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Characterization of regulatory mechanism of *Poncirus trifoliata* microRNAs on their target genes with an integrated strategy of newly developed PPM-RACE and RLM-RACE

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ABSTRACT

MicroRNAs (miRNAs) play an important role in post-transcriptional gene regulation that involved various biological and metabolic processes. Many extensive studies have been done in model plant species, to discover miRNAs' regulating expression of their target genes and analyze their functions. But, the function of *Poncirus trifoliata* miRNAs has not been properly investigated. In this study, we employed the RNA ligase-mediated 5′ rapid amplification of cDNA ends (RLM-RACE) and the newly developed method called poly (A) polymerase-mediated 3′ rapid amplification of cDNA ends (PPM-RACE), which mapped the cleavage site of target mRNAs and detected expression patterns of cleaved fragments that could in turn indicate the regulatory functions of the miRNAs on their target genes. Furthermore, the spatiotemporal expression levels of target genes were analyzed by qRT-PCR, with exhibiting different expression trends from their corresponding miRNAs, thus indicating the cleavage mode of miRNAs on their target genes. The expression patterns of miRNAs, their target mRNAs and cleaved target mRNAs in different organs of juvenile and adult trifoliate orange were studied. The results showed that the expression of miRNAs and their target mRNAs was in a trade-off trend. When the miRNA expression was high, its corresponding target mRNA expression was low, while the cleaved target mRNA expression was high; when the miRNA expression was low, its target mRNA expression was high, while the expression of cleaved target mRNAs follows that of the miRNA. The validation of the cleavage site of target mRNAs and the detection of expression patterns of cleaved fragments can further broaden the knowledge of small RNA-mediated regulation in *P. trifoliate*.

1. Introduction

Micro-RNAs (miRNAs) are endogenous non-coding regulatory RNAs of 20–24 nt in length and are widely distributed in both plants and animals. They play important role in post-transcriptional gene regulation through the degradation of target mRNAs or repression of translation in targeted genes by base pairing with their target genes (Bartel, 2004). Different miRNAs can be found in different plant tissue types or developmental stages, indicating spatially and temporally regulated expression patterns of plant miRNAs (Wang et al., 2009; Wu et al., 2009). The miRNAs played an essential role in regulating diverse plant development processes by targeting mRNAs for translational repression or cleavage (Bartel, 2004; Bartel and Chen, 2004; Miranda et al., 2006; X. Sun et al., 2012; L.M. Sun et al., 2012; Wang et al., 2013), as well as juvenile to adult transition period, root cap development, flower development, and flowering time control (Aukerman and Sakai, 2003; Chen, 2004; Chuck et al., 2007; Mallory et al., 2004; Millar and Gubler, 2005; Wang et al., 2005; Wu and Poethig, 2006; Xie et al., 2006). Computational analysis based on sequence similarity has definitely been proven to be a reliable and successful means of identifying miRNA target genes in plants, since the number of mismatches allowed between the miRNAs and their targets is low (Mallory and Vaucheret, 2004; Schwab et al., 2005). The weakness of this approach may not easily distinguish between false predictions and real targets, substantial effort and time is spent trying to validate false predictions. The miRNAs and their target sites are evolutionarily conserved across genomes (Bonnet et al., 2004), an approach to use the conservation of target complementarity across genomes to reduce false-positives in plant miRNA target

Abbreviations: cDNA, complementary DNA; miRNA, microRNA; NCBI, National Center for Biotechnology Information; PAP, poly (A) polymerase; PCR, polymerase chain reaction; PPM-RACE and RLM-RACE, poly (A) polymerase-mediated 3′ RACE and RNA ligase-mediated 5′ RACE; qRT-PCR, quantitative RT-PCR; RACE, rapid amplification of cDNA ends; RLM-5′ RACE, RNA ligase-mediated 5′ rapid amplification of cDNA ends; RNA, ribonucleic acid; RT-PCR, reverse transcription polymerase chain reaction.

