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Flexible microRNA arm selection in rice

Wangxiong Hu [a,b,c,1], Tingzhang Wang [b,1], Erkui Yue [b], Shu Zheng [c,d,*], Jian-Hong Xu [b,*]

[a] College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China
[b] Institute of Crop Science, Zhejiang Key Laboratory of Crop Germplasm, Zhejiang University, Hangzhou, Zhejiang 310058, China
[c] Zhejiang-California International Nanosystems Institute, Zhejiang University, Hangzhou, Zhejiang 310058, China
[d] Cancer Institute, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310009, China

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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

* Corresponding authors. Addresses: Cancer Institute, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310009, China (S. Zheng), Institute of Crop Science, Zhejiang Key Laboratory of Crop Germplasm, Zhejiang University, Hangzhou, Zhejiang 310058, China, Fax: +86 571 88982406 (J.-H. Xu).
E-mail addresses: zhengshu@zju.edu.cn (S. Zheng), jhxu@zju.edu.cn (J.-H. Xu).
[1] These authors contributed equally to this work.

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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-

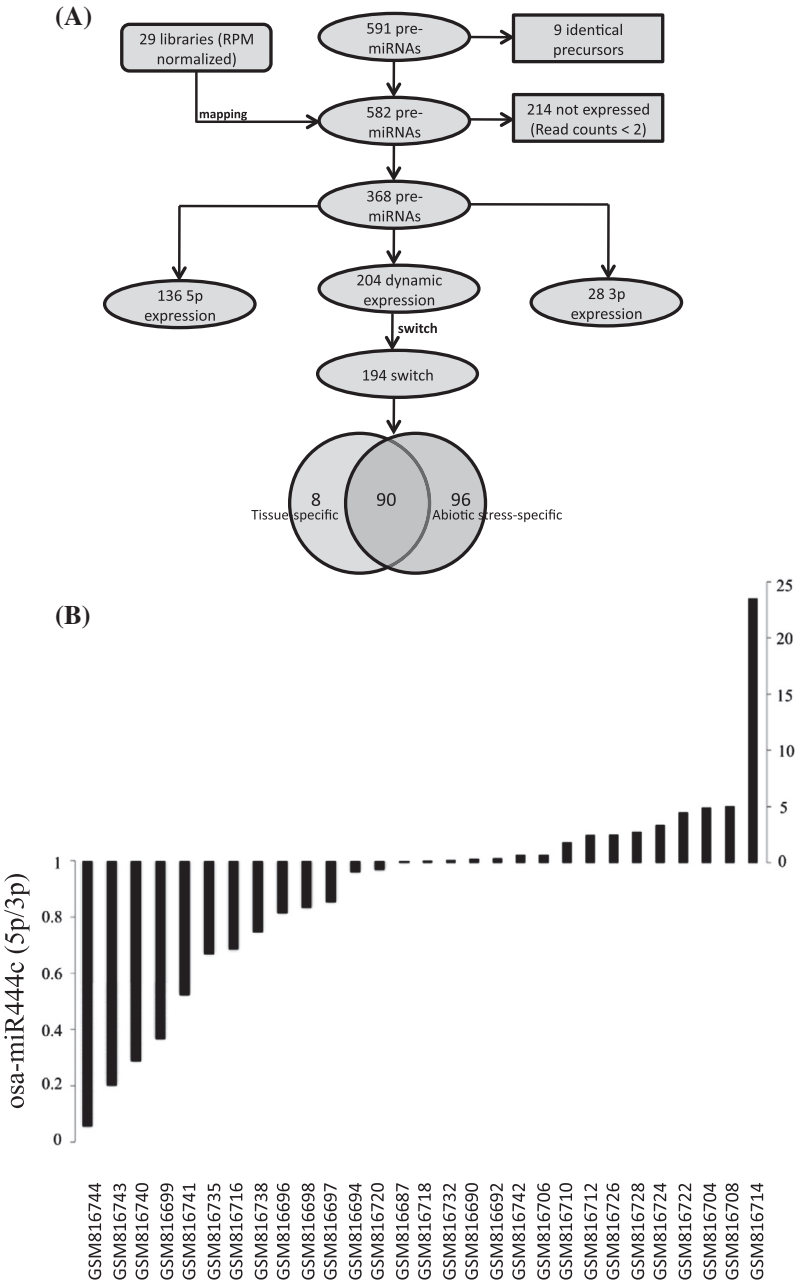

Fig. 1. Identification of flexible miRNA expression in rice. (A) Flowchart of the characterization of expression of miRNAs derived from 591 rice pre-miRNAs. (B) Dynamic arm expression of osa-miR444c in different libraries.

