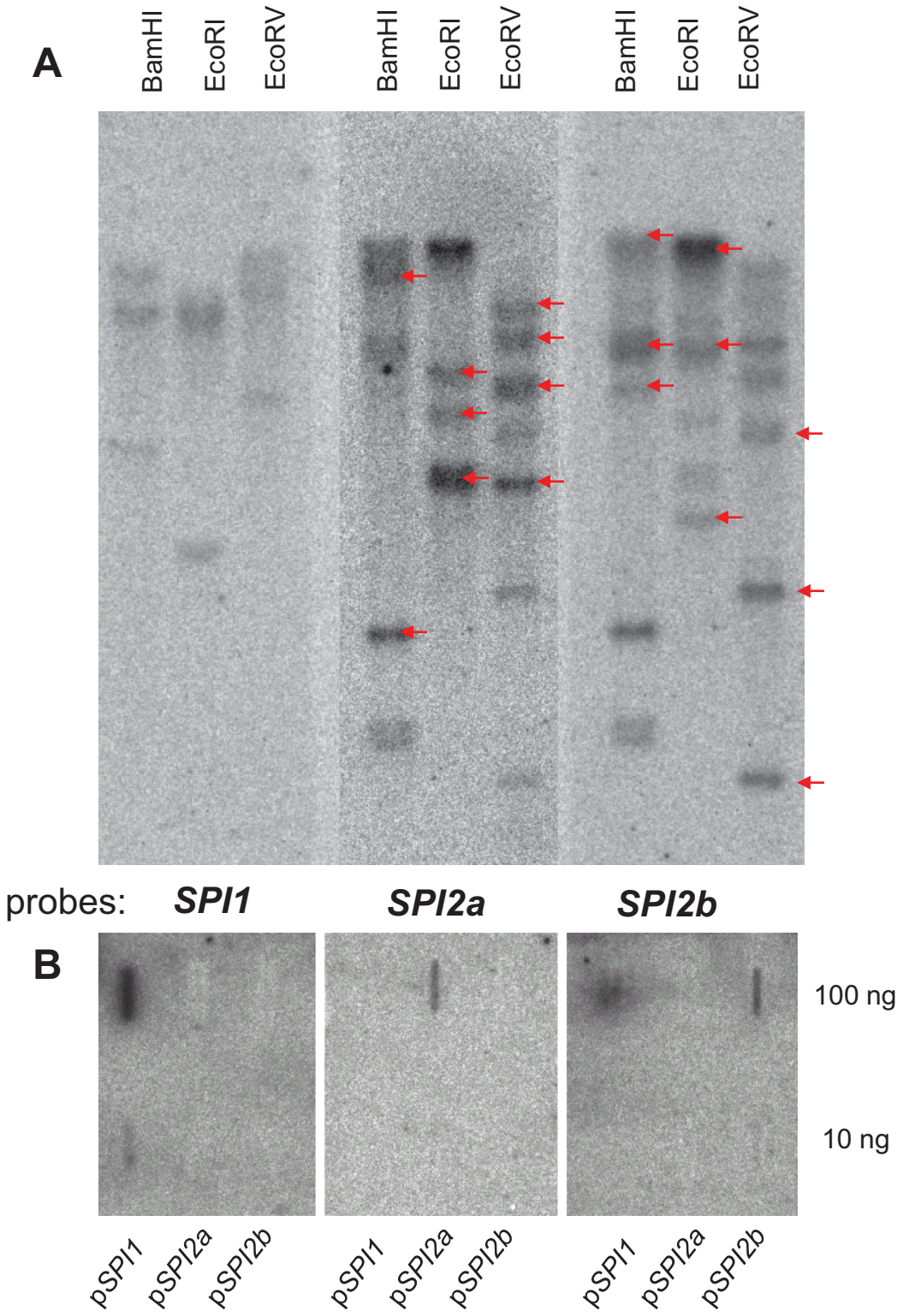
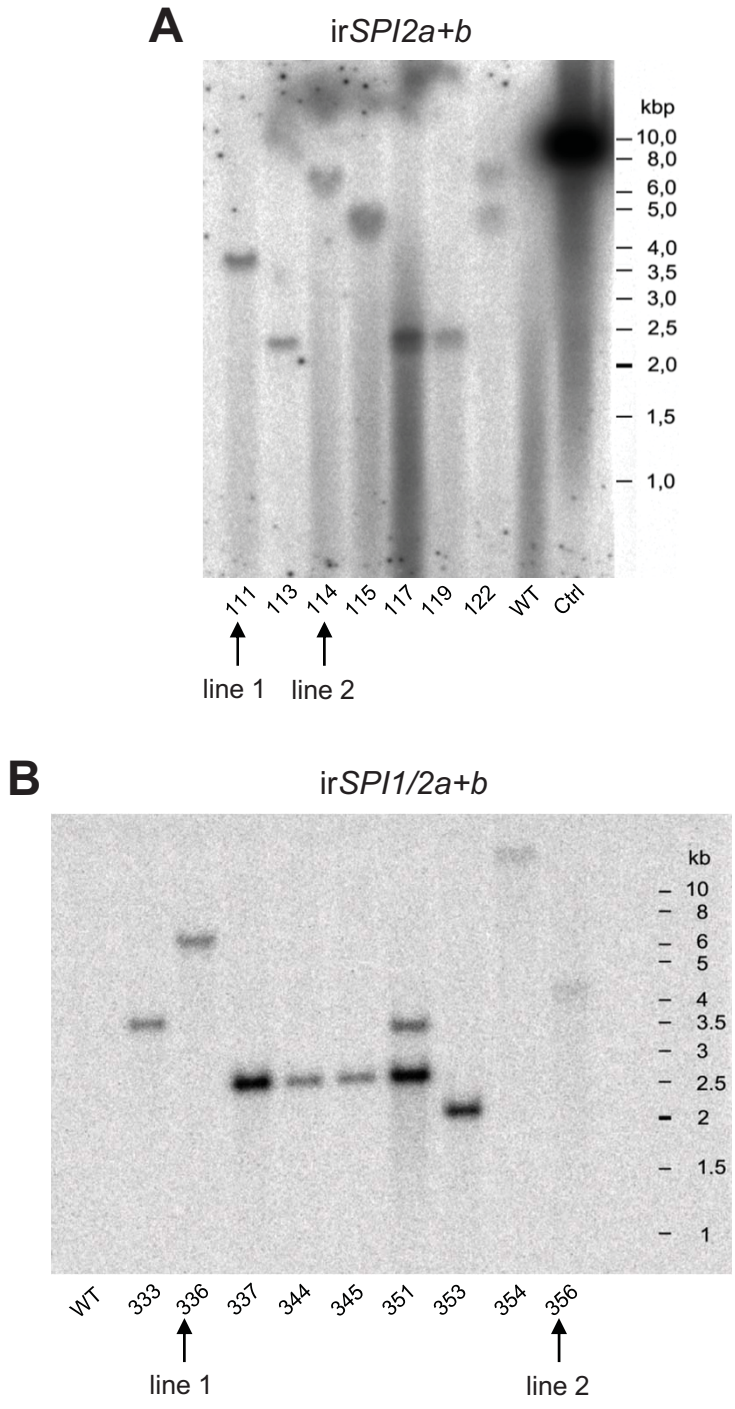
