Genomics 102 (2013) 472–478

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Identification and characterization of microRNAs in the developing maize endosperm

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ARTICLE INFO

Article history:
Received 12 October 2012
Accepted 22 August 2013
Available online 7 September 2013

Keywords:
Maize
Endosperm
miRNA
Identification
Sequencing

ABSTRACT

MicroRNAs (miRNAs) are non-coding RNAs that are approximately 20–22 nucleotides long. miRNAs have been shown to be important regulators that control a large variety of biological functions in eukaryotic cells. To investigate the roles of miRNAs in maize endosperm development, two small RNA libraries of maize endosperm at two developmental stages were sequenced. A total of 17,773,394 and 18,586,523 small RNA raw reads were obtained, respectively. Further analysis identified and characterized 95 known miRNAs belonging to 20 miRNA families. In addition, 18 novel miRNAs were identified and grouped into 11 families. Potential targets for 5 of the novel miRNA families were successfully predicted. We had also identified 12 corresponding miRNAs* of these novel miRNAs. In summary, we investigated expression patterns of miRNA in maize endosperm at key developmental stages and identified miRNAs that are likely to playing an important role in endosperm development.

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1. Introduction

MicroRNAs (miRNAs) are important regulators that control various types of biological functions in eukaryotic cells, including development, viral defense, metabolism and apoptosis [1]. In both animals and plants, miRNAs are transcribed by RNA polymerase II into primary transcripts (pri-miRNAs) [1,2]. In animals, the pri-miRNA is processed to the miRNA precursor (pre-miRNA) by an RNase III-like enzyme called Drosha [3] and then the pre-miRNA is processed by Dicer to produce the mature miRNAs [4,5]. In plants, there are no Drosha homologs. The pri-miRNA is processed by a Dicer-like protein, DCL1, to the miRNA/miRNA* duplex [6–8], which is then methylated by HEN1 [7,9–11]. HASTY, the plant homolog of exportin 5, exports the miRNA/miRNA* duplex to the cytoplasm [12]. Then, the miRNA strand is incorporated into the RNA-induced silencing complex (RISC), which guides the sequence-specific post-transcriptional repression of the target mRNA by degradation or translational repression [1,13,14]. So far researchers have developed a variety of computational approaches to predict targets and identify known/novel miRNAs. The secondary structure, the Dicer cleavage site and the minimum free energy of the unannotated small RNA tags which could be mapped to genome were mainly used to predict novel miRNA. Parameters of mismatches between miRNA & target, mismatch position, and minimum free energy (MFE) of the miRNA/target duplex were mainly used to predict target genes.

Changing miRNA expression levels and disruption of the complementarity between the miRNA and its target mRNA can result in developmental defects or phenotypic changes [15,16]. Furthermore, the targets of several miRNAs have been shown to play important roles in many regulatory pathways containing endosperm development. For example, miR164 negatively regulates ORE1, a NAC (NAM, ATAF, and CUC) transcription factor that positively regulates aging-induced cell death in Arabidopsis leaves [17]; miR156 targets SPLs that play critical roles in cell size [18], fertility [19], and embryonic development [20]; miR396 attenuates cell proliferation in developing leaves, through the repression of the transcription factors for the growth-regulating factor (GRF) [21]; and miRNA159 mediates cleavage of the *MYB101* and *MYB33* transcripts that function as positive regulators of the ABA response to desensitize hormone signaling [22]. Many ABA signal transduction proteins are involved in seed development and germination [23].

A genome-wide analysis and characterization of the microRNAs in maize has helped accelerate our understanding of the regulatory roles of miRNAs in critical biological processes [24]. Maize endosperm development involves a range of biological processes many of which may be regulated, at least in part, by miRNAs. We sequenced two small RNA (sRNA) libraries from developing maize endosperm using high-throughput sequencing technology to investigate expression difference of know miRNAs in the two libraries and to identify

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http://dx.doi.org/10.1016/j.ygeno.2013.08.007

novel miRNAs in maize endosperm development. From these data sets, we characterized the expression levels of 95 known miRNA in the two sRNA libraries and identified 11 novel miRNA families. These data sets will provide insights into the known miRNA involved in endosperm development and significantly enrich the repertoire of maize miRNAs.

2. Results and discussion

2.1. The developing maize endosperm has a complex small RNA population

To study the role of miRNA during maize endosperm development, we sequenced sRNAs from two sets of endosperm tissues, one collected at 4–6 DAP and the second collected from 7 to 23 DAP (see Material and methods), to determine the expression levels of the known miRNAs and to identify novel miRNAs. A total of 17,773,394 and 18,586,523 small RNA raw reads were obtained from the 4–6 DAP and 7–23 DAP tissues, respectively. After removing the low quality sequences and adapter sequences, 16,672,051 and 17,481,949 clean reads from 18 to 30 nucleotides (nt) long including 7,244,120 and 5,104,699 unique sequences were obtained (Table 1 and Supplementary Table 1). In the 4–6 DAP and 7–23 DAP tissues, 93.46% and 89.62% of the sRNAs, respectively, were between 20 and 24 nt in length (Fig. 1); the majority of which were between 21 and 24 nt which may be related to by-products of cleavage by Dicer-like enzymes [26]. The most abundant sRNAs were 24 nt long and accounted for 62.47% and 40.76% of the total reads in the 4–6 DAP and 7–23 DAP tissues, respectively (Fig. 1). This result is consistent with recent studies in *Zea mays* [27–29], *Oryza sativa* [30], *Phaseolus vulgaris* [31], and *Arabidopsis thaliana* [32]. The next most abundant sRNAs were the 21, 22, 23 and 20 nt long sequences in that order (Fig. 1). All the clean reads were mapped to the maize genome (B73 RefGen_v2, release 5a.57). The results indicated that 11,942,344 (71.63%) and 13,734,753 (78.57%) reads from the 4–6 DAP and 7–23 DAP tissues, respectively, were perfectly matched to the maize genome. Small RNAs (sRNA) from Solexa deep sequencing cover almost every kind of RNA, including miRNA, siRNA, piRNA, rRNA, tRNA, snRNA, snoRNA and so on. Around 3.73% and 5.59% of the unique reads in the 4–6 DAP and 7–23 DAP tissues, respectively, matched other non-coding RNAs that made up 11.44% and 23.44% of the total number of clean reads (Table 1).

