The 19th Crucifer Genetics Workshop and Brassica 2014
“Genetic Improvement of Brassicaceae Crops in the Era of Genomics”

Comparative epigenomic analysis between model plant and crop

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Institute of Genetics and Developmental Biology
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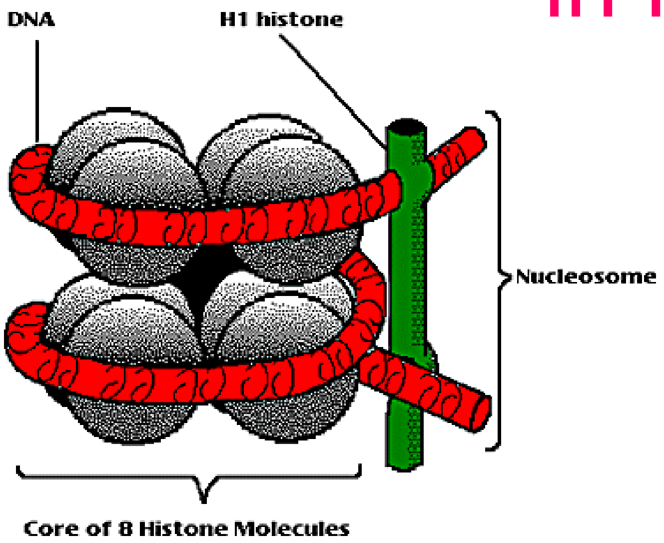
Mechanisms of epigenetic regulation in higher plants

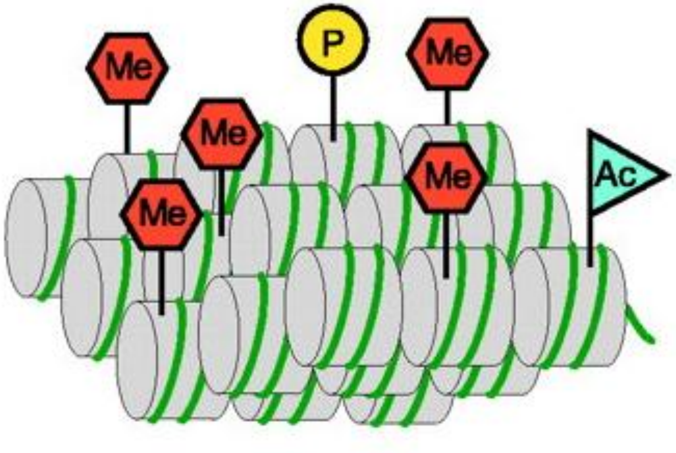


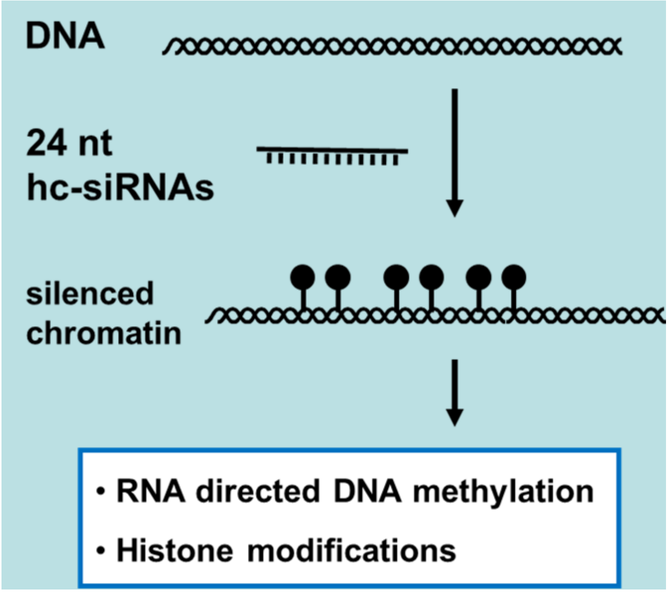
Nucleosome

-**DNA methylation**

-**Histone modification and variants**

-**Regulatory non-coding RNAs**

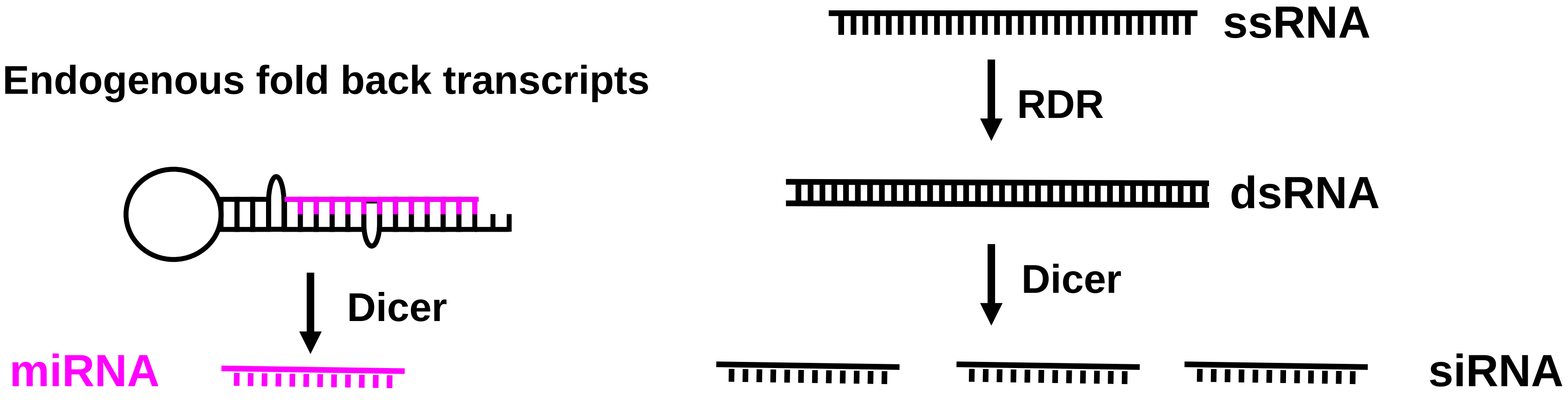




- RNA directed DNA methylation
- Histone modifications

Endogenous small RNA in plants

- **Higher plants are enriched in two types of endogenous small RNAs: microRNAs (miRNAs) and small interfering RNA (siRNAs)**
- **21-24nt in length of non-coding RNAs**
- **Play key roles in regulation of development, various biotic and abiotic stress responses and genome stability**



