

The *MdmiR156n* Regulates Drought Tolerance and Flavonoid Synthesis in Apple Calli and *Arabidopsis*

Guo Chen ^{1,2,†}, Yaping Wang ^{1,2,†}, Xueli Liu ^{1,2}, Siyue Duan ^{1,2}, Shenghui Jiang ^{1,2}, Jun Zhu ^{1,2}, Yugang Zhang ^{1,2,*} and Hongmin Hou ^{1,2*}

¹ College of Horticulture, Qingdao Agricultural University, Qingdao 266109, China

² Engineering Laboratory of Genetic Improvement of Horticultural Crops of Shandong Province, Qingdao Agricultural University, Qingdao 266109, China

* Correspondence: ygzhang@qau.edu.cn (Y.Z.); hmhou@qau.edu.cn (H.H.); Tel.: +86-0532-860-80752 (H.H.)

† These authors contributed equally to this work.

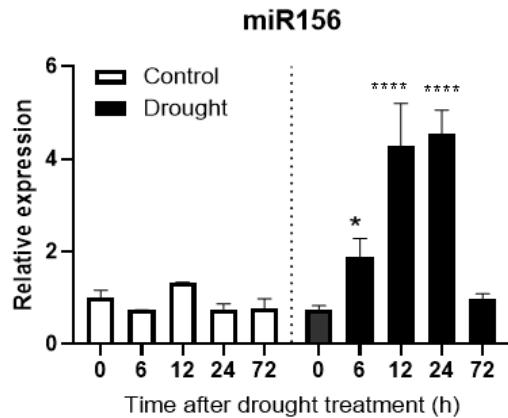


Figure S1. Response of *MdmiR156* to drought stress in apple. Asterisks indicate statistically significant differences (* $p < 0.05$ and **** $p < 0.0001$, one-way ANOVA).