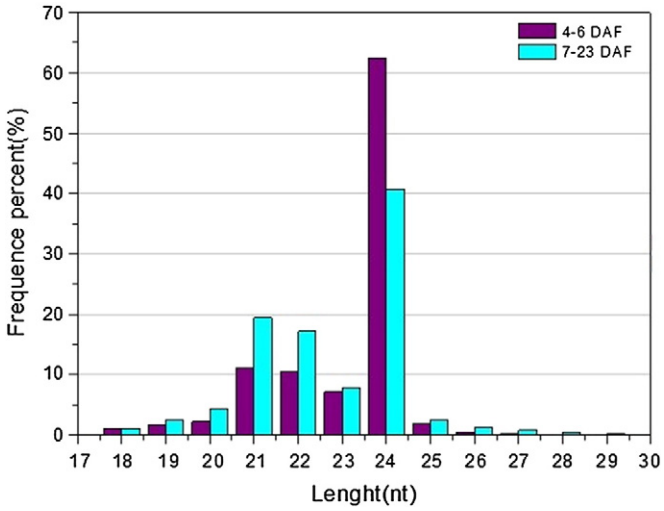

Fig. 1. Total reads of 18–30 nt small RNAs. The most abundant sRNAs were 24 nt long and accounted for 62.47% and 40.76% of the total reads in the 4–6 DAP and 7–23 DAP tissues, respectively.

2.2. Identification of known maize miRNAs

The 172 maize miRNAs registered at miRBase Release 20 can be grouped into 28 miRNA families which were defined by grouping miRNAs that share a common conserved seed region and have different precursors in different genome positions. BLASTN searches against the known maize mature miRNAs and their precursors identified 95 known miRNAs belonging to 20 families in our data sets (Supplementary Table 2). We found that the expression levels between different miRNA family members varied drastically in the 4–6 DAP and 7–23 DAP data sets, suggesting that there was functional divergence within the families at the stage of endosperm development. For example, there were 125,858 clean reads for miR156d and only 164 clean reads for miR156k in the 7–23 DAP endosperm data set (Supplementary Table 2). Eight of the known miRNA families, miR162, miR395, miR399, miR482, miR529, miR1432, miR2118 and miR2275 were not detected in our data sets, suggesting that these miRNAs may be tissue-specific. In our sequencing data, miR398a/b were detected only in the 7–23 DAP endosperm. We found that a few miRNA* reads were more abundant. For example, in the 7–23 DAP data set, there were 81 miR396a/b reads and 657 miR396a*/b* reads, and 36 miR408b reads and 100 miR408b* reads. In the 4–6 DAP data set, there were 13 miR396a/b reads and 57 miR396a*/b* reads, and one miR408b read and six miR408b* reads (Fig. 2). This result is consistent with a recent study in maize [29]. It is possible that these miRNAs* can either down-regulate the expression of its target gene consistent with miRNA in function or be actually a real miRNA. The two miRNAs* may play an important role in endosperm development.

Table 1
Summary of small RNA sequencing.

	4–6		7–23	
	Unique reads	Total reads	Unique reads	Total reads
Non-coding RNAs				
rRNA	57,231 (0.79%)	577,283 (3.46%)	105,712 (2.07%)	1,787,673 (10.23%)
tRNA	10,605 (0.15%)	354,300 (2.13%)	20,373 (0.4%)	1,182,563 (6.76%)
siRNA	196,790 (2.72%)	942,099 (5.65%)	155,553 (3.05%)	1,115,097 (6.38%)
snRNA	3679 (0.05%)	25,189 (0.15%)	2121 (0.04%)	6390 (0.04%)
snoRNA	1456 (0.02%)	8211 (0.05%)	1316 (0.03%)	5201 (0.03%)
Protein-coding RNAs				
Exon	336,054 (4.63%)	943,772 (5.66%)	269,746 (5.28%)	852,225 (4.87%)
Intron	835,842 (11.54%)	2,234,447 (13.40%)	584,988 (11.46%)	2,438,814 (13.95%)
Known miRNAs				
miRNA	599 (0.01%)	1,005,693 (6.03%)	610 (0.01%)	1,651,662 (9.45%)
Other sRNAs	5,801,864 (80.09%)	10,581,057 (63.47%)	3,964,280 (77.66%)	8,442,324 (48.29%)
Total	7,244,120 (100%)	16,672,051 (100%)	5,104,699 (100%)	17,481,949 (100%)

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