involved in this genetic interaction.

Acknowledgements

We sincerely thank Prof. Martin HÜLSKAMP (University of Cologne, Germany) for providing the *sim* mutant for this study.

Compliance with ethics guidelines

References

An, L., Zhou, Z., Su, S., Yan, A., Gan, Y., 2012. *GLABROUS INFLORESCENCE STEMS* (*GIS*) is required for trichome branching through gibberellic acid signaling in *Arabidopsis*. *Plant Cell Physiol.*, **53**(2):457-469. [doi:10. 1093/pcp/pcr192]

Bao, S.J., An, L.J., Su, S., Zhou, Z.J., Gan, Y.B., 2011. Expression patterns of nitrate, phosphate, and sulfate transporters in *Arabidopsis* roots exposed to different nutritional regimes. *Botany-Botanique*, **89**(9):647-653. [doi:10.1139/B11-053]

Bertram, J.G., Bloom, L.B., Turner, J., O'donnell, M., Beechem, J.M., Goodman, M.F., 1998. Pre-steady state analysis of the assembly of wild type and mutant circular clamps of *Escherichia coli* DNA polymerase III onto DNA. *J. Biol. Chem.*, **273**(38):24564-24574. [doi:10. 1074/jbc.273.38.24564]

Bramsiepe, J., Wester, K., Weinl, C., Roodbarkelari, F., Kasili, R., Larkin, J.C., Hulskamp, M., Schnittger, A., 2010. Endoreplication controls cell fate maintenance. *PLoS Genet.*, **6**(6):e1000996. [doi:10.1371/journal.pgen.1000 996]

Cebolla, A., Vinardell, J.M., Kiss, E., Olah, B., Roudier, F., Kondorosi, A., Kondorosi, E., 1999. The mitotic inhibitor *ccs52* is required for endoreduplication and ploidy-dependent cell enlargement in plants. *EMBO J.*, **18**(16): 4476-4484. [doi:10.1093/emboj/18.16.4476]

Churchman, M.L., Brown, M.L., Kato, N., Kirik, V., Hulskamp, M., Inze, D., de Veylder, L., Walker, J.D., Zheng, Z., Oppenheimer, D.G., *et al.*, 2006. SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in *Arabidopsis thaliana*. *Plant Cell*, **18**(11): 3145-3157. [doi:10.1105/tpc.106.044834]

Clough, S.J., Bent, A.F., 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.*, **16**(6):735-743. [doi:10.1046/j. 1365-313x.1998.00343.x]

Folkers, U., Berger, J., Hulskamp, M., 1997. Cell morphogenesis of trichomes in *Arabidopsis*: differential control of primary and secondary branching by branch initiation regulators and cell growth. *Development*, **124**(19): 3779-3786.

Gan, Y.B., Filleur, S., Rahman, A., Gotensparre, S., Forde, B.G., 2005. Nutritional regulation of *ANR1* and other root-expressed MADS-box genes in *Arabidopsis thaliana*. *Planta*, **222**(4):730-742. [doi:10.1007/s00425-005-0020-3]

Gan, Y.B., Kumimoto, R., Liu, C., Ratcliffe, O., Yu, H., Broun, P., 2006. *GLABROUS INFLORESCENCE STEMS* modulates the regulation by gibberellins of epidermal differentiation and shoot maturation in *Arabidopsis. Plant Cell*, **18**(6):1383-1395. [doi:10.1105/tpc.106.041533]

Gan, Y.B., Liu, C., Yu, H., Broun, P., 2007. Integration of cytokinin and gibberellin signalling by *Arabidopsis* transcription factors GIS, ZFP8 and GIS2 in the regulation of epidermal cell fate. *Development*, **134**(11):2073-2081. [doi:10.1242/dev.005017]

Gan, Y.B., Zhou, Z.J., An, L.J., Bao, S.J., Liu, Q., Srinivasan, M., Goddard, P., 2010. The effects of fluctuations in the nutrient supply on the expression of ANR1 and 11 other MADS box genes in shoots and roots of *Arabidopsis thaliana. Botany-Botanique*, **88**(12):1023-1031. [doi:10. 1139/B10-075]

Gan, Y.B., Zhou, Z.J., An, L.J., Bao, S.J., Forde, B.G., 2011. A comparison between northern blotting and quantitative real-time PCR as a means of detecting the nutritional regulation of genes expressed in roots of *Arabidopsis thaliana. Agric. Sci. China*, **10**(3):335-342. [doi:10.1016/ S1671-2927(11)60012-6]

Hülskamp, M., Misra, S., Jurgens, G., 1994. Genetic dissection of trichome cell development in *Arabidopsis. Cell*, **76**(3):555-566. [doi:10.1016/0092-8674(94)90118-X]

Hülskamp, M., Schnittger, A., Folkers, U., 1999. Pattern formation and cell differentiation: trichomes in *Arabidopsis* as a genetic model system. *Int. Rev. Cytol.*, **186**:147-178. [doi:10.1016/S0074-7696(08)61053-0]

