



Chapter 5

Small RNA analysis

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House-keeping noncoding RNAs:

- rRNA
- tRNA
- snRNA
- snoRNA

Small noncoding RNAs:

- siRNA
- miRNA

Long or large noncoding RNAs (>200nt):

- Precursor of small noncoding RNA
- Pol IV- and Pol V-dependent long noncoding RNA (plant-specific)
- mRNA-like long noncoding RNA

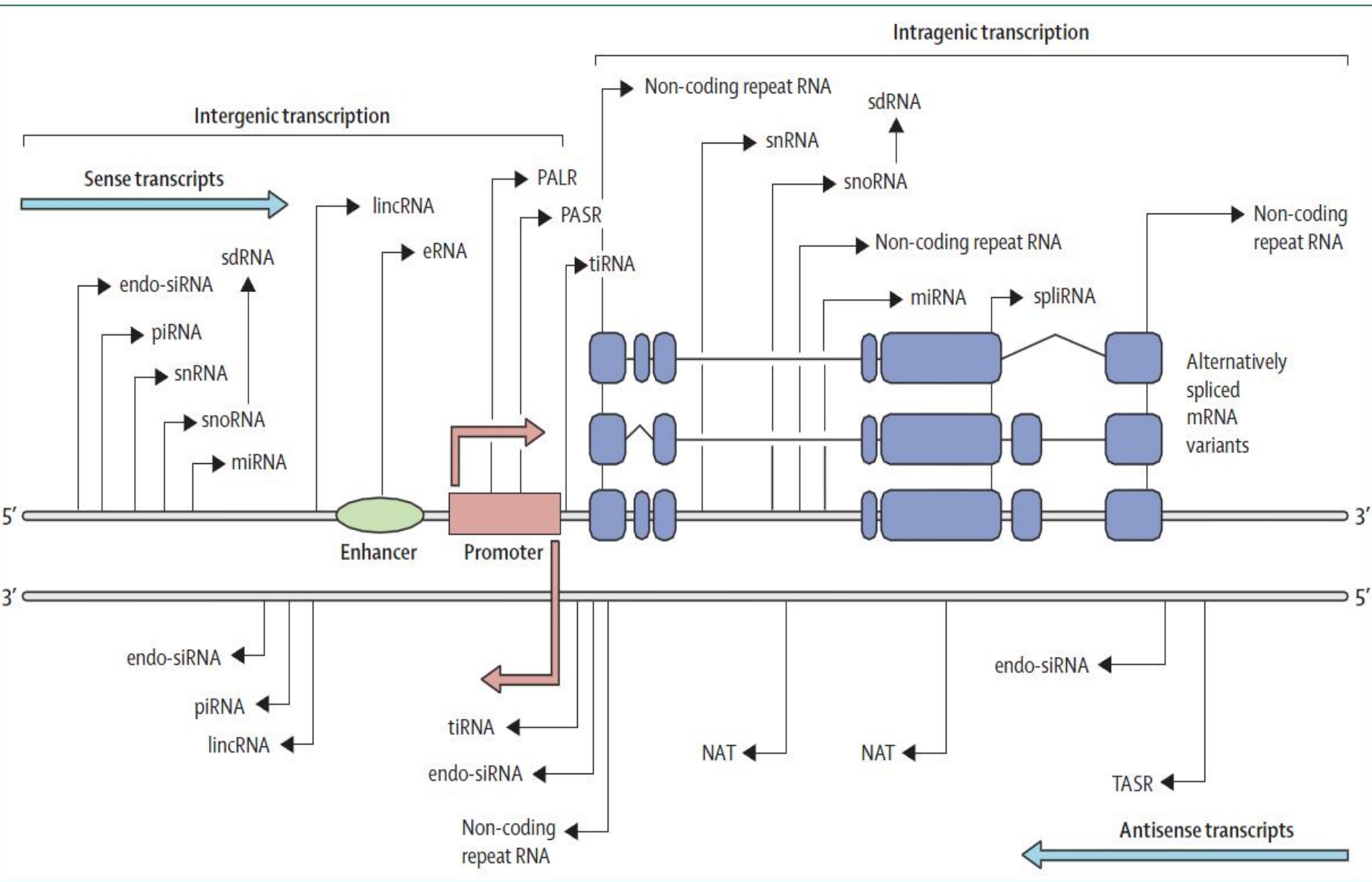
New types

- Circular RNA

<http://rfam.xfam.org/>




Complexity of transcription patterns of ncRNAs



1. Small non-coding RNAs in plants

■ Discovery of microRNA

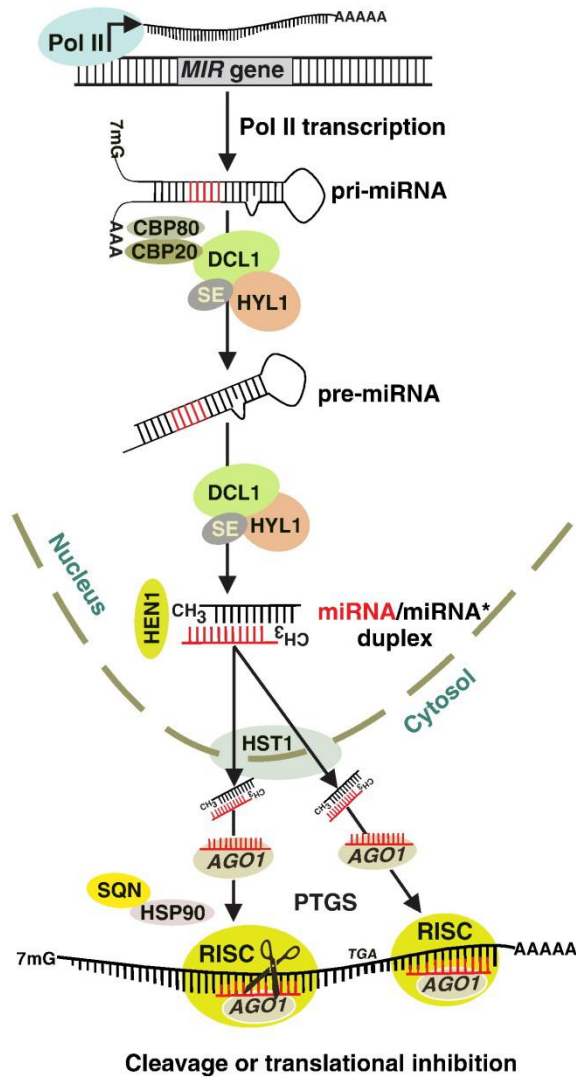
- MicroRNAs were discovered in 1993 by Victor Ambros, Rosalind Lee and Rhonda Feinbaum during a study of the gene *lin-14* in *C. elegans* development.
- However, miRNAs were not recognized as a distinct class of biological regulators with conserved functions until the early 2000s. Since then, miRNA research has revealed multiple roles in negative regulation (transcript degradation and sequestering, translational suppression) and possible involvement in positive regulation (transcriptional and translational activation). By affecting gene regulation, miRNAs are likely to be involved in most biological processes