Endogenous fold back transcripts

Dicer

miRNA

ssRNA

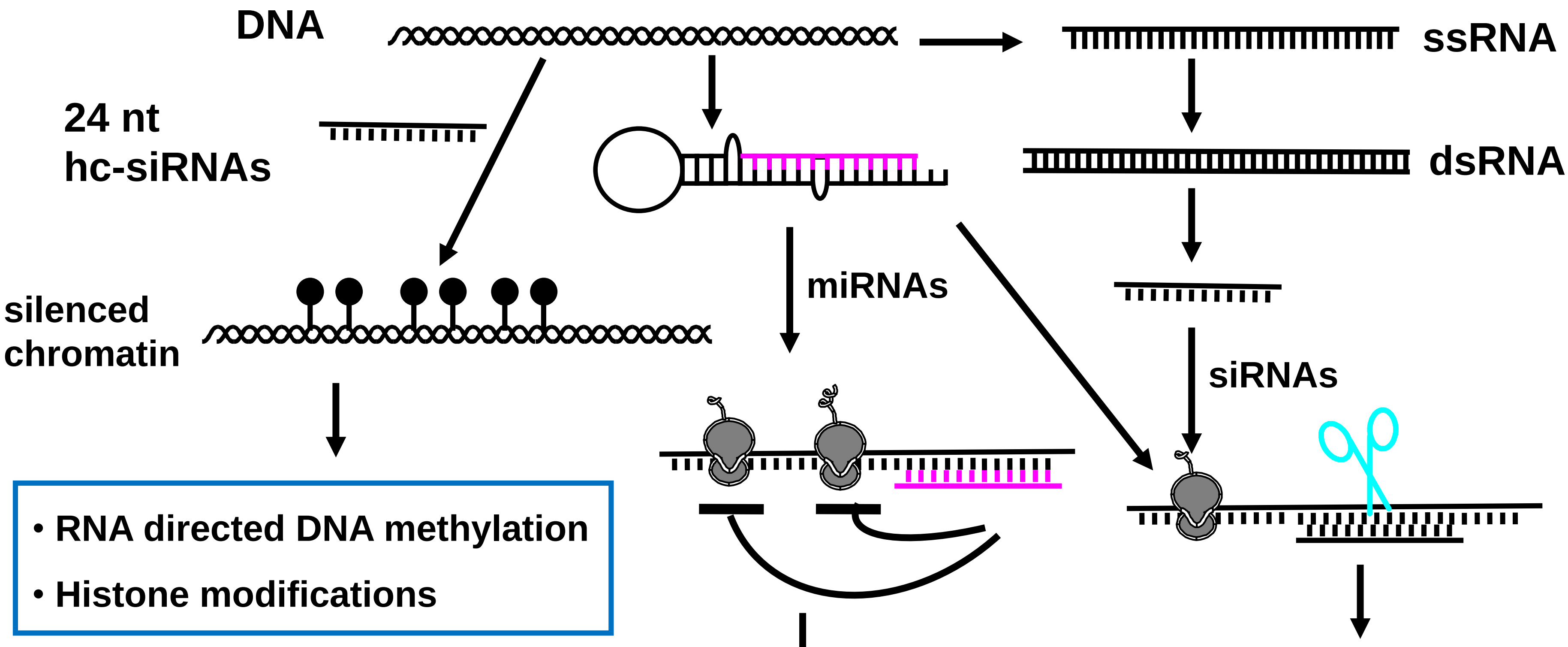
RDR

dsRNA

Dicer

siRNA

Mechanisms of small RNAs silencing



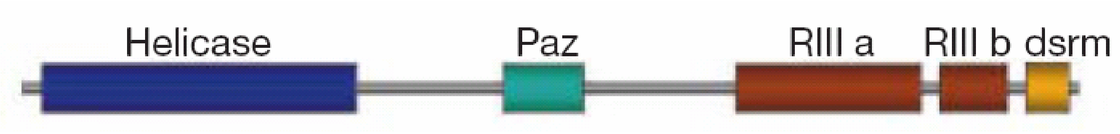
- RNA directed DNA methylation
- Histone modifications

miRNAs recognize 3’UTR sites and inhibit translation

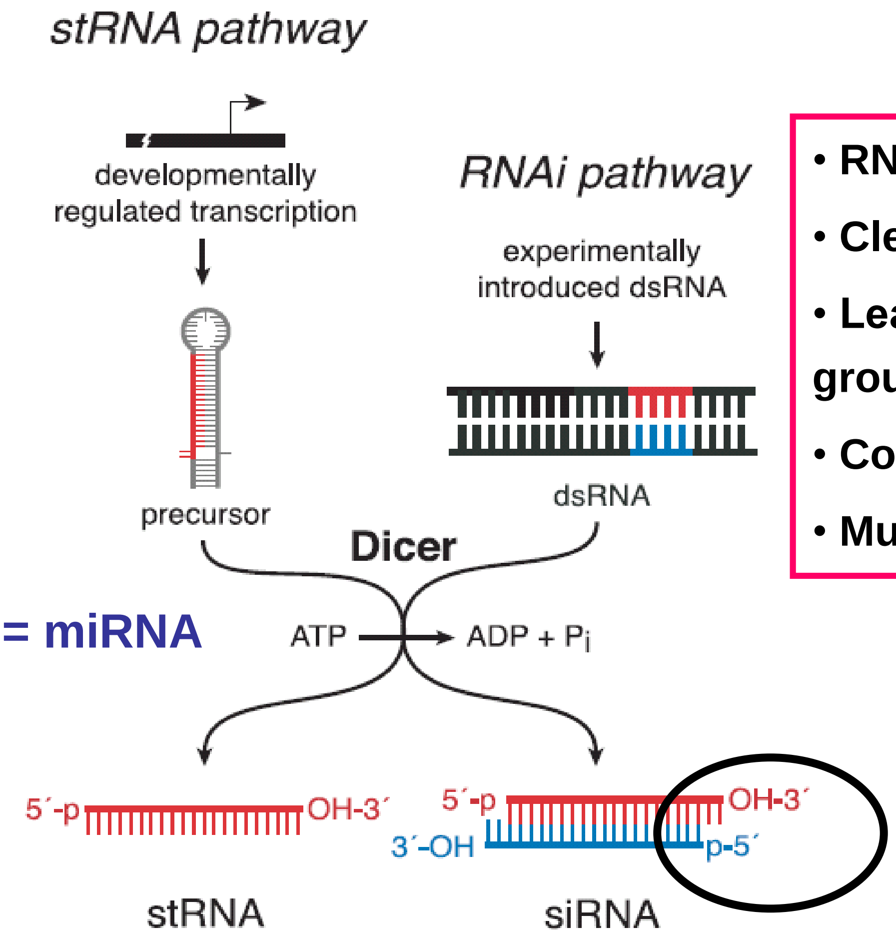
miRNAs /siRNAs pairing with mRNAs to direct cleavage and degradation of mRNAs