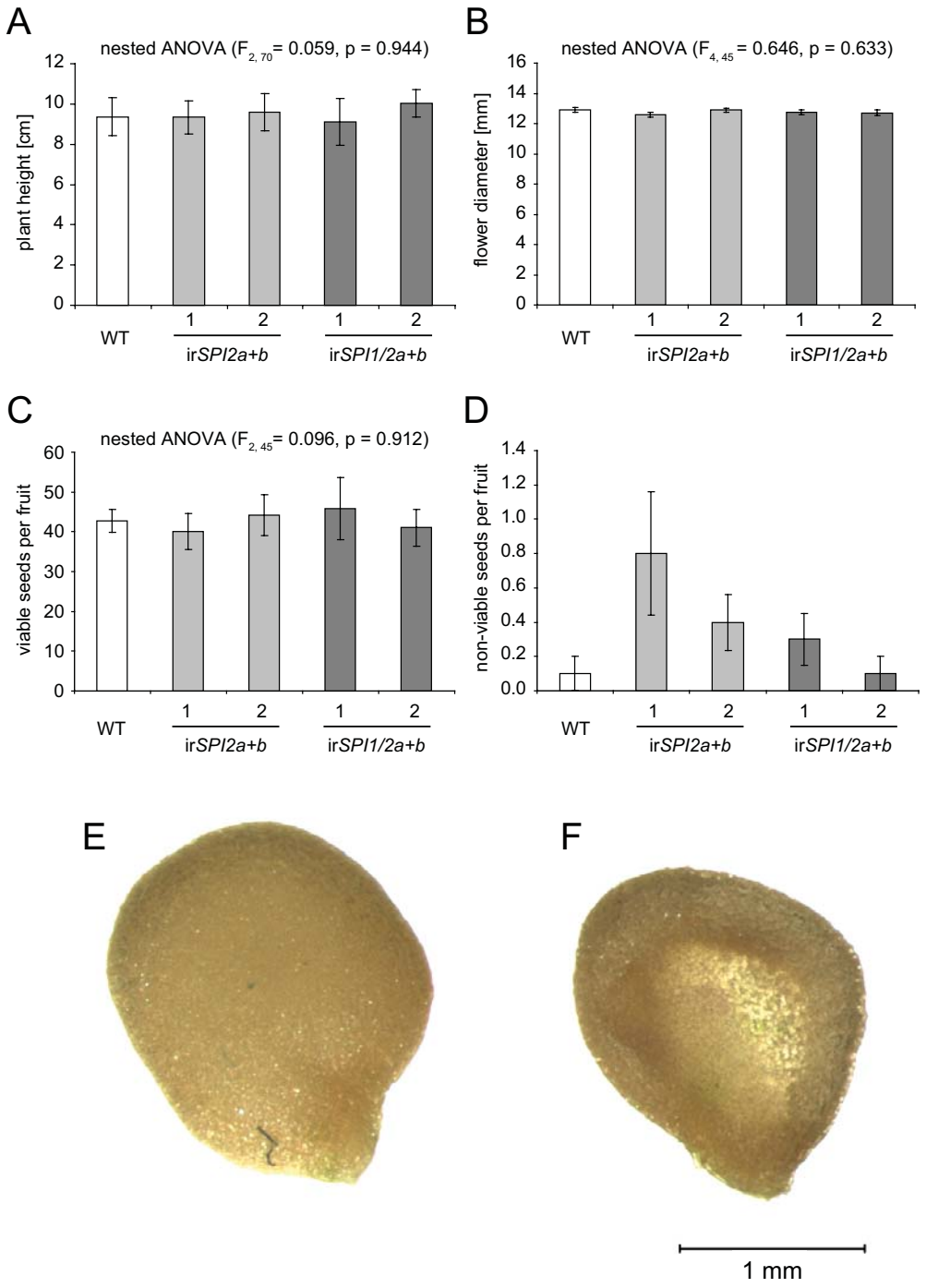


