IDENTIFICATION OF MICRORNAS IN 12 PLANT SPECIES OF FABACEAE

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ABSTRACT

MicroRNAs (miRNAs) are tiny, non-coding and regulatory RNAs approximately 21 nucleotides in length. They are reported in various plants but still needs discovery in important plant species. The 12 plant species of Fabaceae were subjected this time to identify their miRNAs. The comparative genomics approaches with combination of various bioinformatics' tools were applied to find the novel miRNAs. This research leads to the finding of 29 miRNAs belonging to 13 miRNA families. From the 29 miRNAs, nine belongs to *Arachis duranensis*, four to *Lotus japonicas*, three to each *Pisum sativum* and *Arachis hypogaea*, two to each *Arachis ipaensis* and *Phaseolus vulgaris* and one to each *Cicer arietinum*, *Phaseolus acutifolius*, *Lupinus luteus*, *Glycyrrhiza uralensis*, *Robinia pseudoacacia* and *Lathyrus odoratus*. These findings will be useful in the future to design and develop desirable traits in the 12 plant species of Fabaceae.

Keywords: Comparative genomics; Fabaceae; MicroRNAs

Introduction

MicroRNAs (miRNAs) are endogenous, small RNAs, ~21 nt long and are found in a large number of animals and plants, such as *Caenorhabditis elegans*, *Oryza sativa*, *Arabidopsis thaliana*, Cotton, Mouse and human beings [1]. They are found in large number of non-coding RNAs [2], and play a central role in post-transcriptional gene regulation by degrading or by repressing target messenger RNAs in eukaryotes [3]. Since the discovery of the first miRNA, lin-4, in *Caenorhabditis elegans* [4],

thousands of miRNAs has been identified by experimental cloning or computational approaches [5, 6].

In plants, miRNA genes are transcribed by RNA polymerase II, and primary miRNA transcripts are subsequently capped, spliced and poly-adenylated [7]. Plant miRNAs are processed endogenously while the mature miRNAs are transferred into cytoplasm with the help of HASTY, a miRNA transporter [8]. In plants, miRNAs are involved in various features of plant growth and development, including leaf

morphology and polarity, lateral root formation, hormone signaling, transition from immature to adult vegetative stage and vegetative to flowering phase, flowering spell, floral organ identity and reproduction [9].

The miRNAs are also taking part in plant stress responses. A number of miRNAs are regulated in response to different stress conditions, which suggests that miRNA-directed post-transcriptional regulation of their respective target genes is important to cope with the stress conditions [10]. Plant miRNAs are conserved in nature which provides us a good basis for the identification of novel miRNAs in a number of plant species [11, 12].

In this research paper we identified total of 29 newly miRNAs (out of 13 miRNAs families; 156, 159, 160, 162, 164, 166, 167, 168, 390, 393, 394, 5630 and 5658) in 12 diverse plant species (viz; *Cicer arietinum*, *Arachis duranensis*, *Phaseolus acutifolius*, *Pisum sativum*, *Lotus japonicas*, *Arachis hypogaea*, *Lupinus luteus*, *Phaseolus vulgaris*, *Arachis ipaensis*, *Glycyrrhiza uralensis*, *Robinia pseudoacacia*, *Lathyrus odoratus*) of family *Fabaceae*, using all publicly available ESTs (Expressed Sequence Tags) subjected for the computational identification methodology of miRNAs orthologue. These findings will be useful for tracing the evolution of micro

RNAs by examining their expression in common ancestors of different plant species.

Materials and Methods

Retrieval of miRNAs Through Similarity Search

The similar approach as adopted previously [11] was practiced to predict the novel miRNAs in family *Fabaceae*. We subjected plant precursors miRNAs (pre-miRNAs) sequences, retrieved from the microRNA Registry Database (Version Rfam 18 released on November 3, 2011) [13], compared to the publically available *Fabaceae* ESTs from the dbEST (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) released on (1st March, 2012) at <http://blast.ncbi.nlm.nih.gov/Blast.cgi> by the use of blastn [14]. The basic local alignment search tool (BLAST) parameter settings were adjusted, as follows: expect values ~1,000; low density was chosen as the sequence filter, database (others *Fabaceae*) program selection (somewhat similar sequence) and all other parameters were used as default. The consequence results in FASTA formats of all the primary potential candidates' pre-miRNA sequences having maximum 4 mis-matches were saved. The frequented ESTs from the same gene were taken away and produced a single tone EST using blastn with default parameters. For the confirmation that novel miRNAs as non-protein coding RNAs, the

initial potential candidates' pre-miRNA sequences were subjected to BALST against protein database at National Center for Biotechnology Information (NCBI) using blastx (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&BLAST_PROGRAMS) with default parameter to eliminate the protein coding RNAs [15].

