Supplemental Methods

Modification in SA and ABA analysis

1. For humidity shift experiments

Plant materials were lyophilized and ground to a fine powder in 80% (v/v) methanol containing 1% (v/v) acetic acid (10 ml per g FW of tissue). [$^2H_6$]ABA (ICON isotopes) and [$^2H_6$]SA (Sigma) were added as internal standards to a concentration of 10 ng g-1 FW (ABA) and 100 ng g-1 FW (SA). The mixture was incubated for 1 h at 4°C with constant rotation and subsequently centrifuged at 3000g for 20 min at 4°C. MeOH in supernatant were removed and water layer were loaded onto a Oasis HLB cartridge (30 mg, 1 cc; Waters). Before sample loading, the cartridges were washed with 1 ml each of methanol and water and equilibrated with 1 ml of water containing 1% AcOH. 10% of this eluent were dried, dissolved into 20 μl of water containing 1% AcOH, and 10 μl were subjected to the determination of SA by liquid chromatography-tandem mass spectrometry (LC-MS/MS). ABA were eluted with 2 ml of 80% MeOH containing 1% AcOH after washing with 1 ml of water containing 1% AcOH. MeOH in this eluent were removed and water layer were loaded onto an Oasis WAX cartridge (30 mg, 1 cc; Waters), ABA were eluted with 2 ml of 80% MeOH containing 1% AcOH after sequential washing with 1 ml of water containing 1% AcOH and 1 ml of MeOH. Before sample loading, cartridges were washed with 1 ml each of MeCN and MeOH, then regenerated with 500 μl of 0.1 M NaOH, and equilibrated with 1 ml of water containing 1% AcOH. The eluent were dried, dissolved into 20 μl of water containing 1% AcOH, and 10 μl were subjected to the determination of ABA by LC-MS/MS.

The LC-MS/MS system consisted of Agilent 1200 (Agilent) and Agilent 6410 (Agilent). LC separations were performed on an zorbax eclipse C18 (2.1 x 50 mm, 1.8 μm particle size; Agilent) at a flow rate of 200 μl min-1. For the analysis of SA, the mobile phase consisted of water containing 0.1% formic acid (solvent A) and MeCN containing 0.1% formic acid (solvent B), and was programmed as follows: a 10 min linear gradient from 3 to 98% solvent B, kept to 15 min at 98%

solvent B, and a 16 min linear gradient from 98 to 3% solvent B, and kept to 20 min at 3% solvent B. The MS/MS conditions were as follows: capillary voltage, 4000 V; gas temperature, 300°C; gas flow, 9 l min-1; nebulizer, 30 psi; fragmentor, 100; collision energy, 16.0 V. SA and [$^2H_6$]SA showed almost identical retention times, which were 6.2 min. The MS/MS transitions used for SA determination were m/z 137/93 (as SA) and m/z 141/97 (as [$^2H_6$]SA, in water [$^2H_6$]SA converts almost quantitatively to [$^2H_4$]SA). For the analysis of ABA, the mobile phase consisted of water containing 0.01% AcOH (solvent A) and MeCN containing 0,05% (V/V) acetic acid (solvent B), and was programmed as follows: a 15 min linear gradient from 3 to 50% solvent B, 17 min linear gradient from 50% to 98% solvent B, and kept to 20 min, 21 min linear gradient from 98 to 3% solvent B, and kept to 26 min at 3% solvent B. The water and MeCN used were of LC-MS grade (Kanto Chemical). The MS/MS conditions were as follows: capillary voltage, 4000 V; gas temperature, 300°C; gas flow, 9 l min-1; nebulizer, 30 psi; fragmentor, 140; collision energy, 8.0 V. ABA and [$^2H_6$]ABA showed almost identical retention times, which were 10.8 min. The MS/MS transitions used for ABA determination were m/z 263/153 (as ABA) and m/z 269/159 (as [$^2H_6$]ABA).

