Supporting Online Material for

Paternal Control of Embryonic Patterning in *Arabidopsis thaliana*

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Supplementary Online Material

Materials and methods

Plant stocks and growth conditions. Plants for phenotypic analysis were grown in walk-in chambers under constant illumination (65 μmol/m^2/s) at approximately 21°C and 60% relative humidity on commercial potting mix (RediEarth, Sun Gro Horticulture) containing systemic insecticide (Marathon 1% G, Olympic Horticultural Products) and slow-release fertilizer (19/12/6 Osmocote, Scotts Micacle-Gro Co.). In the greenhouse, where culture conditions were more variable, the phenotype of *ssp* embryos was occasionally skewed toward the weaker or stronger side of the spectrum reported in Fig. 1, suggesting an influence of environmental factors. For crosses, the stamens of designated female partners were manually removed prior to pollen dehiscence, and the pistils pollinated two days after emasculation.

The *ssp-1* allele was induced in the Landsberg *erecta* (*Ler*) accession by chemical mutagenesis (S1) and harbors a premature stop codon at amino acid position 141 (C-to-T nucleotide substitution at position +689 of the open reading frame). Additional alleles were found in sequenced-tagged insertion libraries: *ssp-2*, generated by T-DNA transformation in the Columbia (*Col*) accession, harbors an insertion in intron 2 (SALK_051462) (S2); *ssp-3* and *ssp-4*, generated by transposon mutagenesis of *Ler*, harbor insertions after amino acid position 383 (exon 8) and amino acid position 425 (exon 9), respectively (GT11708, ET11486) (S3). Other mutants and marker lines have been described: *yda-1* and hyperactive *YDA* lines (*Ler* accession) (S1), *AtSUC3-GFP* (*C24* accession) (S4), *met1* (also termed *ddm2-1*; *Col* accession) (S5), *ddm1-2* (*Col* accession) (S6), *cmt3* (SALK_148381; *Col* accession) (S7), *drm2* (SALK_150863; *Col* accession) (S8), *rdr1-1*, *rdr 2-1* (SAIL_672F11, and SAIL_1277H08, respectively; *Col* accession) (S9), *rdr6-15* (*Col* accession) (S10), *cdc2a* (*Col* accession) (S11), *fie* (*Ler* accession) (S12).

Map-based cloning. The *ssp-1* mutation maps to an approximately 4cM interval on the top arm of chromosome II, defined by the PCR-based markers *S499* and *T320* (table S2). 97 recombination events within this interval were collected from a mapping population of about 2400 F2 plants as described (S13). Fine-mapping with internal markers (table S2) positioned *ssp-1* between *SNP8866* (7 recombination events) and *S622* (4 recombination events), a 71kbp segment containing 15 predicted genes (At2g17040 to At2g17170). *SNP1809* co-segregated with the mutation. Sequence analysis of candidate genes surrounding *SNP1809* revealed a single base pair substitution in *At1g17090* (see above). A PCR-based marker for this substitution (table S2) also co-segregated with *ssp-1* in the mapping population. 1116 F2 plants were

analyzed, of which 306 were wild-type, 550 heterozygous, and 260 mutant, revealing normal transmission of the *ssp-1* allele through the haploid gametophytic generation.

T-DNA constructs. A 9.8kbp *Sma*I/*EcoR*I fragment covering the *SSP* locus was isolated from the BAC clone F6P23 and transferred to a modified pCAMBIA3300 binary vector (S14). To facilitate genotyping of transgenic plants without interference from SSP transgenes, intron 3 of the *SSP* gene was eliminated by overlapping extension PCR (S15; primer pair I3-F/R, table S3). This construct served as a basis for all further manipulations.

For tagging of the SSP protein, *Apa*I/*Stu*I restriction sites were inserted into two low-complexity regions N- and C-terminal of the kinase domain by overlap extension PCR (primers SSPKT-F/R and SSPMT-F/R, table S3). These sites were then used to insert the coding sequence of YFP (mCitrine variant, described in ref. S16; amplified by primer pair YANS-F&R, table S3).