Prediction of Hairpin Structures

To retrieve the secondary structure of the initial candidate's sequences we used the Zuker folding algorithm with MFOLD (version 3.5) [16], publicly available at <http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi>. Default parameters were used; RNA sequence (linear), folding temperature (37° C), ionic condition (1 M NaCl with no divalent ions), percent sub-optimality number (5); maximum interior/ bulges loop size (30), and all other with preset values. The maximum (- log) free energy structures were chosen on the basis of trial and error, as termed by Barozai et al. [17]. The threshold values used to select a miRNA were same as described by Zhang et al. [18]. The stem portion of the hairpin checked for the mature sequences with at least 12 base pairs involved in Watson–Crick or G/U base pairing between the mature miRNA and the opposite strand (miRNA*).

Filtration of Sequence and Structural Features

For the filtration of sequence and structural features, calculation was made of the GC content, core minimum free energy (mfe), hairpin mfe and core hairpin ratio (ch_ratio) as determined by Li et al. [19]. A small modification for core mfe calculation, was used by Barozai et al. [11]. Drosha, a RNA-III like endonuclease, recognizes and cleaves the primary transcripts of mature miRNAs (pri-miRNA) at 22 nucleotides upstream from the position of the first nucleotide (Pfn - 22) and the 24 nucleotides downstream the terminal nucleotide (Ptn + 24) to yield pre-miRNA in animals [19]. In plant pre-miRNAs, the mature miRNA sequences were located away, so, the Pfn - 24 and Ptn + 26 were included in the core portion of the hairpin structure. The mfe for core and secondary structures were calculated by MFOLD (version 3.5) [16], publicly available at <http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi>. The parameters were adjusted same as reported earlier. For ch_ratio calculation, we divided the core mfe by the hairpin mfe, and the quotient is denoted as the ch_ratio.

Conservation and Phylogenetic Analysis of *Fabaceae* miRNAs

As the miRNAs are conserved in nature among

eukaryotes; subsequently we made conservation and phylogenetic study of the *Fabaceae* miRNAs with others plant miRNAs. The conservation and phylogenetic study of miRNAs were prepared by fetching the FASTA format of pre-miRNAs sequences for *Arabidopsis thaliana* (*ath*), rice (*Oryza sativa*, *osa*) and *Populus trichocarpa* (*Ptc*), from the microRNA Registry Database (Version Rfam 18.0 released November 3rd, 2011) [13] and aligned with *Fabaceae* miRNAs using the publically available weblogo [20] and ClustalW to create cladogram tree [21] respectively. The results were saved.

Prediction of *Fabaceae* miRNA Targets

To predict the *Fabaceae* miRNAs targets, we used the NCBI blastn program [14], by performing the mature miRNA sequences as queries [22]. The parameters were adjusted as, Database; Nucleotide collection (nr/nt), organism; *Fabaceae* (taxid: 3803) and Program Selection; highly similar sequences (megablast). The sequences showing 80% query coverage were selected and giving for the target validation to RNA-hybrid submission algorithm publically available at (<http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html>), a miRNAs target prediction tool [23] for the confirmation of the targets. The results validated by RNAhybrid were saved.

