Unique small RNA (sRNA)-based gene regulatory networks and their potential function in fruit crops

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Introduction & Summary

MicroRNAs (miRNAs) and their regulatory functions have been extensively characterized in model species but whether apple and peach have evolved similar or unique regulatory features remains unknown. We performed deep sRNA-seq and identified conserved, less-conserved and apple- and peach-specific miRNAs or families with distinct expression patterns. The identified miRNAs target over 100 genes representing a wide range of enzymatic and regulatory activities in each species. Importantly, we found that two gene families, *MYB* and *PPR*, are regulated by four different miRNAs, with miR159, miR828 and miR858 colletively targeting up to 81 *MYB* genes potentially involved in diverse aspects of plant growth and development, and miR7122 targeting over 20 *PPRs*. We also found that ten of the 19 miR828-targeted *MYB*s undergo siRNA/phased siRNA (phasiRNA) biogenesis at the 3′ cleaved, highly divergent transcript regions, generating over 100 sequence-distinct siRNAs that potentially target over 70 diverse genes as confirmed by degradome analysis. Similarly, miR7122-mediated cleavage of *PPRs* also triggers robust secondary siRNA/phasiRNA production, and many of siRNAs are involved in cascaded regulation of genes in the same family. Interestingly, miR7122-mediated siRNA biogenesis is indirectly evolved from ancient miR390-TAS biogenesis with several intermediate miRNA pathways, which reveals a novel miRNA-siRNA biogenesis route in plants. Taken together, our work reveals unique miRNA-triggered and phasiRNA-cascaded gene regulatory networks, which likely play an important role in regulation of many agronomically important traits qualitatively and quantitatively in tree fruit and other species

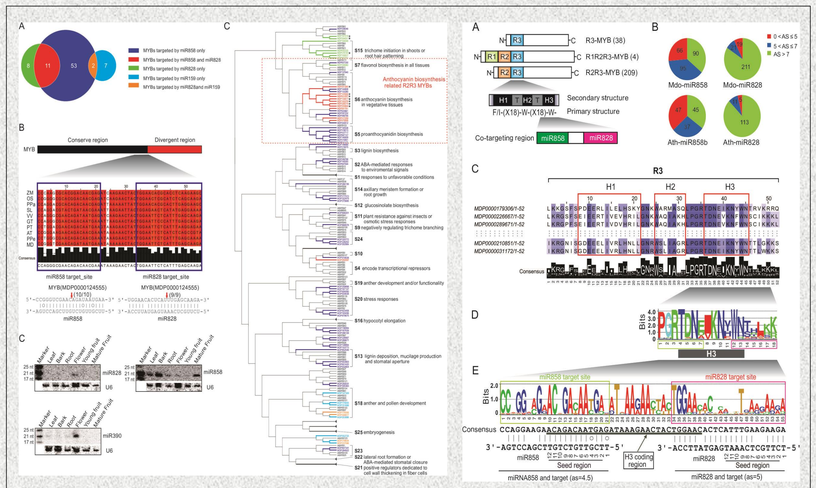


Figure 2. Complex *MYB* regulatory network mediated bymiR159,miR828, and miR858**. (A).** Numbers of *MYB* genes targeted by apple miR159, miR828 and miR858. (**B).** Conservation of the region co-targeted by miR828 and miR858. (**C).** RNA gel blot analysis of expression of miR390, miR828 and miR858 in various tissues. (**D).** Phylogenetic analysis and functional relationship between apple and *Arabidopsis* MYB factors.

Figure 3. The conserved co-targeting sequence of miR858 and miR828. **(A).** Schematic diagram of MYB proteins, and the location of the co-targeting region of miR828 and miR858. (**B).** Distribution of alignment score (AS) between miRNAs (miR828 and miR858) and their target sites among 251 apple (upper pie charts) and 129 *Arabidopsis MYB*s (lower pie charts). (**C).** The H3 helix and its flanking sequences among 251 apple MYB proteins. (**D). The** 18 amino residues encoded by miR828 and miR858 co-targeting region. (**E).** The 55-nt co-targeting region in 251 apple *MYB*s.