Supplemental Figure 1. Copy number of SPI genes in *S. nigrum*.
A, DNA gel blot of genomic DNA digested with the enzymes BamHI, EcoRI, and EcoRV and hybridized with probes specific for *SPI1* (a+b), *SPI2a* , and *SPI2b*. Red arrows indicate signals which are stronger either on the blot hybridized with *SPI2a* or with *SPI2b*. B, specificity of probes. A slot blot of plasmids containing fragments of *SPI1a* , *SPI2a* , and *SPI2b* (pSPI1, pSPI2a, pSPI2b) was hybridized with the same probes as in A. Two concentrations per plasmid were blotted (10 and 100 ng).

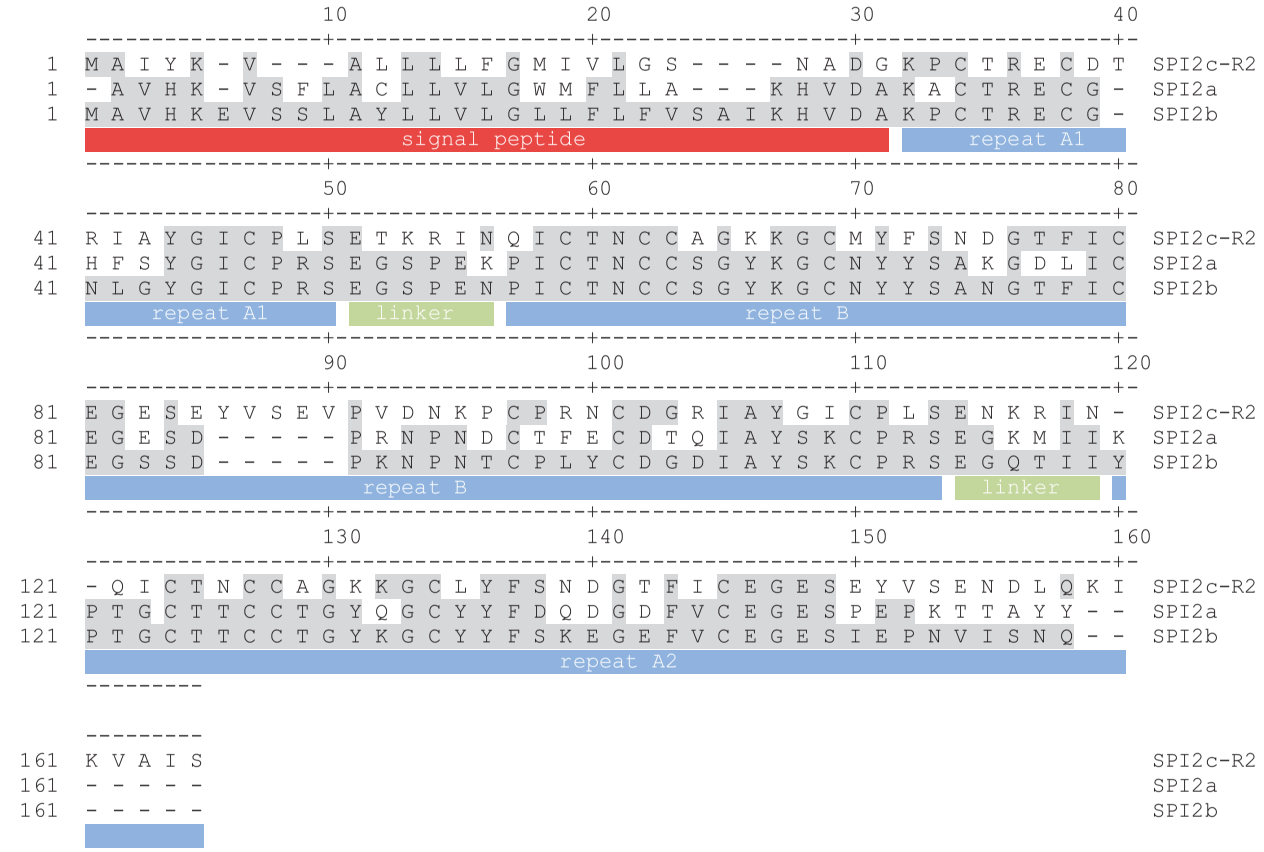


Supplemental Figure 2. Number of T-DNA inserts in the genomes of the transgenic lines ir*SPI2a+b* and ir*SPI1/2a+b*.
A, DNA gel blot of genomic DNA from ir*SPI2a+b* line 1 and 2, hybridized with a probe coding for the hygromycin resistance gene. B, DNA gel blot of genomic DNA from ir*SPI1/2a+b* line 1 and 2, hybridized with the same probe as in A. Arrows indicate the lines selected for further experiments.



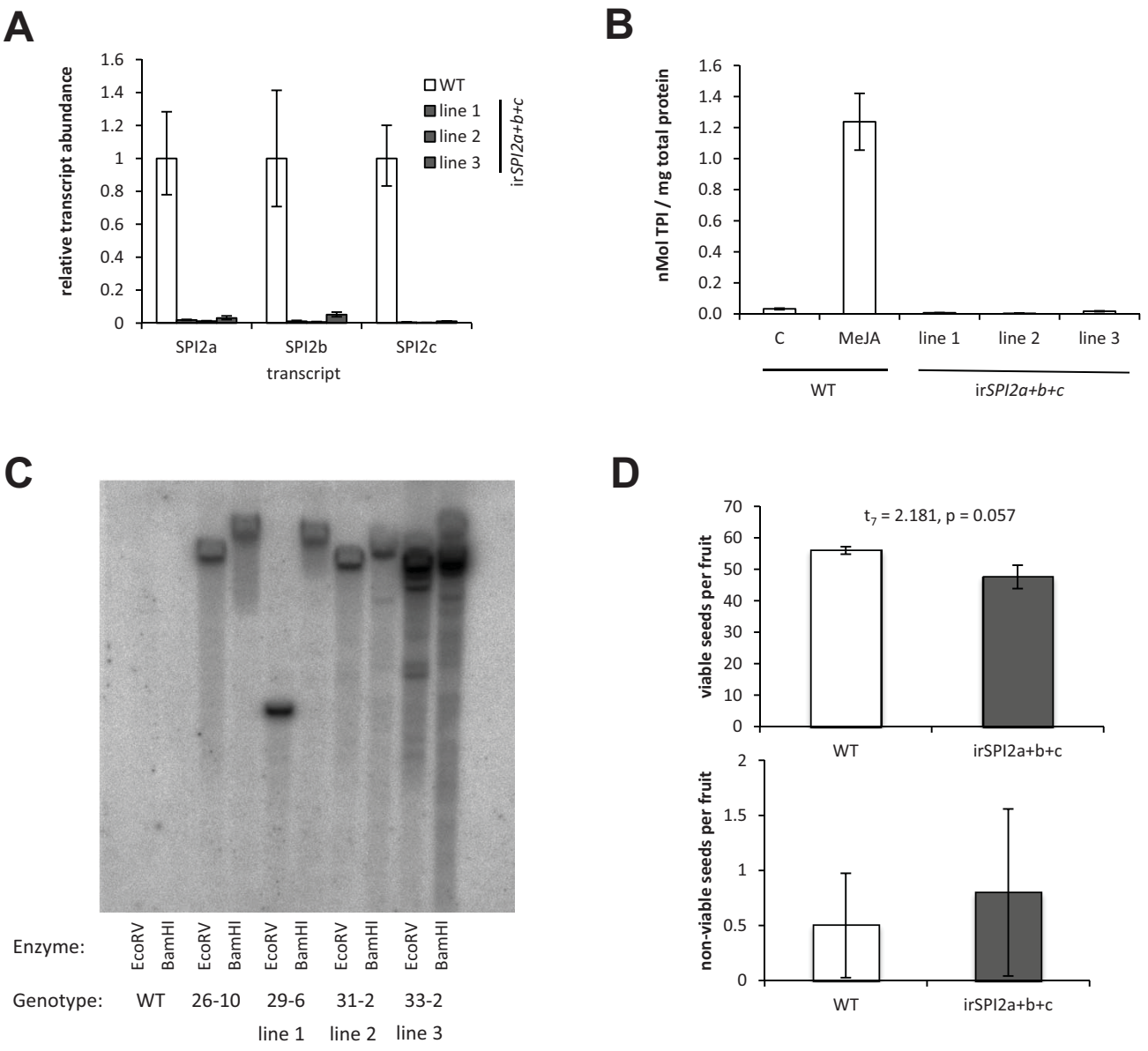
Supplemental Figure 3. Plant height (A), flower diameter (B), and number of viable and non-viable seeds (C, D) in wild-type and SPI-silenced *S. nigrum* plants.

