

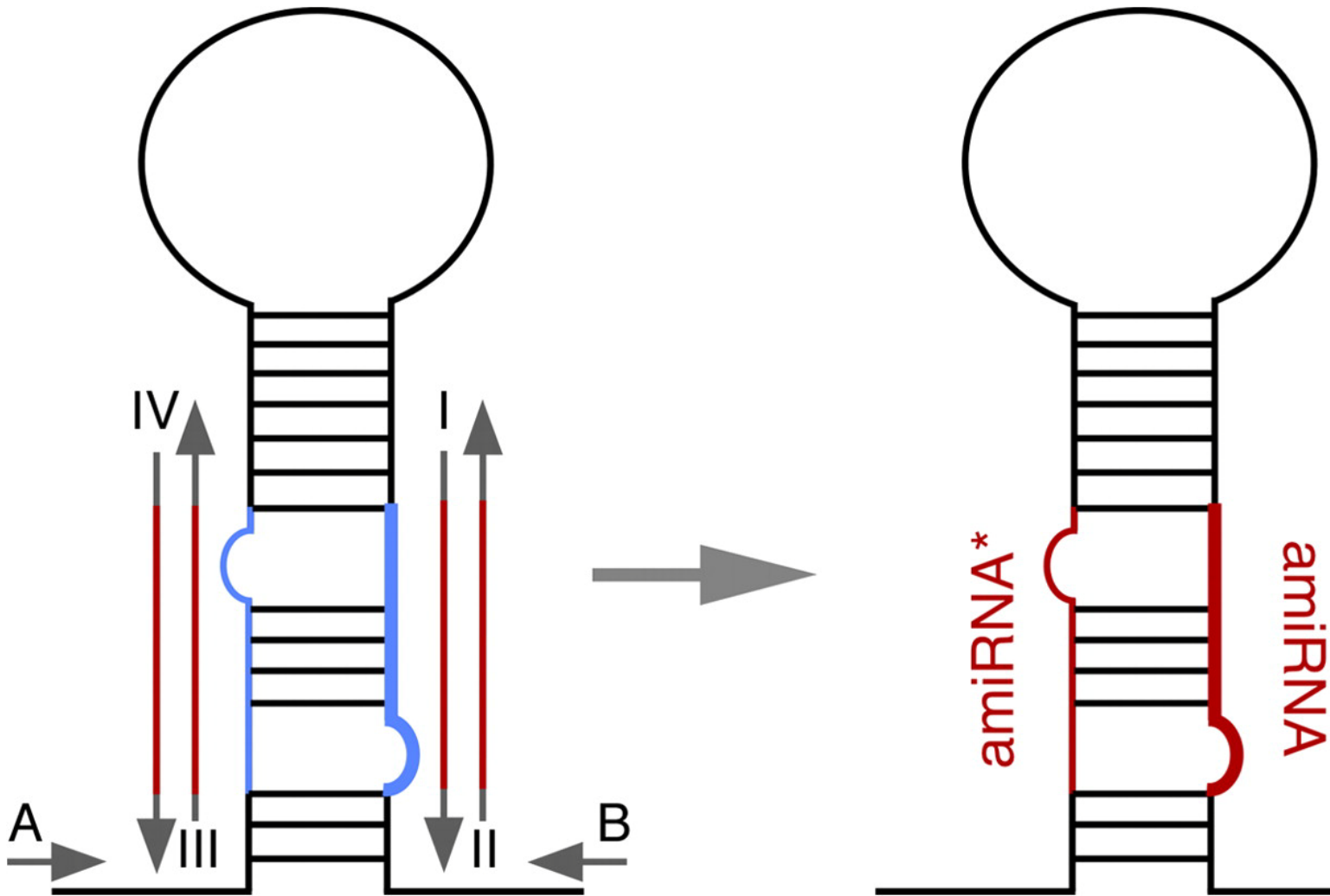
Artificial miRNA (amiR)

- Rebecca Schwab, Stephan Ossowski, Markus Riester, Norman Warthmann, and Detlef Weigel (2006) Highly Specific Gene Silencing by Artificial MicroRNAs in Arabidopsis. *Plant Cell* 18: 1121-1133