Ilgenfritz, H., Bouyer, D., Schnittger, A., Mathur, J., Kirik, V., Schwab, B., Chua, N.H., Jurgens, G., Hulskamp, M., 2003. The *Arabidopsis STICHEL* gene is a regulator of trichome branch number and encodes a novel protein. *Plant Physiol.*, **131**(2):643-655. [doi:10.1104/pp.014209]

Ishida, T., Kurata, T., Okada, K., Wada, T., 2008. A genetic regulatory network in the development of trichomes and root hairs. *Annu. Rev. Plant. Biol.*, **59**:365-386. [doi:10. 1146/annurev.arplant.59.032607.092949]

Johnson, H.B., 1975. Plant pubescence: an ecological perspective. *Bot. Rev.*, **41**(3):233-258. [doi:10.1007/ BF02860838]

Kasili, R., Walker, J.D., Simmons, L.A., Zhou, J., de Veylder, L., Larkin, J.C., 2010. *SIAMESE* cooperates with the CDH1-like protein *CCS52A1* to establish endoreplication in *Arabidopsis thaliana* trichomes. *Genetics*, **185**(1): 257-268. [doi:10.1534/genetics.109.113274]

Larkin, J.C., Brown, M.L., Schiefelbein, J., 2003. How do cells know what they want to be when they grow up? Lessons from epidermal patterning in *Arabidopsis. Annu. Rev. Plant Biol.*, **54**:403-430. [doi:10.1146/annurev.arplant.54. 031902.134823]

Lilly, M.A., Duronio, R.J., 2005. New insights into cell cycle control from the *Drosophila* endocycle. *Oncogene*, **24**(17):2765-2775. [doi:10.1038/sj.onc.1208610]

Luo, D., Oppenheimer, D.G., 1999. Genetic control of trichome branch number in *Arabidopsis*: the roles of the FURCA loci. *Development*, **126**(24):5547-5557.

Marks, M.D., 1997. Molecular genetic analysis of trichome development in *Arabidopsis. Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **48**:137-163. [doi:10.1146/annurev. arplant.48.1.137]

Payne, C.T., Zhang, F., Lloyd, A.M., 2000. *GL3* encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with *GL1* and *TTG1. Genetics*, **156**(3):1349-1362.

Plett, J.M., Mathur, J., Regan, S., 2009. Ethylene receptor ETR2 controls trichome branching by regulating microtubule assembly in *Arabidopsis thaliana. J. Exp. Bot.*, **60**(13):3923-3933. [doi:10.1093/jxb/erp228]

Rao, K.N., Venkatachalam, S.R., 1999. Dihydrofolate reductase and cell growth activity inhibition by the β-carboline-benzoquinolizidine plant alkaloid deoxytu-bulosine from *Alangium lamarckii*: its potential as an antimicrobial and anticancer agent. *Bioorg. Med. Chem.*, **7**(6):1105-1110. [doi:10.1016/S0968-0896(98)00262-4]

Rodney, M., Mark, R.D., 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution*, **51**:1435-1444.

Schellmann, S., Schnittger, A., Kirik, V., Wada, T., Okada, K., Beermann, A., Thumfahrt, J., Jurgens, G., Hülskamp, M., 2002. *TRIPTYCHON* and *CAPRICE* mediate lateral inhibition during trichome and root hair patterning in *Arabidopsis. EMBO J.*, **21**(19):5036-5046. [doi:10.1093/ emboj/cdf524]

Schnittger, A., Schobinger, U., Bouyer, D., Weinl, C., Stierhof, Y.D., Hülskamp, M., 2002a. Ectopic D-type cyclin expression induces not only DNA replication but also cell division in *Arabidopsis* trichomes. *PNAS*, **99**(9): 6410-6415. [doi:10.1073/pnas.092657299]

Schnittger, A., Schobinger, U., Stierhof, Y.D., Hülskamp, M., 2002b. Ectopic B-type cyclin expression induces mitotic cycles in endoreduplicating *Arabidopsis* trichomes. *Curr. Biol.*, **12**(5):415-420. [doi:10.1016/S0960-9822(02)006 93-0]

Szymanski, D.B., Marks, M.D., 1998. *GLABROUS1* overexpression and *TRIPTYCHON* alter the cell cycle and trichome cell fate in *Arabidopsis. Plant Cell*, **10**(12): 2047-2062. [doi:10.1105/tpc.10.12.2047]

Tominaga-Wada, R., Ishida, T., Wada, T., 2011. New Insights into the Mechanism of Development of *Arabidopsis* Root Hairs and Trichomes. *In*: Jeon, K.W. (Ed.), International Review of Cell and Molecular Biology, **286**:67-106.

Walker, J.D., Oppenheimer, D.G., Concienne, J., Larkin, J.C., 2000. *SIAMESE*, a gene controlling the endoreduplication cell cycle in *Arabidopsis thaliana* trichomes. *Development*, **127**(18):3931-3940.

Weinl, C., Marquardt, S., Kuijt, S.J., Nowack, M.K., Jakoby, M.J., Hulskamp, M., Schnittger, A., 2005. Novel functions of plant cyclin-dependent kinase inhibitors, ICK1/KRP1, can act non-cell-autonomously and inhibit entry into mitosis. *Plant Cell*, **17**(6):1704-1722. [doi:10. 1105/tpc.104.030486]