A, mean ± SE plant height of 4-wk-old plants measured from the cotyledonar node to the apex (n = 15). B, mean ± SE flower diameter measured between the tips of the two most distant petals (10 plants per genotype, 3 flower replicates per plant). Nested univariate ANOVA-model: diameter ~ genotype flower-replicate(genotype). C, D mean ± SE number of viable (C) and non-viable (D) seeds per berry (n = 10). E, viable wild-type seed. F, non-viable ir*SPI2a+b* seed (line 1).



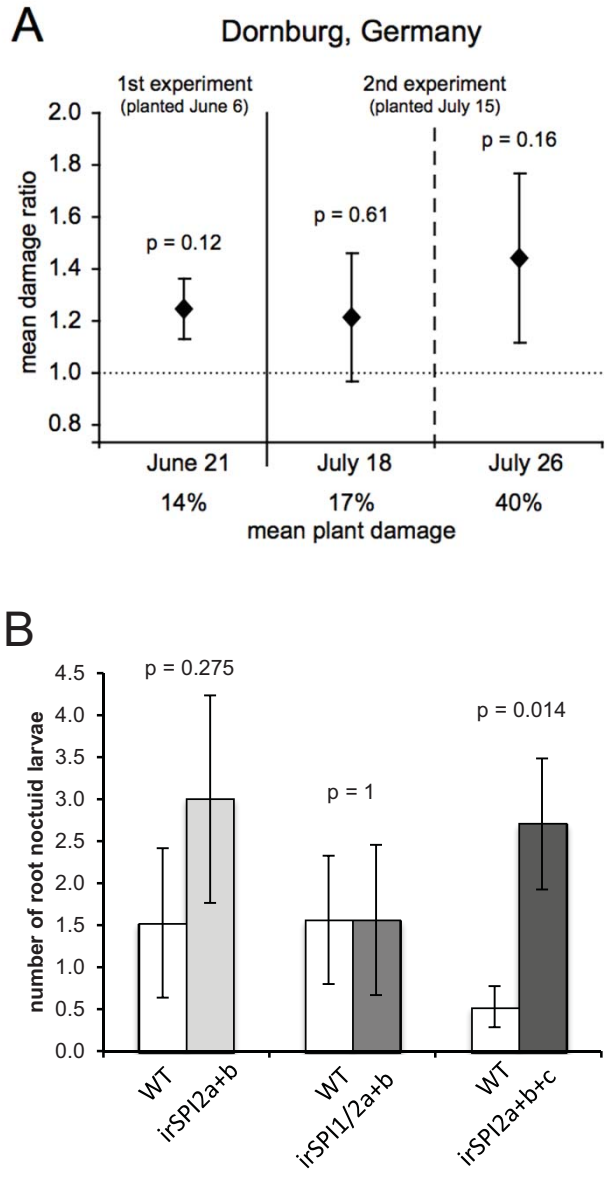
Supplemental Figure 4. Multiple sequence alignment (Clustal W) of translated cDNA sequences from all three *SPI2* genes containing two sequence repeats.

SPI2c-R3, which contains three repeats with 97% identity to *SPI2-R2*, was not included to facilitate the illustration. Residues that match the sequence of *SPI2b* are shaded in gray. Colors show the putative functional units of the proteins as derived from a comparison with other PI-II inhibitors in public databases and literature (Barta et al., 2002; Horn et al., 2005; Kong & Ranganathan, 2008).