- Transcriptional
- Posttranscriptional levels

Dicer: a key protein in small RNA biogenesis



***C. elegan* Dicer**



stRNA = miRNA

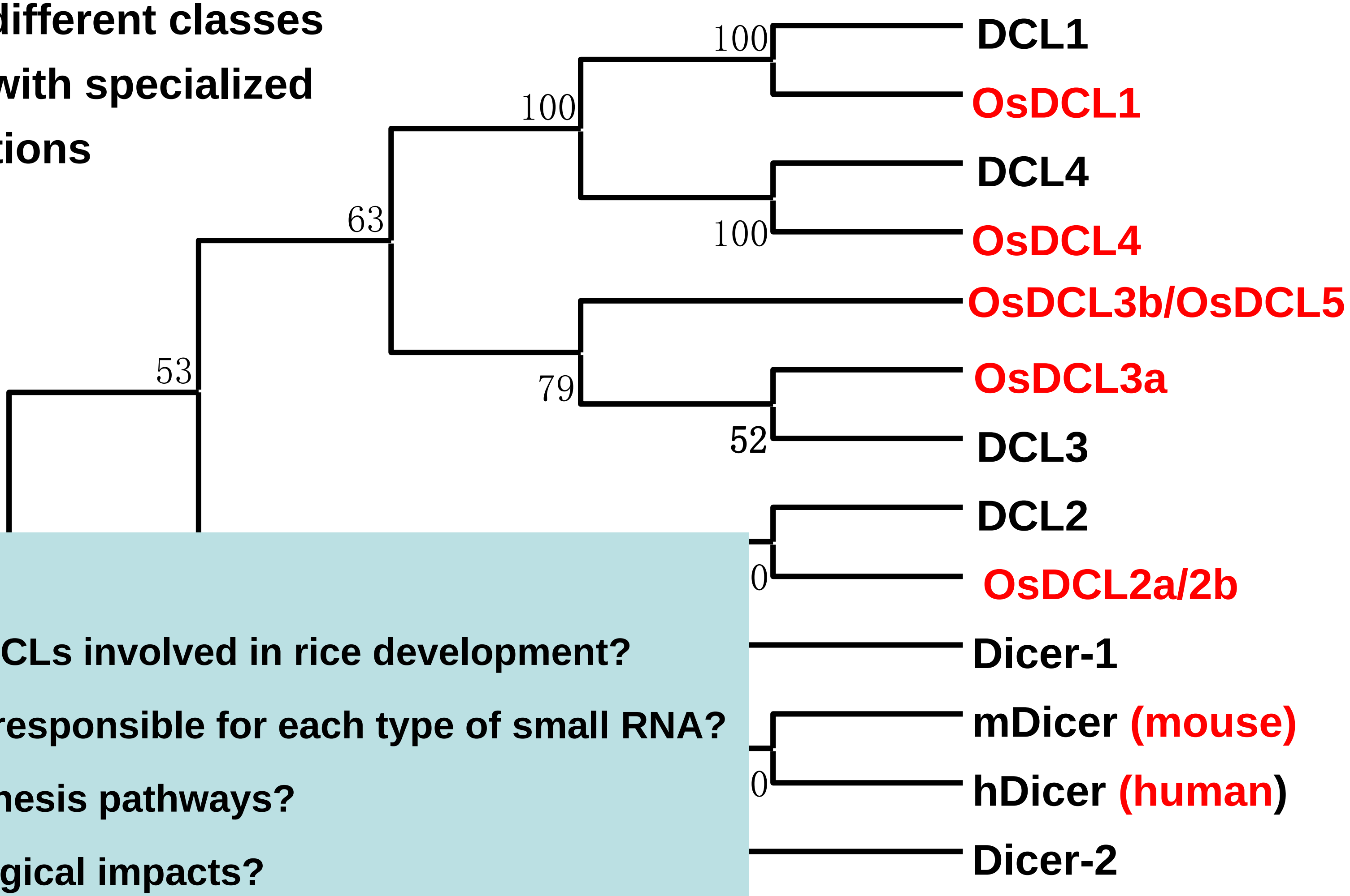
- **RNase III like enzyme**
- **Cleaves dsRNAs or pri/pre-miRNAs**
- **Leaves 3’ overhangs and 5’ phosphate groups**
- **Conserved functional domains**
- **Multiple Dicer genes in fly and plants**

(Bernstein *et al.* 2001)

(Hutvagner *et al.* 2001)

Higher plants have evolved multiple members of RNase III family of proteins

accommodate different classes of small RNAs with specialized molecular functions



Questions:

Are different OsDCLs involved in rice development?

Which OsDCL is responsible for each type of small RNA?

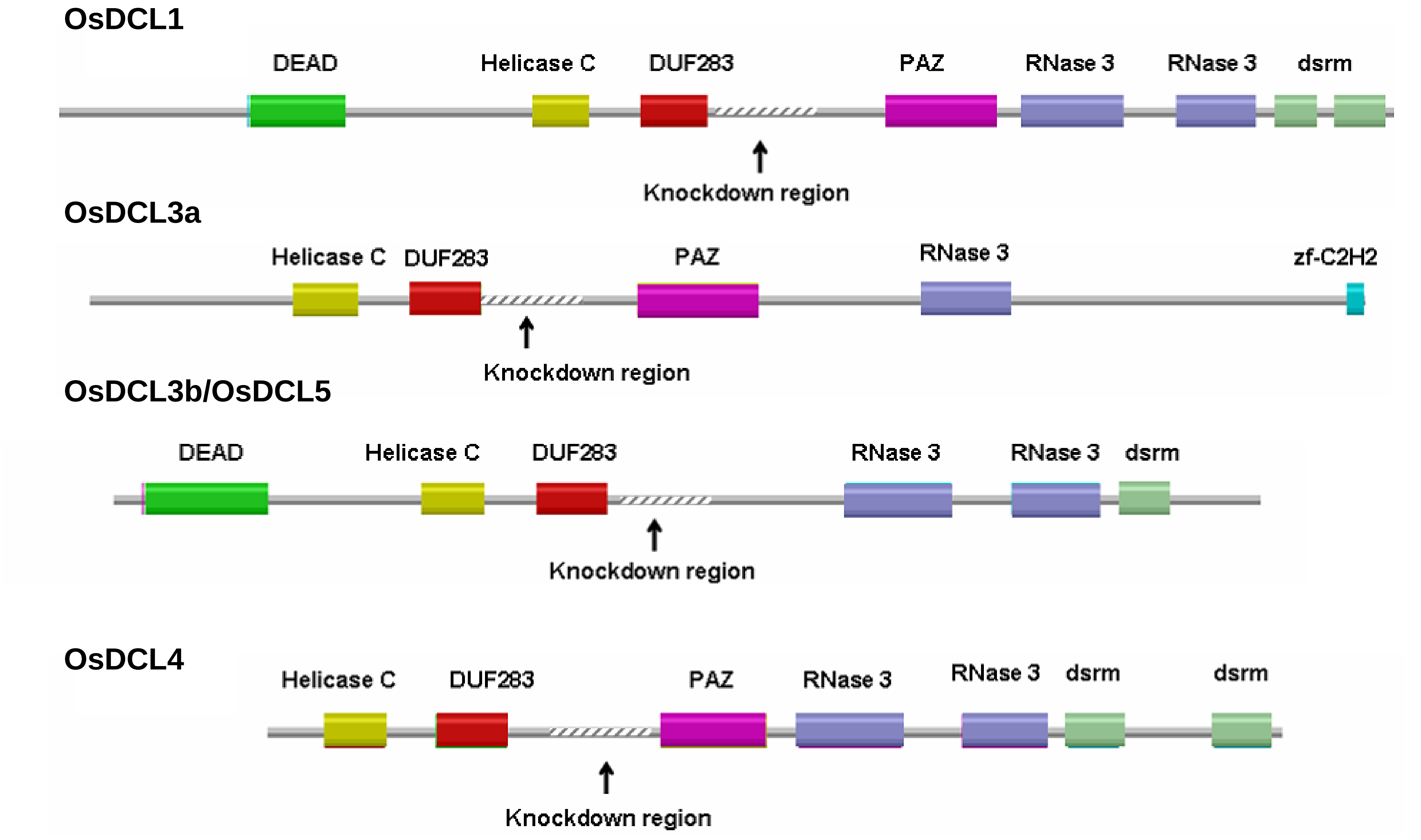
Small RNA biogenesis pathways?