2. Drought stress experiments

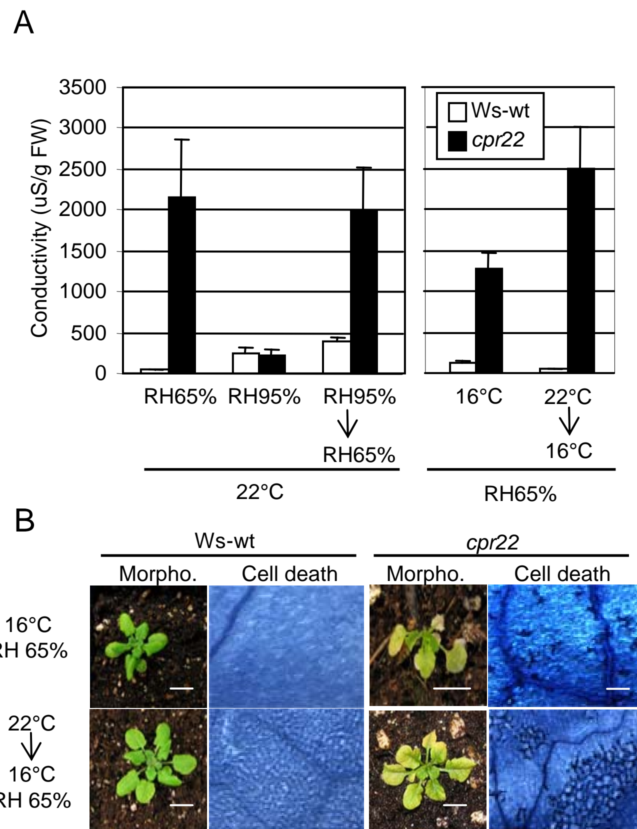
Each sample was homogenized with liquid nitrogen, and extracted with 80% MeOH (3mL x 3) before adding of 100ng of deuterated-ABA (d_4-ABA) as internal standard. After centrifugation at 12,000xg for 10min, the extract was evaporated and partitioned between ethyl acetate and water (pH2.5). The obtained ethyl acetate soluble fraction was purified by a reversed phase HPLC (Intersil ODS C_{18}, 4.6 x 150 mm) eluted with 25% acetonitrile containing 1% acetic acid at a flow rate of 0.5 mL min^{-1}. Fractions were collected every minute. The fractions eluted at 19 min (ABA) was dried, methylated by adding MeOH and ethereal diazomethane at room temperature for 30 min, and analyzed by a capillary GC-MS using a Hewlett-Packard 5973 mass spectrometer (EI, 70 ev) coupled to a 6890 gas chromatograph fitted with a fused silica capillary column (HP-5, 0.25

mm x 30 m, 0.25μm film thickness). The oven temperature was maintained at 100^oC for 2 min, elevated to 260° at a rate of 5°min^{-1} and then maintained at 280°. Helium was used as the carrier gas at a flow rate of 1 mL min-1. Samples were introduced using an on-column injection mode and analyzed by GC-selected ion monitoring (SIM) method. The level of ABA was calculated by ratio of *m/z* at 190 and 194 for the molecular ion of endogenous ABA and d_4-ABA, respectively.

