Supplementary Table S1. List of primers that were used.

List of primers used in plasmid construction, virus detection and RT-PCR.

Table S1

Name	Sequences (5'-3')	Brief Description
OYLT97	CGGAAGCTTGTCGACTGGCGGCCG CTCTAGTGGATC	For CbLCV DNA-A cloning
OYLT98	CGGGTCGACATAGAACGCCACGGG GCATC	
Oligo A	CTGCAAGGCGATTAAGTTGGGTAA C	For all amiRNA cloning
Oligo B	GCGGATAACAATTTCACACAGGAA ACAG	
OTY14	GATCAACATAGACTGATTGGGGCTC TCTCTTTTGTATTCC	For amiR-PDS cloning
OTY15	GAGCCCCAATCAGTCTATGTTGATC AAAGAGAATCAATGA	
OTY16	GAGCACCAATCAGTCAATGTTGTTC ACAGGTCGTGATATG	
OTY17	GAACAACATTGACTGATTGGTGCTC TACATATATATTCCT	
OTY42	GATAAGATCTAACCGTGGCGCACTC TCTCTTTTGTATTCC	For amiR-Su cloning
OTY43	GAGTGCGCCACGGTTAGATCTTATC AAAGAGAATCAATGA	
OTY44	GAGTACGCCACGGTTTGATCTTTTC ACAGGTCGTGATATG	
OTY45	GAAAAGATCAAACCGTGGCGTACT CTACATATATATTCCT	
OWF4I	GATGTATCTGAAACAACTTGGTGTC TCTCTTTTGTATTCC	For amiR-CLA1 cloning
OWF5II	GACACCAAGTTGTTTCAGATACATC AAAGAGAATCAATGA	
OWF6III	GACAACAAGTTGTTTGAGATACTTC ACAGGTCGTGATATG	
OWF7IV	GAAGTATCTCAAACAACTTGTTGTC TAACTATATATTCCT	
OTY34	GATCGGTGCTAAACTAGCACCAGT CTCTCTTTTGTATTCC	For amiR-SGT1 cloning
OTY35	GACTGGTGCTAGTTTAGCACCGATC AAAGAGAATCAATGA	
OTY36	GACTAGTGCTAGTTTTGCACCGTTC ACAGGTCGTGATATG	

OTY37	GAACGGTGCAAAACTAGCACTAGT CTACATATATATTCCT	
OTY77	ACAGTTTGATTGCCAGTCCC	For CbLCV DNA detection
OTY78	ACATCACTCACCCCAGAAGG	
OTY100	TCTGTTTAGCCGGTTTGGTC	For TMV detection
OTY101	TGCAAGCCTGATCGACATAG	
OTY50	CGGGGTACCTCTCGCTCCTATCTAC CA	For miR156b cloning
OTY51	CGGTCTAGACAAGCACCCACTTCC ACA	
OTY54	CGGTCTAGAATAGAAAATACTTCGT TAGC	For miR165a cloning
OTY55	CGGGGTACCACTCATCATTCCCTCA TC	
OTY133	CCTGCATGTGCTCACAGTCTTCTGT CAGTTTTCTC	For miR156b mutaion
OTY134	AACTGACAGAAGACTGTGAGCACA TGCAGGCACTG	
OTY135	AACTGACAGAAAGACTGGTGAGCA CGCACACGCAA	
OTY136	TGCGTGCTCACCAGTCTTTCTGTCA GTTGCCTATC	
OTY137	GTATCCTCGGACCAGTGTTCATCCC CCCCAACATG	For miR 165a mutation
OTY138	TTGGGGGGGATGAACACTGGTCCG AGGATACTCTC	
OTY139	GTTGAGGGGAATGTTCGCTGGATC GAGGATATTAT	
OTY140	ATCCTCGATCCAGCGAACATTCCCC TCAACTGAAA	
X67	TAACCCAAAGGCTAATCGTG	RT-PCR primers for Actin
X68	GTAGTCTCGTGGATTCCTGC	
OKX51	CGGTCTAGAGGGCCCTGACGAGCT TTCGATGCAGTG	RT-PCR primers for PDS
OKX52	CGGAGATCTGGCGCGCCATGGACA TTTATCACAGGAACTC	
OTY77b	GTGCAGGGTCCGAGGT	Universal reward primer for all miRNA end-point stem-loop RT-PCR
OTY169	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACGCCC CA	RT primer for amiR-PDS
OTY170	CCGGCTCAACATAGACTGAT	Forward primer for

		amiR-PDS
OTY173	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACGTGC GC	RT primer for amiR-Su
OTY174	GGGCGTAAGATCTAACCGTG	Forward primer for amiR-Su
OTY175	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACCACC AA	RT primer for amiR-CLA1
OTY176	CCGGCTGTATCTGAAACAAC	Forward primer for amiR-CLA1
OTY177	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACCTGG TG	RT primer for amiR-SGT1
OTY178	CCGGCTCGGTGCTAAACTAG	Forward primer for amiR-SGT1
OTY78b	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACGTGC TC	RT primer for miR156b
OTY79	GCGGCGGTGACAGAAGAGAGT	Forward primer for miR156b
OTY167	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACGGGG GA	RT primer for miR165a
OTY168	TCGCTTCGGACCAGGCTTCA	Forward primer for miR165a
OTY67	GGGACAAGACGAGATGAAGT	RT-PCR primers for SU
OTY68	ACCCGAACCAATTAGGATAA	
OTY98	GTAATGGCTCCTTCCGATGA	RT-PCR primers for CLA1
OTY99	AGCAACATGAGACCCGAAAC	
X1	CGCCGTTGACCTTTACACTC	RT-PCR primers for SGT1
X2	CACCACCTCCTCTGGCTTCT	
OTY69	GTCCTGGTATTATTCCTTCC	RT-PCR primers for TC7909
OTY70	AACACGATGCCATTTATTGA	
OTY71	AGTTCTGCAAGGTTCACCCAC	RT-PCR primers for TC9706
OTY72	CCGTCAACAGACAGCCTCA	
OTY96	GTTAGCAGCACGACCACAGA	RT-PCR primers for DH-ZIP III
OTY97	TCTATTGTCCCTCCATTGCC	

Supplementary Method S1. The protocol for MIR VIGS.