Point mutations in the myristoylation/palmitoylation motif and the ATP binding pocket were introduced by overlap extension PCR with the following primers (table S3): G2-F/R (G2A substitution), C34-F/R (C3,4S substitution), and K78-F/R (K78R substitution). Truncated variants of *SSP*, harboring premature stops at amino acid position 301 and 434, were generated by inserting a synthetic linker with stop codons in all reading frames (5'-CTAGTCTAGACTAG-3') into a blunted *Nde*I site and an *Msc*I site, respectively.

Constructs directing ectopic expression of *SSP* were generated by fusing a *CaMV 35S* enhancer and transcriptional start site to the coding sequence of YFP-tagged as well as G2A and C3,4S variants of *SSP*, utilizing an *MspA1*I site located in the 5' untranslated region of the gene.

A genomic DNA fragment extending from the translational start site of *SSP* to 6.4kbp upstream was PCR amplified using Phusion DNA polymerase (New England Biolabs; primer pair SSP5'-F&R; table S3) and inserted into a plasmid for recombination-based cloning (pDONR221; Invitrogen). This fragment, containing the presumptive *SSP* promoter as well as the 5' untranslated region of the SSP transcript, was then joined with the coding sequence of a nuclear localized triple YFP reporter gene (Venus variant, described in ref. S17) in a modified pGreen binary vector (S18) by multi-site recombination (Gateway, Invitrogen).

RT-PCR and cDNA synthesis. RNA was extracted using the Spectrum Plant Total RNA kit (Sigma). 1 μg total RNA was used as a template for cDNA synthesis primed with oligo dT (annealing at 30 min at 50°C, reverse transcription for 30 min at 60°C with Thermoscript, Invitrogen). *SSP* cDNAs were amplified from aliquots of the reaction with the primer pair SSP-27-F and SSP+939-R (30 cycles, annealing at 62°C; table S3). *YFP* and *actin* cDNAs were amplified with the primer pairs YFP-F/R and Actin-F/R (same conditions; table S3).

A complete *SSP* coding sequence was amplified from a cDNA sample of mature pollen with the primer pair SSP-27-F and SSP+2215-R (40 cycles, annealing at 62°C; table S3) and subcloned into pGEM-T (Promega). Sequencing of individual plasmids revealed the existence of two splice variants. *MspA1*I/*Sph*I restriction sites were utilized to replace the genomic coding sequence with the corresponding sequences of both cDNA variants in T-DNA constructs.

To enable discrimination between the paternal and maternal contribution to *SSP* expression, flowers of the Wassilewskija (*Ws*) accession were hand-pollinated with pollen of *Col* plants. Approximately 2000 immature seed from 70 crosses were dissected 24 hours after pollination and collected in RNAlater solution (Applied Biosystems). RNA extraction and cDNA synthesis were as above, except reverse transcription was primed with random hexamer primers. Nested PCR was performed using the primer pair SSP+204-F/SSP-2215R (30 cycles, annealing at 62^{o}C; table S3). 1μl of the primary PCR product was used as template to amplify a 273 bp fragment of the *SSP* cDNAs with the primer pair cSSP-dCAPS-F& and -R (30 cycles, annealing at 62^{o}C; table S3). The resulting secondary PCR product was incubated with *Bcl*I. *Col*-derived cDNA species remained uncut, whereas species derived from *Ws* were cleaved (245bp and 28bp fragments). This procedure maps the single nucleotide polymorphism *SGCSNP1810* (www.arabidopsis.org), which was originally found in the *Ler* but is also present in the *Ws* accession.

***In situ* hybridization.** Pollen was germinated for 4h on microscope slides as described (S19) and fixed over night by adding 4% formaldehyde directly to the medium. *In situ* hybridization was performed on whole-mount germinated pollen. Siliques were fixed over night at 4^{o}C (4% formaldehyde in PBS) and processed for sectioning and hybridization as described (S20). Digoxigenin-labeled RNA-probes were generated by *in vitro* transcription with T7 polymerase (Stratagene) from purified PCR-generated templates covering non-conserved sequences up- and downstream of the kinase domain (primer pairs SSP+32-F and SSP+409T7-R, SSP+859-F and SSP+1345T7-R, respectively, for *SSP*; PIN7-F and PIN7-T7-R for *PIN7*; table S3). The *SSP* probes were used individually as well as combined to increase signal intensity. Pollen nuclei were counter-stained with DAPI (1mg/l).

