

SUPPLEMENTARY MATERIAL

Quantification of gene transcript abundance

Total RNA was extracted from various plant tissues with Plant RNA extraction Reagent (Carlsbad, CA, Invitrogen). First strand cDNA was synthesized with 500 ng of total RNA by the QuantiTect Reverse Transcription kit (Germantown, MD, Qiagen). The generated cDNA was diluted fifty-fold for qRT-PCR and five-fold for semi-quantitative RT-PCR, respectively.

Root tips and elongation areas were separated by cutting roots from 10 day-old seedlings at 3 mm above the root tip. To obtain RNA from the root maturation area, root tissues between 3 mm and 1 cm from the root tip were used. For the collection of root tissue, Arabidopsis seedlings were grown vertically on 0.5 X MS/1 % agarose plates including 1 % sucrose at 10 days after germination (DAG). Rosette leaves and shoot apical meristems were collected from 21 DAG plants grown in soil under the same light and temperature conditions as described above. RNA from cauline leaves and reproductive tissues were extracted from 28 DAG and 35 DAG plants, respectively.

TaqMan Probe qRT-PCR was performed with TaqMan® Universal PCR Master Mix (Foster City, CA, Applied Biosystems) with thermocycling conditions as follows: 95 °C for 10 min followed by 50 cycles of 95 °C for 15 sec and 60 °C for 1 min. Taq-Man probes for AtCSP3 and actin 2 were prepared from Applied Biosystems and their respective sequences are listed in Supplementary Table S1.

For the confirmation of *atcsp3* T-DNA insertion alleles, cDNA was synthesized with total RNA extracted from leaf tissue of 28 DAG wild type Col-0 and loss-of-function mutant alleles. cDNA synthesis was performed as described above. Primer sequences for genotyping analysis are described in Supplementary Table S2. PCR conditions were set up as follows: 95 °C for 2 min for initial denaturation, followed by 30 cycles of 95 °C for 30 sec, 58 °C for 30 sec, 72 °C for 30 sec, and a final extension for 7 min at 72 °C. Arabidopsis actin 1 (*AAC1*) was amplified as an internal control for 26 cycles with the same PCR conditions as described above.

For semi-quantitative RT-PCR analysis of leaf cell polarity elongation genes, total RNA was extracted from the 5th leaf from three different soil-grown plants under long day conditions for 28 DAG. Primers for *CYCD3;1*, *ROT3*, *LNG1*, *LNG2*, *AN3*, and *AN* were designed by Primer3 software using the cDNA sequence information obtained from the Arabidopsis

Information Resource (www.arabidopsis.org). PCR reactions were performed with a Go-Taq Flexi PCR reaction kit (Madison, WI, Promega). Thermocycling conditions were as follows: 95 °C for 2 min for initial denaturation, followed by 30 cycles of 95 °C for 30 sec, 58 °C for 30 sec, 72 °C for 30 sec, and a final extension of 7 min at 72 °C. *AAc1* was used as an internal control amplified by the same PCR conditions as above. Quantitative real-time PCR analysis was performed to compare transcript abundance of *CYCD3;1* and *LNG1* by using SYBR Green PCR Master kit (Foster City, CA, Applied Biosystems). PCR conditions consisted of 40 cycles of 95 °C for 30 sec at denaturation, 60 °C for 30 sec at annealing, and 72 °C for 30 sec at extension. Melting curve analysis was determined by ramping the temperature from 55 °C to 99 °C with fluorescence measured at every 1 °C interval. A range of six different dilutions of wild type cDNA was used to generate standard curves for transcript abundant conversion. Actin 2 was used as an internal control. All primers for quantification of transcript abundance are listed in Supplementary Table 2.