3. SA for nahG experiment

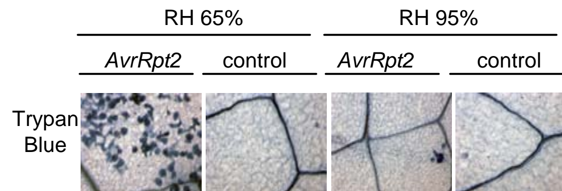
Salicylic acid and its glucoside were quantified using gas chromatography-mass spectrometry. 100 mg of frozen leaf tissue were extracted twice with 800 μl acetone:50 mM citric acid (7:3, v/v) in 2 ml Fast Prep tubes containing ceramic beads using a FastPrep FP 120 tissue homogenizer (Qbiogene, Carlsbad, CA). The samples were spiked with $[^2H_6]$SA as an internal standard (CDN Isotopes, Point-Claire, QC, Canada). After evaporation of the acetone under vacuum the aqueous solutions were extracted twice with 750 μl of diethyl ether. SA-glucoside was extracted from the remaining aqueous solution after acidification with 5 μl of HCl and hydrolysis at 90°C for 1hour by diethyl-ether extraction. All samples were then loaded on 1ml Supelclean LC-NH_2 SPE columns (Supelco, Bellefonte, PA). After washing with 1.2 ml chloroform:2-propanol (2:1, v/v) compounds were eluted with 1.5 ml diethyl-ether:formic acid (98:2, v/v). The eluates were then evaporated to dryness under a stream of N_2, dissolved in 100 μl dichlormethane:methanol (8:2, v/v) and derivatized with 2 μl Trimethylsilyl-Diazomethane (Aldrich, Oakville, ON, Canada) for 20 minutes. The reaction was stopped by adding 2 μl 2M acetic acid in hexane (Schmelz et al., 2004). The resulting methyl esters of SA were analyzed by gas chromatography-mass spectrometry (6890N GC connected to a 5975 mass selective detector, Agilent Technologies, Palo Alto, CA) in isobutene chemical ionization mode following the specifications of Schmelz et al. (2004). The methyl esters were measured using selected-ion monitoring with *m/z* 153 (SA) and *m/z* 157 ($[^2H_6]$SA).

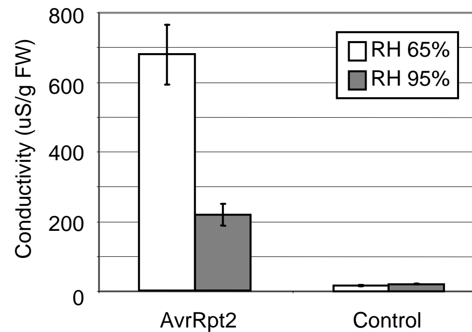
Suppl. Figure 1

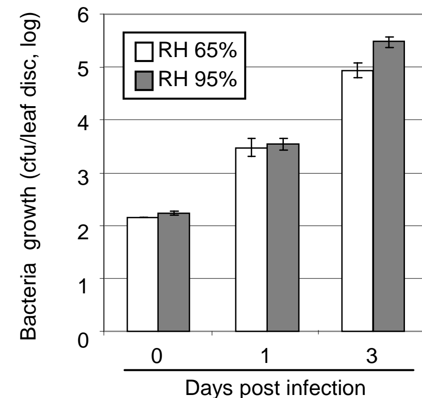


Supplemental Figure 1. Humidity and temperature affect spontaneous cell death formation in *cpr22*. (A) Electrolyte leakage of leaf discs from Ws-wt and *cpr22* plants grown under 65% RH (22°C) conditions, grown under 95%RH (22°C) conditions, grown under 95% RH (22°C) conditions and shifted to 65% RH (22°C) conditions for 2 days, or grown under 16°C (RH65%) conditions, or grown under 22°C (RH65%) conditions and shifted to 16°C (RH65%) conditions for 2 days. Conductivity data is the average ± SE (n=4). Student's T-test shows significant difference between *cpr22* and Ws-wt plants. (B) Photographs show morphology, chlorotic phenotypes and spontaneous cell death by Trypan Blue staining of four-week-old *cpr22* and Ws-wt plants grown under 16°C (65% RH) conditions, or grown at 22°C (65% RH) conditions and shifted to 16°C (65% RH) conditions for 2 days.