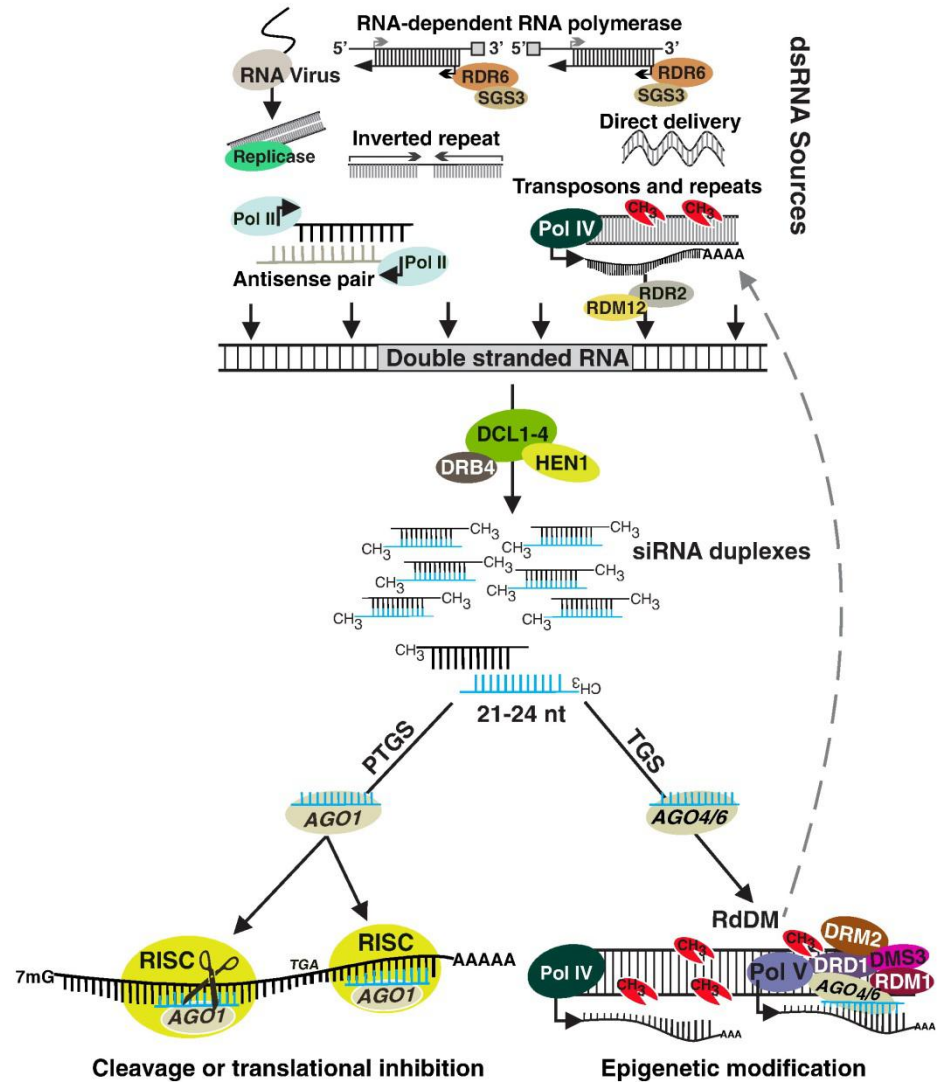
miRNA: miRNAs (microRNAs) are small regulatory RNAs of 20–24 nt that are encoded by endogenous MIR genes. Their primary transcripts form precursor RNAs, which have a partially double-stranded stem-loop structure and which are processed by DCL proteins to release mature miRNAs.

siRNA: siRNAs are generated from perfectly double-stranded RNAs that can originate from different sources such as RNAs transcribed from inverted repeats, natural cis-antisense transcript pairs, the action of RNA-dependent RNA polymerases (RDRs) which convert single stranded RNA into dsRNA, the replication of RNA viruses, and regions of the genome rich in retroelements.

A) miRNA biogenesis and function



B) siRNA biogenesis and function



(Khraiweh *et al.* 2011)

miRNA

- Originate from capped & polyadenylated full length precursors; single chain (pri-miRNA)
- Hairpin precursor 60-400 nt (pre-miRNA)
- Mature miRNA 20-24 nt (miRNA)
- miRNA::miRNA* complex (non-perfect matching)
- Dicer-like RNase III endonuclease (DCL) and Argonaute protein (AGO)
- RISC (RNA-induced silencing complex)
- PTGS (posttranscriptional gene silencing)
- *trans*- or *cis*- acting
- First discovered in 1993 by Victor Ambros at Harvard (*lin-4*)
- *Let-7* discovered in 2000 by Frank Slack as a postdoc at Harvard (Ruvkun lab)
- The first miRNA of plants was discovered in *Arabidopsis*



lin-4 and let-7

(Lee et al. 1993; Wightman et al. 1993)



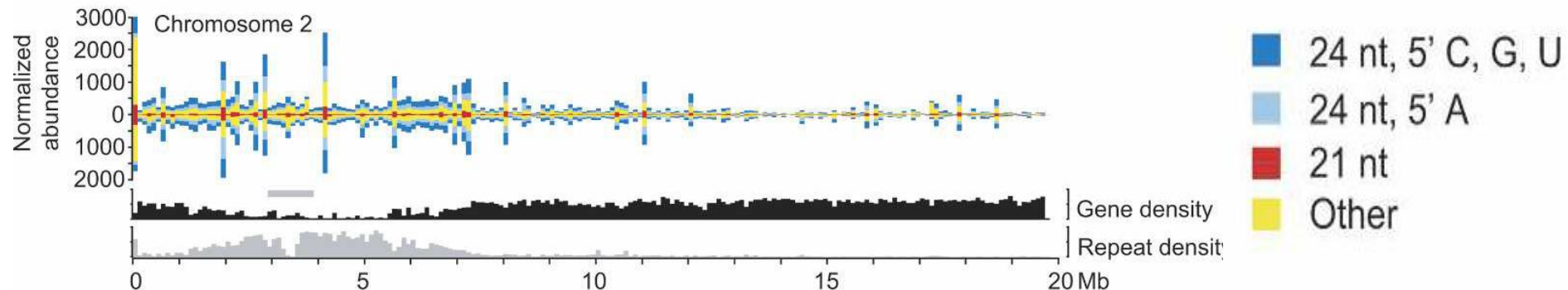
MIR827

```

AACCCUUGAAUGUGUUUGUUGAUUGAUUUCUACACAUGUUGAUCAUCCUGUGUUGAUUCGAUUGGUUUAGAUGACCAUCAACAAACUCUUUCGUGGUUUU
(((((.....)))))).....
.....UUUGUUGAUUGAUUUCUACAC.....
.....TTAGATGACCATCAACAAACT.....
.....TAGATGACCATCAACAAACT.....
  
```

siRNA

- Originate from dsRNA (synthesis by RDR or bimolecular duplexes: NATs, natural antisense transcripts)
- Various types due to the divergence of biogenesis and mechanism processes
- Cleaved by miRNA, sometimes
- Dicer-like RNase III endonuclease (DCL) and Argonaute protein (AGO)
- RISC (RNA-induced silencing complex)
- PTGS and TGS (transcriptional gene silencing)
- Nearly perfect matching with target sites
- *Cis-* acting (also *trans-*)
- New tool for researchers



