The *Arabidopsis CURVY1 (CVY1)* gene encoding a novel receptor-like protein kinase regulates cell morphogenesis, flowering time and seed production

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**Abstract**

**Background:** A molecular-level understanding of the loss of *CURVY1 (CVY1)* gene expression (which encodes a member of the receptor-like protein kinase family) was investigated to gain insights into the mechanisms controlling cell morphogenesis and development in *Arabidopsis thaliana*.

**Results:** Using a reverse genetic and cell biology approaches, we demonstrate that *CVY1* is a new *DISTORTED* gene with similar phenotypic characterization to previously characterized *ARP2/3* distorted mutants. Compared to the wild type, *cvy1* mutant displayed a strong distorted trichome and altered pavement cell phenotypes. In addition, *cvy1* null-mutant flowers earlier, grows faster and produces more siliques than WT and the *arp2/3* mutants. The *CVY1* gene is ubiquitously expressed in all tissues and seems to negatively regulate growth and yield in higher plants.

**Conclusions:** Our results suggest that *CURVY1* gene participates in several biochemical pathways in *Arabidopsis thaliana* including (i) cell morphogenesis regulation through actin cytoskeleton functional networks, (ii) the transition of vegetative to the reproductive stage and (iii) the production of seeds.

**Keywords:** *CURVY1*, Cell morphogenesis, *Arabidopsis thaliana*, Distorted trichome, T-DNA knockout mutant, Actin bundle, Protein kinase, Seed production

**Background**

In plants, cell shape patterning and growth are regulated by multiple genes that are mediated by actin and microtubule cytoskeleton-dependent trafficking pathways [1-3]. The combined activities of the cytoskeleton, endomembrane, and cell wall biosynthetic systems organize the cytoplasm and define the architectural cell patterning [1-3]. Genetic screens have identified a class of mutants known as *DISTORTED* mutants because of their significant actin-related cytoskeletal growth-associated phenotypic defects and overall distorted cell shape patterning and abnormal polarized growth (trichome, epidermis, cell-cell communication) [2,4-6].

Genetic analysis reveals that gene that function in signal transduction cascades controlling local actin polymerization through the ARP2/3 complex [7-10] and the SCAR/WAVE complex [5,11-18] regulate cell patterning/morphogenesis in plants. Most of this knowledge comes from studies of differently distorted trichome mutants generally characterized by irregular cell expansion and polarized growth [2,4,19,20].

In order to decipher the genetic basis of plant cell shape patterning and growth, we employed, in this study, a reverse genetic approach by screening the loss of gene expressions in *Arabidopsis* T-DNA knockout mutants to gain insights into the mechanisms controlling cell morphogenesis in plants. *DISTORTED* mutants are known to display a dramatic cell shape alteration in comparison...
to wild type plants. The overall cell (trichome, pavement cell, root system) morphology of DISTORTED mutants has been well studied [21]. The DISTORTED genes have been reported to function in signal transduction cascades that control actin cytoskeleton assembly through WAVE/SCAR2-ARP2/3 pathway [2,3,20,21].

In this manuscript, we describe a new DISTORTED gene termed CVY1 (CVY1) that encodes a member of the receptor-like kinase (RLK) superfamily. Protein kinases are generally involved in perception of general elicitors initiating signal transduction cascades regulated by protein phosphorylation [22] to activate downstream responses that include the production of reactive oxygen species, ethylene biosynthesis, activation of a MAPK cascade, activation of abiotic or defense gene expression and other biological processes [23-26]. In addition, RLKs have also been recently related to the regulation of unidimensional cell growth, response to nitrate, and transferase activities in eukaryotes [22], several protein kinases and their biological phosphorylation processes are still largely uncharacterized in Arabidopsis thaliana. Among the protein kinase genes, the CVY1 (CVY1) gene appears to have a unique function related to cell morphogenesis, as cvy1 mutant displays phenotypes similar to distorted SCAR/WAVE and ARP2/3 mutant cell morphologies [2,4,16,27]. Using a reverse genetic approach, we examined and characterized a SALK T-DNA knockout cvy1 mutant (cvy1) with respect to cell morphogenesis and growth phenotypes. Knockout mutation in CVY1 caused severe trichome growth defects with relatively mild effects on overall shoot development, demonstrating that CVY1 functions in polarized cell growth and cell shape patterning. In addition, the work demonstrates that CVY1 represents a novel receptor-like kinase that regulates trichome, pavement cell morphogenesis and cell wall biogenesis among other interesting phenotypic features and might function in signal transduction cascades that control local actin assembling through the SCAR2/WAVE-ARP2/3 pathway.