Suppl. Figure 2

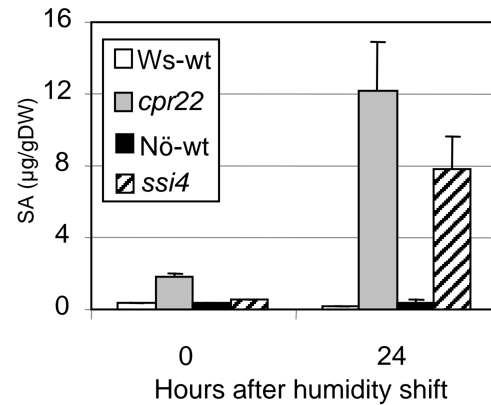






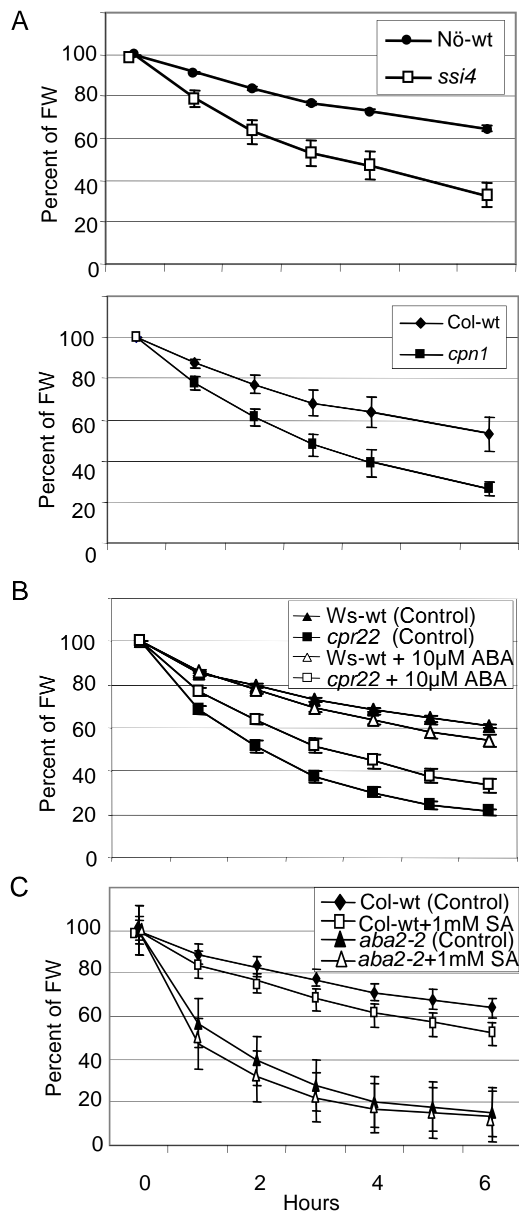
Supplemental Figure 2. High RH attenuates HR formation after infection with *Pseudomonas syringae* DC3000 (*AvrRpt2*). (A) Columbia-wt plants were either kept at 65% RH or shifted for one week to 95% RH prior to infection. They were then infected with *P. syringae* DC3000 at OD600 = 0.04. 10 hpi HR formation was assessed by Trypan Blue staining. (B) Electrolyte leakage of leaf discs from plants grown at 65% RH (open bars) and 95% RH (gray bars) 10 hpi (Average of 25 leaves). Student's T-test shows significant difference between AvrRpt2 65% and RH95%. (C) Bacterial growth count. Experiments were done two times with similar results.

Suppl. Figure 3



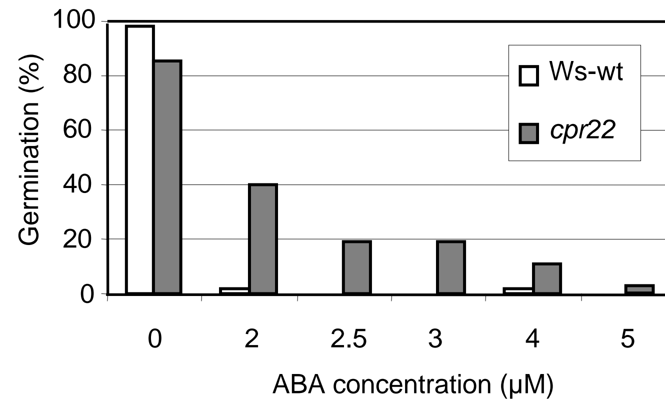
Supplemental Figure 3. SA increase after humidity shift in *cpr22* and *ssi4.* SA levels in Ws-wt (open bars) *cpr22* (gray bars), Nö-wt (black bar) and *ssi4* (hatched bar) at 0 and 24 hours after the shift from 95% RH to 65% RH conditions were measured. Student's T-test shows significant difference between *cpr22* and Ws-wt, and between *ssi4* and Nö-wt plants.

