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Flexible microRNA arm selection in rice

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ABSTRACT

MicroRNAs act at the post-transcriptional level and guide Argonaute proteins to cleave their corresponding target transcripts. However, little attention has been paid to arm selection in miRNA precursors. In this study, small RNA high-throughput sequencing data from 29 different rice libraries were pooled to investigate tissue- and abiotic stress-specific dynamic expression of miRNAs. We found that more than half of pre-miRNAs showed changes in arm selection in different tissues and/or under different abiotic stresses. Our findings suggest that miRNA selection is remarkably prevalent in plants, providing new insights into the role of miRNAs in plant growth and development.

1. Introduction

In the last two decades, the crucial function of microRNAs (miRNAs) in plants and animals has been recognized [1–3]. Through a stepwise maturation process, miRNA/miRNA* duplexes are released by the RNaseIII endonuclease DICER-LIKE1 into the nucleus and are subsequently exported out of the nucleus [4]. Despite equal amounts of the two strands being produced by transcription, their accumulation tends to be asymmetric. The dominant strand, commonly called miRNA, is incorporated into the miRNA-induced silencing complex (miRISC) to execute its function, whereas the other strand, commonly called miRNA* or the passenger strand, is degraded [5,6]. Previous studies proposed that strand selection is mainly based on differences in the free energy of the 5′ end, with the less stable strand selectively incorporated into the miRISC as a mature miRNA [5–7]. Nevertheless, some miRNA* species have also been detected by deep sequencing of plant and animal transcriptomes [8–11]. Furthermore, some miRNA*s are also incorporated into the miRISC and participate in modulating cell activities [12–15].

A plethora of studies have focused on identifying miRNAs and elucidating their biogenesis processes and modes of action [4]. Arm selection has been recently characterized in human tumor and cancer tissues [11,16,17]. Nevertheless, only limited libraries (commonly two libraries) were adopted in the analyses. In plants, however, no studies have been reported with regard to miRNA arm selection. In this study, we systematically analyzed differences in the ratios of miRNA and miRNA* in 29 rice (*Oryza sativa*) deep-sequencing libraries derived from diverse tissues and from plants grown under different abiotic stresses, and found that over half of the expressed precursor miRNAs (pre-miRNAs) showed dynamic arm selection.

2. Methods

2.1. Small RNA (sRNA) libraries

All sRNA libraries were downloaded from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) [18]. The data set was compiled from previously published libraries and included 4 developmental stages and 25 abiotic stresses (Table S1) [19]. Sequences of pre-miRNAs and mature miRNAs were retrieved from miRBase (Release 19, http://www.mirbase.org/) [20].

2.2. 5p and 3p mapping strategy

To compare miRNA expression across tissues, reads were normalized based on the reads per million (RPM) in the library. To obtain higher-confidence results, read counts $\geqslant 2$ were retained and mapped against pre-miRNAs, with no mismatches allowed. Matched reads were further filtered according to whether they

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overlapped with extant mature miRNAs annotated in miRBase. For unannotated potential miRNAs that originated from either miRNA-5p or -3p, reads were calculated as the total number of times that the sequence was partially the reverse complement of the corresponding miRNA annotated in miRBase. Briefly, miRNA-5p and -3p resulting from the same pre-miRNA should share the majority of their sequence with differences only at the 5′ and 3′ overhanging ends.

2.3. Arm switch criteria

The formula $\omega = 5p/(5p + 3p)$ was adopted as a measure of the arm selection, where $0 \leqslant \omega \leqslant 0.1$ indicates 3p dominant expression (3pDE), $0.1 < \omega < 0.9$ indicates co-expression of 5p and 3p (5p3pCE), and $0.9 \leqslant \omega \leqslant 1$ indicates 5p dominant expression (5pDE). Accordingly, when expression of an miRNA shifted from 5pDE to 3pDE or 5p3pCE, or from 3pDE to 5pDE or 5p3pCE or vice versa in at least two libraries, it was considered an arm switch. All basic statistic functions were performed in R.