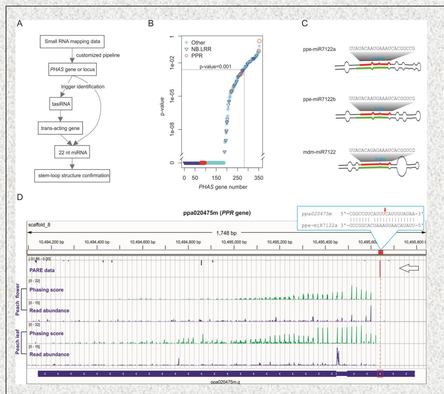


Figure 5. Identification of *PHAS PPR* genes and the trigger miR7122 in peach.**(A)** Workflow of identifying *PHAS* genes/loci and miRNA trigger. **(B)** p-valuedistribution of all putative *PHAS* genes in peach. The p-value threshold of 0.001 is indicated with a dotted line. **(C)** Stem-loop structures of the miR7122 homologues in peach (ppe-miR7122a and b) and apple (mdm-miR7122). Positions of miRNA and miRNA* sequences are marked In red and green, respectively; position of bulge in each stem-loop structure is marked with a light-blue circle. **(D)** Phasingscore and read abundance distribution along a *PHAS PPR* gene in peach flower and leaf tissues.

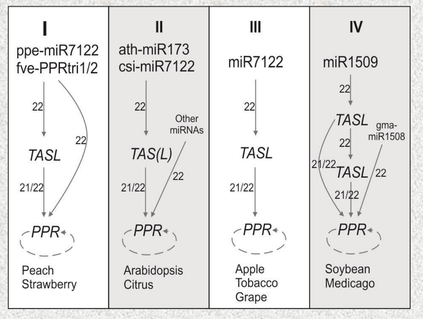
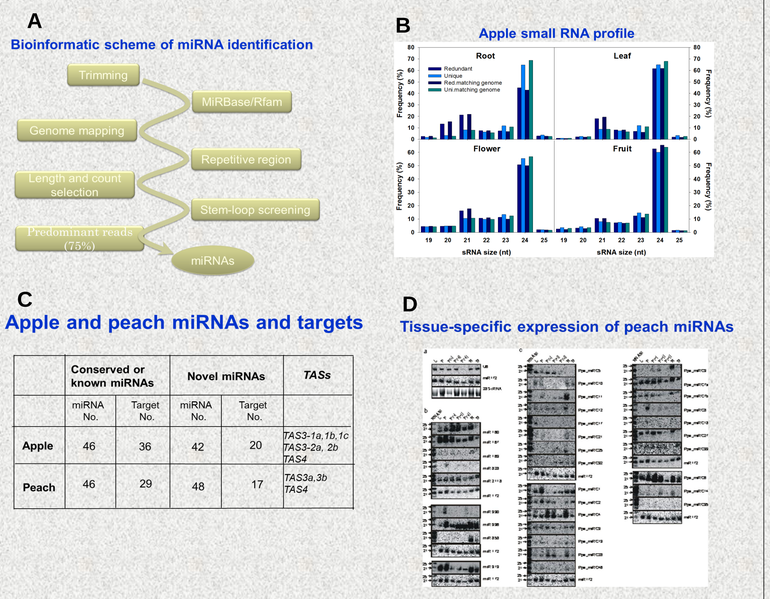


Figure 6. Diverse miR7122-*TAS(L)*-*PPR*-siRNA biogenesis and pathways in plants.



	Conserved or known miRNAs		Novel miRNAs		*TASs*
	miRNA No.	Target No.	miRNA No.	Target No.	
Apple	46	36	42	20	*TAS3-1a,1b,1c TAS3-2a, 2b TAS4*
Peach	46	29	48	17	*TAS3a,3b TAS4*

Figure 1. Characterization of miRNAs in apple and peach. A. Bioinformatic scheme of miRNA identification. B. sRNA profiles. C. The identified miRNAs and their targets D. Tissue-specific expression