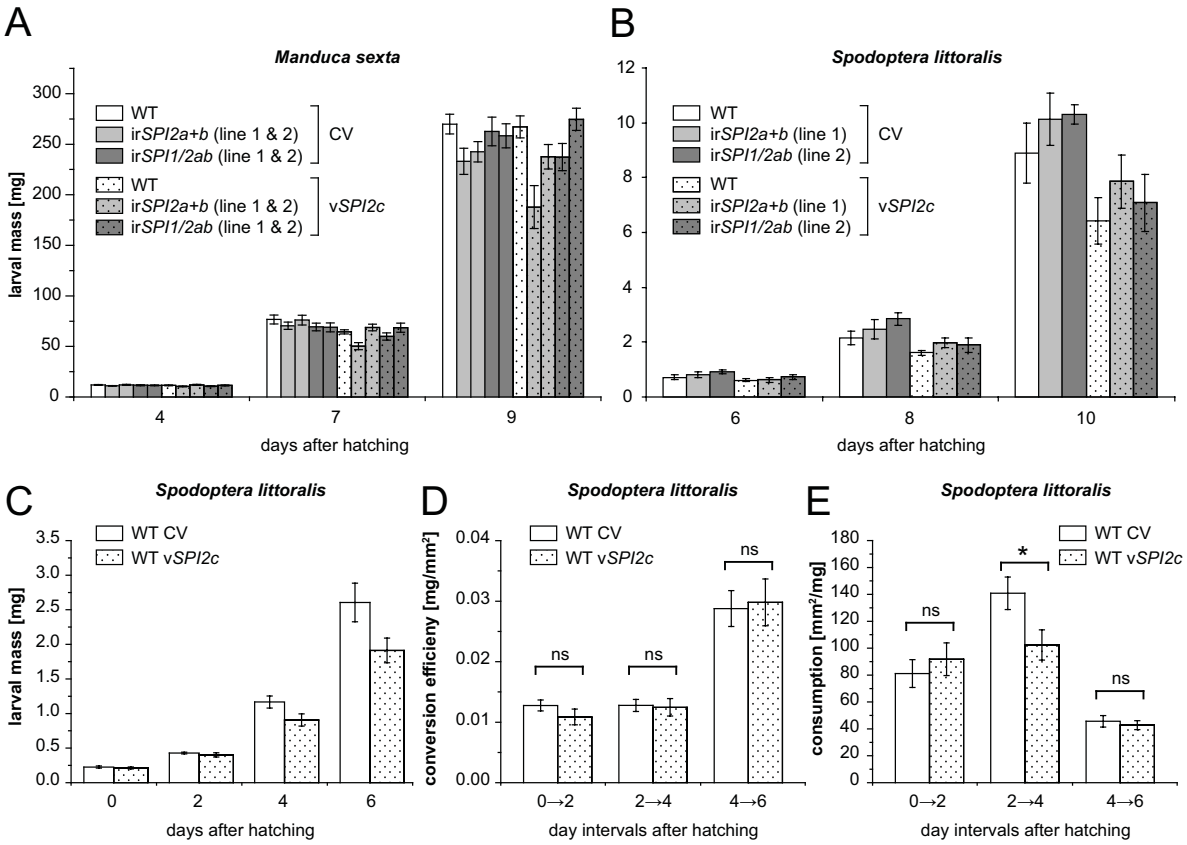
Supplemental Figure 5. **Screening and characterization of ir*SPI2a+b+c* transgenic lines.**

A, mean ± SE relative *SPI2c* transcript abundance as determined via QRT-PCR in MeJA-induced WT and three transgenic lines indepently silenced for SPI2c. Uninduced WT (C) served as a treatment control. B, mean ± SE trypsin PI activity in WT and three SPI2c-silenced lines. C, DNA gel blot of genomic DNA from four ir*SPI2a+b+c* lines, digested with EcoRV and BamHI, and hybridized with a probe coding for the hygromycin resistance gene. D, mean ± SE number of viable and non-viable seeds in irSPI2a+b+c line 1, which was used in all further experiments .



Supplemental Figure 6. Natural herbivore damage on WT and a stably transformed line silenced for different combinations of SPIs.

A, mean ± SE herbivore damage ratio (% damage of line / % damage of WT) of WT and ir*SPI2a*+b (line 1) plants grown in pairs under field- conditions. The two consecutive experiments were carried out in 2005 on a field-site near Dornburg, Germany. Damage was estimated twice in the second experiment. The dotted line indicates a ratio of 1 (when both genotypes were damaged to the same extent). P-values were calculated from a paired t-test after arcsine-transformation: June 21, t_{18} = 1.659; July 18, t_{18} = 0.513; July 26, t_{15} = 1.485. B, mean ± SE number of root feeding noctuid larvae on WT and SPI-silenced plants grown in 2010 on a field plot at the Utah field station. Larvae were counted when removing the plants with their roots from the field plot. Paired t-test: ir*SPI2a+b*, t_{18} = 1.125; ir*SPI1/2a+b*, $t_{15} \approx 0$; ir*SPI2a+b+c*, t_{16} = 2.765.



Supplemental Figure 7. Performance of *M. sexta* and *S. littoralis* larvae feeding on excised leaf discs from WT and SPI-silenced plants, which were additionally silenced for *SPI2c* by virus-induced gene silencing.

WT and independently stably transformed lines (ir*SPI2a+b* line 1 and ir*SPI1/2a+b* line 2), were infected with TRV containing a control vector (CV) or a vector for silencing of *SPI2c* (v*SPI2c*). A, mean ± SE larval mass of *M. sexta* (n = 30). We fitted linear mixed-effects models to the data from day 9 and compared them with a maximum-likelihood ratio test. No significant effect could be found among all treatment groups (for details see next page). B, mean ± SE larval mass of *S. littoralis* over time (n = 12). Silencing of *SPI2c* by VIGS has a significant effect on larval mass, the stable silencing of the other SPI genes has no effect (repeated measures ANOVA, $p < 0.01$, see next page). C, Repetition of the experiment in B (n = 15). Larvae were weighed after hatching, transferred to un-induced plant material, and weighed on alternate days. A repeated measures ANOVA showed a significant 'days*v*SPI2c*' interaction ($p<0.05$, see next page). D, E, mean ± SE food conversion efficiency (D) and consumption (E) corresponding to panel C. Missing values, due to molting of larvae, strongly reduced the power of a repeated measures ANOVA, we calculated individual t-tests for each time-point (D, 0→2: $t_{28} = 1.106$, $p = 0.28$; 2→4: $t_{21} = 0.197$, $p = 0.85$; 4→6: $t_{26} = -0.155$, $p = 0.88$; E, 0→2: $t_{28} = -0.664$, $p = 0.53$; 2→4: $t_{21} = 2.094$, $p = 0.049$; 4→6: $t_{26} = 0.505$, $p = 0.62$; ns: no significant difference; asterisk: $p < 0.05$).