Results and discussion
Genetic and phenotypic characterization of cvy1 mutant
To investigate the role of CVY1 in regulating cell morphogenesis in plants, we initiated a reverse genetic analysis of the gene using the Salk collection of Arabidopsis T-DNA knockout lines of our in-house Arabidopsis seed stock library. CVY1 is here shown to be important not only for polarized cell growth and trichome morphology but also other biological processes including flowering time and seed production. Our data reveals that mutations in CVY1 gene results in strong-distorted trichomes that are similar to the SCAR/WAVE and ARP2/3 mutant phenotypes [2,5,7-18]. To our knowledge, this is the first time that CVY1 has been shown to control cell morphology/patterning (Figure 1). In addition, we investigated the role of CVY1 in other biological processes. We employed a reverse genetic approach using the Arabidopsis T-DNA SALK lines mediating loss of function of CVY1 gene to examine cvy1-knockout phenotypes. The SALK_018797 (curvy1) line harboring a T-DNA insertion in the only exon of CVY1 gene map (Figure 1A) was selected and confirmed as null mutant with loss of CVY1 function. We confirmed the location of the T-DNA using the T-DNA-specific oligonucleotide primer LB1 and the CVY1-specific primer (Table 1) and examined the CVY1 mRNA transcript levels in wild type and cvy1 mutant using RT-PCR. As shown in (Figure 1B), the T-DNA insertion caused a knockout of the CVY1 gene in cvy1 mutant background. The mutation caused significant distortion of trichomes (Figure 1C, D, Table 2) and altered pavement cell morphology (Figure 1E, F, Table 3) compared to wild type. The cvy1 cell patterning (trichomes,

![Figure 1 Physical map of CVY1 gene knockout and phenotypic characterization of cvy1 mutant.](image-url)
epidermal cells) is not obviously different from previously characterized arp2/3 (arp2, arpc4) distorted mutants (Tables 2 and 3). The tissue specific expression pattern of CVY1 (Additional file 1: Figure S1) is consistent with Genevestigator microarray data [28]. The CURVY1 gene is ubiquitously expressed in all tested tissues, but particularly high in polarized cells/tissues such as the trichome, root, root tip, and hypocotyls (Additional file 1: Figure S1), suggesting its importance in plant cell morphogenesis and polarized cell growth.

CURVY1 controls cell morphogenesis in plants

We confirmed that cvy1 morphological phenotype was indeed caused by the described T-DNA insertion by constitutively overexpressing CVY1 gene in cvy1 mutant background. This complementation functionality test was performed by using Agrobacterium tumefaciens mediated transformation to introduce the 35-promoter-CVY1 transgene into cvy1 plants [29]. As expected, overexpression of CVY1 in cvy1 mutant background was sufficient to rescue the cvy1 phenotype (Figure 2A, B), demonstrating that CVY1 gene knockout is indeed responsible for the phenotypic characterization in cvy1 mutant phenotype, and thus providing further confirmation of the correct genetic characterization of CURVY1 as a new “DISTORTED” gene. The T-DNA (SALK_018797) causing knockout mutation in CVY1 (At2g39360) is also present in MIR156A (At2g25095) gene that targets SPL3. However, we ruled out the possibility of cvy1 mutant phenotypes being caused by a plausible insertion on MIR156A gene because homozygous mir156A mutant does not have distorted trichome phenotype and the overall phenotypic complementation tests (trichome phenotype, flowering time, seed production and hypocotyl gravitropism) excluded the implication of MIR156A mutation in the observed/described cvy1 phenotypes (Table 4).