Detail protocol for designing, cloning amiRNAs and MIR VIGS analysis.

1. Artificial microRNA design.

First, a web application called Web MicroRNA Designer (WMD) is used to to design artificial microRNAs (amiRNAs). The new address of WMD website is http://wmd3.weigelworld.org/cgi-bin/webapp.cgi. On its "Designer" page, type the identified gene name (see Note 1) of the gene of interest (e.g. TC14913 for *Nicotiana benthamiana phytoene desaturase* gene) into the "Target genes" field or input the sequence of the gene in a FASTA format if the gene could not be found in transcript library of WMD (e.g. GFP or other non-plant genes). Choose the corresponding sequence library in the "Genome" field (e.g. *Nicotiana benthamiana* EST NbGI-3.0 or *Nicotiana tobacum* EST NtGI-4.0 for genes from *Nicotiana benthamiana*). Input the E-mail address in the "Email" field and press the "Submit" button. Candidate amiRNA sequences will be suggested by WMD through email.

Second, screen the candidate miRNA sequences by "WMD Target Search (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi?page=TargetSearch;project=stdwmd)" to find their potential target gene and only the candidate amiRNA that only targets the gene of interest will be chosen for further cloning. This step will reduce potential off-target effect of amiRNA.

Third, "WMD Oligo" is used to design the primers for amplifying individual amiRNA gene using Arabidopsis miR319a precursor gene (see Note 2) as backbone (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi?page=Oligo;project=stdwmd). Paste the chosen amiRNA sequence into the "MicroRNA sequence" field and press "Submit" button. Four special primers (I, II, III, and IV) are provided by for each designed amiRNAs. In addition, Oligo A (5'-CTGCAAGGCGATTAAGTT GGGTAAC-3') and B (5'-GCGGATAACAATTTCACACAGGAAACAG-3') are designed, which are located at 5'- or 3'-end of miR319a precursor gene.

2. Cloning of amiRNA genes

Six oligoes (four special oligoes, I, II, III and IV, and two universal oligoes, A and B) and the plasmid pRS300 (kindly provided by Dr. Detlef Weigel) are used to PCR amplify amiRNA gene (see Note 3). Two round of PCR are needed to produce the final amiRNA-containing construct. In the first round of PCR, three different reactions are performed simultaneously using plasmid pRS300 as template and primer sets A and IV, II and III, or I and B, respectively. In the second round of PCR, these three PCR products are mixed (see Note 4) and used as template to amplify the full-length amiRNA gene using oligos A and B. The final PCR products are digested by *Kpn*I and *Xba*I, and ligated into *Kpn*I-*Xba*I-digested pCVA vector.

3. Agroinfiltration and MIR VIGS analysis

Transform the amiRNA-containing pCVA and pCVB separately into Agrobacterium GV3101 strain using freeze-thaw method. Grow this two Agrobacterium separately in 5mL of Luria-Bertani medium containing antibiotics (30mg/L rifampicin, 50 mg/L gentamycin and 50 mg/L kanamycin) overnight in a 28°C shaker. Harvest the two Agrobacterium cultures, re-suspend in infiltration buffer (10 mM $MgCl_2$, 10 mM MES, and 200 mM acetosyringone) and adjust to an $O.D._{600}$=2.0. Keep the cultures at room temperature for 3 to 4 h, and mix at 1:1 ratio just before infiltration. Grow *Nicotiana benthamiana* plants in pots at 25°C under 16 h light/8 h dark cycle to six-leaf stage. Infiltrate the agrobacterium culture mix into the stem nodes and petioles of plants using a 1-mL syringe. Plant phenotype is observed since 2 weeks post agroinoculation

Note 1: The identified gene name can be found out by using the blast tool in the WMD site. If no result is found in blast, just input the sequence of the gene in a FASTA format into the “Target genes” field.

Note 2: The backbone sequence of Arabidopsis miR319a precursor gene (NC_003075, 12353276-12352872) is:

XbaI-CCATGGCGATGCCTTAAATAAAGATAAACCCAAAATGTTAATTTTACCAGAACTATATAT

ACGAAGGCAGCATATATGTCACTTAGTGGATCAAGCATGTTTTTGTGCAGGAAAGATTAATCAAG

AAAATTGGAATACAAAAGAGAGANNNNNNNNNNNNNNNNNNNNTCAAAGAGAATCAATGATCCA

ATTTGTCTACCGCATCATTCATTCATTTAACGAGTCTAGTTTGAATTTTGGCGACTCGGTATTTG

GATGAATGAGTCGGAAGCTAATTGAATCATATCACGACCTGTGANNNNNNNNNNNNNNNNNNNN

TCTACATATATATTCCTAAAACATCAATTCAAAACAGCGAGTATTAAGTGTATGAACATGTGTAA

TATGCGTCCGAGCGTGTGTTTG-*Kpn*I

The region in purple is the 21 nt amiRNA and the region in green is the 21 nt amiRNA*.

Note 3: pRS300 is a pBluescript-based plasmid containing Arabidopsis miR319a precursor gene. Any two oligoes flanking the MCS would be suitable as universal oligoes.

Note 4: We recommend preforming gel recovery to the PCR products before mix or using *Dpn*I to digest residual plasmid pRS300 in the mix.