RESULTS AND DISCUSSION

The Newly identified *Fabaceae* miRNAs

Data mining, homology search and comparative genomics resulted many putative and interesting findings in various plant species [24, 25]. Publically available ESTs were computationally considered for the prediction of novel miRNAs in family *Fabaceae*. Twenty nine (29) putative pre-miRNAs of *Fabaceae* were identified by similarity search followed by the filtration and completion strategy. The 29 newly miRNAs belong to 13 miRNA families from the known *Fabaceae* ESTs (Figure#1). The 13 miRNA families (miR-156, 159, 160, 162, 164, 166, 167, 168, 390, 393, 394, 5630 and 5658) are observed for the first time in *Fabaceae*. The species were observed in; *Arachis duranensis* miR-156, 162, 166(a,b), 168(a,b), 390, *Lotus japonicas* miR-160(a,b), 162(a,b), *Arachis hypogaea* miR-162, 166(a,b,c), *Pisum sativum* miR-159, 160, 164, *Phaseolus acutifolius* miR-159, 5658, *Phaseolus vulgaris* miR-167, 168, *Arachis ipaensis* miR-162, 390, *Glycyrrhiza uralensis* miR-393, *Cicer arietinum* miR-156, *Lupinus luteus* miR-162, *Robinia pseudoacacia* miR-394, *Lathyrus odoratus* miR-5630. All the novel *Fabaceae* miRNAs considered as a valid candidates after satisfying the validation formula for biogenesis and expression of the miRNAs, given by Ambros et al. [26]. The novel *Fabaceae* miRNAs fulfilled the criteria B, C and D.

According to Ambros et al. [26] only the criterion D is enough for homologous sequences to validate as new miRNAs in different species.

Characterization of the *Fabaceae* miRNAs

The Table #1 characterizes the novel *Fabaceae* miRNAs in terms of reference miRNA families, accession numbers, plant names, mature sequences, minimum free energies (MFEs), mature sequence arms, number of miss-matches, total scores, query courage, expected values, maximum identities percentage(%), mature sequence lengths, GC content % and precursor length. MFEs play a crucial role in the prediction of stem-loop structure. The newly identified *Fabaceae* pre-miRNAs' MFEs ranges from $-12.30 \text{ kcal mol}^{-1}$ to -87.70 with in an average of $\sim -39.95 \text{ kcal mol}^{-1}$. The majority 55.17% (16 out of 29) *Fabaceae* pre-miRNAs showed mature sequences at 5' arm, and the remaining 44.82% (13 out of 29) pre-miRNAs have 3' arm sequences as illustrated in (Figure#1). The novel identified *Fabaceae* majority miRNAs, 69% miRNAs (20 out of 29) are perfectly (100%) matched with their corresponding reference miRNAs, whereas 27% (8 out of 29) showed one nt mismatched and the remaining 3.44%(1 out of 29) have a difference of two nt from their homologs. Similar results have been reported in different organisms by Reinhart et al. [27] and Barozai et al. [11]. Total score of the pre-miRNAs were showed,

maximum were 141 and minimum were 46.4 with an average of 98.18. Query coverage of the pre-miRNAs was calculated with the maximum value 100% and minimum value 54% with an average of 86%. Expect value and maximum identities were also presented in (Table#1). Three (out of 29) mature miRNA sequences consist of 20 nucleotides (nts), 25 consist of 21 nts and one consists of 22 nts. Majority of the mature sequences 17 (out of 29) consist of 51-66% GC content and 12 (out of 29) consist of 33-50% GC content. The *Fabaceae* pre-miRNA length range from 60 to 180 nt with an average of 100 nt Maximum, 15 out of 29 pre-miRNAs have a sequence length range of 60-90 nt, following by 9 from 91-120 and 5 from 121-180 nt sequence length range. All the mature sequences of *Fabaceae* miRNAs are in the stem regions of the stem-loop structures, as shown in (Figure#1). These findings are same as described by many researchers [18, 11, 28, 29]. The predicted miRNA stem-loop structures showed that 14 mature sequences are 100% hybridized and the remaining 15 mature sequences have (71-95%) hybridization with the opposite arms (miRNAs*) in the stem region and the hairpin precursors do not contain large internal loops or bulges (Figure#1). Similar results were given for the different organisms' miRNAs [13, 17, 29]. The

Table # 1: *Fabaceae* miRNAs characterization in terms of SM, source miRNAs, Acc. # accession number, PN plant name, MS mature sequence, MFE minimum free energy, MSA mature sequence arm, NM number of mismatches (showed in color and enlarged font size), TS total score, QC query coverage, EV expect value, MI maximum identities, MSL mature sequence length, GC% guanine and cytosine percentage and PL precursor miRNA length.