miRNAs (204) possessed mature miRNAs expressed from both arms in at least two of the 29 selected libraries (Fig. 1A and B). Further analysis showed that 194 pre-miRNAs possessed arm switch. All dynamically expressed pre-miRNAs were then further divided into tissue-specific and abiotic stress-specific for arm switching investigation.

3.2. Tissue-specific miRNA arm selection

Among the 194 dynamically expressed pre-miRNAs, 8 exclusively showed differential arm expression in different tissues, and 90 showed both tissue and abiotic stress-related differential arm selection (Figs. 1A, 2A and D). The average frequency of arm switching in all tissues was 19.33% (Fig. 2C). In particular, the highest frequency of arm switching was observed between panicle and seedling (28%), and the lowest frequency of arm switching was observed between shoot and root (15%, Fig. 2C).

For example, flexibility in strand selection was observed for miR396c, miR166e, and miR1423b. Although only miR396c-3p was expressed in shoots, this arm preference disappeared in other tissues and comparable expression of the two arms was detected in seedling and panicles (Fig. 2A and B). More varied tissue expression patterns were seen for some other pre-miRNAs. For example, for pre-miR1423b, miR1423b-5p was more highly expressed than miR1423b-3p in root, but comparable expression of the two arms was detected in seedling (≈60 RPM) and panicles (≈12 RPM), and miR1423b-3p was the dominantly expressed arm in shoot (30.28 RPM compared with 14.42 RPM for miR1423b-5p). In the case of pre-miR166e, miR166e-5p was preferentially expressed in seedlings ($\omega > 0.9$) and roots ($\omega = 1$), whereas miR166e-3p was the dominant strand in panicle. These results suggested that arm selection differed considerably among tissues, and this flexibility in arm selection is probably associated with plant plasticity during development.

3.3. Coordination of miRNA-5p/miRNA-3p under abiotic stress

Many miRNAs induced by diverse abiotic and biotic stresses have been identified in plants. Here we explored arm selection in 25 sRNA libraries from rice exposed to different abiotic stresses. Of the 194 pre-miRNAs showing arm switch, 96 pre-miRNAs showing arm switching were specifically induced by various abiotic stresses. For example, miR1320-3p was detected under sulfate and potassium deficiency in roots and shoots but not under phosphate and nitrogen deficiency. In contrast, miR1320-5p was preferentially expressed under phosphate and nitrogen deficiency as well as under other stresses like drought, high salt, and cold in seedlings. Pre-miR1425 displayed particularly dynamic changes in arm selection under abiotic stresses; miR1425-5p was preferentially expressed under all stresses in seedlings (highest at 234.96 RPM), whereas expression switched to miR1425-3p under phosphate (43.02 RPM, $\omega \approx 0.2$) and potassium (51.63 RPM, $\omega \approx 0.2$) deficiency in shoots. Furthermore, the preference for miR1425-3p expression was even more dramatic in panicles exposed to high salt (124.66 RPM, $\omega < 0.1$) or combined heat and drought (18.71 RPM, $\omega = 0$; Fig. 3A and B). As another example, arm switching was also apparent for pre-miR160a. The expression of miR160a-3p was much higher than that of miR160a-5p in roots, except under potassium deficiency and combined potassium deficiency and high salt. Nevertheless, in panicles, miR160a-5p was preferentially selected under drought, heat, and combined heat and drought, whereas there appeared to be no arm preference in

