

Fig. S1. Downregulation of *ACS* genes in hybrids of various *Arabidopsis* ecotypes. Relative expression levels (R.E.L.) of *ACS* genes at ZT0 in hybrids of various *Arabidopsis* ecotypes (C24, Ler, Ws, Est, and Col). Asterisks indicate down-regulation in the hybrids at statistical significance levels of P < 0.05, compared with MPV.



Fig. S2. Downregulation of *ERF73 and ERF1A* in hybrids of various *Arabidopsis* ecotypes. Relative expression levels (R.E.L.) of *ACS* genes at ZT0 in hybrids of various *Arabidopsis* ecotypes (C24, Ler, Ws, Est, and Col). Asterisks indicate down-regulation in the hybrids at statistical significance levels of P < 0.05, compared with MPV.



Fig. S3. Downregulation of *ACS* genes at different time points in hybrid. Relative expression levels (R.E.L.) of *ACS* genes every 4h in a 24h period (ZT0 = dawn) in the reciprocal F1 hybrids (ColXC24 and C24XCol) and the parents (Col and C24). Asterisks indicate down-regulation (arrows) in the hybrids at statistical significance levels of P < 0.05, compared with MPV.



Fig. S4. Indirect roles of CCA1 on expression regulation of *ACS* genes.(*A*) Relative expression levels of *ACS2*, *ACS4*, *ACS7*, *ACS9* and *ACS11* in WT (Ws) and *cca1 lhy* mutant every 4h under a diurnal cycle (24h) followed by constant light (LL) (48h) conditions. Black, white, and grey boxes indicate dark, light, and subjective night, respectively. Asterisks indicate down-regulation in *cca1 lhy* mutant at statistical significance levels of *P* < 0.05, compared with wild type. (*B*) Distribution of CCA1-binding site (CBS) and G-box (PIF-binding motif) elements in promoters of *ACS2*, *ACS6*, *ACS7*, *ACS8* and *ACS9*. (*C*) Western blot confirmed specific binding activity of anti-CCA1 antibodies to CCA1 protein.



Fig. S5. Relative expression levels (R.E.L.) of *CCA1* every 4h in a 24h period (ZT0 = dawn) in the reciprocal F1 hybrids (CoIXC24 and C24XCoI) compared with MPV. Asterisks indicate down-regulation (arrows) in the hybrids at statistical significance levels of P < 0.05, compared with MPV.



Fig. S6. *PIFs* were repressed in hybrids of various *Arabidopsis* ecotypes. (*A*) Relative expression levels to the mid-parent level of *PIF5* at ZT18 in hybrids of various *Arabidopsis* ecotypes. Asterisks indicate down-regulation in the hybrids at statistical significance levels of P < 0.05, compared with MPV. (*B-C*) Relative expression levels to the mid-parent level of *PIF4* at ZT0 (*B*) and ZT18 (C) in hybrids of various *Arabidopsis* ecotypes. Asterisks indicate down-regulation in the hybrids at statistical significance down-regulation in the hybrids at statistical significance levels of *P* < 0.05, compared with MPV. (*D*) Relative expression levels of *P* < 0.05, compared with MPV. (*D*) Relative expression levels to the mid-parent level of CO-0 and C24.



Fig. S7. ACS genes were regulated by *PIFs*. Relative expression levels of ACS4, ACS5, ACS6, ACS7, ACS8 and ACS11 in WT (Col-0), PIF5-OX and *pif1345* mutant in diurnal conditions (dark, D and light, L). Asterisks in orange and grey respectively indicate upregulation in PIF5-OX and downregulation in *pif1345* at statistical significance levels of P < 0.05, compared with WT.



Fig. S8. *ACS* genes were directly activated by PIF5. (*A*) Transient expression assays showed that PIF5 directly activated expression of ACS7 as in Fig. 2*F*. (*B*) Relative luminescence intensity (Y-axis) for each comparison in (*A*).



Fig. S9. Ethylene production in ACS6-OX lines and plant growth reduction in *PIF* lines. (*A*) Ethylene production in the transgenic plants that over-expressed $ACS6^{DDD}$ in Col_ACS6-OX, C24_ACS6-OX, F1 hybrid (ColXC24_ACS6-OX), and reciprocal hybrid (C24XCOL_ACS6-OX) lines. A dashed line indicates an average level of ethylene in the wild-type plant. (B) Representative seedling images of the wild type (WT), *pif1345* mutant, and PIF5-OX line at 21 days after sowing. (*C*) Quantitative analysis of rosette diameter of the plants in (*A*). Asterisks indicate a statistical significant level (P < 0.05) compared with WT.