Size measurements of embryonic cells. Whole-mount immature seed were dissected from siliques, cleared in modified Hoyer's solution (70% w/v chloralhydrate, 4% w/v glycerol, 5% w/v gum arabic) and examined by DIC microscopy (Leica DMRB compound scope equipped with Qimaging MicroPublisher 5.0RTV digital camera). Measurements were taken from digital images and calibrated by comparison to a stage micrometer.

Fluorescence and confocal laser scanning microscopy. Embryos, immature seed, leaves, and roots were mounted in water containing 10μM propidium iodide as a counter stain and imaged with a confocal laser-scanning microscope.

Mature pollen was harvested in 12% sucrose and germinated on glass slides for about 4 hours (S19). Nuclei were stained with DAPI (1mg/l) in germination medium, and the pollen tubes imaged with a fluorescence compound scope (Zeiss Axioplan2 microscope equipped with a Zeiss Axiocam HRm camera).

Assay for N-myristoylation. Recombinant *AtNMT1* with an N-terminal 10xHis tag was expressed from pET16b (Novagen) in bacteria and purified as described (S21). Myristoylation activity was assayed indirectly, by coupling the reaction to pyruvate dehydrogenase and measuring the produced NADH (S22). Absorbance at 340nm was monitored over time using a spectrophotometer equipped with a temperature control unit (Ultrospec-4000, AP Biotech). The reaction mixtures contained 50mM Tris at pH8.0,

1mM $MgCl_2$, 0.193mM EGTA, 0.32mM DTT, 0.2mM thiamine pyrophosphate, 2mM pyruvate, 0.1mg/ml bovine serum albumin, 0.1% Triton X-100, 5–1000µM peptide, 2.5mM NAD^+, 0.125U/ml porcine heart pyruvate dehydrogenase (0.33U/mg) and were incubated at 30°C. Peptides were custom-synthesized (Genscript Corporation; >95% purity) and dissolved in water at a final concentration of 4mM. The value for kcat/Km was obtained from the experimental data by iterative non-linear least square fits of the Michaelis–Menten equation as described (S23).

Genetic analysis of methylation effects. Pollen of *ssp-1* plants was crossed to pistils of DNA methylation-deficient mutants to test whether expression of the maternal *SSP* allele can be restored by abolishing maintenance methylation (*met1* and *ddm1*) (S24) or small RNA-mediated *de novo* methylation (*cmt3*, *drm2*, and *rdr1*;*-2*;*-6* triple mutants) (S24). All embryos from these crosses showed an *ssp* phenotype.

Generation and analysis of *fie* seed generated by *cdc2a* pollen. Seed with unfertilized endosperm was generated using *cdc2a* and *fie* mutant strains as described (S25). Briefly, loss of *CDC2a* affects cell division in the male gametophyte, resulting in pollen with a single sperm. This sperm fertilizes the egg cell. As the central cell remains unfertilized, a normal, triploid endosperm cannot develop to sustain zygotic development (S11). Second mutations in *FERTILIZATION-INDEPENDENT SEED (FIS)*-class Polycomb-group genes suppress this zygotic arrest by triggering the formation of a diploid endosperm. While this endosperm lacks any paternal contribution, is able to support essentially normal growth of the embryo (S25). Double heterozygous *cdc2a*+/-;*fie*+/- plants were identified by PCR (S11, S25) and allowed to self-fertilize. In the progeny, *fie* seed resulting from pollination with *cdc2a* pollen were recognized by their diploid endosperm, resulting in the presence of two instead of three nucleoli. Zygote division and suspensor formation was not affected in these seed.

References

S1. W. Lukowitz, A. Roeder, D. Parmenter, C. Somerville, Cell **116**, 109 (2004).