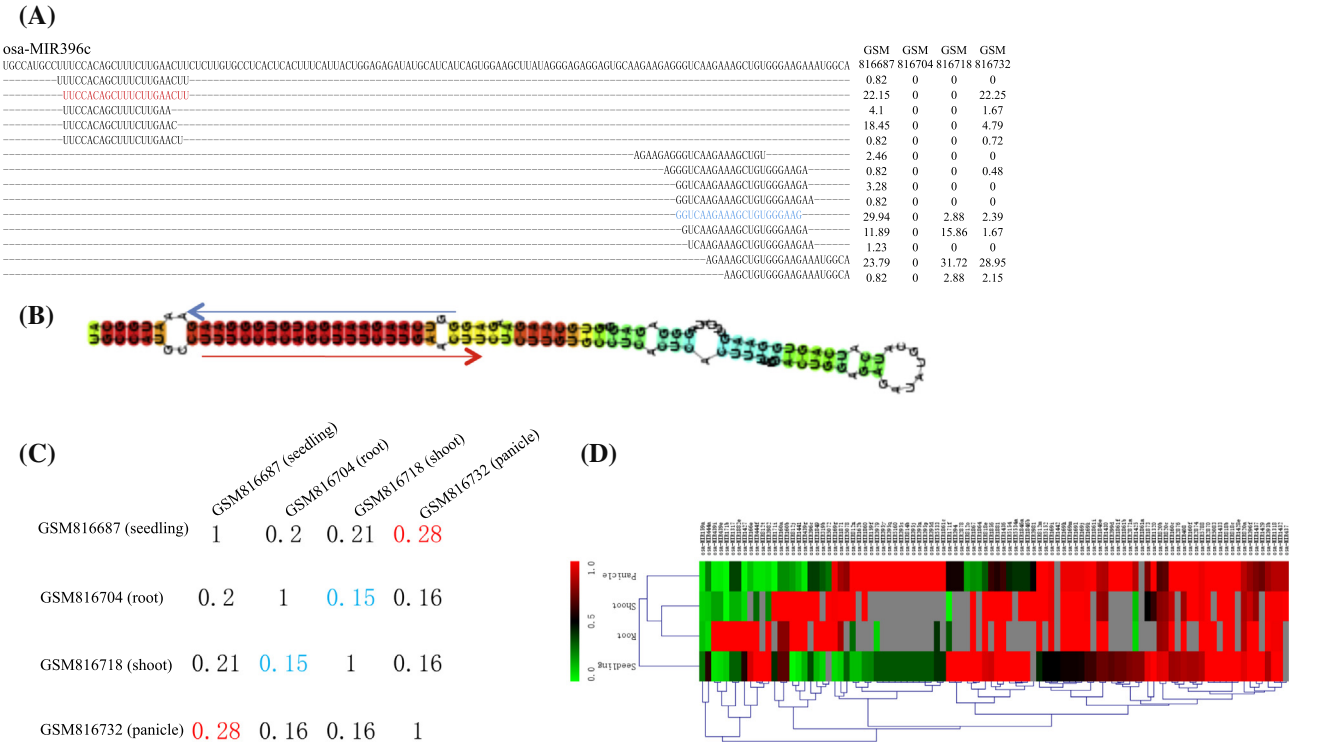

Fig. 2. Identification and characterization of miRNA arm switching in different tissues. (A) Identification of miR396c-5p and miR396c-3p in libraries from 4 different tissues. Values below each library are in RPM. Perfectly matched short reads highlighted in red (miRNA-5p) and blue (miRNA-3p) indicate that both strands are annotated in miRBase. (B) Putative secondary structure of pre-miR396c. The red and blue arrows indicate reads originating from miRNA-5p and -3p, respectively. (C) Pairwise comparison of the frequency of arm switching in different tissues. Numbers shaded in red and blue corresponding to the high and low frequency of arm switching. (D) Heatmap of osa-miRNA arm switching observed in at least two libraries from the 4 tissues. Red indicates miRNA-5p dominant expression, green indicates miRNA-3p dominant expression, black indicates co-expression of both arms, and gray indicates unavailable data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