Statistics for:

***Manduca sexta* (Supplemental Figure 7A).** Likelihood ratio test on linear mixed-effects models (a) and a corresponding ANOVA (a) to test effects of silencing *SPI2c* using VIGS in wild-type plants and SPI-silenced genotypes (ir*SPI1/2a+b*, ir*SPI2a+b*). a, the models included 'genotype' and 'v*SPI2c*' as fixed effects and 'box' and 'line' as random effects. The factor 'box' indicated the container in which caterpillars were pre-reared in batches for three days before being transferred in individual containers. Three such boxes containing 10 to 15 caterpillars per genotype and VIGS construct were used (30 boxes in total). The factor 'line' accounted for possible diffferences between the two lines per genotype. Another possible random effect could have emerged from differences in VIGS efficiency between plant individuals. However, when this 'plant'-effect was included in the model comparison it did not show any effect and was thus removed for further analysis, so that the final model was: (larval mass ~ genotype + v*SPI2c* + genotype*v*SPI2c* + (1|box) + (1|line)). b, as an alternative we computed a univariate ANOVA, neglecting the 'line' factor and just using 'box' as an error term (larval mass ~ genotype + v*SPI2c* + genotype:v*SPI2c* + Error(box)) as multiple error terms are not allowed in ANOVA. For both types of analyses the data was transformed to the power of 1.5 to meet requirements of homoscedasticity.

a. Linear mixed-effects model for larval mass on day 9

	logLik	Chisq	Chi df	P
genotype	-2421.6	5.5343	2	0.06284
v*SPI2c*	-2421.2	0.8272	1	0.36309
genotype*v*SPI2c*	-2421.1	0.3158	2	0.85393

b. ANOVA for larval mass on day 9

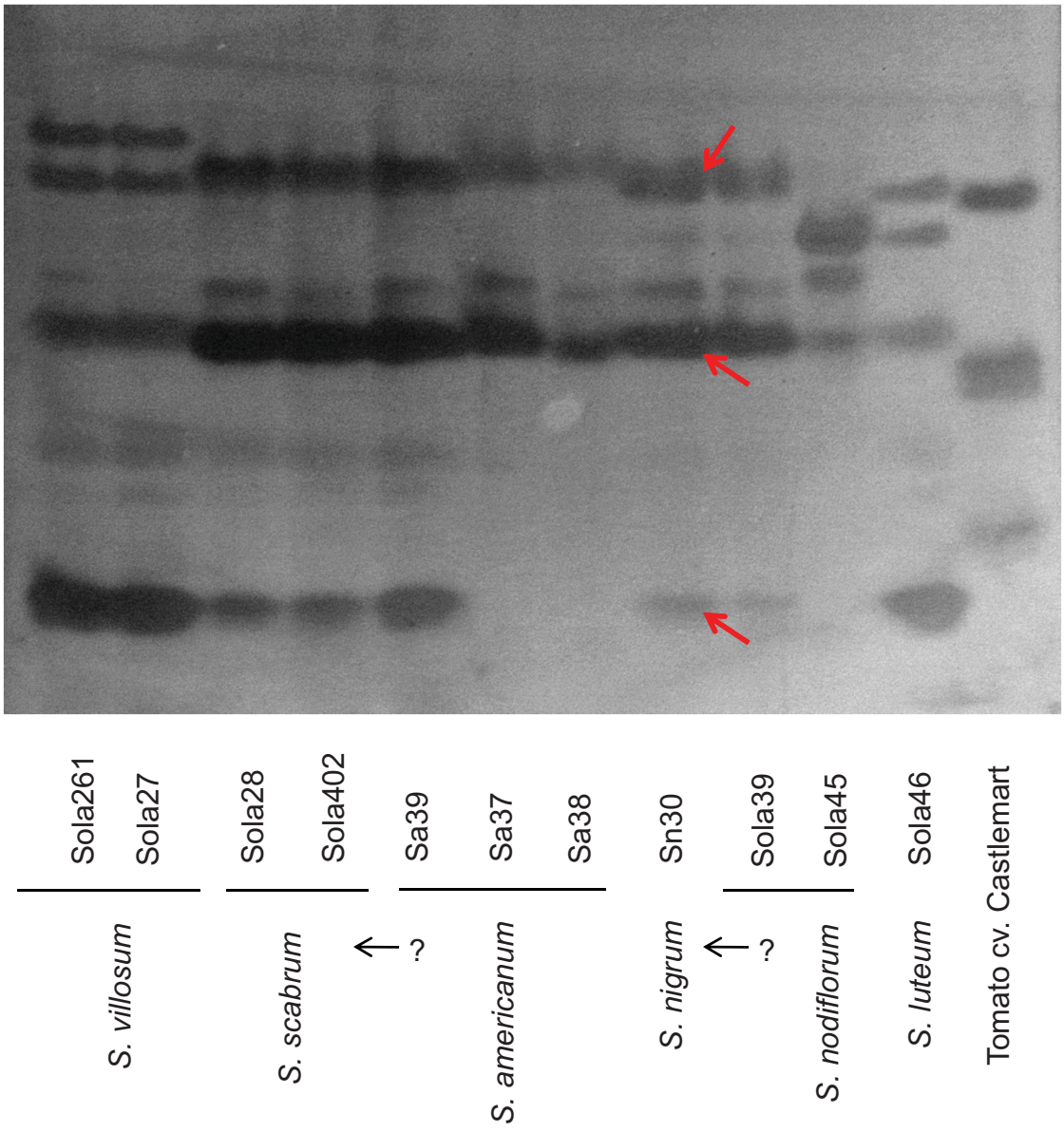
	df	SS	MS	F	P
genotype	2	41533183	20766592	2.4995	0.1033
v*SPI2c*	1	3976912	3976912	0.4787	0.4957
genotype*v*SPI2c*	2	1478833	739417	0.0890	0.9151
Residuals	24	199396260	8308178		
Error: within	248	464566587	1873252		

***Spodoptera littoralis* (Supplemental Figure 7B)**. Repeated measures ANOVA to detect effects on larval mass of *Spodoptera littoralis* after feeding on plant material silenced in *SPI2c* using VIGS in different genetic backgrounds (wild-type or SPI-silenced genotypes ir*SPI1/2a+b*, ir*SPI2a+b*). We defined 'genotype' (WT, ir*SPI2a+b*, ir*SPI1/2a+b*) and 'v*SPI2c*' as factors in the model: genotype + v*SPI2c* + genotype*v*SPI2c* (using sum of squares type III). Mauchly's test indicated a violation of the assumption of sphericity ($\chi^2_{(5)} = 148.9$; $p < 0.001$). Therefore Huynh-Feldt estimates ($\varepsilon = 0.566$) were used to correct the degrees of freedom. (SS, sum of squares; df, degrees of freedom; MS, mean squares; F- and p-value; s indicates significant differences $p < 0.05$)