Suppl. Figure 4



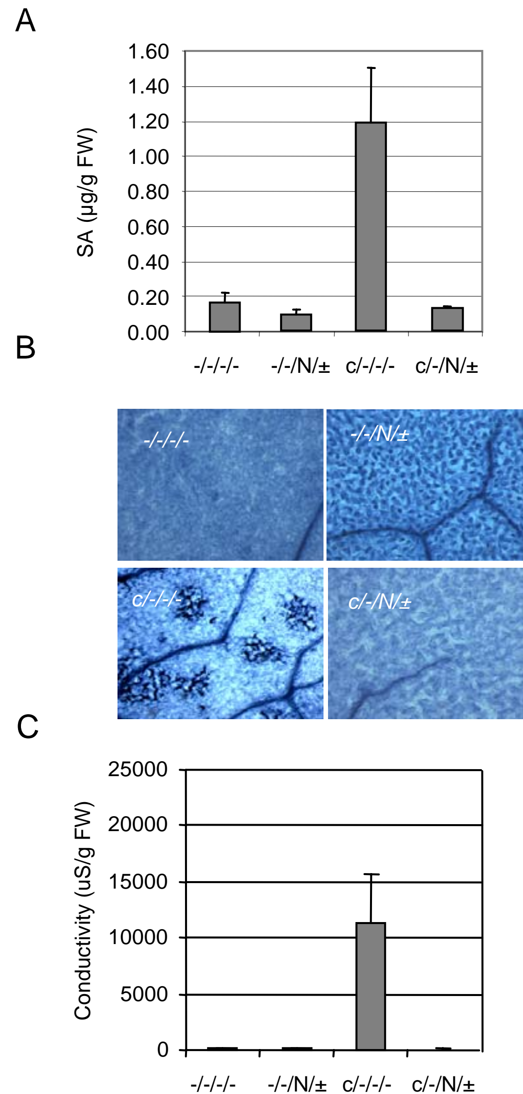
Supplemental Figure 4. Water loss phenotypes in various mutants and treatments. Plants were weighed at various times after detachment from their roots. (A) *ssi4* (open square), Nö-wt (filled circle), Col-wt (filled diamond) and *cpn1*(filled square); (B) Ws-wt plants after soil drenching with control solution (filled triangle) or with 10μM ABA (open triangle), *cpr22* heterozygous plants after soil drenching with control solution (filled square), or with 10μM ABA (open square), (C) *aba2-2* 16h after soil drenching with control solution (closed diamond), or with 1mM SA (open square). Col-wt 16h after soil drenching with control solution (filled triangle) or with 1mM SA (open triangle). All experiments were repeated at least three times with similar results. Control solution: 0.1% MeOH in water that was used to dissolve ABA or SA.

Suppl. Figure 5



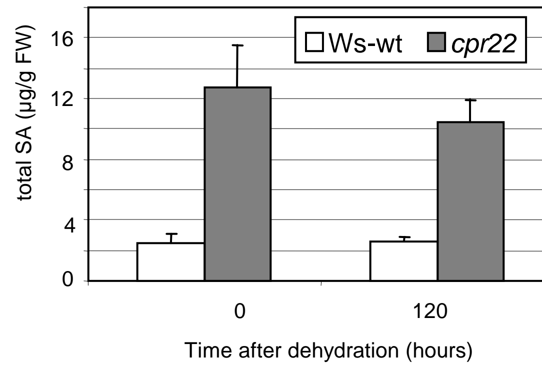
Supplemental Figure 5.
Effect of higher concentrations of ABA on germination of Ws-wt (open bars) and *cpr22* (gray bars) seeds. Experiment was conducted with approximately 100 seeds in one plate. The experiment has been repeated more than three times with similar results.