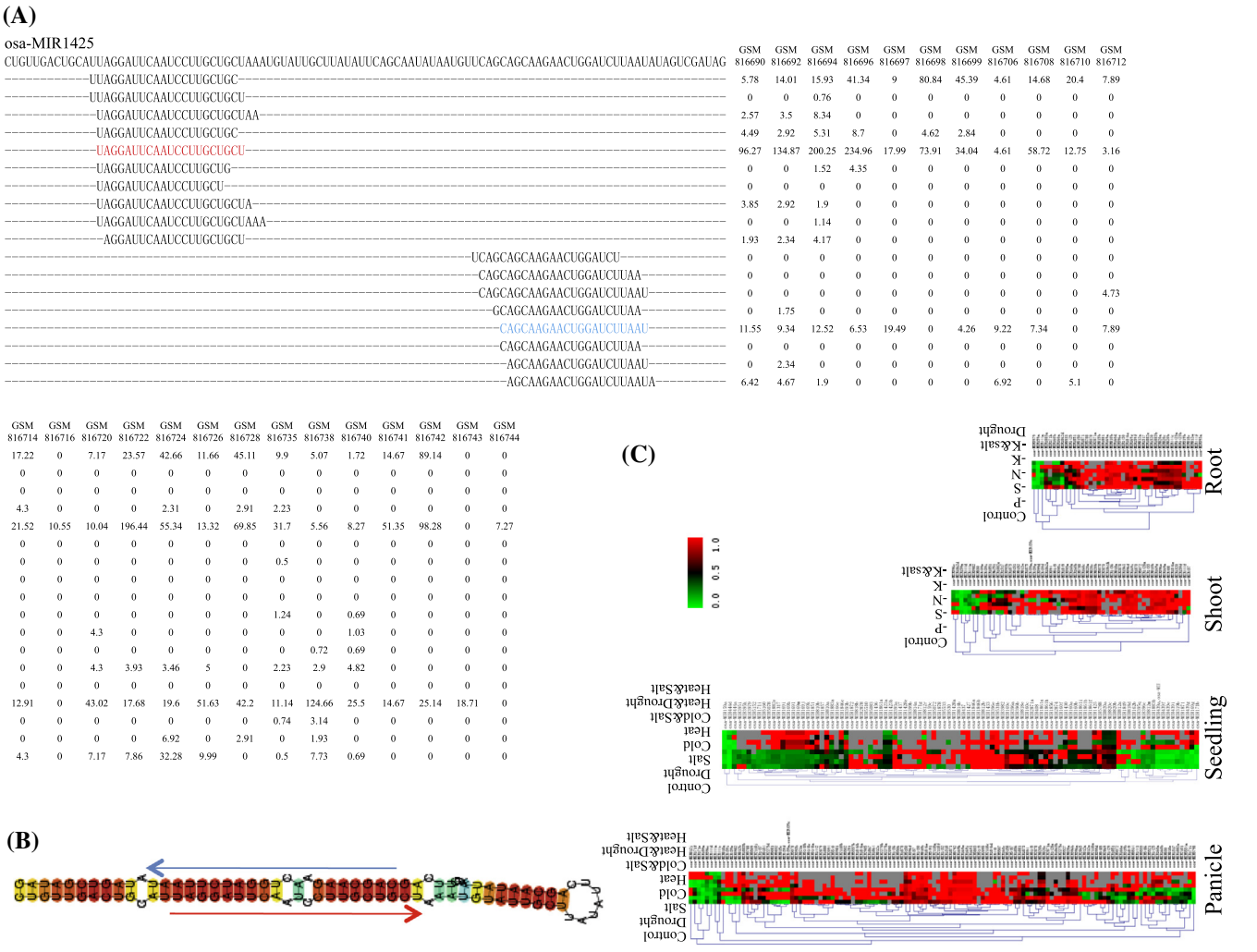

Fig. 3. Identification and characterization of miRNA arm switching under abiotic stress. (A) Identification of miR1425-5p and miR1425-3p in libraries from 25 different abiotic stresses. Values below each library are in RPM. Short reads highlighted in color indicate that both the miRNA-5p (red) and miRNA-3p (blue) are annotated in miRBase. (B) Putative secondary structure of pre-miR1425. Red and blue arrows indicate reads originating from miRNA-5p and -3p, respectively. (C) Heatmap of arm switching of osa-miRNAs under different abiotic stresses in panicles, seedlings, shoots, and roots. Red indicates miRNA-5p dominant expression, green indicates miRNA-3p dominant expression, black indicates co-expression of both arms, and gray indicates unavailable data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

panicles under high salt, and miR160a-3p was preferred in panicles exposed to cold.

