

Additional methods

miRNA sequence analysis

MicroRNA mature and precursor sequences were obtained from miRbase (<http://www.mirbase.org>) and Tomato Functional Genomic Database (<http://ted.bti.cornell.edu/>). *MIR* gene sequences were derived by BLAST searching the sequence against tomato genomic resources in Sol Genomics Network (<https://solgenomics.net/>). Sequences were aligned using CLUSTALW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2>) web tool. The mfold web server (University of Albany, New York) has been used to generate stem-loop structure of all precursors. Gene specific primers (Supplementary table 1) were designed manually and IDT Oligo analyzer tool was used to verify the primers.

Cloning and sequencing of mature miR167

Total small RNA was isolated using pure link miRNA isolation kit (Invitrogen). The small RNA was then quantified and assessed for quality using a Nanodrop ND-1000 spectrophotometer followed by 10% urea-PAGE analysis. Presence of a distinct tRNA band corresponding to 100 bp band of ladder and absence of higher molecular weight rRNA bands indicated that small RNA was pure. About 300 ng of small RNA was then converted to cDNA by using NcodeVILO-cDNA synthesis kit (Invitrogen) according to the manufacturer's protocol. Mature miR167 specific amplification was done and the product was cloned in TA-vector for sequencing.

Transient Promoter activity assays

Protoplast isolation, transformation and promoter activity assay

Tomato mesophyll protoplast was isolated from young leaves of three weeks old tomato plants and promoter:reporter constructs were transformed using the standard protocol (Yoo *et al.*, 2007). One µg of pure plasmid DNA was electroporated (200 V, 50 ms) using a electroporator (BTX). Total protein was isolated and firefly luciferase activity assay was performed using the Luciferase assay kit (Promega). Luminescence was measured by Varioskan Flash multimode reader (Thermo Scientific).

Agroinfiltration and reporter activity assay

LBA4404 *Agrobacterium* strain harbouring recombinant binary vectors were used for agroinfiltration of fully expanded young leaves of 4-5 week old tomato plants following standard protocol (D'Aoust *et al.*, 2009). In brief, actively growing culture of *Agrobacterium* was suspended in MES buffer (10 mM 2-N-morpholinoethanesulfonic acid, pH-5.5, 10 mM MgSO₄ and 100 µM Acetosyringone) and adjusted to a concentration of 0.8 OD₆₀₀. Bacteria were pressure infiltrated into the intercellular space of the abaxial surfaces of leaflets using a needleless 1 ml plastic syringe. About 100 µl of bacterial suspension was infiltrated in each spot, typically 2-3 cm² infiltrated area and 2-4 spots per leaflet. Infiltrated leaves (~60 mg) were harvested after 48 hours for total protein extraction.

GUS extraction buffer (50 mM NaHPO₄ ; pH- 7, 10 mM βME, 10 mM Na₂EDTA; pH-8, 0.1% Sarcosyl, 0.1% Triton X-100), 10 µl/mg tissue was used for final suspension of total protein for GUS activity assays. The assay was conducted for 1 h in the same extraction buffer supplemented with 2 mM MUG. Fluorescence was measured using Varioskan Flash multimode reader (Thermo Scientific) at 365 nm excitation and 455 nm emission wavelengths. Fluorescent measurements were converted into yield of MU from a standard curve. GUS activity was expressed as pmol of MU/µg protein/hour.

Generation of transgenic tobacco lines

Tobacco leaf discs were transformed with *Agrobacterium* LBA4404 harbouring *PMIR167a::GUS* constructs in pCambia1304 vector. Plant regeneration and selection was carried out in complete MS media supplemented with 1 mg/l zeatin, 25 mg/l hygromycin and 500 mg/l cefotaxim. After several cycles of selection, transformed lines were rooted on hygromycin containing MS media. The transgenic T₀ generation plantlets were also tested for GUS activity by histochemical GUS staining. Blue coloration was considered as the indicator of a positively transformed plant. Confirmed transformed plants were hardened before transferring to soilrite and maintained in glass house for seed production (T₁). T₁-seeds were surface sterilized and selected on Hygromycin (25 mg/l) containing MS-plates. Seedlings showing robust growth were hardened and transferred to soil and maintained in a glass house until seeds (T₂) were obtained.

Confirmation of transgene integration by Southern blotting

About 20 µg of genomic DNA was digested with *EcoRI* (New England Biolabs, USA), resolved in a 0.8% agarose-TAE gel, and capillary transferred onto HybondTM-XL membrane (GE Healthcare, USA). Blots were probed with radiolabelled fragment of the coding region of GUS gene. Probe was prepared by random priming method using Klenow fragment of DNA polymerase I (Thermo scientific) in the presence of α -³²P-dCTP. Probes were purified using illustra MicroSpin G-25 Columns (GE Healthcare) and ~20 X 10⁶ cpm probe was used per blot. Hybridization and visualization were performed as described in RNA gel blot analysis.