S2. J. M. Alonso, A. N. Stepanova, T. J. Leisse, C. J. Kim, H. Chen, P. Shinn, D. K. Stevenson, J. Zimmerman, P. Barajas, R. Cheuk, C. Gadrinab, C. Heller, A. Jeske, E. Koesema, C. C. Meyers, H. Parker, L. Prednis, Y. Ansari, N. Choy, H. Deen, M. Geralt, N. Hazari, E. Hom, M. Karnes, C. Mulholland, R. Ndubaku, I. Schmidt, P. Guzman, L. Aguilar-Henonin, M. Schmid, D. Weigel, D. E. Carter, T. Marchand, E. Risseeuw, D. Brogden, A. Zeko, W. L. Crosby, C. C. Berry, J. R. Ecker, Science **301**, 653 (2003).

S3. V. Sundaresan, P. Springer, T. Volpe, S. Haward, J. D. G. Jones, C. Dean, H. Ma, R. Martienssen, Genes Dev **9**, 1797 (1995).

S4. S. Meyer, C. Lauterbach, M. Niedermeier, I. Barth, R. D. Sjolund, N. Sauer, Plant Physiol. **134**, 684 (2004).

S5. H. Saze, O. Mittelsten Scheid, J. Paszkowski, Nature Genet. **34**, 65 (2003).

S6. J. A. Jeddeloh, T. L. Stokes, E. J. Richards, Nature Genet. **22**, 94 (1999).

S7. L. Bartee, F. Malagnac, J. Bender, Genes Dev. **15**, 1753 (2001).

S8. X. Cao, S. E. Jacobsen, Curr. Biol. **12**, 1138 (2002).

S9. Z. Xie, L. K. Johansen, A. M. Gustafson, K. D. Kasschau, A. D. Lellis, D. Zilberman, S. E. Jacobsen, J. C. Carrington, PLoS Biol. **2,** 642 (2004).

S10. E. Allen, Z. Xie, A. M. Gustafson, G. H. Sung, J. W. Spatafora, J. C. Carrington, Nature Genet. **36**, 1282 (2004).

S11. M. K. Nowack, P. E. Grini, M. J. Jacobi, M. Lafos, C. Koncz, A. Schnittger, Nature Genet. **38**, 63 (2006).

S12. A. M. Chaudhury, L. Ming, C. Miller, S. Craig, E. S. Dennis, W. J. Peacock, Proc. Natl. Acad. Sci. USA 94, 4223 (1997)

S13. W. Lukowitz, C. S. Gillmor, W.-R. Scheible, Plant Physiol. **123**, 795 (2000).

S14. D. McElroy, D. A. Chamberlain, E. Moon, K. J. Wilson, Mol. Breed. **1**, 27 (1995).

S15. W. Ito, H. Ishiguro, Y. Kurosawa, Gene **102**, 67 (1991).

S16. O. Griesbeck, G. S. Baird, R. E. Campbell, D. A. Zacharias, R. Y. Tsien, J. Biol. Chem. **276**, 29188 (2001).

S17. M. G. Heisler, C. Ohno, P. Das, P. Sieber, G. V. Reddy, J. A. Long, E. M. Meyerowitz, Curr. Biol. **15**, (2005).

S18. J-Y. Lee, J. Colinas, J. Y. Wang, D. Mace, U. Ohler, P. N. Benfey, Proc. Natl. Acad. Sci. USA **103**, 6055 (2006).

S19. M. K. Thorsness, M. K. Kandasamy, M. E. Nasrallah, J. B. Nasrallah, Plant Cell **5**, 253 (1993).

S20. W. Lukowitz, U. Mayer, G. Juergens, Cell **84**, 61 (1996).

S21. M. Pierre, J. A. Traverso, B. Boisson, S. Domenichini, D. Bouches, C. Giglione, T. Meinnel, Plant Cell **19**, 2804 (2007).

S22. B. Boisson, T. Meinnel, Anal. Biochem. **322**, 116 (2003).

S23. A. Martinez, J. A. Traverso, B. Valot, M. Ferro, C. Espagne, G. Ephritkhine, M. Zivy, C. Gaglione, T. Meinnel, Proteomics **8**, 2809 (2008).