Trichome branch length assay and pavement cell phenotypes are generally the most sensitive assays to describe phenotypic similarity among different distorted mutants [2,20]. The trichome phenotypes (Table 2) of cvy1 mutants were indistinguishable from the well characterized ARP2/3 distorted mutants (ARPC2 and ARPC4). In addition, cvy1 shape complexity of pavement-cells was significantly reduced compared to WT (Figure 1E, F), but was also indistinguishable from arpc2 and arpc4 pavement cells (Figure 3A-D, Table 3), suggesting a conserved cell shape regulatory relationship between CURVY1 and ARP2/3 in plants. The data suggests that CURVY1 belongs to the “distorted” group of genes. ARP2/3 gene mutations are associated with actin cytoskeleton defects [2], suggesting that

### Table 1 Sequences of oligonucleotide primers used in this study

| Name   | Primer sequence | Description               |
|--------|-----------------|---------------------------|
| CVY1-F1| 5′CCAGTGGGAACTTTCCGGTGG3′ | For RT-PCR               |
| CVY1-R1| 5′ATGGTGGGAACTTTCCGGTGG3′ | For RT-PCR               |
| TDNA-LB| 5′CCCGTCCTACTGGAACAG3′ | For T-DNA insertion       |
| CRV1-F2| 5′ATGCTCCGCGATATTTCCGGG3′ | For complementation test |
| CRV1-R2| 5′GAAGCTCTTTCG3′       | For complementation test |
| Act2-F | 5′CGCTCGACGACCGACAG3′ | For RT-PCR               |
| Act2-R | 5′GAGATGACGAAGACACAG3′ | For RT-PCR               |

### Table 2 Comparative quantitative phenotypic analysis of cvy1 trichomes to well characterized arp2/3 trichome mutants

| Trichome  | WT        | cvy1         | arpc2 (dis2) | arpc4         |
|-----------|-----------|--------------|--------------|---------------|
| Branch 1  | 286 ± 31  | 82 ± 27      | 87 ± 31      | 78 ± 26       |
|           | (n = 16)  | (n = 10)     | (n = 14)     | (n = 12)      |
| Branch 2  | 256 ± 50  | 30 ± 10      | 29 ± 12      | 28 ± 12       |
|           | (n = 16)  | (n = 10)     | (n = 10)     | (n = 12)      |
| Branch 3  | 194 ± 46  | 22 ± 12      | 18 ± 7       | 20 ± 8        |
|           | (n = 16)  | (n = 10)     | (n = 10)     | (n = 12)      |

The numbers in the parentheses indicate the number of samples analyzed. Mean values with different letters are significantly different from each other, and mean values with the same letter in the group are not significantly different (P <0.05).

### Table 3 Comparative quantitative analysis of cvy1 pavement cell shape phenotype to well characterized arp2/3 pavement cells

| Pavement cell | WT        | cvy1         | arpc2 (dis2) | arpc4         |
|---------------|-----------|--------------|--------------|---------------|
| Size (μm²)    | 210 ± 9   | 156 ± 3     | 170 ± 6     | 162 ± 3      |
|              | (n = 25)  | (n = 24)     | (n = 20)     | (n = 28)      |
| Circularity*  | 0.25 ± 0.06 | 0.38 ± 0.05 | 0.34 ± 0.06 | 0.30 ± 0.03  |
|              | (n = 25)  | (n = 24)     | (n = 20)     | (n = 28)      |

The numbers in the parentheses indicate the number of samples analyzed. Mean values with different letters are significantly different from each other, and mean values with the same letter in the group are not significantly different (P <0.05). *Circularity describes the cell shape complexity.

