Disruption of miRNA Sequences by TALENs and CRISPR/Cas9 Induces Varied Lengths of miRNA Production

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Supplementary Information
(Figures S1 to S3, Table S1 and S2)
Supplementary Figure S1. Analysis of the mir160a mutants by CRIPSR/Cas9 with single guide RNA. a. Sanger sequencing results for T1 and T2 plants. Sequencing confirmed homozygous single “T” insertions and the biallelic Line 13 in T1. Sequencing results of the T2 plants of Line 13 confirmed single “T” insertion (T2-1) and 5-bp deletion (T2-2). b. Genome browser views of small RNAs generated from ARF10, ARF16 and ARF17 in wild type and mir160a*+1 plants.
Supplementary Figure S2. Characterization of the mir160a mutant with 5-bp deletion.

a. The 2-3 week wild type, mir160a*+1, and mir160a*-Δ5 plants; scale bars represent 1 cm. b. qPCR analysis of ARF10, ARF16, ARF17, and PRI-MIR160A transcript levels in wild type mir160a*+1, and mir160a*-Δ5 plants. c. Flower phenotypes of the mir160a*-Δ5 mutants. d. Developing seeds of the wild type and mir160a*-Δ5 mutants. Blue arrows indicate delayed or aborted developing seeds; white arrows indicate unfertilized ovules.
Supplementary Figure S3. Agarose gel analysis of the PCR products from CRISPR/Cas9-transformed plants with double guide RNAs. Genomic DNA of the T1 plants were extracted for PCR analysis with primers (Supplemental Table S1) to amplify the region surrounding the edit sites.
Table S1. Primers and synthesized DNA sequences used in this study

| Primer name | Sequence (5’ to 3’) | Usage |
|-------------|---------------------|-------|
| Rice (*Oryza sativa*) | | |
| MIR390-F | ATGGGATCCTAGAGCAAGAGGCACCACCTG | PCR-amplification of *miR390* genomic region for mutant detection |
| MIR390-R | CATTCTAGATGCCCATCTCATGGACAGTAG | |
| miR390-F1 | AATGAATCTCTGACTGACTACAAACATCCACTG | PCR-amplification of *miR390* gene for complementation |
| miR390-R1 | TGGGAATCCAGGTACCTGACTGATAAACAGGCTTC | Forward primers for 2-step PCR-amplification of edited *miR390* |
| MIR390-F2 | AATCCCTGAAGCTCAGGAGGG | |
| MIR390-F3 | AATCCCTGAAGCTCAGGAGAT | |
| MIR159b-F1 | ATGAATTCTCAGATGCCTGTAGTGC | Genotyping of MIR159b mutants |
| MIR159b-R1 | CATTCTAGATCCCATGAAATGCTGCT | |
| MIR408-F1 | ATGGGAATCTCAGAGCAAGCAGGACAT | Genotyping of MIR408 mutants |
| MIR408-R1 | TCAATCAGACAGCCCCACGAGTGCA | |
| MIR394-F1 | ATGAATTCTCAGATGCCTGTAGTGC | Genotyping of MIR394 mutants |
| MIR394-R1 | TACTCTAGACAGCCCCACGAGTGCA | |
| MIR398b-F1 | TCAATCAGACAGCCCCACGAGTGCA | Genotyping of MIR398b mutants |
| MIR398b-R1 | AGGTGTCATGTGTCGTTAC | |
| Arabidopsis (*Arabidopsis thaliana*) | | |
| MIR160a-q-F | TGTCATGACGCATATCATATGAGATG | qRT-PCR primers for *ath-MIR160a* |
| MIR160a-q-R | CTCATCAACACAAATCATTGGTACC | |
| MIR160a-F | GATGAGGCAATGAAAGCAAGACAGAC | Genotype the T1 plants edited by CRISPR/Cas9 with double guide RNA |
| MIR160a-R | CACCAGCGAATTTAGTTTCTTACATA | |
| ARF10-F | CATTCTGAGCTTGGATTCC | qPCR primers for *ARF10* |
| ARF10-R | ACAAAGAGGGAGATGGTGC | |
| ARF16-F | CAACTGGATCAATCCAGATC | qPCR primers for *ARF16* |
| ARF16-R | GAAGATCGAAGATGATAACCC | |
| ARF17-F | GTTGACTGTCTAGTGACAG | qPCR primers for *ARF17* |
| ARF17-R | TATTTGTGATAGCTGCGGGAG | |
| sgRNA | GTTAAGACCTTTTCTGTTGAAACACGGAACTTCTGACCTG | Synthesized DNA fragment for CRISPR/Cas9 vector construction. PmeI sites are highlighted in blue. The target-specific sequence of the guide RNA is highlighted in red. |

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Table S2. **Small RNA library data for miRNA mutants.** (please see the Excel file)

Each mutant sRNA library was screened against both wt and mutant miRNA sequence. In **miR390** mutant, heterozygous **miR390** mutant was used as control since wt rice does not contain mutant **miR390**. The result from heterozygous **miR390** mutant shows that wt allele produces significant more miRNA than mutant allele, while homozygous mutant **miR390** produces more mutant **miR390** than heterozygous mutant.