Targets and biological impacts?

Different functions of DCLs in Arabidopsis

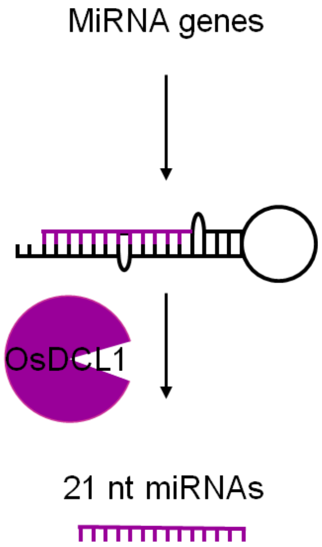
- DCL1: 21-22nt miRNA, developmental control
- DCL2: 22nt siRNA, virus induced siRNAs
- DCL3: 24nt siRNA, RdDM/chromatin
- DCL4: 21nt siRNA and ta-siRNA, RNA degradation

Schematic representation of conserved motifs among DCL proteins in rice

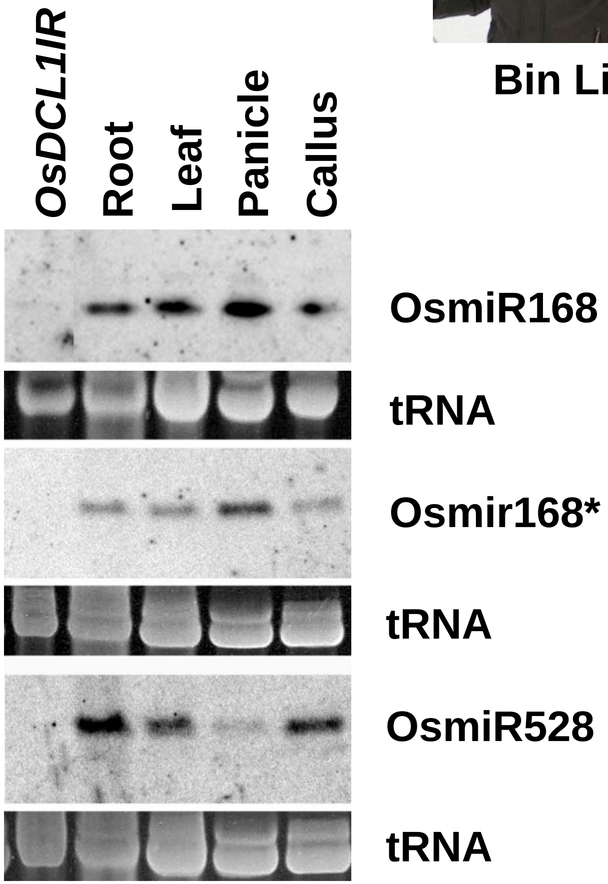


Morphology of *OsDCL1IR* plants showing pleiotropic phenotypes

Bin Liu



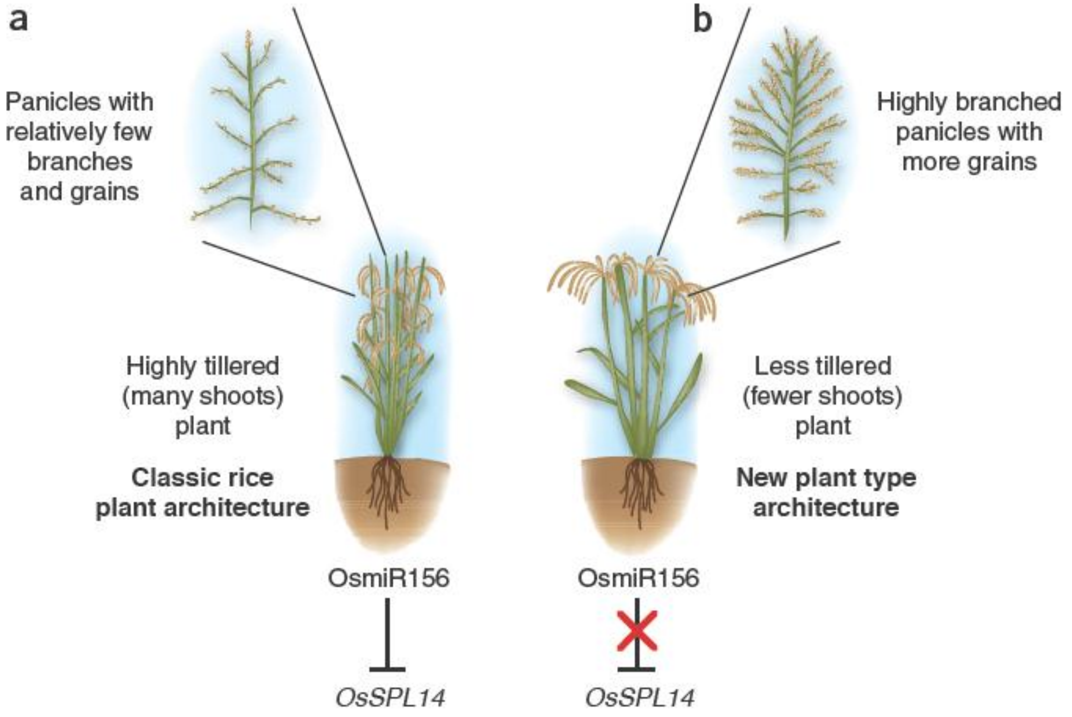
- ***The dlc1*** **null in *Arabidopsis* and rice are lethal.**
- **The weak *OsDCL1IR* lines: narrow, rolled, outward-folded leaves; fewer adventitious roots; fewer tillers; No homozygous obtained in later generations**

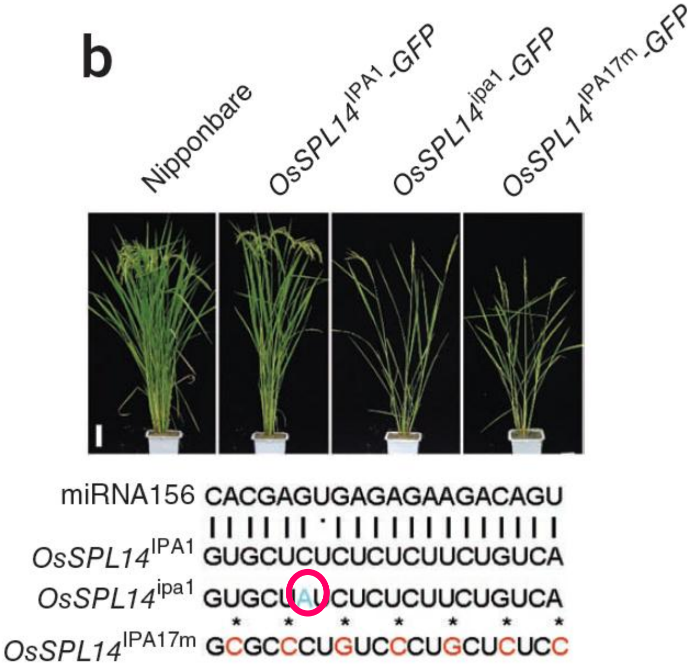


OsDCL1 **is required for miRNA accumulation**

Liu *et al*., (2005）Plant Physiology

Rice miRNA156/*OsSPL14* pair has significant impacts on breeding: ‘ideal’ plant architecture (IPA)