Figure 2 Overexpression of CVY1 gene rescues the cvy1 trichome phenotype in a complementation test. A) Distorted trichome phenotype of cvy1 mutant. B) The distorted trichome phenotype in cvy1 mutant is perfectly rescued by 35S:CVY1 gene expression.
CURVY1 might regulate cell morphogenesis through signal transduction cascades that control local actin assembly through the ARP2/3 complex or the SCAR/WAVE complex [2,20]. In addition, we scored the stomata surface areas and found WT-stomata overall to be bigger than the stomata of mutants (Figure 3E). We analyzed the growth of wild type, curvy1 mutants and the well characterized arp2/3 (arp2, arpc4) mutants under latrunculin B (LatB), an actin filament depolymerization drug [30]. The wild type (n = 22 seedlings), curvy1 (n = 28 seedlings) and arp2/3 (n = 12 seedlings) were affected by LatB (5 nM) treatment. However, we observed a significantly stronger effect of LatB on curvy1 mutants that was indistinguishable from the effect of LatB on arp2/3 (arp2 and arpc4) mutants (Table 5), supporting the notion that CURVY1 might regulate cell morphogenesis through actin cytoskeleton networks [2,30]. To further make the link between CURVY1 and the actin cytoskeleton, we tested the sensitivity of cvy1 rescue lines to the actin depolymerization drug LatB. As expected, the effect of LatB on cvy1 rescue lines (n = 20 seedlings) were similar to that of the wild type (n = 22 seedlings). Overall, these data demonstrate that CURVY1 regulates cell morphogenesis through actin cytoskeleton functional network.

CURVY1 encodes a member of the receptor-like kinase (RLK) protein family

The RLKs are integral plasma membrane associated proteins with an extracellular domain that mainly binds to a carbohydrate, a transmembrane domain, and an intracellular Ser/Thr kinase domain [31]. Overall, plant RLKs have been reported to regulate various signaling pathways, including meristem function, brassinosteroid perception, floral abscission, ovule development and embryogenesis, plant defense, and plant morphology [32]. Previous studies showed that selected members of Arabidopsis CrRLK gene family including FERONIA (FER: At3g51550) [33-36], THESEUS1 (THE1: At5g54380) [37], HERCULES1 [35], ANXUR1 and ANXUR2 (ANX1 and ANX2) [38,39] regulate cell growth processes in different tissues under different development conditions.

Table 4 Overexpression of CVY1 gene rescues the overall cvy1 phenotypes in complementation tests

| Phenotypes                          | WT          | curvy1       | cvy1_35S:CVY1 |
|------------------------------------|-------------|--------------|--------------|
| Flowering time (in number of rosette leaves) | 14.0 ± 1.5  | 10.0 ± 1.1   | 15.5 ± 2.0   |
| (n = 22)a                          | (n = 28)b   | (n = 12)a    |             |
| Number of siliques/seed production at 31 days | 12.5 ± 2.0  | 45.0 ± 5.0   | 14.0 ± 4.0   |
| (n = 22)a                          | (n = 28)b   | (n = 12)a    |             |
| Dark grown phenotype               | GG (n = 22) | LGG (n = 28) | GG (n = 12)  |

Flowering time, siliques/seed production and dark phenotypes of cvy1 mutant were rescued by 35S:CVY1 gene expression in a complementation test. Numbers in the parentheses indicate the number of samples analyzed. Mean values with different letters are significantly different from each other, and mean values with the same letter in the group are not significantly different (P <0.05). GG = Grow against gravity; LGG = Loss of growth against gravity.

Table 5 The effect of latrunculin B (LatB) on wild type arp2/3 and cvy1 seedlings

| Treatment                  | Root length (mm) |
|----------------------------|------------------|
|                            | WT               | curvy1         | arpc2 (dis2) | arpc4 |
| Control                    | 15.5 ± 0.5       | 15.0 ± 0.3     | 10.0 ± 0.6   | 9.0 ± 0.3 |
| (n = 22)a                  | (n = 28)a        | (n = 12)b      | (n = 12)b    |       |
| LatB (5 nM)                | 7.0 ± 0.05       | 5.0 ± 0.05     | 4.5 ± 0.06   | 4.0 ± 0.03 |
| (n = 22)c                  | (n = 28)d        | (n = 12)d      | (n = 12)d    |       |

The numbers in the parentheses indicate the number of samples analyzed. Mean values with different letters are significantly different from each other, and mean values with the same letter in the group are not significantly different (P <0.05). The data was generated from vertical plate grown seedlings for 12 days under continuous illumination.