**Table 1.** Predicted amiRNA Sequences and Targets

amiRNA	Predicted Mature Sequence (5'–3')	Predicted Target(s)	Known Target Functions	Reference
amiR-lfy-1	<b>U</b> AACAGUGA <b>A</b> CGUACUGUCGC	<i>LFY</i>	Master regulator of floral identity	1
amiR-lfy-2	<b>U</b> UACGAUAAA <b>A</b> CGGUUGCUCGC			
amiR-white-1	<b>U</b> UAGUGAGA <b>A</b> UGUUGC GCCGG	<i>GUN4</i>	Cofactor in chlorophyll biosynthesis	2
amiR-white-2	<b>U</b> UUAACCAG <b>A</b> UUUUGCUCGC			
amiR-ft-1	<b>U</b> AUUCUCGG <b>A</b> GGUGAGUGUUG	<i>FT</i>	Promotion of flowering	3
amiR-ft-2	<b>U</b> UGGUUAUA <b>A</b> AGGAAGAGGCC			
amiR-trichome	<b>U</b> CCCAUUCG <b>A</b> UACUGCUCGCC	<i>TRY, CPC, ETC2</i>	Trichome patterning	4, 5
amiR-mads-1	<b>U</b> UUUGGAGA <b>A</b> AGUGACUUGUC	<i>SOC1, MAF1-3, ANR1, and three others</i>	Regulation of flowering, nutrient uptake	
amiR-mads-2	<b>U</b> UGUUCUCU <b>A</b> UCCUCUUCAGC	<i>SEP1-4, SHP1-2, AP1, CAL, and 10 others</i>	Patterning of floral organs	6
amiR-yabby-1	<b>U</b> ACUGAAAG <b>C</b> UUCUCUGUGGG	<i>INO, YAB3, and three others</i>		
amiR-yabby-2	<b>U</b> GUAUGCUG <b>A</b> UGGGACUCUCG	<i>CRC</i>	Regulation of adaxial polarity	7

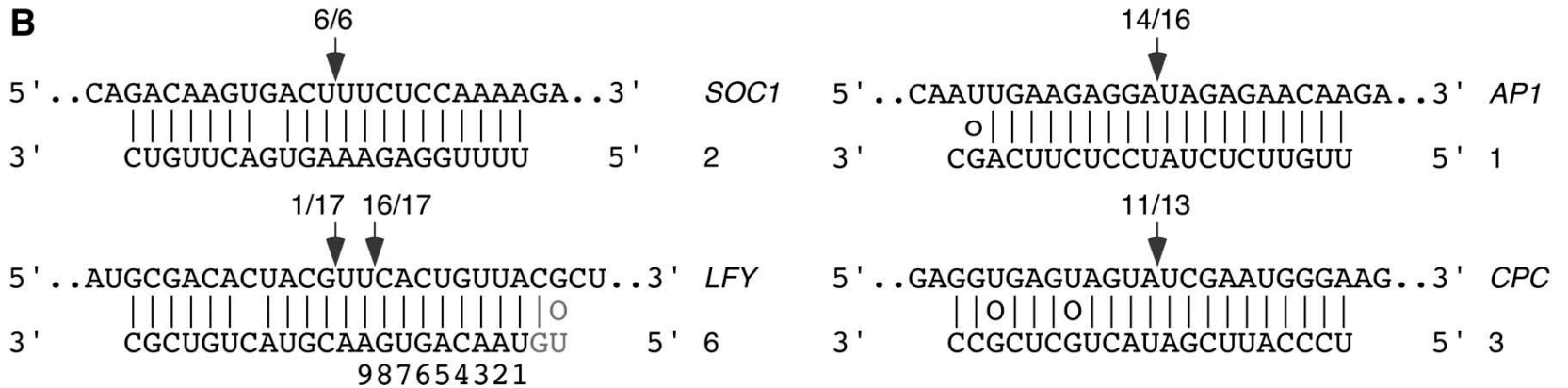
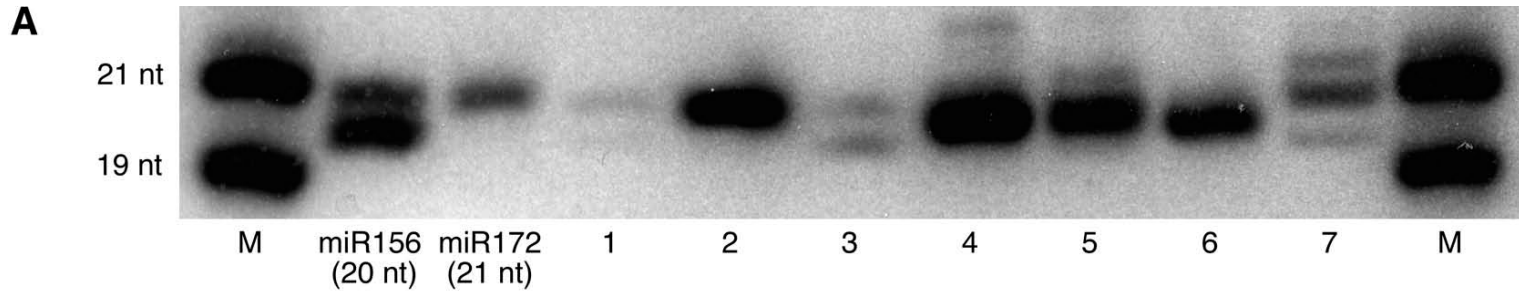
Positions 1 and 10 of small RNAs are marked in bold. A complete list of targets together with alignments to the respective amiRNAs is shown in Supplemental Figure 1 online. References: 1, Weigel et al. (1992); 2, Larkin et al. (2003); 3, Kardailsky et al. (1999); 4, Schellmann et al. (2002); 5, Kirik et al. (2004); 6, Becker and Theissen (2003); 7, Engstrom et al. (2004).