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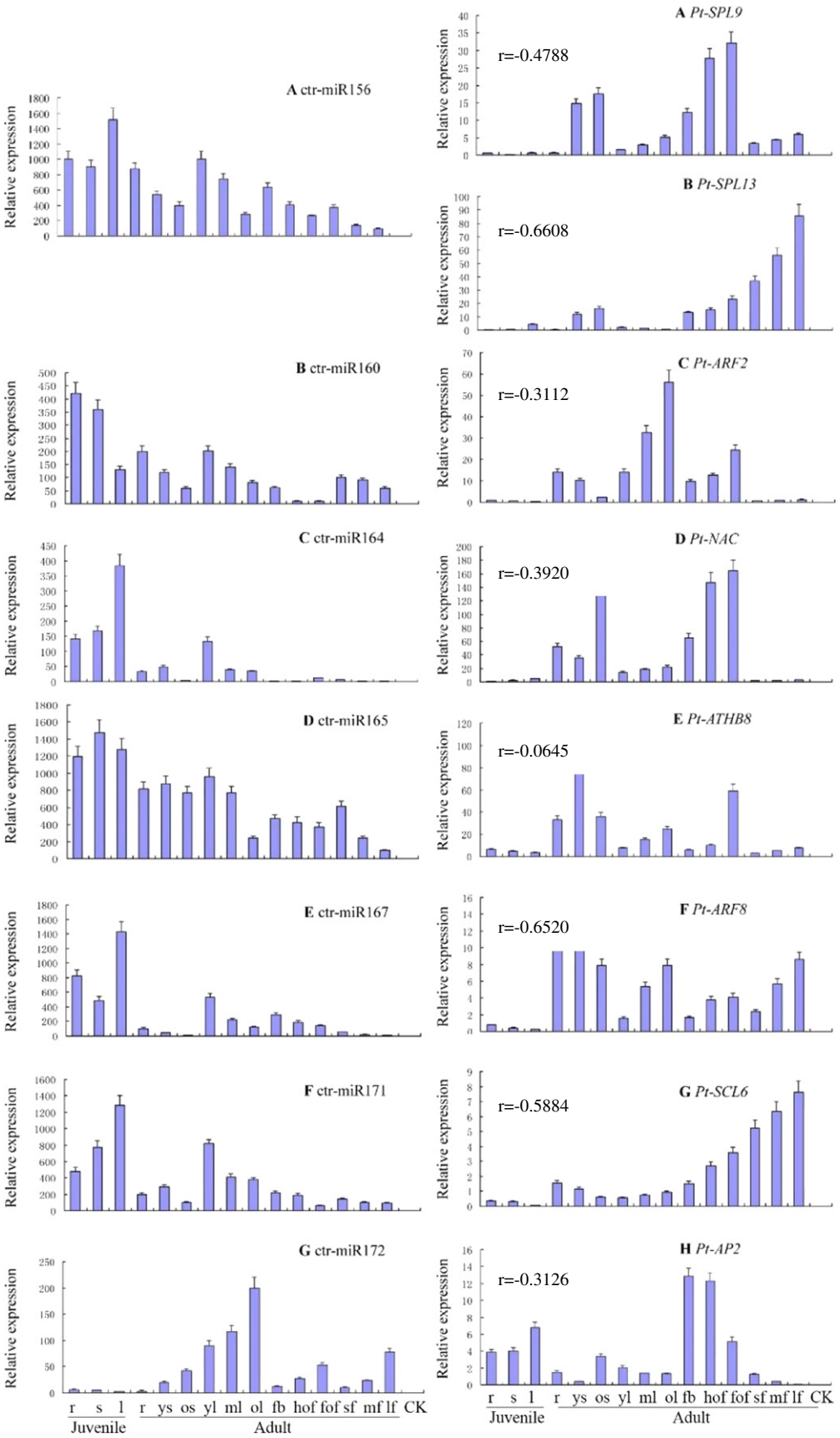

Fig. 1. Expression patterns of miRNA predicted from *P. trifoliata* and their target mRNA by qRT-PCR. The left side was the expression patterns of miRNAs and the right side was the expression patterns of their target mRNA. s, t and l are samples of roots, stems and leaves respectively, from juvenile phase trifoliate orange; r, ys, os, yl, ml, ol, fb, hof, fof, sf, mf, lf and CK are the adult trifoliate roots, young stems, old stems, young leaves, mature leaves, old leaves, flower buds, half open flowers, full open flowers, small fruits (0.50 cm in diameter), middle fruits (1.50 cm in diameter), large fruits (2.50 cm in diameter), and control for water, respectively; "r" denoted the correlation coefficient. Each reaction was repeated three times and the standard error plotted.