Figure 3 curvy1 cell shape phenotype is indistinguishable from arp2/3 cell shape mutants. (A-D) Wide-field fluorescence images of fields of cotyledon epidermal pavement cells of wild-type (A), cvy1 (B), arpc2 (C) and arpc4 (D). (E) Wild type-stomata are bigger than the mutant-stomata. Stomata mean values with different letters are significantly different from each other, and mean values with the same letter in the group are not significantly different (P <0.05). Bars = 50 μm.
Likewise, CURVY1 has been found to control plant cell morphology and overall growth including flowering time, cell polarity, and actin cytoskeleton network.

CURVY1 gene encodes a receptor-like kinase (RLK) that belongs to the Catharanthus roseus RLK (CrRLK)-like family [40,41]. RLKs represent a large diverse family of proteins with approximately 600 members in Arabidopsis thaliana [42]. However, the CrRLK-like family comprises a conserved extracellular carbohydrate-binding malectin-like domain [40] with 17 members in Arabidopsis (Figure 4) and 20 in rice [40]. As expected, CURVY1 displayed all protein features (malectin-like domain, serine/threonine-protein kinase active site, protein kinase catalytic domain) of well characterized CrRLK-like family [40,41]. Interestingly, all 17 Arabidopsis members of CrRLK gene family are structurally well conserved. They are exclusively made of a single exon flanked with a variable UTR length structure at both 3’ and 5’ ends. Nine out of the 17 Arabidopsis members of CrRLK1-like gene family are located on chromosome 5, three on chromosome 2 and 3 respectively and one on chromosome 1 and 4 respectively (Figure 4B). Phylogenetic analysis revealed four subclasses with CURVY1 belonging to the larger subclass composed of 10 members including the well characterized THESEUS1 (THE1: At5g54380) and HERCULES1 (HERK1: At3g46290) (Figure 4B). These four subclasses suggest a diversification of Arabidopsis CrRLK1-like proteins based on functional specifications (Figure 4B).

Actin bundles are disorganized in curvy1 epidermal cells

We examined the organization of the actin cytoskeleton in pavement cells of cvy1 mutant. The wild type (n = 8) generates a significantly higher population of polarized actin bundles extending towards to the peripheral patterning of the pavement cells (Figure 5A). The cvy1 pavement cells (n = 10) displayed the presence of high levels of presumably diffuse and loosely aligned actin monomers and filaments, but lacking in polarized actin bundles (Figure 5B). The actin cytoskeleton phenotype of cvy1
mutant is similar to what has been reported for arp2/3 mutants [2]. The actin cytoskeleton phenotype (Figure 5) suggests that the CURVY1 gene might function in a common WAVE/SCAR2-ARP2/3 pathway [2,3,6,20,30]. To further support the function of CURVY1 through actin cytoskeleton network, we used the ImageJ analysis tool to quantify the number of actin bundles (AB) in pavement cells after thresholding the stacked image to easily track/count the actin bundles. Using a grid system (of 25 μsq surface area as unit of the grid) covering the entire pavement cell (Additional file 2: Figure S2), we obtained a significantly (P <0.05) higher number of actin bundles in WT (AB = 6.333 ± 1732, n = 9 samples) compared to cvy1 mutant (AB = 2.333 ± 1414, n = 9 samples) per surface unit of the grid (Additional file 2: Figure S2). Consistent with the diffused and loosely aligned actin cytoskeleton phenotype of curvy1 (Figure 5), the dark grown cvy1 mutant displayed a loss of gravity and polarized growth orientation compared to WT (Figure 6A, B). In addition, the etiolated phenotype was rescued by overexpressing CURVY1 gene in cvy1 mutant plants (Figure 6C, Table 4), suggesting that CURVY1 regulates cell morphology and polarized growth through functional actin cytoskeleton network in Arabidopsis thaliana.