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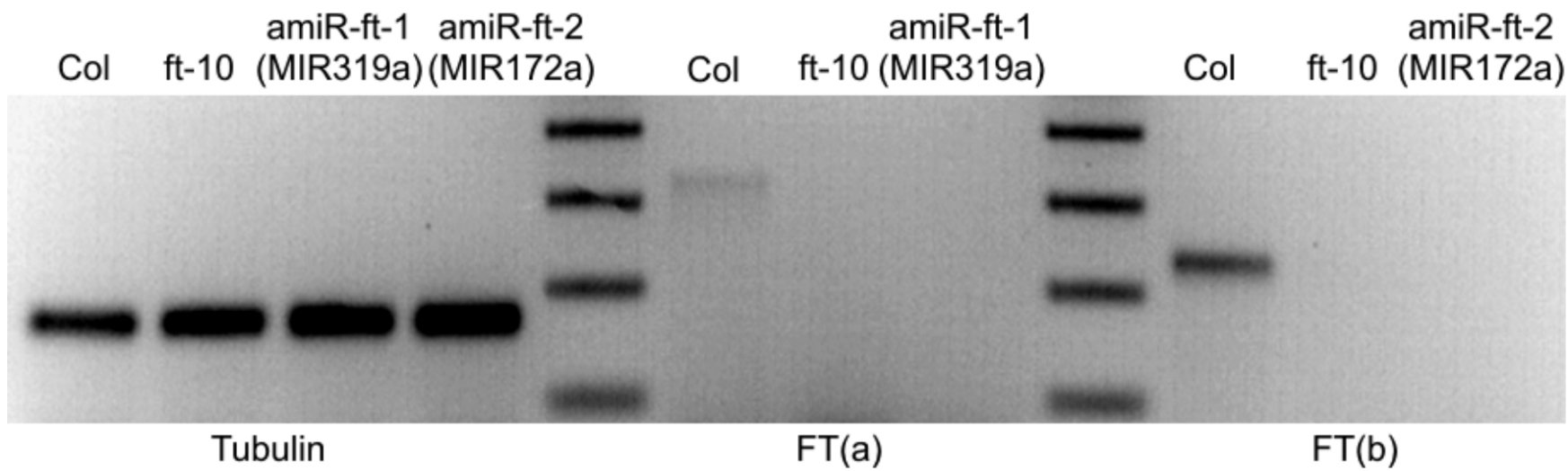
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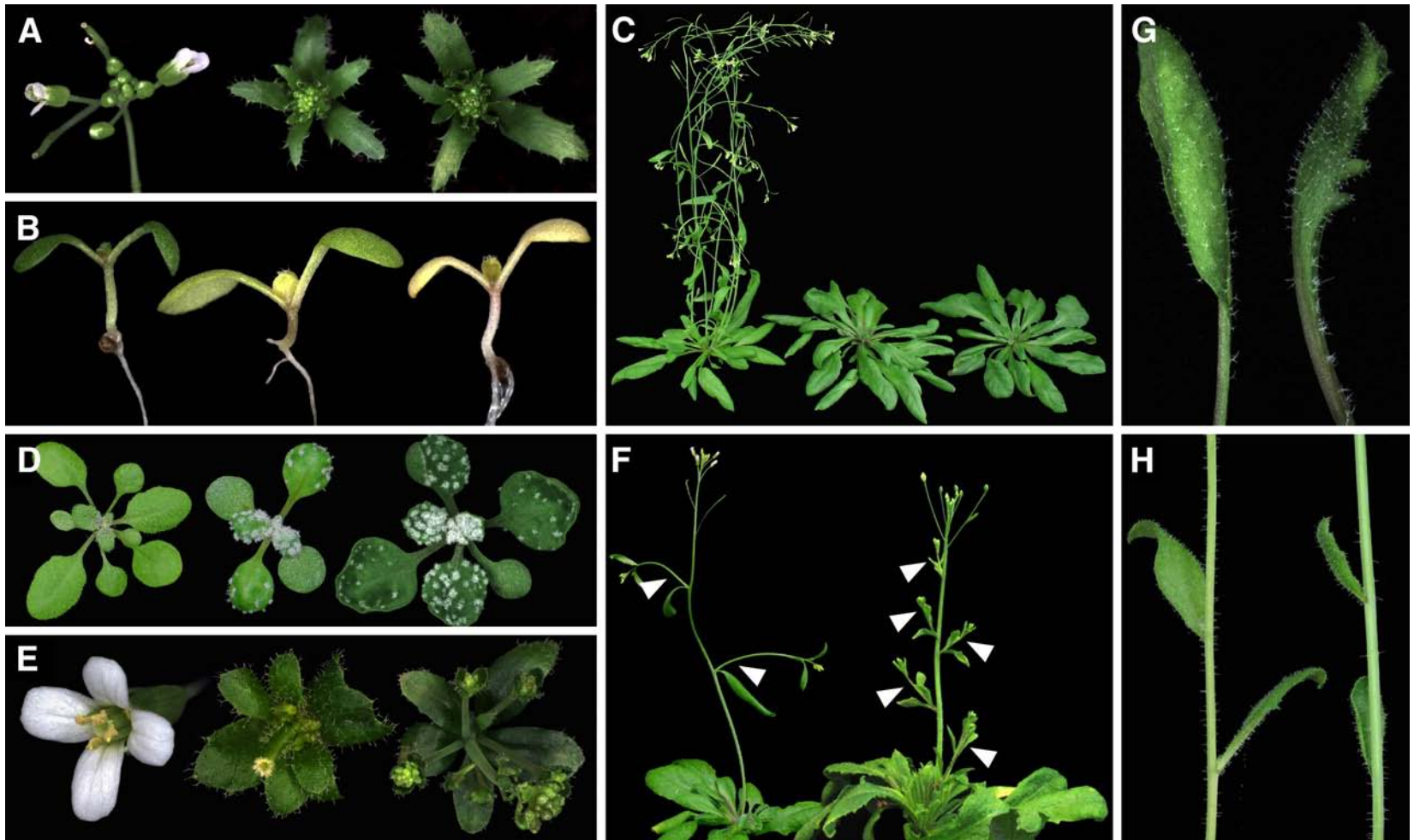