	SS	df	MS	F	P	
Within-Subjects Effects						
day	2116.64	1.13	1871.1	424.99	0.000	s
day * genotype	9.23	2.26	4.08	0.93	0.411	
day * v*SPI2c*	53.42	1.13	47.23	10.73	0.001	s
day * genotype * v*SPI2c*	1.39	2.26	0.61	0.14	0.893	
Error(day)	288.87	65.61	4.40			
Between-Subjects Effects						
Intercept	2734.47	1	2734.47	532.05	0.000	s
genotype	15.51	2	7.75	1.51	0.230	
v*SPI2c*	66.53	1	66.53	12.94	0.001	s
genotype * v*SPI2c*	1.68	2	0.84	0.16	0.849	
Error	298.09	58	5.14			

***Spodoptera littoralis* (Supplemental Figure 7C-E)**. Repeated measures ANOVA to detect effects on larval mass of *Spodoptera littoralis* after feeding on wild-type plant material silenced in *SPI2c* using VIGS. Although log-transformation improved homogeneity of variances Mauchly's test indicated a violation of the assumption of sphericity ($\chi^2_{(5)} = 101.54$; $p < 0.001$). Therefore Huynh-Feldt estimates ($\varepsilon = 0.728$) were used to correct the degrees of freedom. (SS, sum of squares type III; df, degrees of freedom; MS, mean squares; F- and p-value; s indicates significant differences $p < 0.05$)

	SS	df	MS	F	P	
Within-Subjects Effects						
day	15.916	1.986	8.014	696.138	0.000	s
day * v*SPI2c*	0.084	1.986	0.042	3.691	0.032	s
Error(day)	0.594	51.637	0.012			
Between-Subjects Effects						
Intercept	3.963	1	3.963	86.818	0.000	s
v*SPI2c*	.166	1	0.166	3.642	0.067	
Error	1.187	26	0.046			



Supplemental Figure 8. Native PAGE (12%) of extracts from different *Solanum spp.* visualized with GXCP for TPI activity.

The red arrow indicates the location of the SPI2c-type bands in *S. nigrum*. All seeds except *S. nigrum* and tomato were obtained from the seedbank of IPK Gatersleben, Germany, and the corresponding accession numbers are given in the figure. The taxonomic situation of the black nightshades is difficult and for this reason the species identities are not completely certain. For example Sa39 is listed in the database as *S. americanum* but when compared morphologically and in the PI profile it rather resembles *S. scabrum*. Similarly Sola39 is classified as *S. nodiflorum* but it is more likely to be *S. nigrum*. This putatively correct taxonomic classification is indicated by black arrows.

Supplemental Table 1. Statistical analysis for data in Figure 7A. Repeated measures ANOVA to detect effects of stably silencing SPIs, of methyl jasmonate elicitation, and of their interaction on larval mass of *Manduca sexta* . We defined 'genotype' (WT, ir*SPI2a+b* , ir*SPI1/2a+b, irSPI2a+b+c*) and 'treatment' (control or MeJA) as factors in the model: genotype treatment genotype*treatment. Data were log-transformed to improve homogeneity of variances. Mauchly's test indicated a violation of the assumption of sphericity, therefore Huyn-Feld-estimates were used to correct the degrees of freedom ($\chi^2_{(9)} = 183.862$, $\varepsilon = 0.709$). (df, degrees of freedom; F- and p-value)

Within-Subjects Effects

	Type III Sum of Squares	df	Mean Square	F	P
day	399.191	2.837	140.727	5658.300	0.000
day * genotype	0.049	8.510	0.006	0.231	0.988
day * treatment	0.916	2.837	0.323	12.978	0.000
day * genotype * treatment	0.158	8.510	0.019	0.746	0.659
Error(day)	13.052	524.777	0.025		

Between-Subjects Effects

	Type III Sum of Squares	df	Mean Square	F	P
Intercept	2332.544	1	2332.544	13912.080	0.000
genotype	0.374	3	0.125	0.744	0.527
treatment	1.292	1	1.292	7.704	0.006
genotype * treatment	0.138	3	0.046	0.275	0.844
Error	31.018	185	0.168		

Supplemental Table 2. Statistical analysis for data in Figure 8A. Repeated measures ANOVA to detect effects of stably silencing SPIs, of methyl jasmonate elicitation, and of their interaction on larval mass of *Spodoptera littoralis* . We defined 'genotype' (WT, ir*SPI2a+b* , ir*SPI1/2a+b, irSPI2a+b+c*) and 'treatment' (control or MeJA) as factors in the model: genotype treatment genotype*treatment. Data were log-transformed to improve homogeneity of variances. Mauchly's test indicated a violation of the assumption of sphericity, therefore Huyn-Feld-estimates were used to correct the degrees of freedom ($\chi^2_{(9)} = 272.495$, $\varepsilon = 0.651$). (df, degrees of freedom; F- and p-value)

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	P
day	742.885	2.605	285.130	17282.334	.000
day * genotype	.380	7.816	.049	2.948	.003
day * treatment	2.181	2.605	.837	50.734	.000
day * genotype * treatment	.167	7.816	.021	1.299	.243
Error(day)	9.328	565.378	.016		

Tests of Between-Subjects Effects

	Type III Sum of Squares	df	Mean Square	F	P
Intercept	575.490	1	575.490	4332.638	.000
genotype	.332	3	.111	.832	.477
treatment	2.785	1	2.785	20.969	.000
genotype * treatment	.371	3	.124	.931	.426
Error	28.823	217	.133		

Supplemental Table 3. Statistical analysis for data in Figure 8D. Repeated measures ANOVA to detect effects of stably silencing SPIs, of methyl jasmonate elicitation, and of their interaction on larval mass of *Spodoptera exigua* (a, b). We defined 'genotype' (WT, ir*SPI2a+b*, ir*SPI1/2a+b*), 'line' nested within 'genotype', and 'treatment' (control or MeJA) as factors in the model: genotype line(genotype) treatment genotype x treatment (a). For *S. exigua* feeding on un-induced tissue only one line per plant genotype was used. This reduced the power to detect a genotype-specific effect in the complete model. Thus we computed a second ANOVA for larvae feeding on un-induced material only (b). Data were log-transformed to improve homogeneity of variances. Mauchly's test indicated a violation of the assumption of sphericity ($\chi^2_{(5)} = 72.99$ (a), $\chi^2_{(5)} = 30.16$ (b), $p < 0.05$). Therefore Huyn-Feld-estimates ($\varepsilon = 0.728$ (a), $\varepsilon = 0.724$ (b)) were used to correct the degrees of freedom. (SS, sum of squares type IV; df, degrees of freedom; MS, mean squares; F- and p-value; s indicates significant differences $p < 0.05$)