Suppl. Figure 6



Supplemental Figure 6. *nahG* trangsgene suppresses SA levels and cell death formation after humidity shift in *cpr22*. Nö-wt, Ws-wt, Nö-*nahG*, *cpr22* (heterozygous), F2 progeny of cross pollination of *nahG* transgenic (Nö background) and *cpr22* (Ws background) plants were used (c/-/-/-; plants that are heterozygous for *cpr22*, but do not carry *nahG*, c/-/N/± ; plants that are heterozygous for *cpr22* and carry *nahG*). All plants were grown under 95% RH conditions and then shifted to 65% RH conditions. (A) SA levels. Student's T-test shows significant difference between *c/-/-/-* and c/-/N/±. (B) Trypan Blue staining for cell death detection 2 days after shift, (C) Electrolyte leakage of leaf discs 2 days after shift. Experiments were done three times with similar results.

Suppl. Figure 7



Supplemental Figure 7
cpr22 plants did not show significant changes in SA accumulation under moderate drought stress. Ws-wt (open bars), *cpr22* heterozygous plants (gray bars). The data represent the mean levels of three extracts. Each extract was made from 3-5 plants. The experiment has been repeated three times with similar results.

Supplemental Table1: Fold differences in *cpr22/ssi4* (compared to wt) of ABA- and Defense/SA-related genes at high humidity (95%) and 24 hours after shift to ambient humidity (65%). (Microarrays were preprocessed together using GC-robust multiarray analysis (gcrma) (Wu et al., 2004). Expression data were filtered to remove probe sets with low expression and low variance across all arrays (genefilter, Gentleman et al., 2009; minimum intensity of 100, on a minimum of 25% of arrays, and minimum interquartile range of 0.5 on the log2 scale).

			cpr22				*ssi4*			
Gene Annotation	probe ID	Gene ID	before shift		24 h after shift		before shift		24 h after shift	
			Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
ABA-related genes										
NCED3	257280_at	At3g14440	0.6	0.3	10.5	7.4	1.3	1.2	8.5	3.1
AAO3	263570_at	At2g27150	2.2	2.9	3.1	4.0	1.8	1.1	4.9	2.3
ERA1	249405_at	At5g40280	1.7	1.5	3.0	2.5	1.1	1.0	2.7	2.1
ABI1	253994_at	At4g26080	1.2	1.0	3.9	3.0	2.2	0.8	5.0	1.9
HAB1	259922_at	At1g72770	0.8	0.9	3.1	2.8	1.1	1.0	2.8	1.8
PP2c	247723_at	At5g59220	1.9	0.6	52.3	37.2	1.3	0.9	41.4	5.6
ANAC072_RD26	253872_at	At4g27410	0.6	0.8	5.7	4.2	3.4	1.4	8.0	3.2
RD20	255795_at	At2g33380	0.9	0.6	4.5	6.1	1.9	0.6	2.9	1.5
CYP707A4	257035_at	At3g19270	3.2	1.1	9.9	9.3	0.9	1.5	2.7	2.2
Snrk3.22	255872_at	At2g30360	0.9	2.2	2.5	3.0	1.5	1.5	4.0	1.7
PYR1	254705_at	At4g17870	0.7	0.4	0.3	0.2	1.0	1.0	0.5	0.6
PYL2	248865_at	At5g46790	0.9	0.7	0.6	0.4	0.8	0.9	0.4	0.6
PYL4	267034_at	At2g38310	1.1	1.2	0.6	0.4	1.1	1.1	0.5	0.7
AtMYC2_RD22BP1	261713_at	At1g32640	0.9	0.6	1.2	1.1	1.9	0.8	1.6	0.9
ABF3_DPBF5	253263_at	At4g34000	0.7	0.8	1.1	2.3	1.3	0.8	1.6	1.5
ABF4	258026_at		1.0	1.1	2.2	1.3	1.9	0.8	1.4	1.4
RD22	246908_at	At5g25610	0.6	0.7	0.05	0.1	1.2	1.0	0.5	0.5
RD29A	248337_at	At5g52310	0.4	0.5	0.2	0.6	1.6	0.6	1.9	1.0
ERD3	254563_at	At4g19120	1.5	2.9	0.5	0.8	0.8	0.8	0.4	0.5
KIN1/KIN2	246481_s_at	At5g15960/ At5g15970	0.8	0.8	0.5	0.5	1.3	1.0	2.0	0.9
Defense/SA-related genes										
WRKY70	251705_at	At3g56400	1.0	1.7	8.8	5.1	1.8	1.1	9.2	3.2
EDS1	252373_at	At3g48090	5.9	10.3	7.9	13.9	1.5	1.1	7.2	3.1
PAD4	252060_at	At3g52430	15.4	24.4	76.5	62.6	0.7	1.5	74.4	5.3
PR-1	266385_at	At2g14610	18.1	19.5	78.2	45.1	2.3	1.0	19.6	4.1
disease resistance gene "*SSI4*"	249264_s_at	At5g41740/ At5g41750	3.8	3.0	9.3	9.9	1.1	0.9	8.8	3.5
disease resistance gene	249393_at	At5g40170	1.6	2.0	7.2	5.9	1.1	0.8	2.2	1.6
disease resistance gene	254851_at	At4g12010	1.9	3.6	4.2	6.8	1.4	1.1	2.7	1.9
ICS1_EDS16_SID2	262177_at	At1g74710	14.0	30.0	16.7	33.3	1.0	1.6	22.5	4.9
SID1__EDS5	252921_at	At4G39030	75.8	96.2	152.8	280.4	1.7	2.0	74.5	7.0