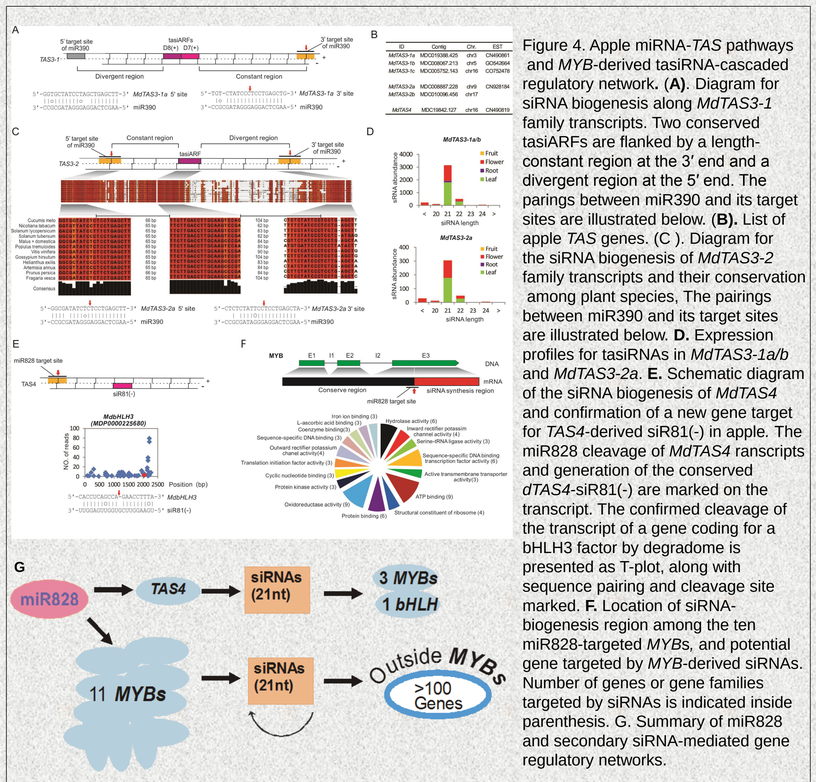


Figure 4. Apple miRNA-*TAS* pathways and *MYB*-derived tasiRNA-cascaded regulatory network**. (A)**. Diagram for siRNA biogenesis along *MdTAS3-1* family transcripts. Two conserved tasiARFs are flanked by a length-constant region at the 3′ end and a divergent region at the 5′ end. The parings between miR390 and its target sites are illustrated below. (**B).** List of apple *TAS* genes. (C). Diagram for the siRNA biogenesis of *MdTAS3-2* family transcripts and their conservation among plant species, The pairings between miR390 and its target sites are illustrated below. **D.** Expression profiles for tasiRNAs in *MdTAS3-1a/b* and *MdTAS3-2a*. **E.** Schematic diagram of the siRNA biogenesis of *MdTAS4* and confirmation of a new gene target for *TAS4*-derived siR81(-) in apple. The miR828 cleavage of *MdTAS4* ranscripts and generation of the conserved *dTAS4*-siR81(-) are marked on the transcript. The confirmed cleavage of the transcript of a gene coding for a bHLH3 factor by degradome is presented as T-plot, along with sequence pairing and cleavage site marked. **F.** Location of siRNA-biogenesis region among the ten miR828-targeted *MYB*s, and potential gene targeted by *MYB*-derived siRNAs. Number of genes or gene families targeted by siRNAs is indicated inside parenthesis. G. Summary of miR828 and secondary siRNA-mediated gene regulatory networks.

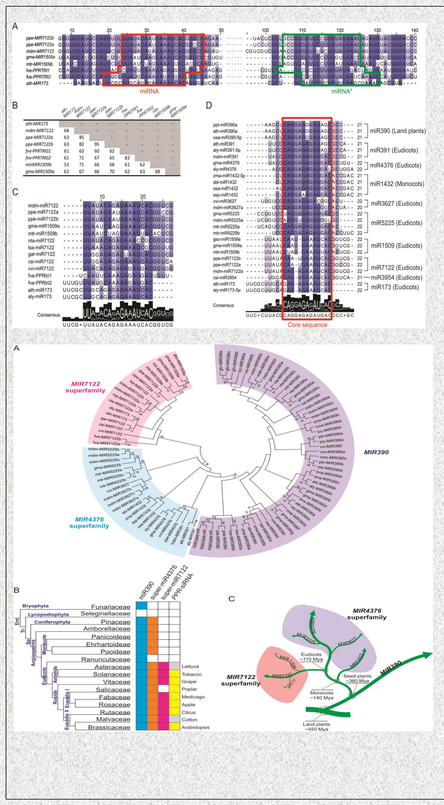


Figure 7. miRNA triggers share a common origin and show sequence similarity to many other miRNAs. **(A)** Conservation profile of foldback sequences of eight *MIRNA* genes with miRNA and miRNA* marked in red and green boxes, respectively. **(B)** Pairwise alignment scores (percentage) for each *MIRNA* gene.**(C)** Conservation profile of all the miR7122 omologues identified. Consensus sequence and conservation logo are included below. **(D)** Conservation profile of miR7122-related miRNAs. All the miRNA sequences were retrieved from miRbase (version 19). Position of the core equences is marked with a red box.

Figure 8. *Super-MIR7122*s are potentially evolved from *MIR390*s via *super-MIR4376*s.

Acknowledgements – Partial financial support provided by NIFA, USDA-ARS, Virginia Tech.

Dennis Bennett, Chris Dardick, Ann Callahan, Blake Meyers, Eric Beers, and Zhongchi Liu are thanked for their inputs or technical support.

Related papers:

Xia et al., (2012), Genome Biology 13:R47, doi:10.1186/gb-2012-13-6r47.

Xia et al., (2013) Plant Cell 25: 1555-1572.

Hong Zhu et al., (2012) BMC Plant Biology 12:149, doi:10.1186/1471-2229-12-149.