S24. S. W-L. Chan, I. R. Henderson, S. E. Jacobsen. Nature Rev. Genet. **6**, 351 (2005)

S25. M. K. Nowack, R. Shrizadi, N. Dissmeyer, A. Dolf, E. Endl, P. E. Grini, A. Schnittger, Nature **447**, 312 (2007).

S26. Several websites are available for searches, such as the *Arabidopsis* eGFP browser (bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi).
S27. M. L. Engel, R. Holmes-Davis, S. McCormick, Plant Physiol. **138**, 2124 (2005).
S28. J. Friml, A. Vieten, M. Sauer, D. Weijers, H. Schwarz, T. Hamann, R. Offriga, G. Jürgens, Nature **426**, 147 (2003).
S29. P. Lu, R. Porat, J. A. Nadeau, S.D. O'Neill, Plant Cell **8**, 2155 (1996).

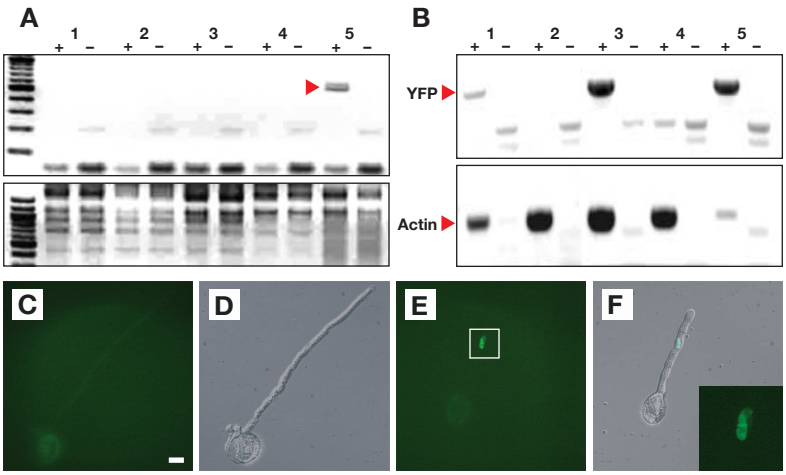

Fig. S1. Expression of *SSP* in pollen. (**A**) **Top panel**: RT-PCR with *SSP*-specific primers from total RNA of whole seedlings (1), rosette leaves (2), stems (3), whole inflorescences (4), and mature pollen (5); arrowhead in (5) points to a double band representing two *SSP* splice forms; lanes marked with a minus sign show control reactions without reverse transcriptase. **Bottom panel**: sample of total RNA used for RT-PCR reactions stained with ethidium bromide. Pollen-specific expression of SSP is in coincordance with microarray data in the public domain (S26). (**B**) **Top panel**: RT-PCR with YFP-specific primers from total RNA prepared from pollen of transgenic *ssp-1* plants harboring a functional YFP-tagged *SSP* variant (lane 1), *ssp-1* plants, transgenic *Col* wild type plants harboring the SSP::3xVenus reporter construct (3), and *Col* plants (4); RNA from transgenic seed expressing an inactive YFP-tagged *SSP* variant from a strong, broadly active promoter shown as positive control (lane 5); arrowhead marks position of YFP-specific band; although the assay was not meant to be quantitative, a stronger signal was consistently obtained with the triple-YFP transgene; lanes marked with a minus sign show reactions without reverse transcriptase. **Bottom panel**: RT-PCR with actin-specific primers from the same RNA samples. (**C–F**) Fluorescence microscopy of germinated pollen harboring a functional YFP-tagged *SSP* variant (**C**), and a reporter construct based on the promoter of the *GEX2* gene (S27) (**E**); overlays with DIC images of pollen tubes shown in (**D**, **F**); inlet: detail of two sperm cells in close proximity, boxed in (**E**); note the absence of specific fluorescence in (**C**), although *SSP* and *GEX2* show similar patterns of RNA expression and report similar normalized expression levels by microarray profiling (S26); scale bar 20μm.