Fabaceae MiRNAs	SM	Acc. #	PN	MS	MFE	MSA	NM	TS	QC%	EV	MI %	MSL	GC %	PL
car-miR156	ath-miR156e	HO063979	<i>Cicer arietinum</i>	TGACAGAAGAGAGTGAGCAC	-52.50	5'	0	104	99%	5e-21	81	20	50	88
adu-miR156	ath-miR156h	GW954185	<i>Arachis duranensis</i>	TGACAGAAGAATAGAGAGCAC	-41.30	5'	1	116	94%	2e-13	90	20	45	98
pac-miR159	ath-miR159b	HO805845	<i>Phaseolus acutifolius</i>	TTTGGATTGAAGGGAGCTCTA	-87.70	3'	1	69.8	94%	1e-10	69	21	42	180
psa-miR159	ath-miR159b	FG534720	<i>Pisum sativum</i>	TT TGGATTGAAGGGAGCTCTA	-21.70	3'	1	89.1	55%	1e-04	95	21	42	78
lja-miR160a	ath-miR160a	DC595011	<i>Lotus japonicus</i>	TGCCTGGCTCCCTGTATGCCA	-51.90	5'	0	53.6	97%	8e-06	76	21	61	83
lja-miR160b	ath-miR160a	FS344717	<i>Lotus japonicus</i>	TGCCTGGCTCCCTGTATGCCA	-48.70	5'	0	93.3	96%	9e-18	85	21	61	83
psa-miR160	ath-miR160a	FG538087	<i>Pisum sativum</i>	TGCCTGGCTCCCTGTATGCCA	-53.30	5'	0	75.2	96%	3e-12	80	21	61	83
adu-miR162	ath-miR162b	GW949510	<i>Arachis duranensis</i>	TCGATAAACCTCTGCATCCAG	-31.90	3'	0	93.3	90%	9e-18	81	21	47	90
ahy-miR162	ath-miR162b	GO323824	<i>Arachis hypogaea</i>	TCGATAAACCTCTGCATCCAG	-35.90	3'	0	98.7	90%	2e-19	82	21	47	90
aip-miR162	ath-miR162b	GW990392	<i>Arachis ipaensis</i>	GCGATAAACCTCTGCATCCAG	-33.50	3'	1	95.1	90%	3e-18	81	21	52	94
lja-miR162a	ath-miR162b	GO007141	<i>Lotus japonicus</i>	TCGATAAACCTCTGCATCCAG	-33.60	3'	0	87.8	88%	4e-16	80	21	47	88
lja-miR162b	ath-miR162b	GO015722	<i>Lotus japonicus</i>	TCGATAAACCTCTGCATCCAG	-32.70	3'	0	87.8	88%	4e-16	80	21	47	90
llu-miR162	ath-miR162b	BG149136	<i>Lupinus luteus</i>	TCGATAAACCTCTGCATCCAG	-36.20	3'	0	141	90%	1e-16	79	21	47	82
psa-miR164	ath-miR164a	FG530884	<i>Pisum sativum</i>	TGGAGAAGCAGGGCACGTGCA	-34.20	5'	0	92.7	100%	7e-07	80	21	47	86
adu-miR166a	ath-miR166b	GW939731	<i>Arachis duranensis</i>	TCGGACCAGGCTTCATTCCCC	-38.20	3'	0	121	100%	4e-04	92	21	61	112
adu-miR166b	ath-miR166b	GW951206	<i>Arachis duranensis</i>	TCGGACCACGCTTCATTCCC	-38.10	3'	1	117	100%	0.004	92	21	61	112
adu-miR166c	ath-miR166d	GW955263	<i>Arachis duranensis</i>	TCGGACCAGGCTTCATTCCCC	-39.80	3'	0	124	100%	0.015	96	21	61	71
ahy-miR166a	ath-miR166b	GO323023	<i>Arachis hypogaea</i>	TCGGACCAGGCTTCATTCCCC	-36.60	3'	0	126	100%	4e-04	92	21	61	112