Comparison of miRNAs and siRNAs

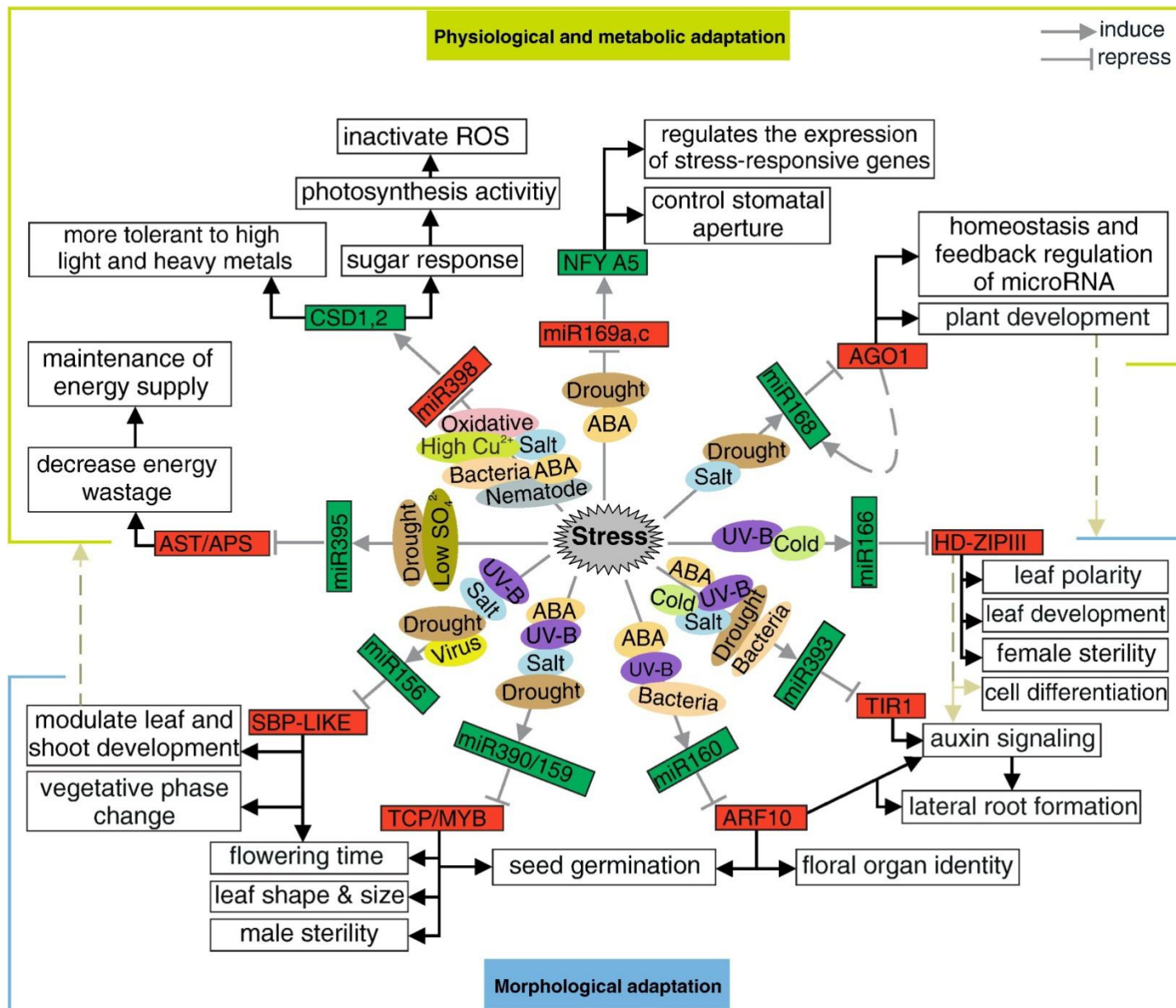
	miRNAs	siRNAs
Dicer	DCL1	DCL1-4
5' end	Mostly U	Mostly A for 24-mers
Origin	Mostly intergenic region	Transposable-element, repeat sequence, heterochromatic sequence
Precursor	Hairpin pri-miRNA	Long dsRNA
Copy number	One pre-miRNA, one miRNA (one arm of the foldback structure)	One precursor, many siRNA (both strands of the precursor)
Conservation	Yes	No
Target gene	<i>trans</i>	<i>cis</i> and <i>trans</i>
Function	developmental regulation and stress response	Chromatin modification and stress response
Mechanism	mRNA cleavage or translational repression	DNA methylation and mRNA cleavage

	1	10	20	30	40	50	60	70	80	90	100	110
ath-MIR156a	---	---	---	---	---	---	---	---	---	---	---	---
ath-MIR156c	---	---	---	---	---	---	---	---	---	---	---	---
gna-MIR156a	---	---	---	---	---	---	---	---	---	---	---	---
ath-MIR156d	---	---	---	---	---	---	---	---	---	---	---	---
ath-MIR156b	GCTAGAGAGGGAGAGATGGT	GATTGAGGATGCAC	AGAAACTGACAG	AGAGAGTGA	CACA-C	AAAGGCART	---	---	---	---	---	---
osa-MIR156b	TTGCTTTGAGAGGGG	AGAGATCTC	---	---	---	---	---	---	---	---	---	---
osa-MIR156j	TTGGGGCGGGCGGGATC	GAGCATGAA	AGATTTG	---	---	---	---	---	---	---	---	---
ath-MIR156e	---	---	---	---	---	---	---	---	---	---	---	---
ath-MIR156f	---	---	---	---	---	---	---	---	---	---	---	---
osa-MIR156c	GGAGGAGAGAGGGGGT	GGAGGGT	GGGCTG	ACAGAGAGT	GAGCACA	ACGGTGG	---	---	---	---	---	---
zna-MIR156c	GCTGGAGAGAGGGT	---	---	---	---	---	---	---	---	---	---	---
sof-MIR156	GCTGGAGAGAGGGT	---	---	---	---	---	---	---	---	---	---	---
zna-MIR156b	AGATGAGTTTTTT	GAAGGTTT	---	---	---	---	---	---	---	---	---	---
sbi-MIR156c	---	---	---	---	---	---	---	---	---	---	---	---
osa-MIR156a	GGAGGGT	GACAGAGAGT	GAGCACA	ACGGTGG	---	---	---	---	---	---	---	---
ath-MIR156g	ATAACGAGGGC	GACAGAGAGT	GAGCACA	ACGGTGG	---	---	---	---	---	---	---	---
sbi-MIR156b	---	---	---	---	---	---	---	---	---	---	---	---
osa-MIR156d	GGAGAGCT	CTCATGAGA	---	---	---	---	---	---	---	---	---	---
zna-MIR156d	AGGCT	CTCGAGAGA	---	---	---	---	---	---	---	---	---	---
zna-MIR156k	GGCTTAGGCC	CCGAGAGA	---	---	---	---	---	---	---	---	---	---
sbi-MIR156e	CGATTAGGCC	CTGAGAGA	---	---	---	---	---	---	---	---	---	---
osa-MIR156e	---	---	---	---	---	---	---	---	---	---	---	---
zna-MIR156e	CCGGCGGGCG	CGCGCGGG	---	---	---	---	---	---	---	---	---	---
zna-MIR156h	CGGGCTCGCG	CGCGCGGG	---	---	---	---	---	---	---	---	---	---
zna-MIR156i	CGCG	CGCGCGGG	---	---	---	---	---	---	---	---	---	---
osa-MIR156i	---	---	---	---	---	---	---	---	---	---	---	---
sbi-MIR156a	---	---	---	---	---	---	---	---	---	---	---	---
osa-MIR156k	TTGAGAGTGA	---	---	---	---	---	---	---	---	---	---	---
sbi-MIR156d	TGGACCTCTGGAGT	GAGTGA	---	---	---	---	---	---	---	---	---	---
zna-MIR156j	CGAGTGGACCTCGGG	AGCGA	---	---	---	---	---	---	---	---	---	---
Consensusg.....	TGACAGAGAGAGT	GAGCACA	ACGGTGG	...	g.ggc...	..t..GCAaT