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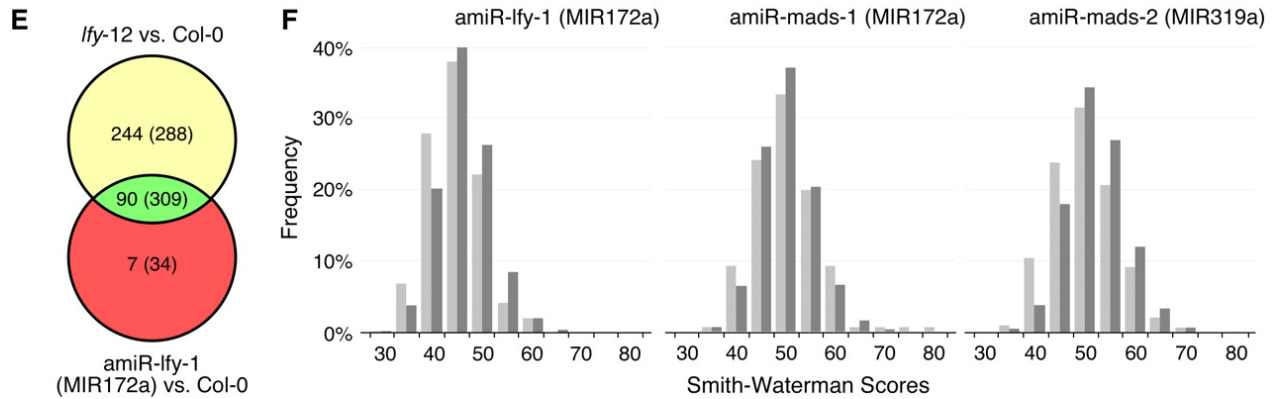
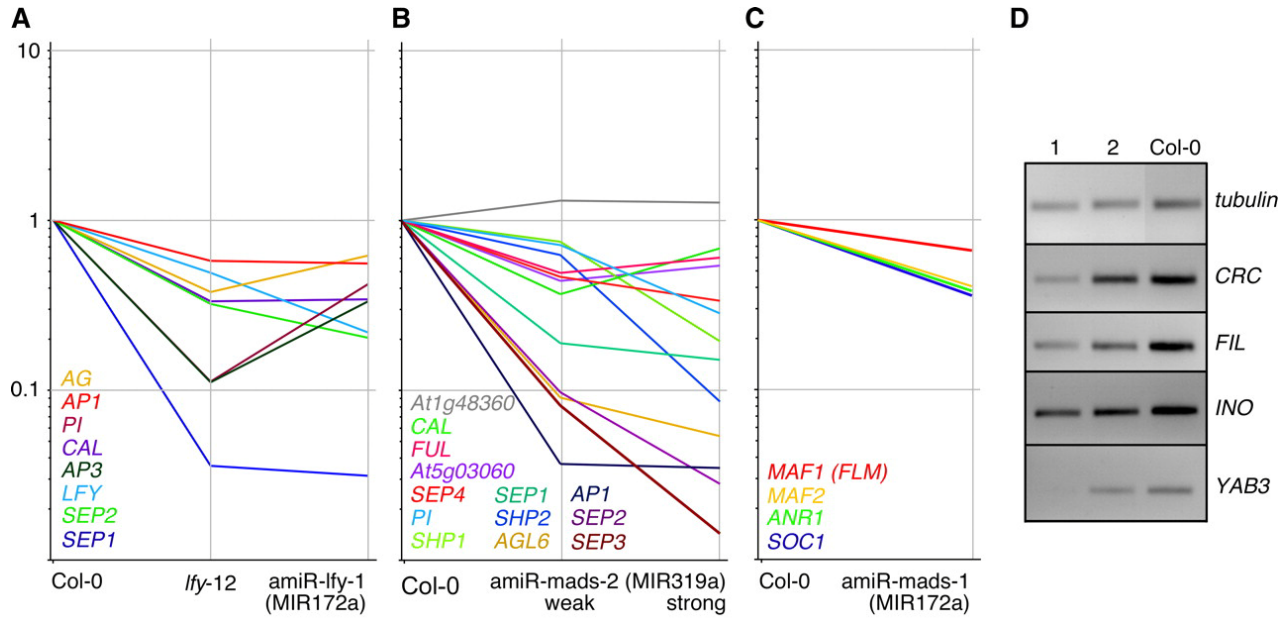