ahy-miR166b	ath-miR166b	GO330519	<i>Arachis hypogaea</i>	TCGGACCAGGCTTCATTCCCC	-34.30	3'	0	131	100%	8e-06	92	21	61	116
pvu-miR167	ath-miR167b	FE692413	<i>Phaseolus vulgaris</i>	TGAAGCTGCCAGCATGATCTA	-43.70	5'	0	95.1	99%	3e-18	81	21	61	99
adu-miR168a	ath-miR168b	GW927363	<i>Arachis duranensis</i>	TCGCTTGGTGCAGGTCGGGAA	-21.70	5'	0	99	60%	2e-06	92	21	61	60
adu-miR168b	ath-miR168b	GW945909	<i>Arachis duranensis</i>	TCGCTTGGTGCAGGTCGGGCA	-52.00	5'	1	85.5	54%	0.004	90	21	66	120
pvu-miR168	ath-miR168b	FE674450	<i>Phaseolus vulgaris</i>	TCGCTTGGTGCAGGTCGGGAA	-35.80	5'	0	98.2	57%	8e-06	94	21	61	140
adu-miR390	ath-miR390a	GW943473	<i>Arachis duranensis</i>	AAGCTCAGGAGGGATAGCGCC	-58.50	5'	0	114	71%	2e-08	97	21	57	126
aip-miR390	ath-miR390a	GW966457	<i>Arachis ipaensis</i>	AAACTCAGGAGGGATAGCGCC	-37.20	5'	1	110	94%	4e-10	76	21	57	80
gur-miR393	ath-miR393a	FS253174	<i>Glycyrrhiza uralensis</i>	TCCAAAGGGATCGCATTGATCC	-52.60	5'	0	133	57%	4e-04	91	22	50	123
rps-miR394	ath-miR394a	BI642560	<i>Robinia pseudoacacia</i>	TTGGCATTCTGTCCACCTCC	-40.00	5'	0	92.7	62%	4e-04	87	20	55	109
lod-miR5630	ath-miR5630	GO319187	<i>Lathyrus odoratus</i>	GCTGAGAGCGGTTCTGATGGC	-22.90	5'	2	80.6	100%	6e-14	85	21	61	80
ahy-miR5658	ath-miR5658	GO330337	<i>Arachis hypogaea</i>	ATGATGATGATGATGATGAAG	-12.30	5'	1	46.4	73%	0.001	74	21	33	140

Table # 2: Comparison of *Fabaceae*, *Ath* and *Li et al.*, reference values of GC content, core mfe, hairpin mfe and ch_ratio

Reference	GC contents	Core mfe	Hairpin mfe	Ch_ratio
Li et al.	30–60 (93%)	-42 to -17 (99%)	-50.2 to -24.2 (99%)	50–96 (99%)
<i>Ath</i>	36.4–51.1	-54.2 to -23.5	-79.4 to -48.3	42–93
<i>Fabaceae</i>	42-61 (93%)	-49.70 to -17.2(72%)	-58.50 to -31.90(83%)	42-98(82.75%)

Fabaceae pre-miRNAs were subjected through BLAST against the protein database at NCBI using blastx and found no homology with known proteins. This result has confirmed our identified pre-miRNAs as strong candidates in *Fabaceae*. These findings are in agreement with the repeated works [18, 11, 28, 29].

Sequence and Structural Features Filter

It is an important step to ensure the pre-miRNAs from false positives. The filter is composed of four indices, namely GC content, core mfe, hairpin mfe and the ratio of core mfe to hairpin mfe (ch_ratio) (Barozai et al., 2008). As shown in (Table # 2.), identified miRNAs of *Fabaceae* have a range of GC content 42-61% (93%), core mfe (-49.70 to -17.2 kcal mol⁻¹, 72%), hairpin mfe -58.50 to -31.90 kcal mol⁻¹, 83%)) and ch_ratio (42-98, 82.75%). The GC content and ch_ratio are within the range given by Li et al. [19] and Barozai et al. [11].

Conservation and Phylogenetic Analysis of *Fabaceae* miRNAs

The conservation and phylogenetic analysis were made among the novel identified *Fabaceae* miRNAs with *Arabidopsis lyrata* (aly) and *Brassica rapa* (bra) (dicotyledonous), *Brachypodium distachyon* (bdi) and *Triticum aestivum* (tae) (monocotyledonous). The miRNAs 160 was considered with more conservation than others, as shown in (Figure#2.) These findings were similar to Zhang et al. [18] and

Barozai et al. [11]. The conservation made them strong candidates of miRNAs in *Fabaceae* and suggests their conserved physiological function. The Phylogenetic tree showed that on the basis of pre-miRNA (miR-160) sequences, the *Fabaceae* is more closed to *Triticum aestivum* (tae), *Brachypodium distachyon* (bdi), *Brassica rapa* (bra) and *Arabidopsis lyrata* (aly) (Figure # 3). The results are in agreement with the reported works [11, 6].