	111	120	130	140	150	160	170	180	190	200	205
ath-MIR156a	ACTTGGCTTCTTT	GGGTGCTCACT	GCTCTTTCTGTC	GATTCCGGT	---	---	---	---	---	---	---
ath-MIR156c	ATTTGGCTTCTTT	GGGTGCTCACT	GCTCTTTCTGTC	GATTCCGGT	---	---	---	---	---	---	---
gna-MIR156a	ATTGCGG	---	---	---	---	---	---	---	---	---	---
ath-MIR156d	GATGGTTGCTTT	GGGTGCTCACT	CTCTTTCTGTC	---	---	---	---	---	---	---	---
ath-MIR156b	GGG	---	---	---	---	---	---	---	---	---	---
osa-MIR156b	GCTGGGA	---	---	---	---	---	---	---	---	---	---
osa-MIR156j	CCTGCC	---	---	---	---	---	---	---	---	---	---
ath-MIR156e	GCTTGA	---	---	---	---	---	---	---	---	---	---
ath-MIR156f	GCTTGA	---	---	---	---	---	---	---	---	---	---
osa-MIR156c	GCTTGA	---	---	---	---	---	---	---	---	---	---
zna-MIR156c	GCTCGAA	---	---	---	---	---	---	---	---	---	---
sof-MIR156	GCTCGAA	---	---	---	---	---	---	---	---	---	---
zna-MIR156b	GCTAGGA	---	---	---	---	---	---	---	---	---	---
sbi-MIR156c	GCTCGAA	---	---	---	---	---	---	---	---	---	---
osa-MIR156a	GCTTGA	---	---	---	---	---	---	---	---	---	---
ath-MIR156g	GCTCGAA	---	---	---	---	---	---	---	---	---	---
sbi-MIR156b	GTTGGGA	---	---	---	---	---	---	---	---	---	---
osa-MIR156d	CCC	---	---	---	---	---	---	---	---	---	---
zna-MIR156d	GCC	---	---	---	---	---	---	---	---	---	---
zna-MIR156k	GCC	---	---	---	---	---	---	---	---	---	---
sbi-MIR156e	GCC	---	---	---	---	---	---	---	---	---	---
osa-MIR156e	GCC	---	---	---	---	---	---	---	---	---	---
zna-MIR156e	GCC	---	---	---	---	---	---	---	---	---	---
zna-MIR156h	GCC	---	---	---	---	---	---	---	---	---	---
zna-MIR156i	GCC	---	---	---	---	---	---	---	---	---	---
osa-MIR156h	GCTGCC	---	---	---	---	---	---	---	---	---	---
osa-MIR156i	GCC	---	---	---	---	---	---	---	---	---	---
sbi-MIR156a	GTC	---	---	---	---	---	---	---	---	---	---
osa-MIR156k	TTCTGCC	---	---	---	---	---	---	---	---	---	---
sbi-MIR156d	TTCTGCC	---	---	---	---	---	---	---	---	---	---
zna-MIR156j	TTCTGCC	---	---	---	---	---	---	---	---	---	---
Consensus	Gct..G.....	...	CGGGT	GCTCACT	CTCT	CTGTC	...	CCcct	..G.....

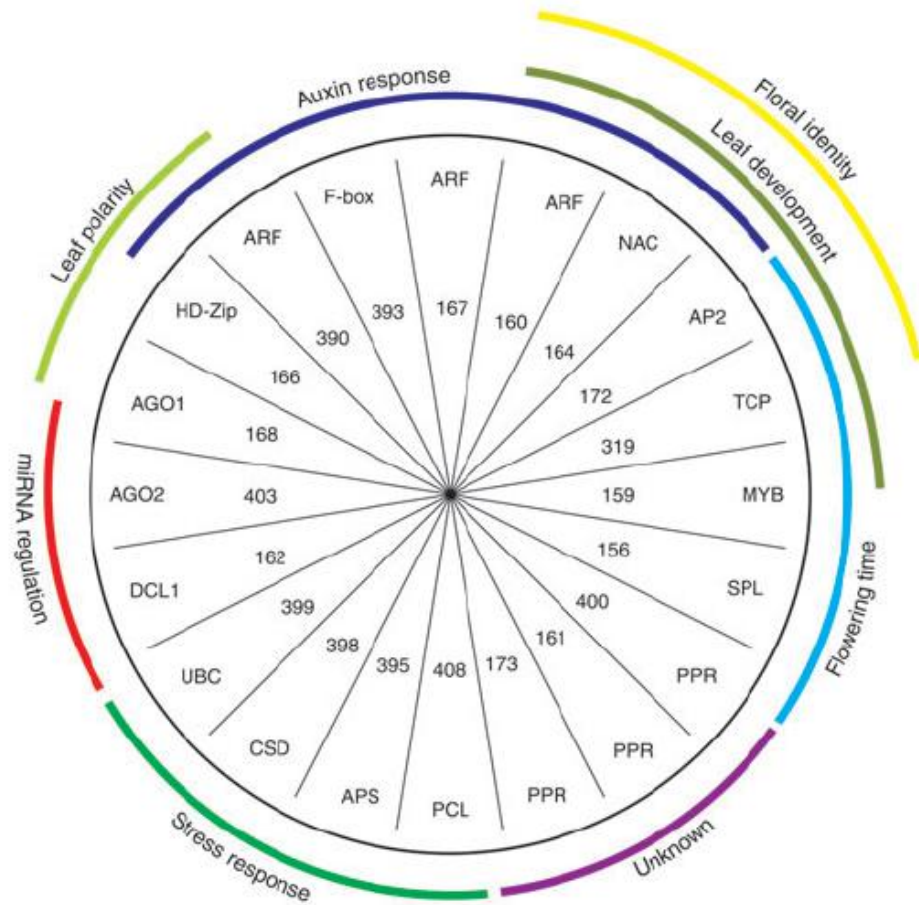
Homology Between mature miRNAs and divergence between pre-miRNAs

Role of small RNAs in crops



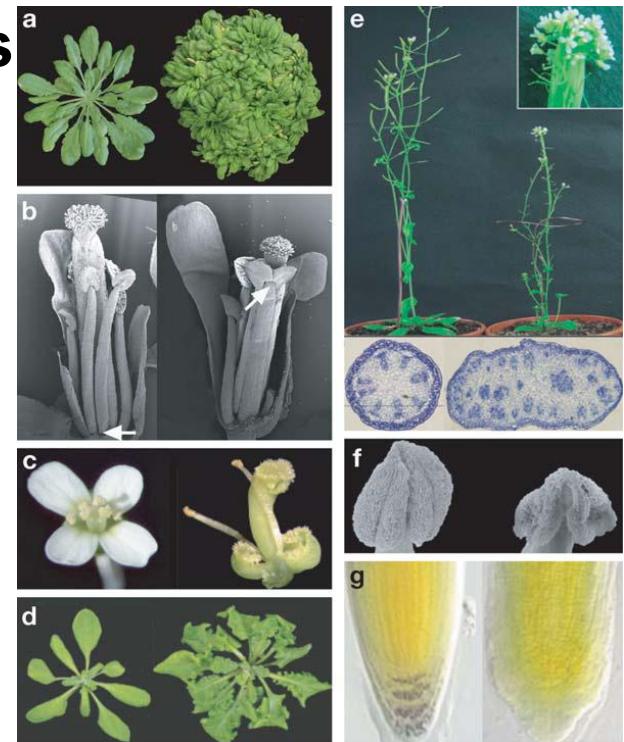
(Khraiweh *et al.* 2011)

Targets of conserved miRNAs

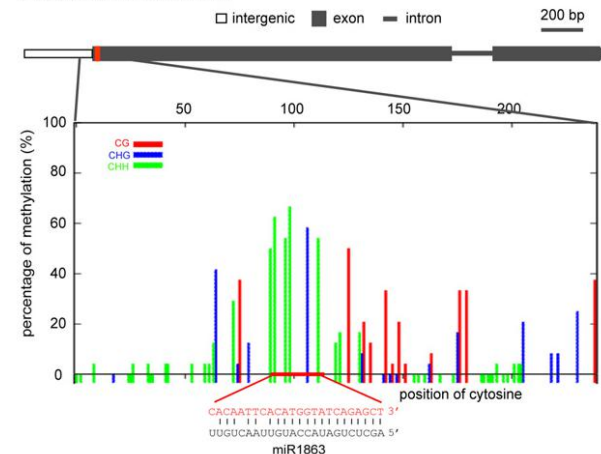


Regulatory Roles of Plant MicroRNAs

- miRNA and vegetative organ development
- miRNA and floral development and vegetative phase change
- miRNAs regulate miRNA and siRNA biogenesis and function
- miRNAs involved in signal transduction
- miRNAs involved in plant disease
- miRNAs involved in environmental stress responses
- Even more, miRNAs and DNA-methylation



Os06g38480 (miR1863 target)