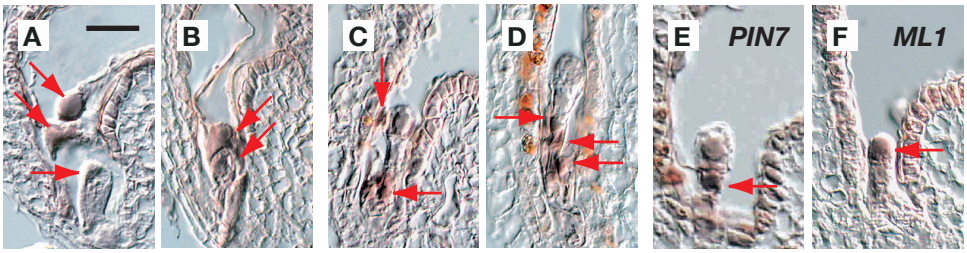

Fig. S2. Presence of *SSP* transcripts in the zygote and endosperm. *In situ* hybridization with *SSP*-specific probes to histological sections of seed containing zygotes (**A**, **B**) and 1-cell embryos (**C**, **D**); arrows point to weak signals in the zygote and the micropylar region of the endosperm; note that signals persist beyond the first division in the endosperm but not in the embryo; non-specific staining was independently assessed using control probes directed against *PIN7* mRNA, preferentially accumulating in the basal cell after division of the zygote (**E**, arrow) (S28) and *ML1* mRNA, preferentially accumulating in the apical cell (**F**, arrow) (ref. S29); scale bar 20μm.

	Classes of suspensor phenotypes					
	Exaggerated	Normal	Short	Rudimentary	Cone-shaped	Unrecognizable
Self-fertilized *ssp-1/ssp-1*	0	0	75 (16%)	246 (53%)	114 (25%)	30 (6%)
Self-fertilized *yda-1/+*	0	344 (60%)	67 (12%)	25 (4%)	20 (4%)	116 (20%)
Self-fertilized *ssp-1/ssp-1*; *yda-1/+*	0	0	4 (1%)	145 (47%)	99 (32%)	60 (19%)
Self-fertilized *D-YDA/+*; *yda-1/yda-1*	242 (45%)	142 (27%)	149 (28%)			
Self-fertilized *D-YDA/+*; *ssp-1/ssp-1*	305 (60%)	94 (18%)	113 (22%)			

Tab. S1. Genetic interactions of *SSP* and *YDA*. Frequency of suspensor phenotypes observed in the progeny of self-fertilized plants of the listed genotype; "exaggerated" suspensors were longer than wild type, contained more cells, and were often associated with aberrant development of the proembryo (see ref. S1 for images). Fig. 2 shows representative examples of the "short" (I), "rudimentary" (H), "cone-shaped" (G), and "unrecognizable" (F) suspensor classes; for the analysis of plants containing hyperactive *YDA* variants, the phenotypic classes of "short" to "unrecognizable" suspensors combined in one; hyperactive *YDA* variants are associated with a number of dominant effects that eventually become lethal, and can only be propagated in the heterozygous state.

Marker	Position	Primer pair	Comments
S499 (CEREON449449)	At2g16400/10	CATTGCTTGACCGAGCAGAG GTGAGAATAGAAGTGCGTTGG	Length polymorphism *Col*: ~200bp; *Ler>Col*
S643 (CEREON459643)	At2g16780/90	CCTCCTTACCTTCATCTGCC GCTGTTTCGAAACAGTAAAACG	Length polymorphism *Col*:~180bp; *Ler>Col*
SNP8866 (SGCSNP8866)	AtAt2g17036/40	TTTGGCCTCCACCTCC CAAGGTGACTGTATTTCACTGG	Length polymorphism *Col*: ~610bp; *Ler>Col*
SNP1809 (SGCSNP1809)	At2g17090	CAAACAAGCCTCTCCTAGTGG GCCTCTCAATGTTTGAAGtACGAGG	dCAPS marker, creates polymorphic *Box*I site *Col*: ~240bp+20bp; *Ler*: ~260bp
S622 (CEREON444622)	At2g17170/80	GAAGAAACACCATAATCCATGC GAGGAAGAGCATAACATAGCC	Polymorphic *Cla*I site: *Col*: ~490bp+280bp; *Ler*: ~770bp
S500 (CEREON459500)	At2g17230/40	GTTCGTAATCCTTCCATGTCC GTCTAACAAATTTGCTTCCAGC	Length polymorphism *Col*: ~350bp; *Ler<Col*
T650 (CEREON458650)	At2g17660	GAAACGAGTCCATTTCGTAGG CCTTCCAGCTTCATTCGACG	Length polymorphism *Col*: ~180bp;
T320 (CEREON460320)	At2g18380/90	GTGACATTGTCACTCACGTGG CAAAGATGTCTTCAGTGTCTGG	Length polymorphism *Col*: ~200bp; *Ler<Col*
ssp-1 (EMS-induced allele)	At2g17090	TTAGAGACCACACGAGAAGGC TAACATGGCTTGGTCTGATCC	Polymorphic *Ssp*I site Wild type: ~620bp; *ssp-1*: ~440bp+180bp