We also explored the arm selection for separate tissues under different stresses and found that abiotic stress-induced changes in arm selection occurred more frequently in seedlings (34.6%, 98/283) and panicles (37.7%, 121/321) relative to roots (22.4%, 38/170), shoots (24.4%, 49/201), and control tissues (Fig. 3C). And this was mainly contributed by seedlings- or panicles-specific switched pre-miRNAs (Fig. 4). We further tested whether conserved miRNAs (based on the conservation between rice and *Arabidopsis*) are less apt to display arm switch because they have been incorporated into plant regulatory network than non-conserved miRNAs. The assumption held in panicle under different abiotic stresses ($P < 0.001$, χ^2 test) and marginally significant in shoot ($P = 0.0499$, χ^2 test), whereas no significant difference was observed in root and seedling ($P > 0.05$, χ^2 test). Nevertheless, we did find that non-conserved miRNAs harboring much more frequently arm switching across different tissues ($P < 0.001$, χ^2 test, Fig. 4). A wide variety of abiotic stresses gave rise to reciprocal expression of

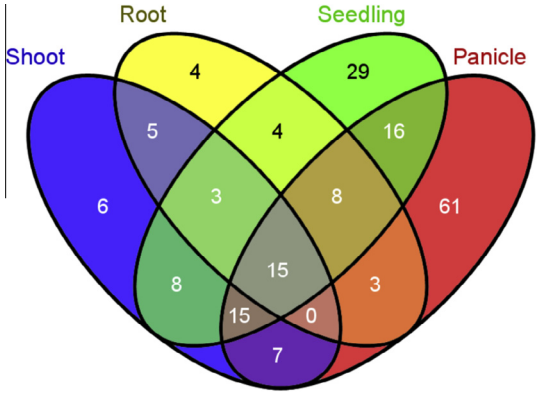

Fig. 4. Tissue-specific and overlap of pre-miRNA harboring arm switching under abiotic stress.

miRNA-5p and miRNA-3p, indicating that a subset of miRNAs can alternate arm selection in response to different stresses.

4. Discussion

miRNA was thought to be quite distinct from miRNA*, and miRNA* was thought to be required only to maintain the structure of precursor for interactions with Dicer [21]. Our results, however, showed that miRNA* can replace miRNA to become the dominantly expressed strand under specific conditions (Fig. 1A). Dynamic changes were observed during plant development and under different abiotic stresses, indicating that the expression of miRNAs is flexible and can be modulated to accommodate changing cellular microenvironments in the regulatory network. In addition, dominant strand selection may have changed during the evolution of different species [22,23]. In parallel, both strands of miRNAs show strong sequence conservation (stringent constraints) across distant evolutionary species indicating that they are both functional [24,25].

Notably, we demonstrated that the arm switching of miRNA was not only tissue-specific, but also was frequent under abiotic stress (Fig. 2D and Fig. 3C), suggesting that miRNA5p/3p selection is coordinated during plant development and under stress. The selection of miRNA5p/3p may benefit from being under the control of the same promoter, which could maximize the effect of regulatory elements. Recently Guo et al. [17] proposed that arm switching may contribute to isomiR expression and thus complicate the regulatory network. Furthermore, the miRNA5p/3p switch mechanism may favor keeping the genome more compact, because miRNA-5p and miRNA-3p rarely target the same gene families. This is more evident in plants than in animals because of the nearly perfect match between miRNAs and their targets in plants [4]. Nevertheless, the mechanisms of strand selection are still poorly understood. Notably, the preference for miRNA-5p or miRNA-3p was inconsistent among the different tissues and abiotic stresses and cannot be explained solely by a hydrogen bonding-based selection rule [5], suggesting there must be other mechanisms involved in controlling strand selection. It is widely accepted that miRNAs and their targets share simultaneous spatial expression [4], and from this we assume that target expression and *cis* elements within the precursor could be responsible for dominant strand switching. De et al. [26] showed that the guide miRNA loads onto the miRISC protein Argonaut 2 (AGO2) to resist nuclease degradation and that it dissociates from AGO2 when the complementary target mRNA is presented. Because AGO2 can bind and release RNA duplexes continuously until the guide miRNA strand is determined and is responsible for passenger strand removal [7,27], the presence of the target mRNA could contribute to the strand selection. In *Drosophila melanogaster*, most miRNAs are bound to AGO1 and miRNA*s, in contrast, are bound to AGO2, although both delivery pathways result in RNA interference [28,29]. Incorporation of miRNA*s into miRISC in this species further underscores the crucial role of arm selection in these regulatory networks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.036.

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