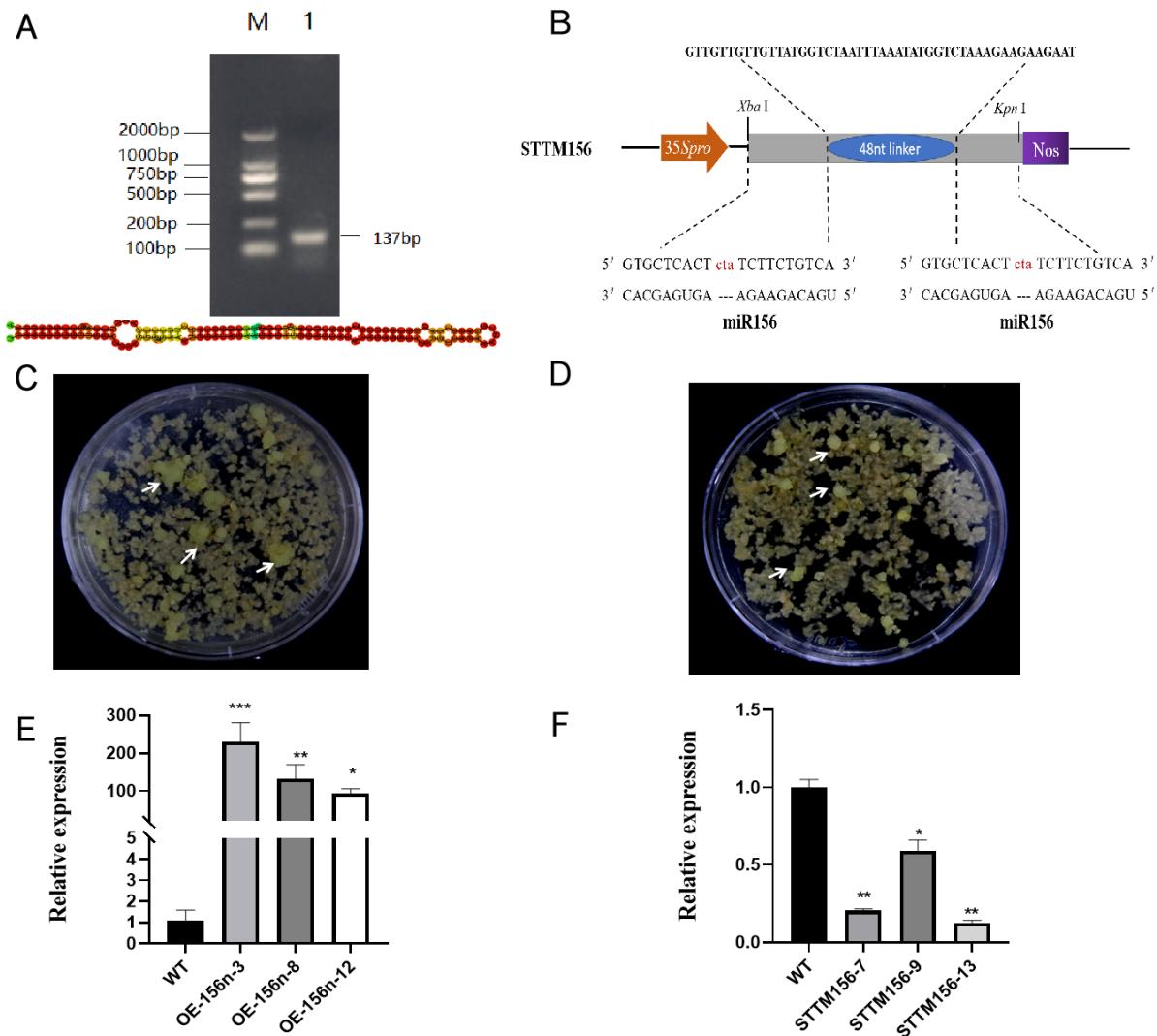


Figure S2. Screening and identification of the OE-156n and SSTM-156n transgenic resistant apple 'Orin' callus. (A) Cloning of *MdmiR156n* precursor genes. (B) The diagram of SSTM-miR156 structure with 48 nt imperfect stem-loop. (C, D) Screening the OE-156n and SSTM-156n transgenic resistant apple 'Orin' callus with kanamycin. The arrows indicate newly generated callus. (E, F) Identification of the OE-156n and SSTM-156n transgene positive lines by qRT-PCR. Values are means SD of three independent biological replicates. Asterisks indicate statistically significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, one-way ANOVA).