3. Results

3.1. The abundance of miRNA-5p and/or miRNA-3p

A total of 29 publicly available small RNA (sRNA) libraries (4 from different tissues, 25 from different abiotic stresses) generated by next-generation sequencing of rice were used to investigate the abundance of miRNA-5p and/or miRNA-3p species (Table S1). Quite a few pre-miRNAs (214) were removed from further analysis because they were not covered by any miRNAs. We found that 136 pre-miRNAs specifically expressed only miRNA-5p and 28 specifically expressed only miRNA-3p in one or more library. In other words, no corresponding miRNA*s were detected for these miRNAs in the given libraries (Fig. 1A). Nevertheless, most of expressed pre-

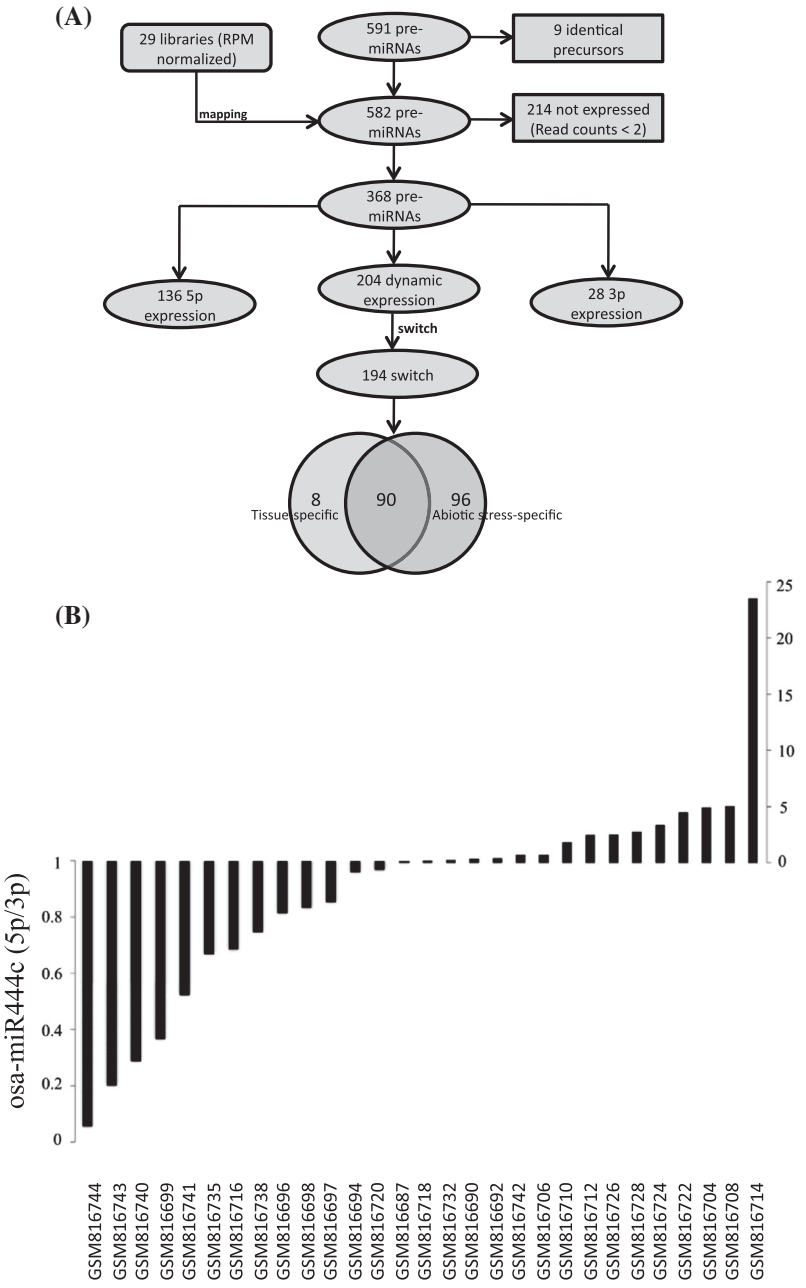

Fig. 1. Identification of flexible miRNA expression in rice. (A) Flowchart of the characterization of expression of miRNAs derived from 591 rice pre-miRNAs. (B) Dynamic arm expression of osa-miR444c in different libraries.