Number of miRNAs

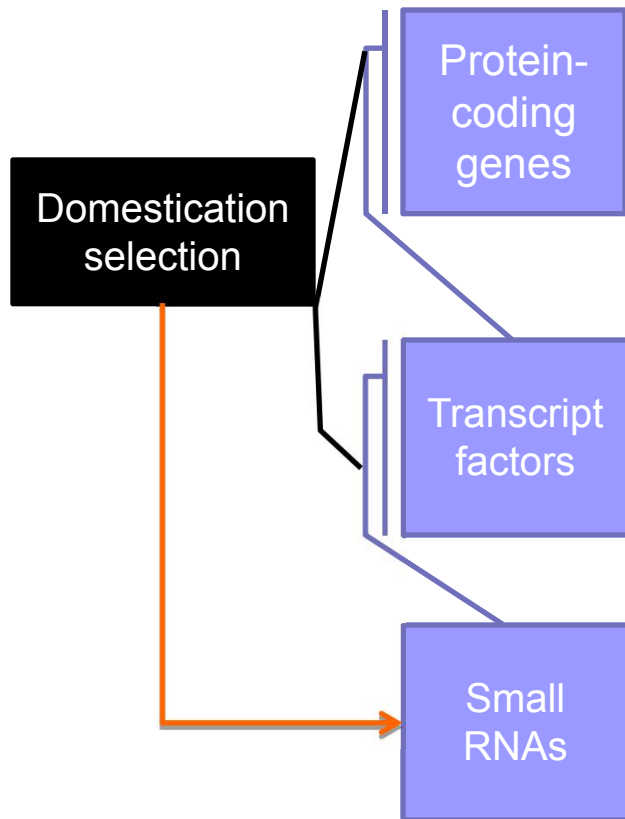
- miRNAs (miRBase, release 22, March 2018)
 - Rice: 604 precursors, 738 mature
 - Soybean: 684 precursors, 756 mature
 - Maize: 174 precursors, 325 mature

Identification of miRNAs by us

- Rice: long miRNA-like (Zhu et al. 2008)
- Tobacco: >200 miRNAs (Tang et al. 2012)
- *O. rufipogon*: >300 miRNAs (Wang et al. 2012); >3000 phasiRNAs (Liu et al. 2013)
- *B. napus*: 360 conserved and 533 novel miRNAs (Shen et al, 2014; Shen et al., 2015)
- *N. benthamiana*: ~200 miRNA (Xiao et al. 2014)

2. Evolution of small RNAs: artificial selection

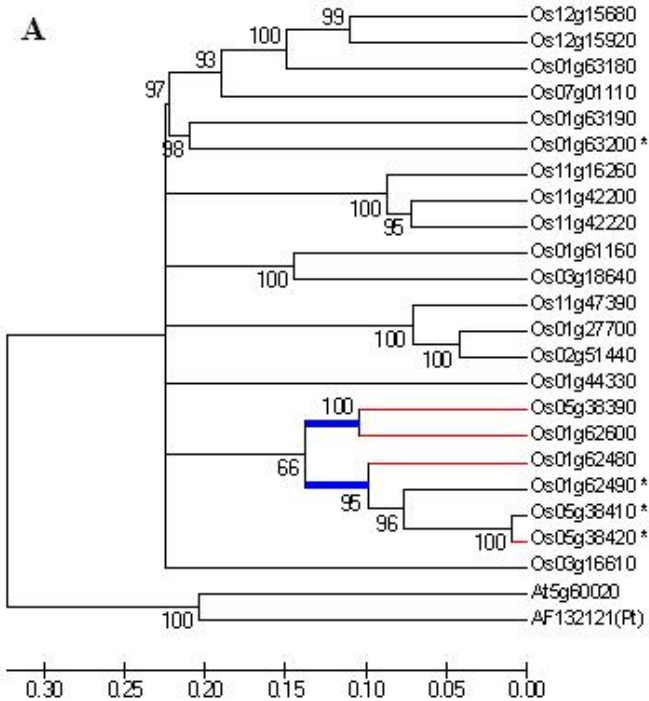
- Role of small RNAs in crop domestication and genetic improvement



- ✓ **Transcript factor targets: protein-coding genes**
- ✓ **Non-coding small RNA targets: transcript factor**
- ✓ **Domestication targets: transcript factor**

Question:

Targets of domestication selection: small RNAs?



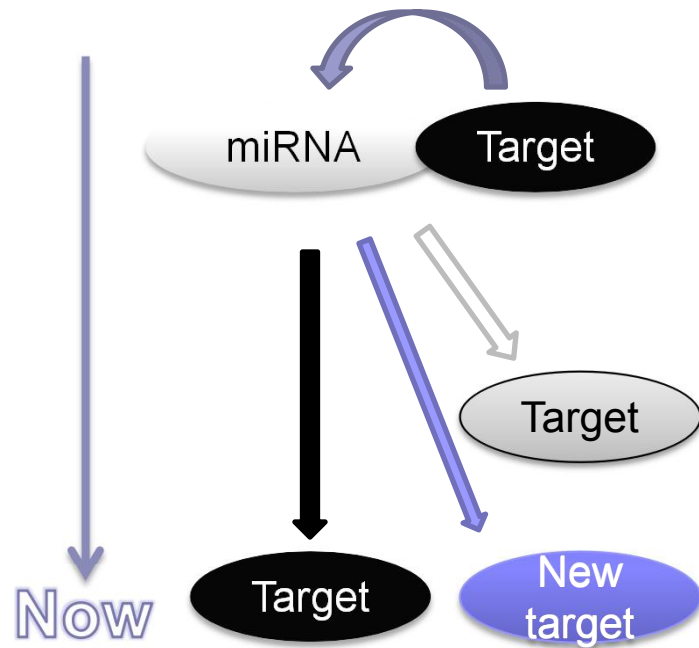
B

```

miR397a 5' GUAGUUGCGACGUGAGUUACU 3'
          |||
Os05g38420 CAUCAACGCGCGCAUCAACGA (1) *
Os01g62480 CAUCAACGCGCGCAUCAACGA (3)
Os05g38390 GAUCAACGCGCGCAUCAACGA (4)
Os01g62600 GAUCAACUCGCGCAUCAACGA (5)
  
```

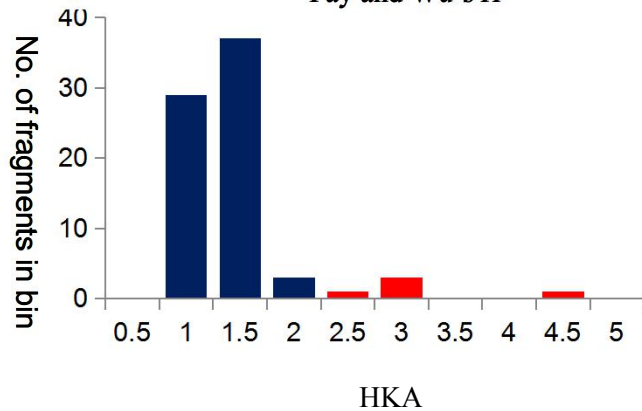
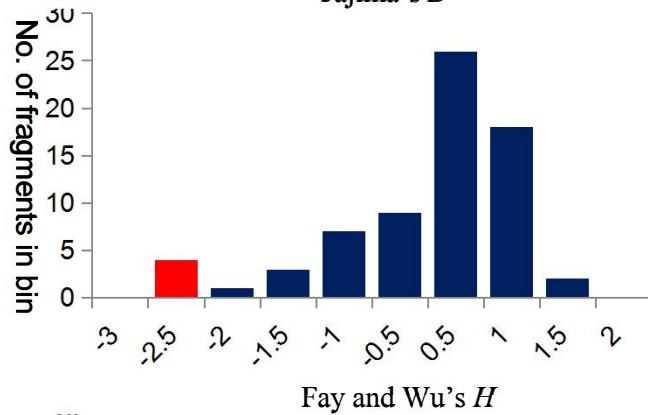
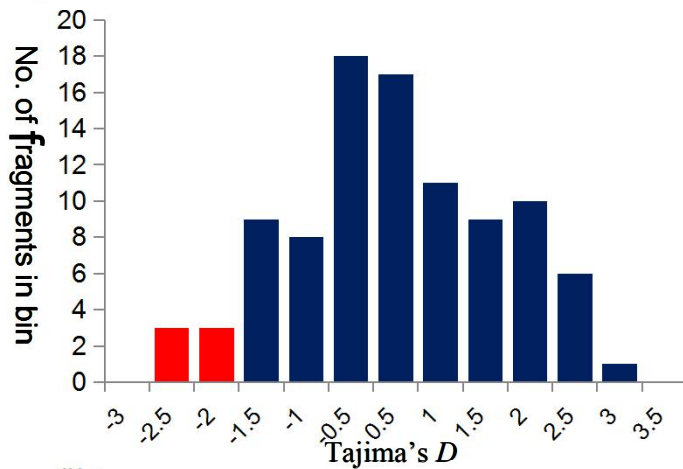
The loss of miR397 binding site in its target family

