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Small RNAs in tomato fruit and leaf development

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Abstract

Tomato fruit and leaf development offers excellent systems to study the evolution of gene regulation underlying development of different organs. We have identified over 350 and 700 small RNAs from tomato fruit and leaf, respectively. Except for conserved microRNAs, more than 90% of the small RNAs are unique to tomato. We confirmed expression of some conserved as well as novel putative microRNAs by Northern hybridization. Our results help form a basis for comparative studies on how small RNA-mediated gene expression has contributed to the evolution of common and distinct developmental pathways of fruits and leaves. We have established a website (http://ted.bti.cornell.edu/digital/sRNA/) for public access to all of our small RNA sequences, their expression patterns in respective tissues, and their matching genes or predicted target genes in a searchable manner.

Keywords: Tomato; Small RNA; miRNA; siRNA; Fruit development; Leaf development

1. Introduction

Understanding the mechanisms regulating fruit development has practical and basic importance. Tomato is an excellent model to study fleshy fruit development because of the rich genetic and molecular toolkits available and its economic importance [1]. Its development undergoes several stages [2,3]: i) floral development and fruit set, ii) cell division after anthesis and fertilization, iii) cell expansion, and iv) fruit ripening. The substantial changes in cellular and biochemical events during tomato fruit development allow integrative analyses of many aspects of plant biology. These include hormone biosynthesis and function, cell division control, cell-to-cell communication, cell wall dynamics, and various metabolic pathways [2–5].

Plant hormones [4] and metabolites [5] play important roles in tomato fruit development. Several genes that determine fruit characteristics have been cloned [1]. Transcript expression profiles [6–11] as well as proteome [12,13] are available. Despite these advances, how gene expression is regulated for the development of a fruit remains poorly understood. One aspect of fruit development, which bears great significance in the evolution of plant forms, is the ontogenic relationship between fruits and leaves. Such a relationship is supported by structural similarity, the expression of certain genes in both fruits and leaves, and genetic evidence that some MADS-box genes affect the development of both leaves and fruits [2]. Comparative analyses of gene regulation underlying fruit and leaf development should help further understand the evolution of plant organs.

It is possible that RNA silencing mediated by the 20–24 nt small RNAs such as microRNAs (miRNAs) and small

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interfering RNAs (siRNAs) regulates common and distinct developmental processes in the tomato fruit and leaf, in light of their demonstrated roles in many aspects of plant developmental processes based on studies with model systems such as *Arabidopsis*, maize and rice [14–18]. Small RNAs are produced as duplexes (e.g., miRNA:miRNA* duplex) from precursor RNAs via cleavage by dicer (in animals) or dicer-like (DCL; in plants) RNases. One strand of the duplex is loaded into RISC (RNA-induced silencing complex) to mediate sequence-specific mRNA cleavage or translation inhibition, whereas the other strand (e.g., miRNA*) is degraded [19,20]. Furthermore, small RNAs can mediate chromatin modification to regulate gene expression at the transcriptional level [21,22].

Indeed, miRNAs were identified in tomato by computation [23] and detected in tomato seeds [24,25] and leaves [26] by Northern hybridization, miRNAs and other small RNAs were identified in mature green tomato fruit by sequencing [27], and miR319-regulated expression of *LANCEOLATE* is necessary for compound leaf development in tomato [28]. Here we report our analyses of small RNA profiles in tomato leaf as well as fruit at various developmental stages.

2. Materials and methods

2.1. Cloning and sequencing of tomato small RNAs

The procedure was essentially the same as previously described [29]. Briefly, total RNA was isolated from tomato (*Solanum lycopersicum* cv. Rutgers) leaf and fruit samples. Small RNAs were enriched by polyethylene glycol differential precipitation. Small RNAs of 15–30 nt were gel-isolated from 15% PAGE, ligated to 3′ and 5′ adapters, reverse transcribed and amplified by polymerase chain reaction (PCR). The PCR products were cloned into the TOPO TA vector (Invitrogen, Carlsbad, California) and sequenced at the Plant-Microbe Genomics Facility, Ohio State University and at the Samuel R. Noble Foundation Genomics Facility.

2.2. Small RNA analyses

Adaptor sequences were removed to obtain small RNA sequences and the resulting sequences were filtered for lengths between 15 and 30 nt as described [29]. The small RNA sequences were BLAST searched to identify matching sequences in the following databases: the NBCI Genbank (http://www.ncbi.nlm.nih.gov/Genbank/index.html), the SGN tomato genome and Unigene databases (http://sgn.cornell.edu/), the Arabidopsis small RNA database (http://asrp.cgrb.oregonstate.edu/), the MicroRNA (release 9.2) and MicroRNA hairpin databases (http://microrna.sanger.ac.uk/sequences/), TAIR Arabidopsis genome database (http://www.arabidopsis.org/), TIGR Rice genome database (http://www.tigr.org/tdb/e2k1/osa/), and JGI Popular genome database (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html). Target gene prediction was performed based on the parameters previously established [30].

2.3. Identification of target gene cleavage

RNA ligase-mediated 5′ RACE (Rapid Amplification of cDNA Ends) was performed on poly-adenylated RNA of respective samples as previously described [31], except that a nested PCR reaction was performed. The sequences of reverse primers used for RT-PCR and nested PCR were as follows. For AP2, the RT primer, TCAAGAAGGTCTCATGAGTGAATG and the nested primer, ATGGAAACCATTTTCTGAGGAC. For AGO1, the RT primer, ACAAGGCCACTGGGTATGCTGAAT and the nested primer, AACAAACCCATAAGTTTCTCG. For ARF8, the RT primer, GGCCCTGTTTGCCCATCTGCTG and the nested primer, GATACTCTCTCCACTTGAAGTG.

2.4. Prediction of precursor genes

The small RNAs were mapped to corresponding regions in SGN BAC, BAC-end and Unigene sequences (http://sgn.cornell.edu/) by BLASTN algorithm. The sequences that matched at least 90% of a given small RNA (with up to one mismatch) were extracted with flanking sequences (110 nt on both sides) and further screened by RNA fold prediction program for their potential hairpin structure(s) [32]. Genes

A

Sequence	Length	Number of reads			
		Leaf	Flower bud	Fruit at 10 DPA	Mature fruit
TGCCTGGCTCCCTGTATGCCA	21	1	0	0	0

B

databases	Number of hits (≤3 mismatch)
miRNA database (release 9.2)	33
miRNA hairpin database	34
Arabidopsis small RNA database	15
GenBank nucleotide database (nt)	150
Tomato finished BAC database (v95)	No hits found
SGN Tomato unigene database	2
Arabidopsis genome - TAIR	4
Rice genome - TIGR	10
Poplar genome - JGI	17

C

mRNA	Alignment	Score	Mismatch	Wobble	Indel	mRNA Direction
SGN-U332548	mRNA (415 – 435) mRNA 5' AGGCAUACAGGGAGCCAGGCA 3' sRNA 3' ACCGUAUGUCCCUCGGUCCGU 5'	1	1	0	0	+
SGN-U324618	mRNA (598 – 618) mRNA 5' GGGCAUGCAGGGAGCCAGGCA 3' sRNA 3' ACCGUAUGUCCCUCGGUCCGU 5'	1.5	1	1	0	+

Fig. 1. Search functions in the tomato small RNA database. (A) and (B) The result page from a query for small RNA SlsmR-301. The page displays the sequences, the abundance in each tissue (A) and the hits from different databases (B) of SlsmR-301. (C) Tomato target genes returned by search using the sequence of SlsmR-301. The score is described in Jones-Rhoades and Bartel [30]. Indel, insertion/deletion.