Supplemental Table 2 Primer sequences

Experiment	Gene	Primer name	Sequence
Real Time PCR	*RD29A*	rd29A-F	5’-TGGATCTGAAGAACGAATCTGATATC-3’
		rd29A-R	5’-GGTCTTCCCTTCGCCAGAA-3’
	KIN1/2	KIN1-F	5’-ACCAACAAGAATGCCTTCCA-3’
		KIN1-R	5’- CCGCATCCGATACACTCTTT-3’
	NCED3	NCED3-F	5’- CCGGTGGTTTACGACAAGAA-3’
		NCED3-R	5’- CCCAAGCGTTCCAGAGATG-3’
	PP2C	PP2C-F	5’- TGGAGATCCGGAGGTTTAAG-3’
		PP2C-R	5’- TCTCCTCCGCCTCTGTAAGT-3’
	AAO3	AAO3-F	5’-GAAGGTCTTGGAAACACGAAGAA-3’
		AAO3-R	5’-GAAATACACATCCCTGGTGTACAAAAC-3
	18SrRNA	18s rRNA-F	5’- AAACGGCTACCACATCCAAG-3’
		18s rRNA-R	5’- CCTCCAATGGATCCTCGTTA-3’
		’	
RT-PCR	*RD29A*	RD29A-F	5’-ATTCCGGTGGGAGATCAAACTC-3’
		RD29A-R	5’-AACAGAATGTGGCGATTCTGGC-3’
	RD29B	RD29B-F	5’-TGGGAAGAGACTTACCGACGGAACTCATG-3’
		RD29B-R	5’-CGGAGAGAGGTAGCTTTGTCATCACCGTTG-3’
	RAB18	RAB18-F	5’-TTCACCGCTCCGGATCTGGAT-3’
		RAB18-R	5’-GCTCATTACACACTCATGTAAGTA-3’
	ACT2	ACT2-F	5’-CTAAGCTCTCAAGATCAAAGGCTTA-3’
		ACT2-R	5’-TTAACATTGCAAAGAGTTTCAAGG-3’
Detection of the *AtCNGC11/12* chimera gene		D2-F1	5’-GTTGCAACTGTGCATGATGG-3’
		GSP14-R3	5’-TCATGAGATCCGGTTGTACTTTTCA-3’
Heterozygosity of the *AtCNGC11/12* chimera gene		D2DT-F1	5’-CTCTTGATCCCACTTCC-3’
		D2DT-R1	5’-GACCGTGAGATTGCAGT-3’
Detection of the *NahG* transgene		*NahG*-F2	5’-AAAGCCACCACGTTGAT
		NahG-R2	5’-ATCCAGGTACAGCTGTT