CURVY1 controls other biological processes in plants
Interestingly, we noticed an early flowering phenotype in cvy1 mutants (n = 18) supported by a significantly (P <0.05) reduced number of rosette leaves (9 ± 0.8) compared to the wild type (13 ± 0.5, n = 16) at bolting time. Unlike cvy1 mutants, arp2/3 mutants (arpc2 rosette leaves = 18 ± 1.3, n = 14 and arpc4 rosette leaves = 22 ± 1.5, n = 12, at bolting time) showed a significantly (P <0.05) delayed flowering phenotype compared to WT and cvy1 mutant (Figure 7A), suggesting that CURVY1 might regulate growth development through distinct signal transduction cascades to control transition from vegetative to reproductive stage. The homozygous cvy1 mutant (n = 9) displayed a faster growth rate and higher seed pod (siliques) production compared to the wild type (n = 9) and arp2/3 mutants (n = 9) (Figure 7B-D), indicating that CURVY1 negatively regulates cell division and growth in meristemic regions as well as the overall production of seeds. Under similar growth conditions, cvy1 mutants produced about three-fold more seed pods (siliques; yield) compared to WT (Figure 7B) and 10-fold more siliques than arpc2 mutant (Figure 7C). Manipulating CURVY1 gene might be a promising target to improve crop yield in higher plants. To support this observation, we weighed all the seeds of each genotype at harvest time and found cvy1 seeds weighing two and half times more than those of WT and five times more than seeds of arpc2 mutant. Our data reveals that mutations in CVY1 gene result in early flowering, senescence, and improved seed productivity. The mechanism by which CURVY1 regulates transition processes from vegetative to reproductive phase needs to be investigated in agronomically important crops.

Conclusions
In summary, we present in this work the identification of a new gene, CURVY1 that regulates growth, cell morphogenesis and seed production in Arabidopsis thaliana. This work presents evidence that CURVY1 belongs to
the “distorted group” of genes. Homozygous cvy1 mutant displayed strong morphological phenotypes that are indistinguishable from the well-characterized DISTORTED trichome mutants [2]. The CURVY1 gene encoding a receptor-like protein kinase is ubiquitously expressed in all tissues tested. The distorted trichome phenotype in cvy1 mutant was rescued by expressing CURVY1 gene in the mutant background. Unlike the other DISTORTED mutants, mutation of CURVY1 gene promotes early flowering and seed production in Arabidopsis thaliana. Overall, CURVY1 represents a novel receptor-like kinase gene involved in regulating cell morphogenesis, including trichome and pavement cell shape patterning through local actin cytoskeleton assembling and additionally functions in signal transduction cascades that control flowering time and seed production in plants.

Methods

Plant strain, growth conditions and mutant characterization

Arabidopsis thaliana (ecotype Col-0) and cvy1 knockout mutant (T-DNA SALK_018797) [from Arabidopsis Biological Research Center (ABRC)] were used throughout this work. Appropriate seeds were sown on Murashige and Skoog (1× MS) agar plates or soil and seedlings were allowed to grow under continuous illumination (120–150 μEm⁻² s⁻¹) at 24°C. For cvy1 mutant characterization, T-DNA insertion was PCR-confirmed using CVY1 gene specific primers (Table 1) and T-DNA left border primer Lb (Table 1). To analyze the expression of CVY1 gene in mutant backgrounds, total RNA was extracted from the homozygous T-DNA insertion mutants by TRIzol reagent (Molecular Research Center) and then reversed transcribed.
using qScript cDNA Supermix (Quanta BioSciences, Gaithersburg, MD, USA) as previously described [30]. Thereafter, the cDNA was used as template for PCR using CVY1 gene-specific primers (Table 1), running 30 amplification cycles (linear range of amplification) [30]. PCR fragments were separated on 1% agarose gels containing ethidium bromide. A cDNA fragment generated from ACTIN served as an internal control.