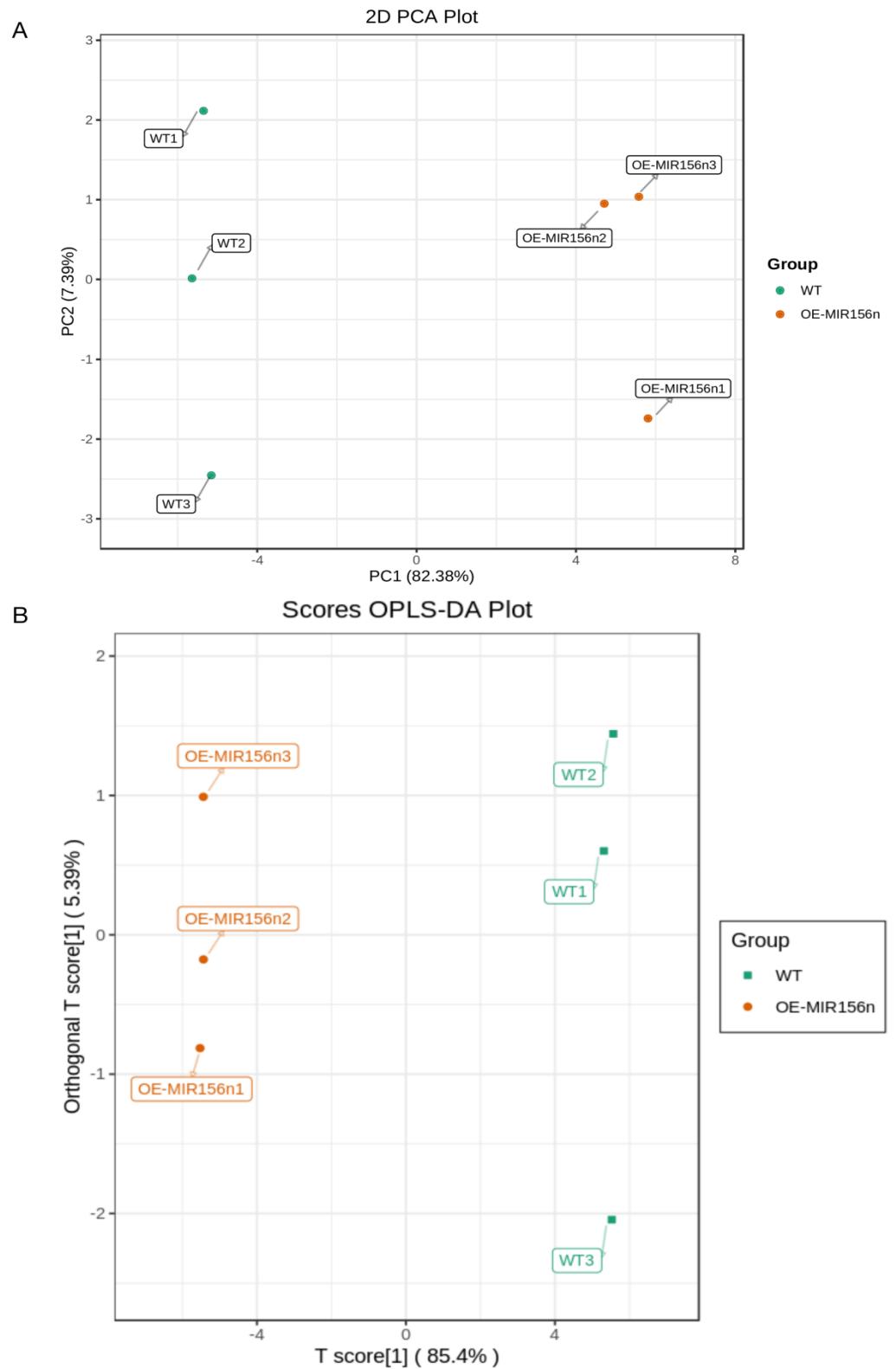


Figure S3. Quality control of metabolomics analysis of WT and OE-MIR156n lines under drought stress. A. PCA analysis. B. OPLS-DA analysis.

Table S1. The information of primers used in this paper. F indicates forward primer, R indicates reversed primer and the underline indicates restriction sites.