Table S1	Primers	used in	this	study	y.
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Name	Primer sequence	Usage	
ACS2F	ATTTGCGTGGATGGATTTGAGACA		
ACS2R	AACGGAAGGAAGAGCCAGGAGACA		
ACS4F	CCGGGTTGGTTTAGAGTTTGTTTC		
ACS4R	TTCGCTTTTACTCTTTTGGCATCT		
ACS5F	TAAATGGAGAACCGGAGCAGAGAT		
ACS5R	CAAGTGGGTTAGATGGATTCGTGA		
ACS6E			
ACSER			
ACSZE			
ACS7P			
ACSOF		RT-qPCR	
ACSOF			
ACSOF			
ACSAR	GICAACCCAACAGAACAAACCA		
ACS11F			
ACS11R	GGAGACCCATTIGTIGATAAGAGA		
ERF1aF			
ERF1aR			
ERF73F	AATAATCCGGACACGCTTCTG		
ERF73R	CGTTGTTGGCTTCTTCACTATCAT		
PIF4F	TTGGGCGTGGAACTTGGACT		
PIF4R	CTGGGTTTGGGTTTGTTCTCTATG		
PIF5F	GTTTCCCGGGGTACAATCATCTCC		
PIF5R	GCTGGTTGTTGTTGCACGGTCTG		
ACS2pF	CTCGAGATCAACTTATTATTTATTGGC		
ACS2pR	TCTAGATTGCTGTGTCAATTCTCACTT		
ACS6pF	CTCGAGAACATCAGTCTGATAAAAAA		
ACS6pR	GGATCCTTTTGTTTCTTCTTTAATA		
ACS7pF	CTCGAGGTACATGAAAAGTGGTAAAAGTAT	Promoter cloping	
ACS7pR	GGATCCTTTTTCTTAGAGCTTCGAACCTGA	Fromoter cioning	
ACS8pF	CTCGAGATATCAAACTAAACATACACAC		
ACS8pR	GGATCCTTTCTTAATTAGCTCTAGAGAT		
ACS9pF	CTCGAGCTAATAGTGACAAGTGAACCT		
ACS9pR	GGATCCTTTTTGATATAAAAATCAAAAAG		
CCA1-CDSF	CTCGAGATGGAGACAAATTCGTCTGGA		
CCA1-CDSR	TCTAGATCATGTGGAAGCTTGAGTTTC	CDS cloning	
PIF5CDSF	TACCGCTCGAGATGGAACAAGTGTTTGCTGATTG		
PIF5CDSR	CGCGGATCCTCAGCCTATTTTACCCATATGAA		
UBQ10ChipF	TCCAGGACAAGGAGGTATTCCTCCG		
UBQ10ChipR	CCACCAAAGTTTTACATGAAACGAA		
TOC1EEF	TTTTATGGCCTGCACTTTTTATTG		
TOC1EER	GGTGGGACTTGGGATATTTTAGG		
ACS2CBSF	TGCTAGCAAAACACAACCATCT		
ACS2CBSR	TGAAAAGTAACAAGCGAACCAA	1	
ACS6CBSF	TTGGTCAAAGTGAAGGCTTCAAAA	ChIP-qPCR for CCA1	
ACS6CBSR	TGATAGTGGCAGACATTGGAC		
ACS7CBSF	TCATACTTAATTAGAGACGAA		
ACS7CBSR	GGCTATCCATTTACACTTTATT		
ACS8CBSF	TACATTAAGACGGTCCAAAGAG		
ACS8CBSR	CTAACAAAAACTATATCGGCAACA		
ACS2GF	AGGAGGATTTGAGTTTTTGACATT		
ACS2GR	GTTGGTGGGTTTGGACTCTTT	ChIP-qPCR for PIF5	
ACS7GF	GGTCACGTCTGCTATATACTC		
ACS7GR	CTTAGAGCTTCGAACCTGACACGT		
ACS9GE			
ACS9GR			
PP2AF			
PP2AR			