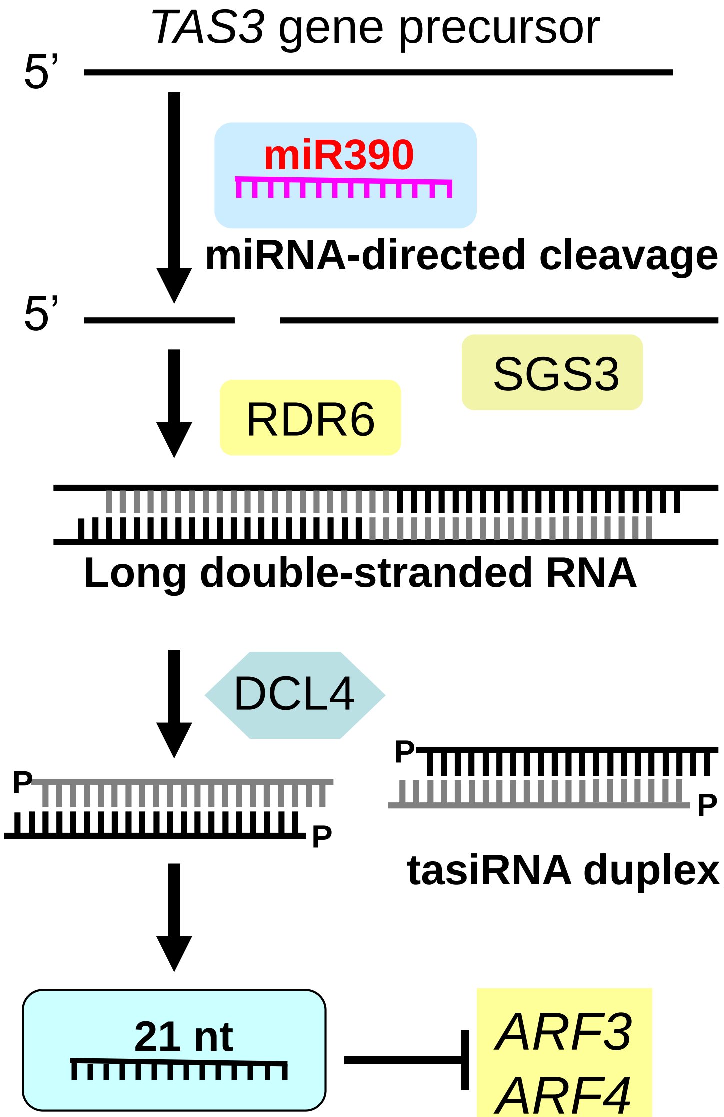




- **Illustrates how regulatory variation at the rice gene *OsSPL14* can lead to altered plant morphology and improve grain yield.**
- **Supports the possibility of improving rice yield through changing miRNA/target pair for altering plant architecture.**

Jiao Y, *et al*. (2010) *Nat Genet 42(6):541-544.*
Miura K, *et al.* (2010) *Nat Genet 42(6):545-549.*
Springer N (2010) *Nat Genet 42(6):475-476.*

ta-siRNA: cross talk between miRNA and siRNA



TAS3 gene precursor

5'

miR390

miRNA-directed cleavage

5'

SGS3

RDR6

Long double-stranded RNA

DCL4

P

P

P

P

tasiRNA duplex

21 nt

ARF3
ARF4

ARF3* and *ARF4* are targets of TAS3 tasiRNAs (tasiR-*ARFs*) in *Arabidopsis

Col-O

dcl4

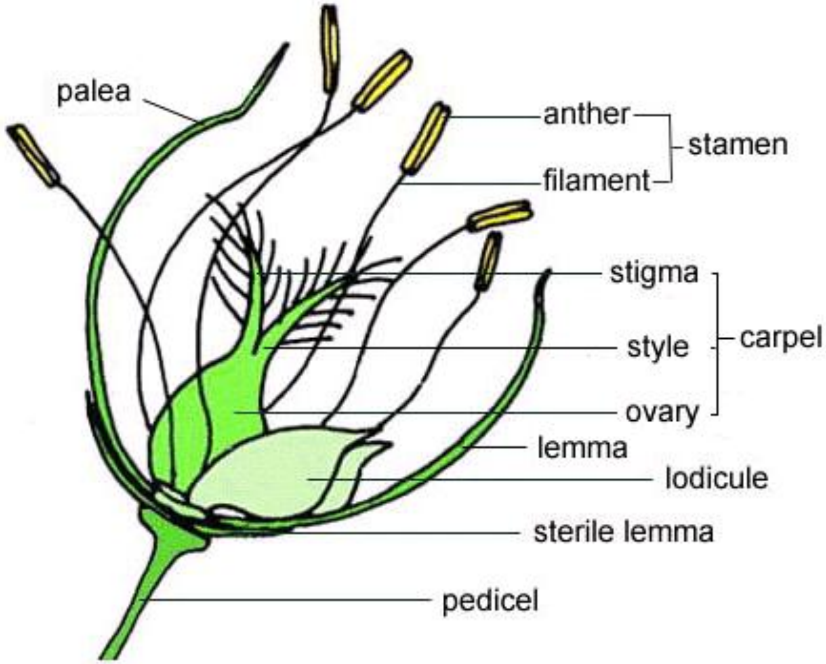
- **Narrow and curly leaves**
- **Accelerated juvenile to adult phase change**