(Guo *et al.* BMC Genomics, 2008)

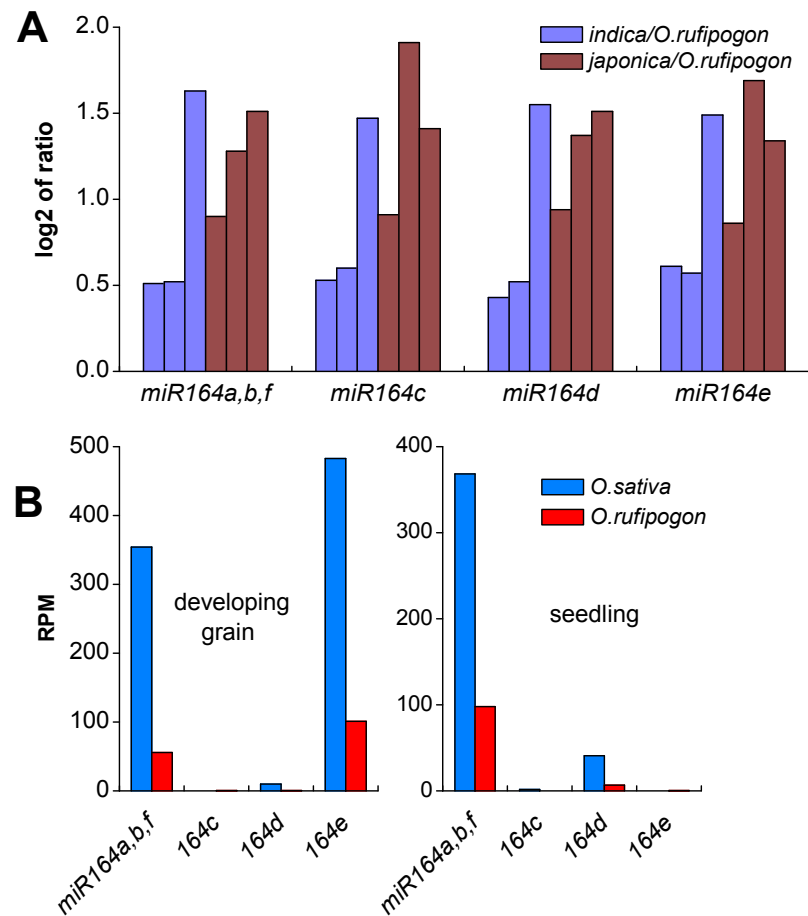


Nucleotide mutations together with insertions/deletions are responsible for the gain/loss of miRNA binding sites during co-evolution of miRNAs and their target genes.

Question: How about domestication selection?



Distribution of Tajima's D , Fay and Wu's H and HKA test values across all small RNA sequencing fragments in cultivated rice populations. Significant ($<0.05\%$) fragments under positive selection are colored red.



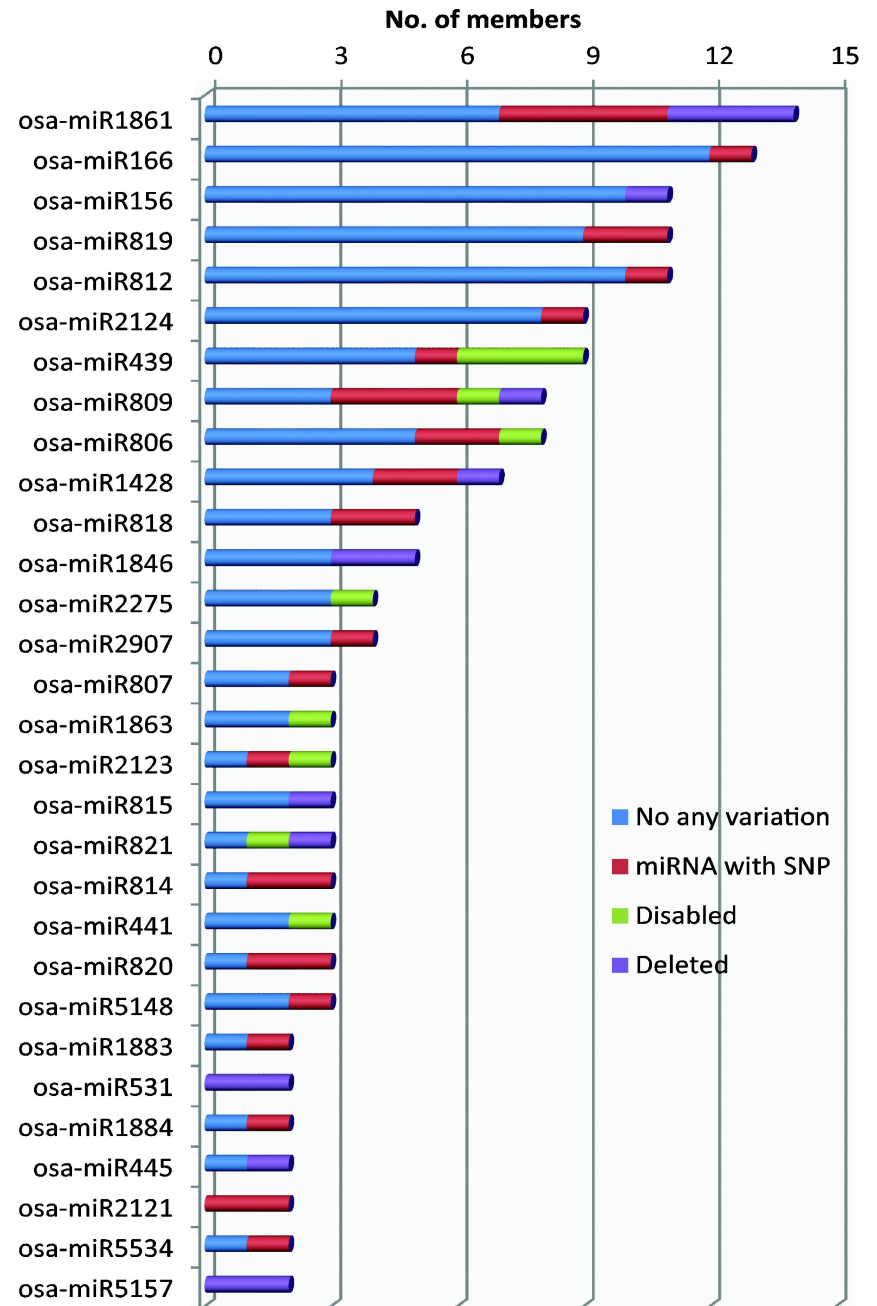
Expression divergence of the miR164 family in cultivated and wild rice. A: Log2 of the expression ratios of miR164 in six microarray chips including six cultivars and six *O. rufipogon* lines; **B:** The expression of miR164 family in developing grain and seedling as indicated by reads per million (RPM) in *O. sativa* (*japonica*) and *O. rufipogon* small RNA populations through Illumina sequencing.

miR164 family: selection evidence

Title	<i>indica</i>			<i>japonica</i>			<i>rufipogon</i>		
	Seq_No.	Tajima'D	P (H test)	Seq_No.	Tajima'D	P (H test)	Seq_No.	Tajima'D	P (H test)
miR164a	25	1.544	0.0772	22	-1.3099	0.7128	11	-0.8448	0.5446
miR164b	23	-1.1361	0.2312	21	-0.0466	0.8038	12	-0.0143	0.3002
miR164c	23	-0.5139	0.021	23	-0.6312	0.001	8	0.4691	0.105
miR164d	25	-0.4764	0.0028	21	-1.2874	0.0966	12	-1.5419	0.2304
miR164e	21	-1.9033	0.003	21	1.1834	0.6054	10	-1.0403	0.905
miR164f	22	NA	NA	22	NA	NA	10	NA	NA

Genomic variations in precursors of 38 previously identified conserved miRNA families in wild rice.