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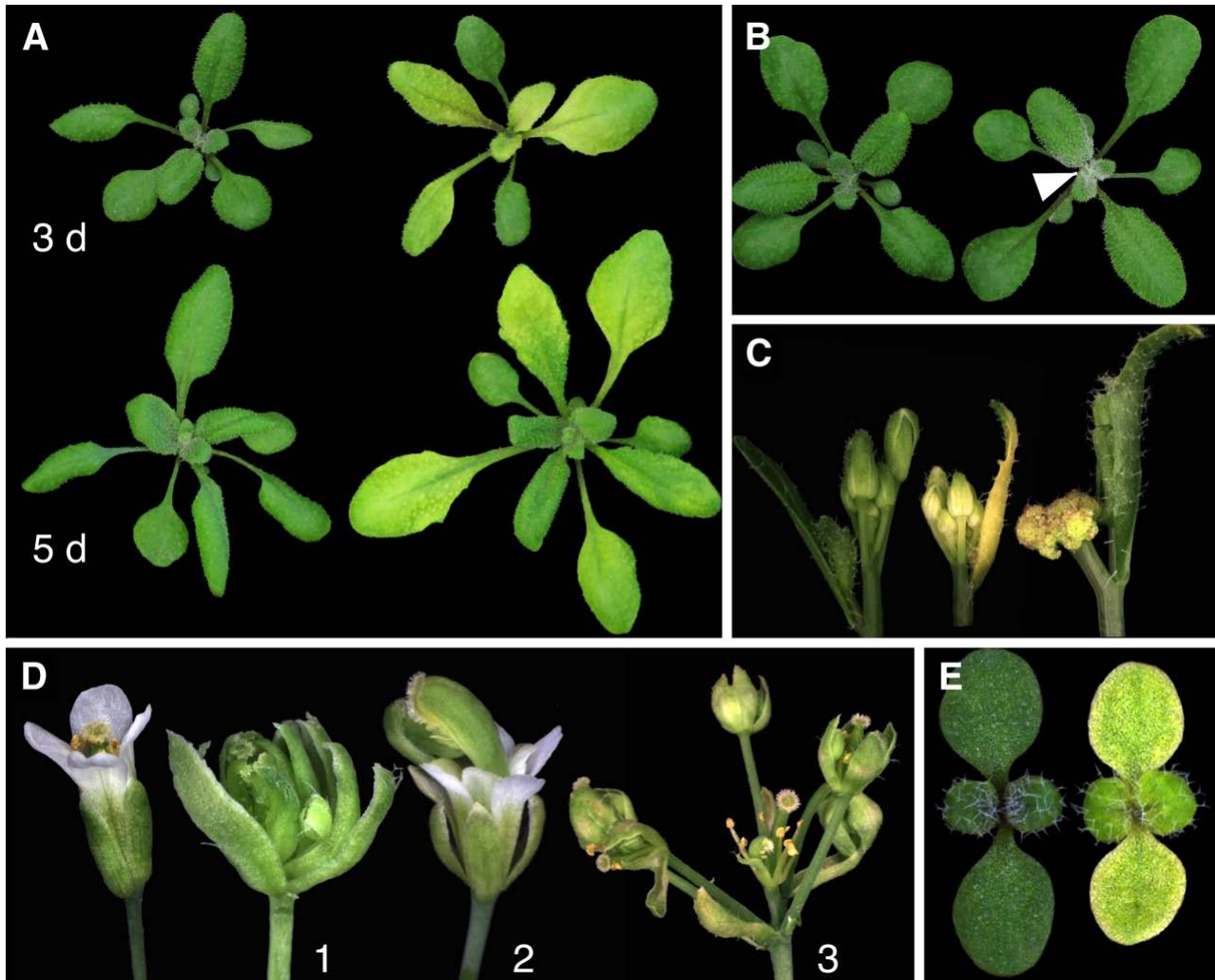
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**Table 2.** Summary of Downregulated Genes with Up to Five Mismatches