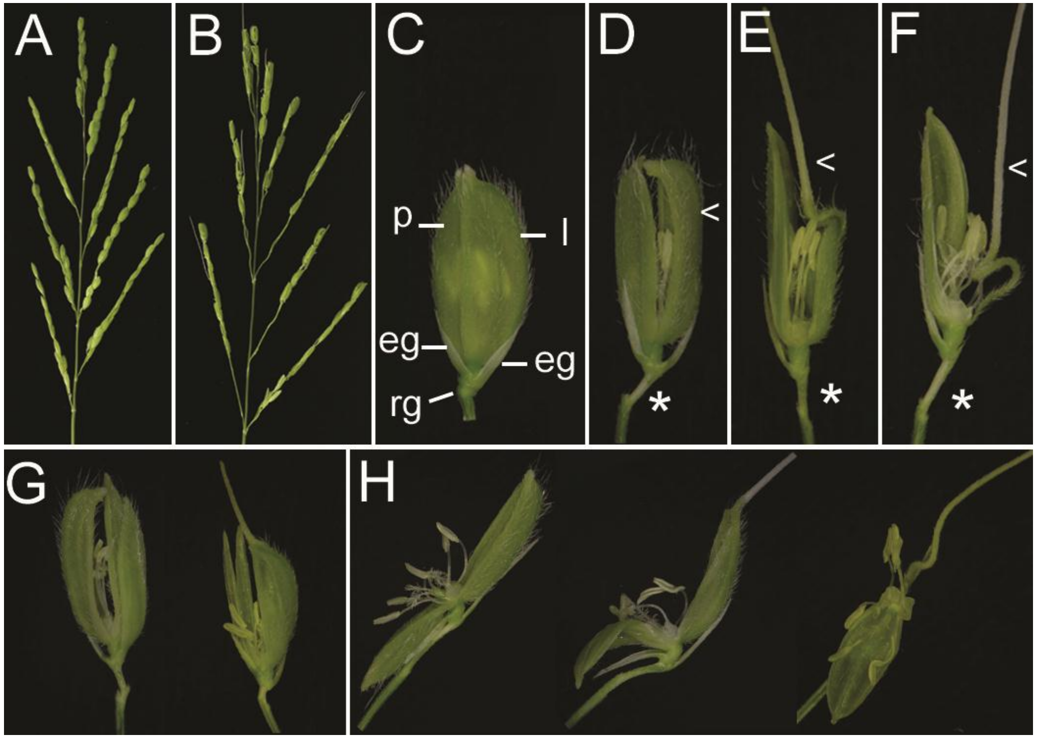
Xie *et al.*, (2005) PNAS

Yoshikawa *et al.*, (2005) Gene & Dev

Knockdown of *OsDCL4* results in abnormal spikelet morphology

Bin Liu

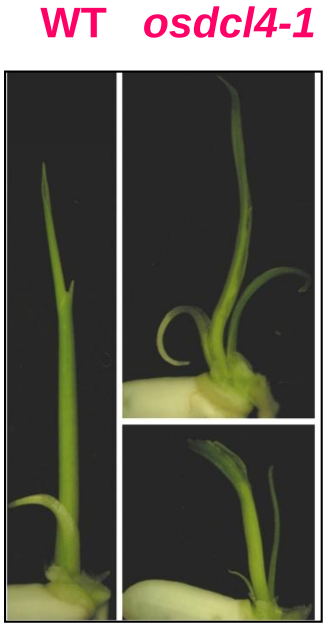


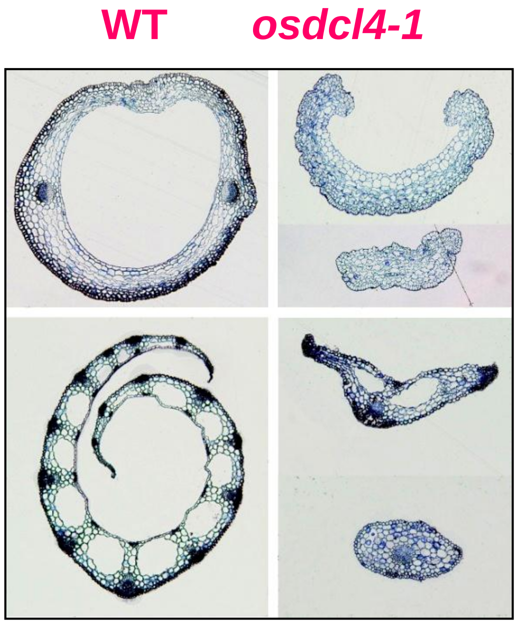


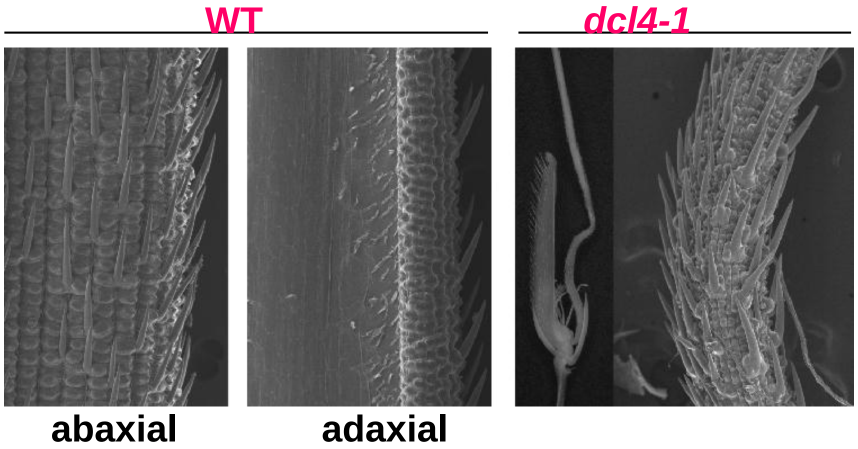
In *Nipponbare* (*Japonica* variety) background

Liu *et al*., (2007) Plant Cell

Rice DCL4 plays broader roles in developmental regulation than its Arabidopsis counterpart



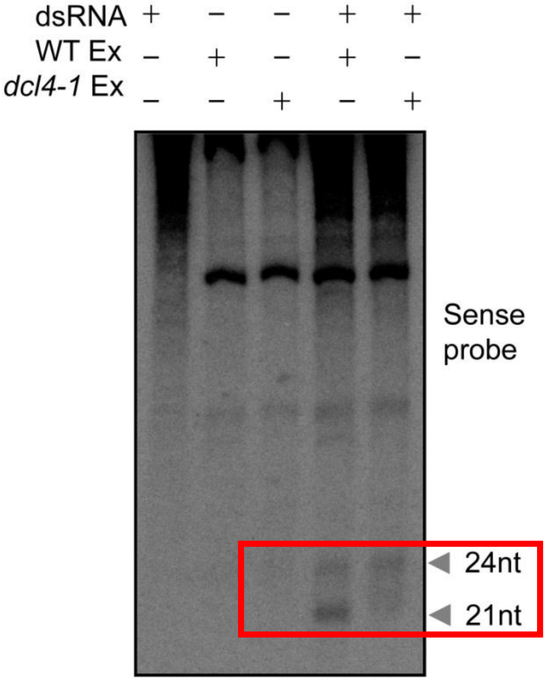




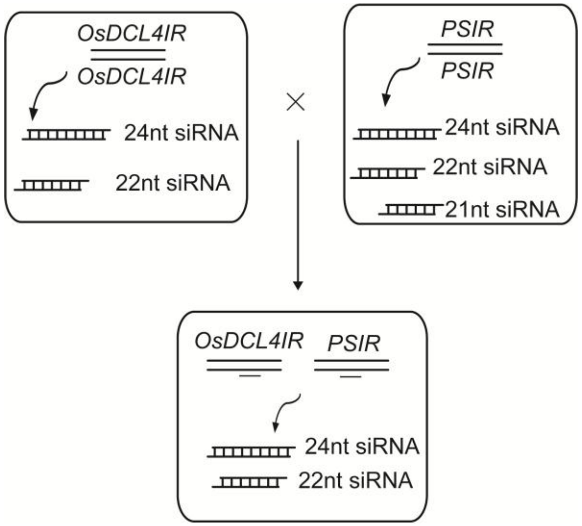
- **Abnormal spikelet with degenerated lemma**
- **Affects coleoptiles and first leaf sheath in early vegetative development**
- **Altered lateral organ polarity and vascular tissue development**