(Wang *et al*, 2012, New Phytologist)



miRNAs are targets of artificial selection

- Our results illustrated *MIRNA* genes, like protein-coding genes, might have been significantly shaped during rice domestication and could be one of the driven forces contributed to rice domestication.

3. Identification of small RNAs

- miRNAs
- phsiRNAs

Prediction of *MIRNA* gene

- 3 stages:

- 1. genetic screening technology; direct cloning of miRNAs → small RNA isolation
- 2. computational approaches
- 3. prediction based on high throughput sequencing data

Data mining based on high throughput sequencing technology

Small RNA sequencing:

- SE, < 50nt
- Millions of reads
- High sensitivity
- FASTQ & FASTA

Pipeline:

- Quality filtering
- Trim adaptors
- eliminate the redundancy
- Mapping to reference sequence
- Removing small RNA mapping to tRNA, rRNA, snRNA, snoRNA, mRNA et al.
- Prediction (software or webserver, based on structure and property of miRNA)

Item	Root			Leaf
	Topping	Wounding	Control	(wounding)
Total				
Raw reads	5,186,473	5,510,747	3,074,948	4,722,862
Unique raw reads	1,959,287	3,083,036	1,018,809	2,178,969
Mapped to GSS[#]				
Reads	2,632,084	2,700,532	1,277,575	2,469,534
Specific in each library	477,279	803,960	206,310	491,809
Conserved in all libraries	1,667,977	1,085,172	782,079	1,312,822
Unique	542,516	965,547	252,425	687,949
Specific in each libraries	385,469	702,522	148,981	442,337
Conserved in all libraries	25,780	25,780	25,780	25,780
Singleton	417,905	761,185	202,567	534,409
Mapped to EST[#]				
Reads	1,293,204	1,218,970	764,235	1,528,346
Specific in each libraries	99,553	137,509	43,758	107,459
Conserved in all libraries	985,513	811,452	563,565	1,165,580
Unique	129,710	186,601	72,750	149,454
Specific in each libraries	77,511	116,666	32,875	84,638
Conserved in all libraries	14,001	14,001	14,001	14,001
Singleton	88,566	131,515	48,486	102,406

Prediction of miRNA's targets

miRU (update to psRNATarget)

<http://bioinfo3.noble.org/miRNA/>

[miRU.htm](http://bioinfo3.noble.org/miRNA/miRU.htm)

(Dai and Zhao, 2011)

Principle

1. ≤ 4 mismatches (at most 7)

2. Nearly none of mismatch allowed between 1st to 10th position from 5' end, especially 9th to 11th position

3. One to many

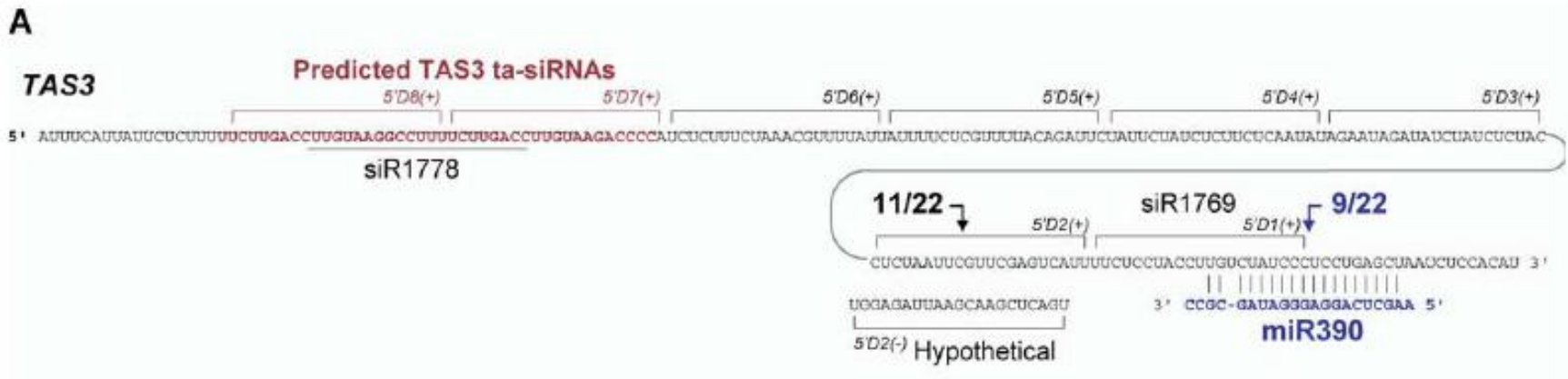
The screenshot shows the psRNATarget web interface. At the top, it identifies the user as 'The Samuel Roberts Noble Foundation' and the user as 'The Zhao Bioinformatics Laboratory'. The page title is 'psRNATarget: A Plant Small RNA Target Analysis Server'. There are navigation links for 'About', 'Citation', and 'Analysis'. The current location is 'Analysis'. There are three tabs: 'User-submitted small RNAs / preloaded transcripts', 'Preloaded small RNAs / user-submitted transcripts', and 'User-submitted small RNAs / user-submitted transcripts'. The main form has a section for 'Upload small RNA sequence(s) in FASTA format:' with a 'Load demo data' link and a 'Browse...' button. Below this is a text area 'or paste sequences below:'. There are two error messages: '- file / input sequence size limit: 20M.' and '- invalid small RNAs will be ignored during analysis.'. A dropdown menu 'Select a preloaded transcript genomic library for target search:' lists various species and genome versions. Below this is a 'Request to add / update a transcript library:' link. There are several input fields for search parameters: 'Maximum expectation' (3.0), 'Please set a more relaxed cut-off threshold' (4.0-5.0), 'Length for complementarity scoring' (20), 'Target accessibility' (25.0), 'Flanking length around target site' (17 bp upstream, 13 bp downstream), and 'Range of central mismatch leading to translational inhibition' (9 - 11 nt). At the bottom, there are 'Submit' and 'Help' buttons.

Prediction of phasiRNA

Howell et al., 2007

$$P = \ln\left[1 + \sum_{i=1}^8 k_i\right]^{n-2}, P > 0,$$

n = number of phase cycle positions occupied by at least one small RNA read within an eight-cycle window,³²
 k = the total number of reads for all small RNAs with consolidated start coordinates in a given phase within an eight-cycle window.
 Phase cycle length was set at 21 nucleotides. A positive phase signal, therefore, was limited by the power function (n-2) to those loci with small RNAs occupying at least three cycle positions in a phased, 21-nucleotide register.



4. Related database

-miRBase

<http://www.mirbase.org/>



Latest miRBase blog posts

[MicroRNA Gene Ontology annotations](#)

You might have noticed some additional information on the mature miRNA pages in the last few weeks. See for example: http://mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT0000123 http://mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT0000069 The new section "QuickGO function" contains a set of high quality manual annotations of Gene Ontology terms for mature miRNAs, the vast majority of which come from the work of Rachael Huntley et [...]