For complementation test, a RT-PCR amplification of 2600 bp fragment containing the 5’ and 3’ untranslated regions as well as CVY1-encoding sequence (At2g39360) from WT cDNA (Table 1) was cloned into the SmaI site of the pROK2 vector [43] in front of CaMV 35S promoter-driven overexpression [43,44] and stably transformed cvy1 mutant background by the floral dip method [29]. For tissue specific gene expression analysis, the cDNA from respective tissues was used to perform real-time qPCR of CVY1 gene expression. Real-time qPCR was performed on Eco real-time PCR system (Illumina, San Diego, CA, USA) using PerfeCTa SYBR green FastMix (Quanta BioScience, Gaithersburg, MD, USA). The relative CVY1 expression level was assessed using ACTIN gene as internal control (Table 1).

Arabidopsis thaliana CrRLK1-like family: structural characterization and phylogenetic analysis

Catharanthus roseus RLK (CrRLK) characteristics were used to retrieve the 17 members of Arabidopsis thaliana CrRLK1-like gene family according to Hemyat and Hofte [31] and used to generate the phylogenetic tree according to Gachomo et al. [30]. CURVY1 (a member of CrRLK1-like family) protein functional domains were studied using different structure-functional motifs and/or patterns databases such as Pfam v25.0 (pfam.sanger.ac.uk), Prosite (prosite.expasy.org/scanprosite) and Conserved Domain Database (CDD) v3.02, CDART (Conserved Domain Architecture Retrieval Tool) to reveal the kinase catalytic domains, the carbohydrate, substrate and ATP binding sites and their 3D structural features according to Gachomo et al. [30].

Scanning electron microscopy (SEM)

SEM images of upper developing leaves, showing mature trichomes of WT and cvy1 mutant were acquired at different magnifications as previously described [30]. SEM images were taken using a LEO 1450 EP SEM [30].

Cell morphological analysis

Confocal image analysis was performed on one week after germination of plate grown plants. Pavement-cell shape analysis was performed by staining the samples with 10 μM of the lipophilic dye, FM464, for 2 hr in darkness under rocking conditions. The images were acquired using confocal microscopy (inverted Leica SP8 confocal microscope at 488 nm, 25% laser power and emission at 600 nm). The F-actin localization was done according to Kotchoni et al. [2]. Images were collected using an inverted Leica SP8 confocal microscope with water-immersion objective. The images were processed and analyzed using ImageJ software.

Determination of flowering time

Flowering time was assessed by counting the number of rosette leaves when flower bolts were 1 cm in length or when floral buds were visible at the center of the rosette as previously reported [30,45].

Statistical analysis

Experiments were performed at least three times. Data were expressed as mean values ± SE. P values were determined by Student’s t test analysis.

Additional files

Additional file 1: Figure S1. Expression of CVY1 in various tissues. qRT-PCR analysis of CVY1 gene (±SE) of three replicate samples per indicated tissues are depicted. A.U. = Relative expression CVY1 using internal Actin control in Arbitrary Unit.

Additional file 2: Figure S2. CVY1 regulates cell morphogenesis through actin cytoskeleton bundles. Actin filament bundles were analyzed with ImageJ after the image was thresholded to obtain filament bundles instead of monomeric actin subunit in the pavement cell. Actin bundles were quantified per grid (each grid measuring 25 μm) as depicted in WT Col-0 (A) compared to cvy1 (B) pavement cells. Actin bundles crossing the grid boundary were counted for both adjacent grids.

Competing interests

The authors declare that they have no competing interest.

Authors’ contributions

SOK conceived the study. SOK, EWG, WMS contributed reagents/materials/analysis tools. All authors read and approved the final manuscript.

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