	SS	df	MS	F	P	
(a) *Spodoptera exigua* larval mass						
Within-Subjects Effects						
day	16.72	2.06	8.14	279.54	0.000	s
day * genotype	0.20	4.11	0.05	1.69	0.152	
day * line (genotype)	0.05	4.11	0.01	.45	0.774	
day * treatment	0.39	2.06	0.19	6.53	0.002	s
day * genotype * treatment	0.21	4.11	0.05	1.76	0.137	
Error(day)	4.85	166.47	0.03			
Between-Subjects Effects						
Intercept	40.70	1	40.70	223.58	0.000	
genotype	1.04	2	0.52	2.84	0.064	
line (genotype)	0.70	2	0.35	1.92	0.153	
treatment	2.35	1	2.35	12.93	0.001	s
genotype * treatment	0.38	2	0.19	1.04	0.360	
Error	14.75	81	0.18			
(b) *Spodoptera exigua* larval mass, un-induced plants only						
Within-Subjects Effects						
day	16.54	2.17	7.61	267.58	0.000	s
day * genotype	0.21	4.35	0.05	1.70	0.152	
Error(day)	2.53	89.10	0.03			
Between-Subjects Effects						
Intercept	49.24	1	49.24	257.16	0.000	
genotype	1.60	2	0.80	4.17	0.022	s
Error	7.85	41	0.19			

Supplemental Table 4. Primer sequences used for cloning, construct generation, and qRT-PCR.

Primer name	Sequence (5' -> 3')	Target	
PIS5-21 (fwd)	GAAAACAAAGGTGGCCAGAAC	SPI1	cloning
PIS6-21 (rev)	CATTATATATAGTAAGAGTAC	SPI1	
Sa_pin2a_F1	TGGCTGTTCACAAAGTTAGCTTC	SPI2a	
Sa_pin2a_R1	AGTCCACATTACAGTAACCAGCA	SPI2a	
Sl_PI2_F4	AATGTGACACTCGAATTGACTATG	SPI2c	
Sl_PI2_R5	CATAGTCAATTCGAGTGTCACATT	SPI2c	
Sl_PI2_R6	CTGCACAACAGTTGGTGCA	SPI2c	
Sl_PI2_R7	TTCAGATTCTCCYTCACAAATAAAAG	SPI2c	
SnPIN2c_F1_3'R	CTATGGGATTTGCCCACTT	SPI2c (3' RACE)	
SnPIN2c_F2_3'R	GTGAGGTACCGGTGGACA	SPI2c (3' RACE)	
SnPIN2c_R2	CGTACACACTTAAGTAGCACAAACC	SPI2c	
PIS1-33 (fw)	GCGGCGCCATGGATTAGAAATAACGTTGGGTTC	SPI2b (for generating pSOL3PIS)	inverted-repeat construct generation
PIS2-32 (rev)	GCGGCGCTGCAGGCTGTTCACAAAGAAGTTAG	SPI2b (for generating pSOL3PIS)	
PIS3-33 (fw)	GCGGCGCTCGAGATTAGAAATAACGTTGGGTTC	SPI2b (for generating pSOL3PIS)	
PIS4-33 (rev)	GCGGCGGAGCTCGGCTGTTCACAAAGAAGTTAG	SPI2b (for generating pSOL3PIS)	
PIS9-32 (fw)	GCGGCGCTGCAGAAAACAAAGGTGGCCAGAAC	SPI1 (for generating pSOL3PIN12)	
PIS10-40 (rev)	GCGGCGCCATGGATGCATGACATTATAATCAACATGAACC	SPI1 (for generating pSOL3PIN12)	
PIS11-33 (fw)	GCGGCGGTATACGAAAACAAAGGTGGCCAGAAC	SPI1 (for generating pSOL3PIN12)	
PIS12-38 (rev)	GCGGCGCTCGAGCTCGACATTATAATCAACATGAACCC	SPI1 (for generating pSOL3PIN12)	
PIN2c1-33	GCGGCGCTCGAGTATGTAAGTGAGGTACCGGTG	SPI2c with restriction site	VIGS construct generation
PIN2c2-33	GCGGCGGAATTCAAGTGGGCAAATTCCATAGGC	SPI2c with restriction site	
Sn_pin1_F1	TACCAGCAAAGCTTGCTAAGG	SPI1 (only for wild-type)	qRT-PCR
Sn_pin1_R1	TTGTGACATTATAATCAACATGAACC	SPI1 (only for wild-type)	
Sn_pin1_F2	TTGATGTAATTAGCAGCCACACA	SPI1	
Sn_pin1_R2	CATTATATATAGTAAGAGTACATTGTGAC	SPI1	
Sn_pin2a_F3	ATTGTACCTTCGAATGTGATAC	SPI2a	
Sn_pin2a_R4	GATAGATAACACAACAGATGATTG	SPI2a	
Sn_pin2b_F2	TGCCCTCTATATTGTGATGG	SPI2b	
Sn_pin2b_R2	ACAGTGATCATTAGCATATATTGC	SPI2b	
Sn_pin2c_F1	GATCTCCAGAAAATCAAGGTTGC	SPI2c	
Sn_pin2c_R1	GCCATGGCAGAAATATATCATCA	SPI2c	
Sol_EF1a_fp	GTTTCACTGCCCAGGTCATCATC	EF1α	
Sol_EF1a_rp	TGGGCTTGGTGGGAATCATC	EF1α	
Sn_pin1_prF1	GCCAGAACTTGTTGGTGTAC	SPI1	DNA gel blot probes
Sn_pin1_prR1	CATGTGTGGCTGCTAATTACA	SPI1	
Sn_pin2a_prF1	TGAACCCAAGACCACTGCTTAT	SPI2a	
Sn_pin2a_prR1	GTCCACATTACAGTAACCAGCAT	SPI2a	
Sn_pin2b_prF2	AATGATATGCGTTGTAGTTTTTA	SPI2b	
Sn_pin2b_prR2	CATATTACAGTGATCATTAGCAT	SPI2b	