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Supplemental data 1. Characterisation of the transgenic lines used in this study.

Suppressor-expressing lines

Binary vector constructs encoding silencing suppressors were mobilized into *Agrobacterium* strain GV3101 and transformed into *Arabidopsis thaliana* ecotype Col-0 according to Bechtold *et al.* (1993). The following lines were generated in two independent rounds of transformations. The molecular data shown in the manuscript are from one representative of each homozygous/hemizygous line expressing a given suppressor. Similar results were obtained with at least two additional lines of each suppressor (except for HcPro class I).

Suppressor	Selection in plant	Total number of primary transformants	Expression of the suppressor	Phenotype[a]	Segregation in T2[b]	Monolocus, lines selected
P1-HcPro	Hygromycin	20	20/20 (Northern)	20/20: class I: 5 class II: 7 classIII: 8	 (2:1) 2/5 (2:1) 3/7 (2:1) 4/8	 1 (hemi) 3 (hemi) 3 (hemi)
P38	Kanamycin	11	10/11 (Western)	None	(3:1) 4/10	4 (homo)
P19	Kanamycin	12	12/12 (Western)	12/12, uniform	(2:1) 4/12	4 (hemi)
P25	Kanamycin	14	13/14 (Northern)	None	(3:1) 5/13	5 (homo)
P15	Kanamycin	8	8/8 (Western)	8/8, uniform	(2:1) 3/8	3 (hemi)

[a] number of primary transformants exhibiting developmental anomalies;
'uniform' means that the same anomalies were observed among primary transformants, independently of the level of suppressor expression. The absence of phenotype in the P38 and P25 transformants was also independent of suppressor expression levels.

[b] for each transformant, 50 T1 seeds were sown and selection was on the appropriate antibiotic. Segregation ratios, indicated in brackets, were based on the number of surviving seedlings. A distortion in the segregation ratio normally expected for the presence of one transgene locus (3:1 ratio) was observed in the case of P1-HcPro (all classes), P19 and P15. Analysis of immature siliques in those plants revealed that approx. 1/4 of the embryos had not developed, suggestive of suppressor dosage-dependent embryo lethality. The observed 2:1 ratio is consistent with the presence of one suppressor locus, which, when brought into homozygous conditions causes abortive embryo development. Consequently, the lines selected for P1-HcPro (classes II and III), P19 and P15 were maintained through backcrosses to wt plants and subsequent antibiotic selection, thereby keeping the suppressor loci in hemizygous conditions. The presence of a single suppressor locus in each of those lines is further suggested by the 1:2 segregation ratio observed in the backcrosses. Note that, as explained in the manuscript, P1-HcPro, P19 and P15 also had altered flower organs, which, in itself, caused a significant reduction in the number of seeds produced upon selfing. P1-HcPro plants of class I did not produce any seed, with the exception of one line for which a few seeds could be recovered.

Crosses to line CHS-RNAi; anthocyanin analysis

Line CHS RNAi was a kind gift from Peter Waterhouse (SCIRO, Australia). This line (ecotype Col-0) is homozygous for a single locus containing a transgene that produces CHS dsRNA and, consequently, silences the CHS endogenous mRNA (Wesley *et al*. 2001). Selection for this locus is on BASTA. Each of the suppressor-expressing line used in this study was crossed with line CHS RNAi. Seeds were harvested and seedlings selected on BASTA and hygromycin (crosses with P1-HcPro class II and class III) or BASTA and Kanamycin (crosses with the other suppressors). Expression of the silencing suppressor was confirmed in those crosses by Northern or Western analysis (see above table). The P38 and P25 plants were selfed and double homozygous lines were identified by co-selection and subsequently analysed. The P1-HcPro, P15 and P19 plants were maintained by backcrossing to line CHS RNAi. Individuals homozygous for CHS RNAi were selected on BASTA. The molecular data shown in Figure 2 are from one representative of each homozygous line expressing a given suppressor.

Anthocyanin analysis

Plants at the 6-8-leaf stage were placed 45 cm below a 400 W sodium lamp (Osram SON E 400 W) with a 24h photoperiod and were watered constantly. After 1 day, anthocyanins were extracted from leaves and quantified by spectrophotometry at 530 nm, according to Gerats et al. (1982). The values were obtained from four independent experiments, each involving a blend of four randomly chosen leaves from five plants expressing one given suppressor.

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