	amiR-lfy-1	amiR-mads-1	amiR-mads-2
Predicted targets, represented on array	1	6	18
Predicted targets, present in control	1	4	13
Predicted targets, present in control and downregulated in amiRNA overexpressers	1	1	6
All genes present in control without predicted targets	15,367	15,364	15,355
All genes downregulated without predicted targets	342	141	287
Genes with five mismatches, present in control	32	89	311
Observed downregulated			
Predicted targets	1	1	5
Others	1	3	5
Expected downregulated without predicted targets	0.7	0.8	5.6
$\chi^2$	0.1	6.4	0.5

Significantly downregulated genes were determined using LIMMA (Smyth et al., 2005), with an expression change of at least 1.5-fold at an estimated 1% false discovery rate (FDR) (Benjamini and Yekutieli, 2001). A  $\chi^2$  test was used to determine statistical significance of the difference between expected and observed downregulated genes among the nontargets (genes with five or less mismatches, but not predicted targets). A minimal  $\chi^2$  value of 6.63 corresponds to significant differences at  $\alpha = 0.01$ . Use of the logit-T algorithm produced qualitatively similar results. A total of 15,368 genes were called present in Col-0 inflorescences. For amiR-lfy-1, we used the sequence of the 21-mer deduced from the mapping of the cleavage product, which was shifted by two nucleotides from the intended amiRNA.



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