Supplementary Table S1. List of context sequence and amplicon length for Taq-Man probes

Primer name	Sequence (5' - 3')	Amplicon length (b.p.)
Actin 2	CTGGATCGGTGGTTCCATTCTTGCT	85
AtCSP3	ATGATATGTGTGCTTGCTTATTAC	91

Supplementary Table S2. Primer list for semi-quantitative RT-PCR and real-time PCR

Primer name	Sequence (5'- 3')
F1	TGGTTGTTTTGGTAGTTTTTG
R1	TCATTGCAAGGAAAGACAAGA
AAc1-for	CGGCGATTCCAGGGAACATTGG
AAc1-rev	GTGCTCGACTCTGGAGATGGTG
ROT3-for	AGATTTTCGTCAGCGGAAAGA
ROT3-rev	CCAAAGGGTGTGAAGCAAAT
AN-for	AAACCTGGGGCTTTTCTTGT
AN-rev	CCTGTTGCCTACTGGTGGAT
LNG1-for	ATGGAGAAGACGCAGCATT
LNG1-rev	GACTGCTTCTCGAACCCAAG
LNG2-for	GAAGGAAGAGGAGCGGCTAT
LNG2-rev	CCGCTTCTGAATTTACCAT
AN3-for	GCTGGTTACTACCCAGCAA
AN3-rev	ATCCAAGCTGGCTATGGTG
CYCD3;1-for	ATGGATGCTTCAGCTCGTTT
CYCD3;1-rev	GATGCGGTCCACTGGTAGTT
Actin2-for	GCTAACATTGTGCTCAGTGGTG
Actin2-rev	GGAACCACCGATCCAGACACTGTA
LNG1for- real-time PCR	TTCCTTCAGGCAAAGCAAGT
LNG1rev- real-time PCR	CATTACTCAAACCGGGCTGT
CYCD3:1 for-real-time PCR	TCGTTGAACAGTCCAAGCTG
CYCD3:1-Rev-real-time PCR	TGCAAAATCGGCTTCTTCTT



Figure S1. Whole plant in soil at 28 DAG

All plants were grown under long day conditions (16/8 hrs for light/dark) at 23 °C. *atcsp3-1* and *atcsp3-2* have a smaller leaf size relative to wild type plants when measured at the onset of flower bolting. This result indicated that the small size phenotype of *atcsp3-1* and *atcsp3-2* was not affected by a retardation of developmental progression. Scale bar = 1 cm.

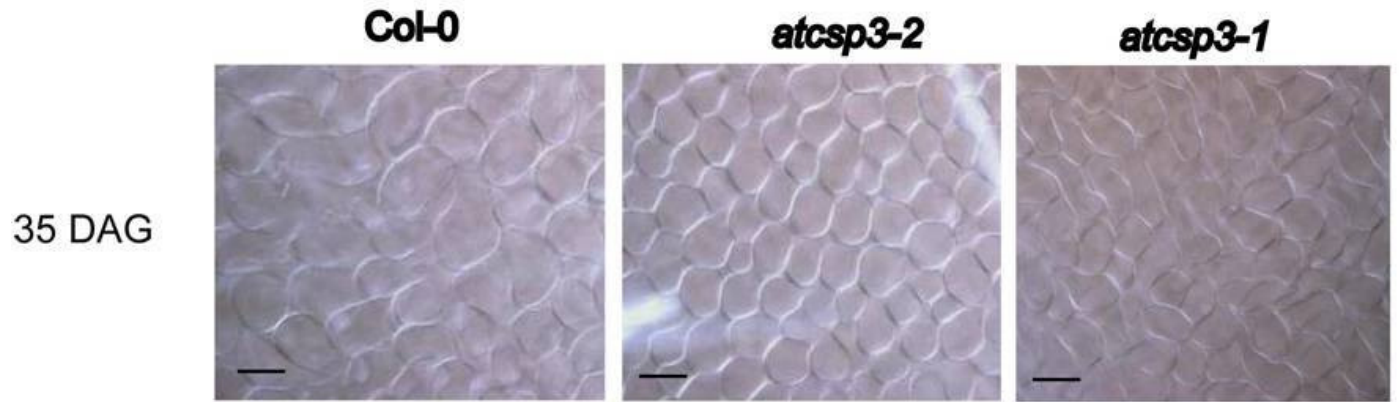


Figure S2. Microscopic images of 35 DAG 5th leaf palisade cells (Bar= 20 μ m).

DIC images were taken from the middle part of 5th leaves at 35 DAG from wild type Col-0, *atcsp3-2* and *atcsp3-1*. Note that the small size phenotype of palisade cells in *atcsp3-1* and *atcsp3-2* was sustained. Scale bars indicate 20 μ m.