The *Fabaceae* miRNAs Putative Targets

The conserved nature of miRNAs in different organisms suggests their conserved function [7]. Total 61 targets were predicted for the 29 novel *Fabaceae* miRNAs, using a combination of BLAST and RNA-Hybrid algorithms (Table # 3). Majority of these putative targets are already reported in different plant species [7, 11, 29, 30]. The Major portion of the predicted *Fabaceae* miRNAs targets are involved in transcription factor and metabolism (40.98%, 26.22%) respectively, followed by signal transduction (24.59%), transporter (04.91%), growth and development (01.63%) and (03.27%) respectively.

Transcription factor (sequence-specific DNA-binding factor) is a key regulating factor that controls the transcription of genetic information from DNA to mRNA. Such targeted proteins were found in the newly identified miRNAs families (miR-156, 159, 160, 164, 166, 167 and 168) of *Fabaceae* in the

current research work and the same results have been found by the other workers [6, 29].

Our findings **were** revealed that many miRNA families of *Fabaceae* take part in the regulation of metabolic path-way protein. These are; miR-160, miR-162, miR-164, miR-390, and miR-5630. These results were in agreement among the works of previously researchers [31, 29, 12].

Experimental and computational studies have shown that miRNAs play a dynamic role in hormone signal transduction [32]. In our results 24.59% miRNAs were found to target *Fabaceae* genes that are involved in signal transduction. For instance miR-168, 393, 394, 5630 and 5658 target the signal transduction proteins like; F-box protein, Protein transport inhibitor response 1, Zinc finger CCCH domain-containing protein and PLTRP2E11 resistance protein PLTR gene.

In *Fabaceae* the miRNAs group, for instance miR-390 and miR-5658 are involved in the transport pathway. These findings are incorporated with the earlier studies [10, 33].

Growth and development of an organism is regulated by gene functioning. The miRNAs are played a vital role in gene regulation during growth and development. Many miRNAs are involved in plant growth and development [17]. The putative *Fabaceae* miRNAs miR-167 and miR-168 respectively were found to target the proteins like; Putative pentatricopeptide repeat-containing protein and DNA repair protein of growth and development. These results have been found in the previous works [7, 34, 35].

Conclusions

In current research work we adopted the similarity search methodology for the prediction of novel 29 miRNAs in 12 various *Fabaceae* species, using ESTs publically available. In addition, to identify their target proteins, we found different targeted mRNAs of the putative miRNAs families regarding transcription factors, signal transduction and growth and development. These newly identified miRNAs will be helpful in understanding various stages of the life cycle of *Fabaceae* plants.

Figure # 2: The *Fabaceae* miRNAs Conservation studies. Alignment of miRNAs (miR-160) of *Fabaceae*, (*Lotus japonicus*, *lja*) with *Arabidopsis lyrata* (*aly*), *Brassica rapa* (*bra*), *Brachypodium distachyon* (*bdi*) and *Triticum aestivum* (*tae*), using Weblogo, showing conserved nature of mature sequence highlighted in a rectangle box.

Figure # 3: The *Fabaceae* miRNA phylogenetic analysis. The Phylogenetic analysis of the pre-miRNA (miR-160) of *Fabaceae* (*Lotus japonicus*, *lja*) with *Arabidopsis lyrata* (*aly*), *Brassica rapa* (*bra*), *Brachypodium distachyon* (*bdi*), *Triticum aestivum* (*tae*), is done with the help of Clustalw and cladogram tree was generated using neighbor joining clustering method. The Phylogenetic tree showed that on the basis of pre-miRNA sequences, the *Fabaceae* (*Lotus japonicus*, *lja*) is more closed to *Triticum aestivum* (*tae*), *Brachypodium distachyon* (*bdi*), *Brassica rapa* (*bra*) and *Arabidopsis lyrata* (*aly*).

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