By [sam](#) (June 7, 2018)

[miRBase 22 release](#)

After repeated and unreasonable delay, miRBase 22 is finally released. As you might expect with such a long gap, the number of sequences in the database has jumped significantly — by over a third. The vast majority of the increase comes from new microRNA annotations in species not previous represented in the database. Indeed, there [...]

By [sam](#) (March 12, 2018)

miRNA count: 38589 entries

[Release 22](#): March 2018

Search by miRNA name or keyword

Download published miRNA data

[Download page](#) | [FTP site](#)

miRBase: the microRNA database

miRBase provides the following services:

- The [miRBase database](#) is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for [searching](#) and [browsing](#), and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also [available for download](#).
- The [miRBase Registry](#) provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the [help pages](#) for more information about the naming service.

To receive email notification of data updates and feature changes please subscribe to the [miRBase announcements mailing list](#). Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

miRBase is managed by the [Griffiths-Jones lab](#) at the [Faculty of Biology, Medicine and Health, University of Manchester](#) with funding from the [BBSRC](#). miRBase was previously hosted and supported by the [Wellcome Trust Sanger Institute](#).

References

If you make use of the data presented here, please cite the following articles in addition to the primary data sources:

[miRBase: annotating high confidence microRNAs using deep sequencing data.](#)

Kozomara A, Griffiths-Jones S.
NAR 2014 42:D68-D73

[miRBase: integrating microRNA annotation and deep-sequencing data.](#)

Kozomara A, Griffiths-Jones S.
NAR 2011 39:D152-D157

[miRBase: tools for microRNA genomics.](#)

Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ.
NAR 2008 36:D154-D158

[miRBase: microRNA sequences, targets and gene nomenclature.](#)

Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ.
NAR 2006 34:D140-D144

[The microRNA Registry.](#)

Griffiths-Jones S.

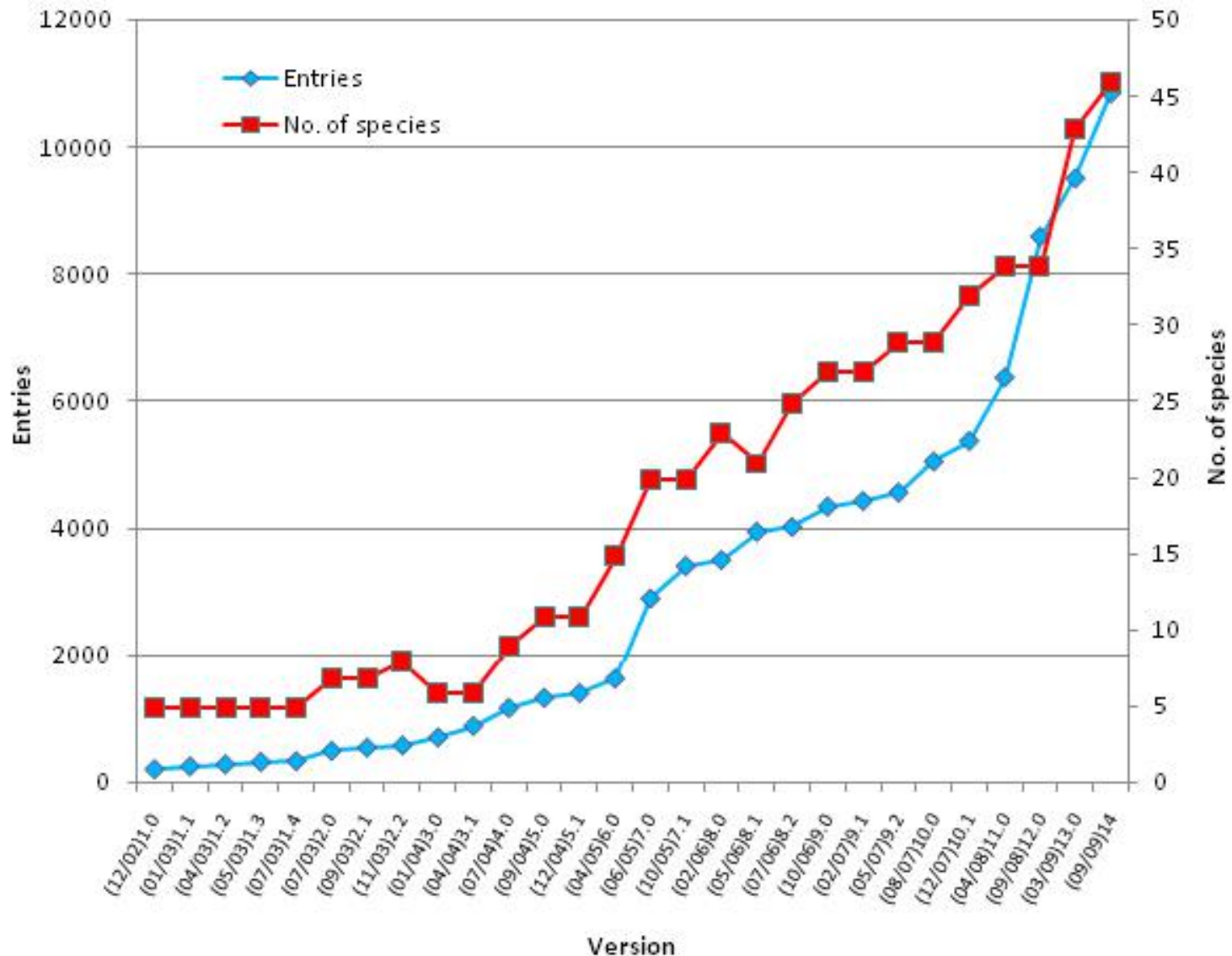
miRBase Genome Browser

<http://www.mirbase.org/cgi-bin/browse.pl>

■ monocotyledons

- [Asparagus officinalis](#) (101 precursors, 101 mature) [Aspof.V1]
- [Aegilops tauschii](#) (88 precursors, 173 mature) [ASM34733v2]
- [Brachypodium distachyon](#) (317 precursors, 525 mature) [Bd21]
- [Elaeis guineensis](#) (6 precursors, 6 mature)
- [Festuca arundinacea](#) (15 precursors, 15 mature)
- [Hordeum vulgare](#) (69 precursors, 71 mature) [Hvul_cvHarunaNijo3HBAC01]
- [Oryza sativa](#) (604 precursors, 738 mature) [MSU7]
- [Sorghum bicolor](#) (205 precursors, 241 mature) [Sorghum_bicolor_NCBIv3]
- [Saccharum officinarum](#) (16 precursors, 16 mature)
- [Saccharum sp.](#) (19 precursors, 20 mature)
- [Triticum aestivum](#) (122 precursors, 125 mature)
- [Triticum turgidum](#) (1 precursors, 1 mature)
- [Vriesea carinata](#) (33 precursors, 65 mature)
- [Zea mays](#) (174 precursors, 325 mature) [B73_RefGen_v4]

The Increasing records in miRBase



Stem-loop sequence MI0000653

Accession	MI0000653	
ID	osa-MIR156a	
Description	Oryza sativa miR156a stem-loop	
Stem-loop	<pre> g - - a uguu uu aau ggagg ugacaga agaga gugagcac cguggu ucc gcaua g ccucc acugucu ucucu cacucgug gcaucg agg cguau u - c u c - - - - uu ccg </pre> <p>Get sequence</p>	
Deep sequencing	1111 reads, 3 experiments	
Comments	The stem-loop sequence represented here is predicted based on homology to miRNAs rice.	
Genome context	<i>Coordinates</i> 01: 22523102-22523201 [-]	<i>Overlapping transcripts</i> intergenic
Gene family	MIPF0000008; MIR156	

Mature sequence MIMAT0000618

Accession	MIMAT0000618
ID	osa-miR156a
Sequence	7 - ugacagaagagagugagcac - 26 Get sequence
Deep sequencing	1110 reads, 3 experiments
Evidence	by similarity; MI0000183

References

- 1 PMID:[12101121](#)
"MicroRNAs in plants"
Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP
Genes Dev. 16:1616-1626(2002).