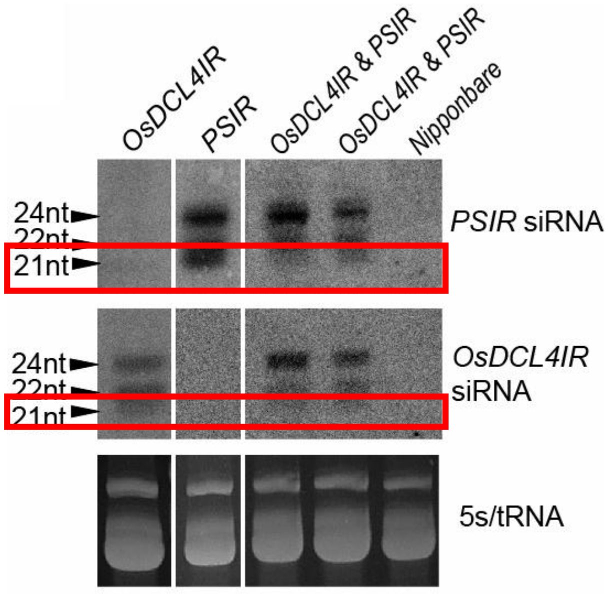
OsDCL4 catalyzes 21nt siRNA production

***In vitro* biochemical analysis**

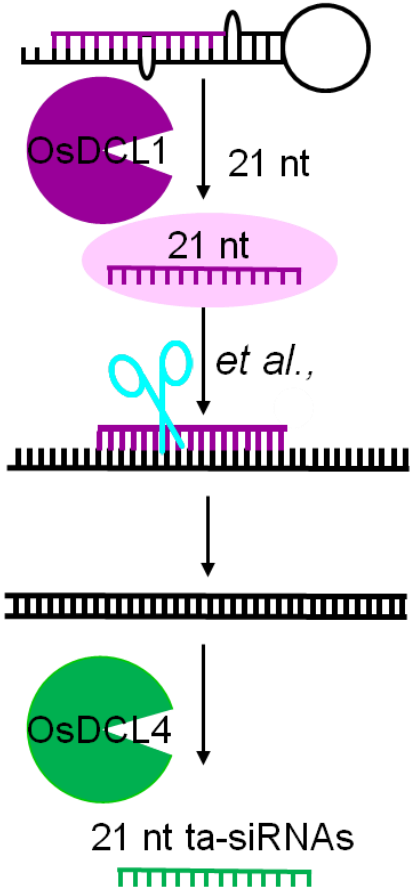


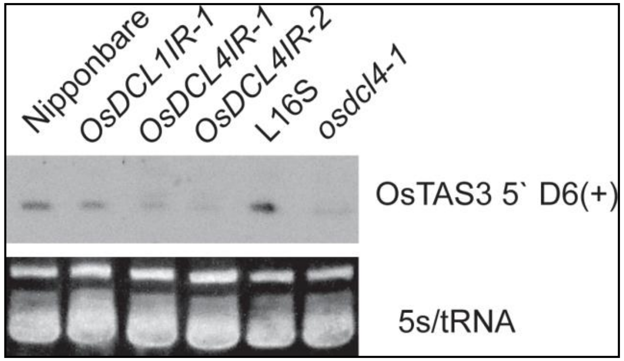
Inverted repeats associated with transgenes or endogenous genes