Tab. S2. Markers for PCR-based mapping and genotyping. Alias of the marker name listed in brackets refers to the designation of the sequence polymorphism in the TAIR database (www.Arabidopsis.org).

Oligonucleotide	Sequence
I3-F	GTTTTGTTTGATGAGGATGGTGATGCATGTCTTTCTTG
I3-R	CATGCATCACCATCCTCATCAAACAAAACCTTGTAAGC
SSPMT-F	CCCATCACCATCACCATCACCCCGGGAGGCCTGAAGATCCACCATTGACAAAATTC
SSPMT-R	GGGTGATGGTGATGGTGATGGGGCCCTCCGCCGTTTCTAGGTTC
SSPKT-F	CCCATCACCATCACCATCACCCCGGGAGGCCTTCAAGTAATCTTTCGCCACTAG
SSPKT-R	GGGTGATGGTGATGGTGATGGGGCCCCGATGATGCATCCTTCTCTTG
YANS-F	GATGACGATAAGGGGCCCATGGTGAGC
YANS-R	CTTGTACAGCTCAGGCCTGCCGAGAGTG
G2-F	GAAGAATCTGATGGCCTGTTGTTACTCAC
G2-R	GTAACAACAGGCCATCAGATTCTTCAGC
C34-F	GAATCTGATGGGCTCGAGTTACTCACTATCTTCAACAG
C34-R	TGAGTAACTCGAGCCCATCAGATTCTTCAGCGG
K78-F	CGTCACTATTCGAAGATTTAATAACATGGCTTGGT
K78-R	CCATGTTATTAAATCTTCGAATAGCGACGAAACCAC
SSP5'-F	CGAATTGGATCCCGGGAGAGGATTATAG
SSP5'-R	TTTTTCTCGAGATTCTTCAGCGGGCA
SSP-27-F	CTCTTCTTTTGCCCGCTGAAGAATCTG
YFP-F	TTGAATTCATGGTGAGCAAGGGCGAG
YFP-R	AAAAGCTTCATGCCGAGAGTGATCCC
Actin-F	GCTCGACTCTTGGAGATGGTGTG
Actin-R	GCGATTCCAGGGAATTGTGG
SSP+939-R	TCTCCTTCATCAAGCCGAAACAAGAAAGAC
SSP+2215-R	CTCATTGTTACTATTTCTTTACTTCAAGAAGAG
cSSP-1R	ATGACCTCTCTTCAACGAACT
cSSP-dCAPS-F	TCGCCTCTCAATGTTTGAAGTACGATG
cSSP-dCAPS-R	CCATTCTTCGAAAGATAACTCG
SSP+32-F	TAGATCCTGTTCAAGACCACACGA
SSP+409T7-R	GAGATAATACGACTCACTATAGGAGGAATAAACGCTTCGCAAGAGT
SSP+859-F	ACGCTTGAAACCTTGCAAACT
SSP+1345T7-R	GAGATAATACGACTCACTATAGGATGTTCATGTCGAGTTTGGCT
PIN7-F	CGCTTCTTCTCTTTCTTTTCG
PIN7-T7-R	GAGATAATACGACTCACTATAGGAGGGTTTAGCTCTGCTGTGGA

Tab. S3. Oligonucleotides for molecular biology.