Primer Name	Sequence (5'-3')
<i>MdActin</i> -F	ATTCAAGTATGCCTGGGTGC
<i>MdActin</i> -R	CAGTCAGCCTGTGATGTTCC
<i>Atactin1</i> -F	AGGCACCTCTAACCTAAAGC
<i>Atactin1</i> -R	GGACAAACGGAATCTCTCAGC
<i>MdNCED3</i> -F	CCCGACTGCTCTGCTTCCA
<i>MdNCED3</i> -R	AGCCGGATTCTGACAAGACG
<i>MdDREB2</i> -F	ATGGCCTATGACGATGCTGC
<i>MdDREB2</i> -R	GAAGTTCAAATGGAAGTGG
<i>MdP5CS1</i> -F	AAGTCGATGTCAGTTGTGG
<i>MdP5CS1</i> -R	ATAAATGTGAGATGTCCAAGCG
<i>MdRD22</i> -F	CACGTGGACTTCGGATC
<i>MdRD22</i> -R	CTCCTGCTCCAGTGGAC
<i>AtNCED3</i> -F	TTGATGCTCCAGATTGCTTC
<i>AtNCED3</i> -R	GGACCCTATCACGACGACTT
<i>AtP5CS1</i> -F	TTCTCAGATGGTTCCAGGTTG
<i>AtP5CS1</i> -R	TGGGAATGTCCTGATGGGTG
<i>AtRD29B</i> -F	GTGAAGATGACTATCTCGTGGTC
<i>AtRD29B</i> -R	TACCAAGAGACTCAGCAATCTCTG
<i>AtPAL1</i> -F	CTTGGAACAGAGCTTTGACCG
<i>AtPAL1</i> -R	CGTGAAAACCTTGTCAACTCTTC
<i>AtCHS</i> -F	GGAGAAGTTCAAGCGCATGTG
<i>AtCHS</i> -R	ATGTGACGTTCCGAATTGTCG
<i>AtCHI</i> -F	CTCTCTTACGGTGCCTTTCG
<i>AtCHI</i> -R	CACCGTTCTCCGATGATAGA
<i>AtFLS1</i> -F	CCACCGTCATGCGTCAATTACAG
<i>AtFLS1</i> -R	TCTCCGCCAGACCTTCTTCAA
<i>AtFLS3</i> -F	GCTTGTGGCAAGTGCCTGG
<i>AtFLS3</i> -R	GCTCGGTGGGATTCCGTGG
<i>AtDFR</i> -F	AGCCGCCAAGGGACGTTATATTG
<i>AtDFR</i> -R	CCGGGAGAAAACCCTTTGACGA
<i>AtF3'H</i> -F	TTCCTTACCTTCAGGCCTTATC
<i>AtF3'H</i> -R	CGAGAGTGGTGTGGATG
<i>AtANS</i> -F	GGCTGTGTTGTGAGCCACCA
<i>AtANS</i> -R	CCTTGGAGGAAACTAGCCGGAGA
<i>AtUGT78D2</i> -F	TTTGCGCATTGTGCTGT
<i>AtUGT78D2</i> -R	TCAGCAAATGCGGAAACG
<i>AtUGT75C1</i> -F	CGGTGTTGGAGAGTGTATCGG
<i>AtUGT75C1</i> -R	TGATCCCCAAAAATGCC
<i>MdPAL</i> -F	ACCCTGGACAGATTGAGGCAGCT
<i>MdPAL</i> -R	GCGTAGCGATCCTGCTTGGCT
<i>MdCHS</i> -F	GTGACTGTCCAGGAAGTCGC
<i>MdCHS</i> -R	GCACACACTGGATTCTCCTTACG
<i>MdCHI</i> -F	GAAGGGTAAGACCGCCGAG
<i>MdCHI</i> -R	CACAATTCTCCGAAACTTCTCAG
<i>MdF3H</i> -F	CGGGATGATGGGAAAACG
<i>MdF3H</i> -R	CGCTGGTTCTGGAATGTG
<i>MdF3'H</i> -F	ACGATGGCGGATGTTACGG
<i>MdF3'H</i> -R	GCTTTGACCCTGCACITGCT
<i>MdDFR</i> -F	GGACCCCGAGAATGAAGTG

<i>MdDFR-R</i>	CTCCACATTACGGTTCCTG
<i>MdANS-F</i>	GAGAAAGTATGCCAATGACCAGG
<i>MdANS-R</i>	GGCGGTTGCCTCAATGTAAT
<i>MdUGT-F</i>	GCTGACGAGTTGGGAGTGC
<i>MdUGT-R</i>	CCTTCCGCTAAGTCTTGATT
<i>MdFLS-F</i>	ACGAGCAACCGGAATCACAAC
<i>MdFLS-R</i>	CCCAGTTGGAGCTGGCCTCAGTA
<i>35S-F</i>	AGATACTGGAAAAGGAAGGTGGC
<i>156n-F</i>	<u>TCTAGATTAATCTGGTTGGACTTAGGGTAG</u>
<i>156n-R</i>	<u>GGTACCTTAATCCGGCAGTGGAAA</u>
<i>Md-5.8S rRNA-F</i>	GCAACGGATATCTCGGCTCT
<i>Md-5.8S rRNA-R</i>	CAACTTGCITCAAAGACTCG
<i>35S-F</i>	AGATACTGGAAAAGGAAGGTGGC
<i>STTM156n-R:</i> Reverse transcription primer	CTGACAGAACGACTAGAGTGAGC
Universal PCR reverse primer	GTCACATCGTATCGTAAGCTGCGCAGCTGATGTGACGTGCTCAC
<i>MdmiR156-F</i>	CACATCGTATCGTAAGCTGC <u>TGCACTAGCGTGTGACAGAACGAGA</u>