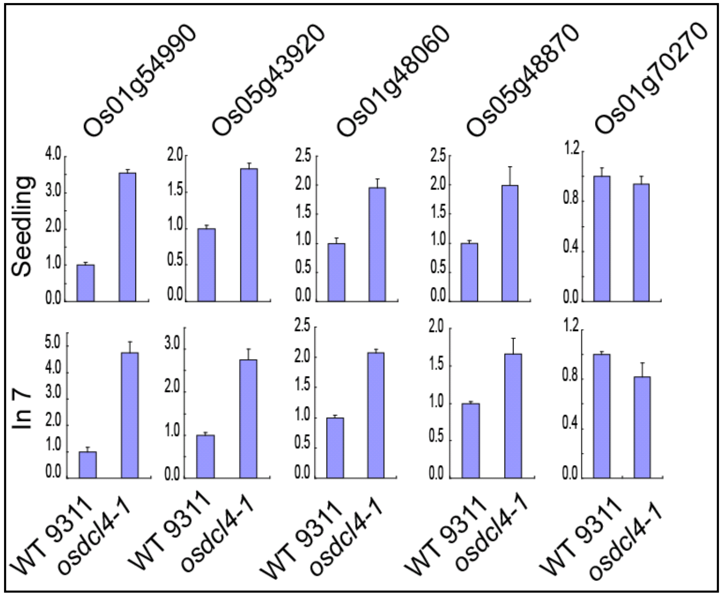




Regulation of *ARFs* by OsDCL4



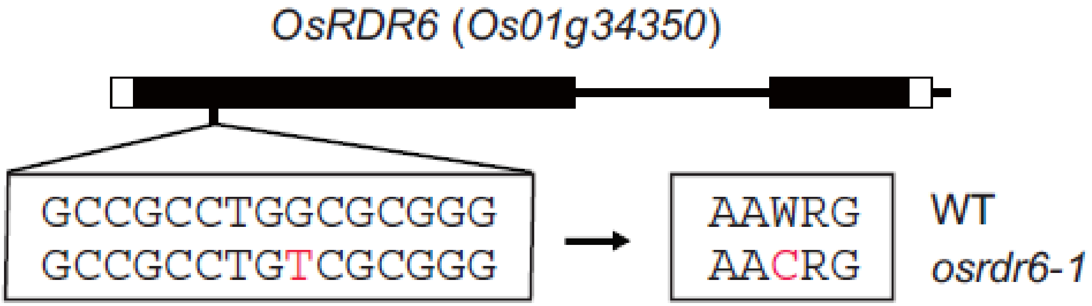


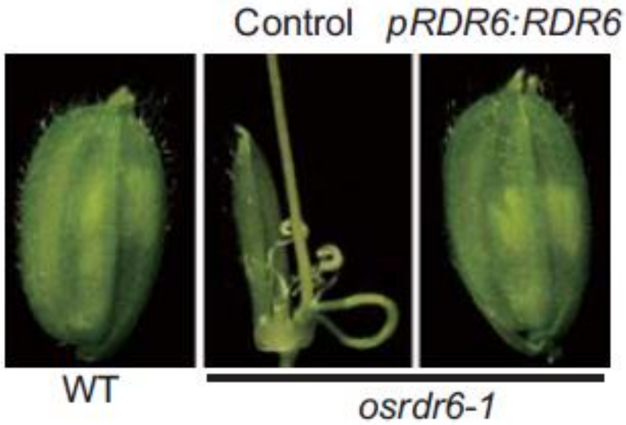


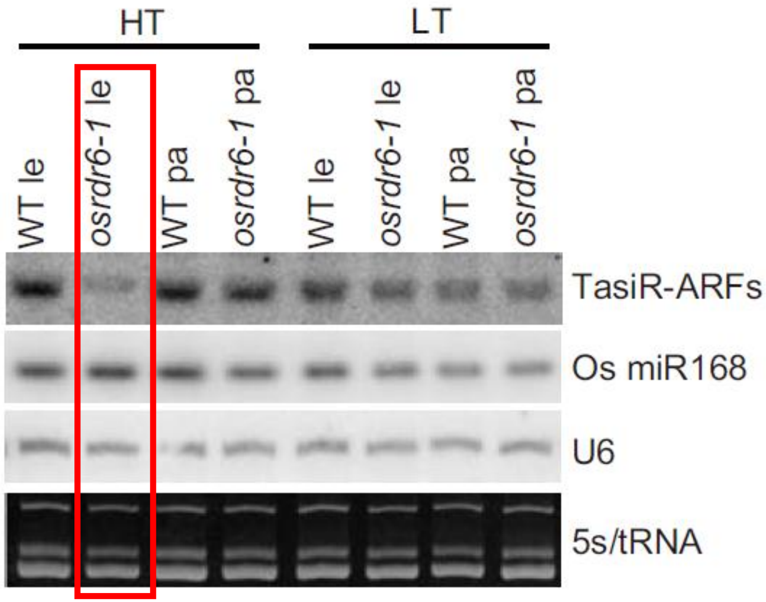
- Biogenesis of tasiR-*ARFs* requires both OsDCL1 and OsDCL4 activities.
- Impaired OsDCL4 results in up regulation of *ARFs*.

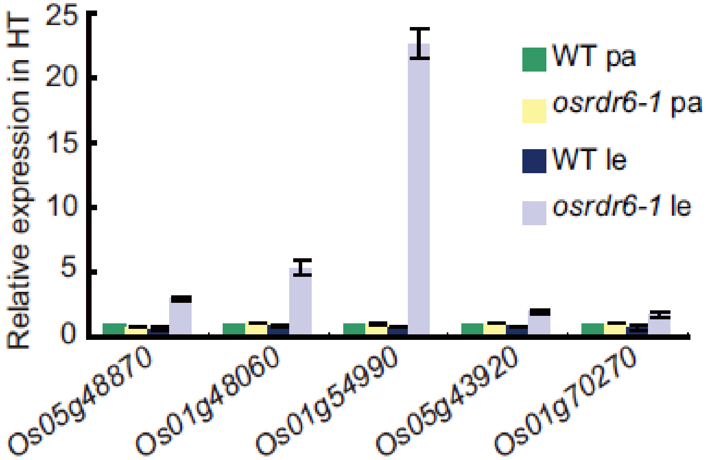
Xianwei Song

A point mutation in *rdr6* is responsible for mutant phenotypes



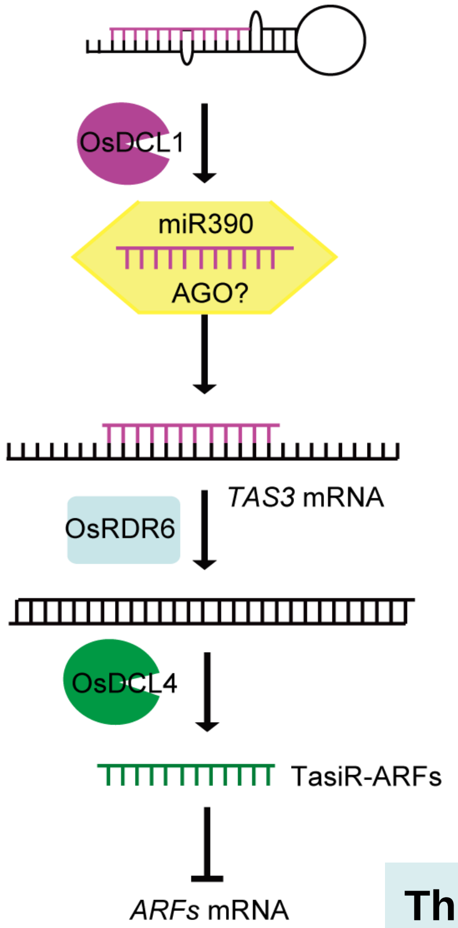


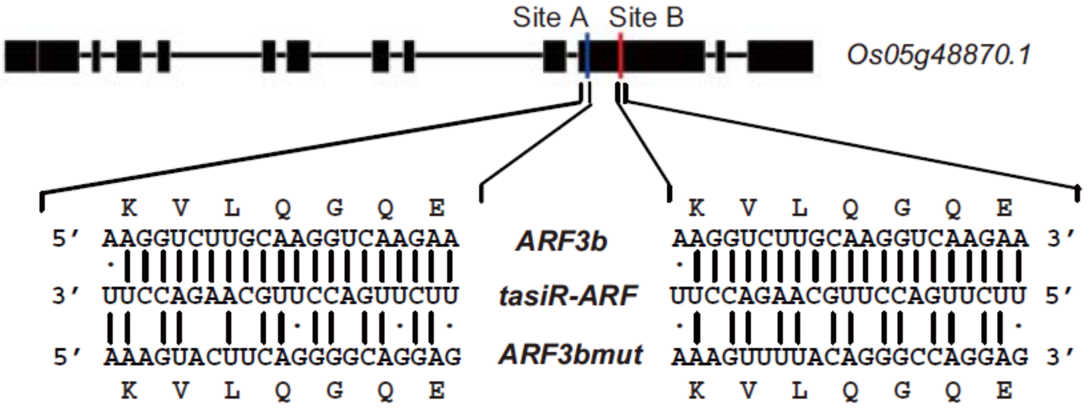


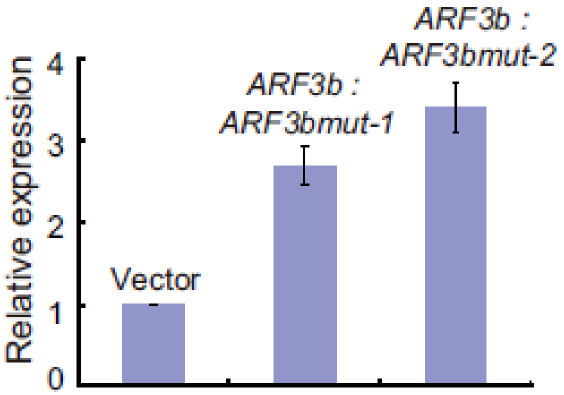


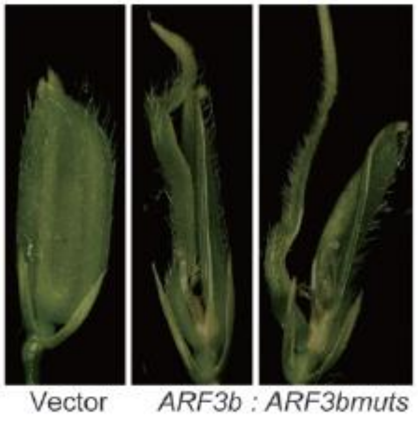
Song *et al*., 2012 (b) Plant Journal

Proper level of *ARF* expression is important for plant development in rice



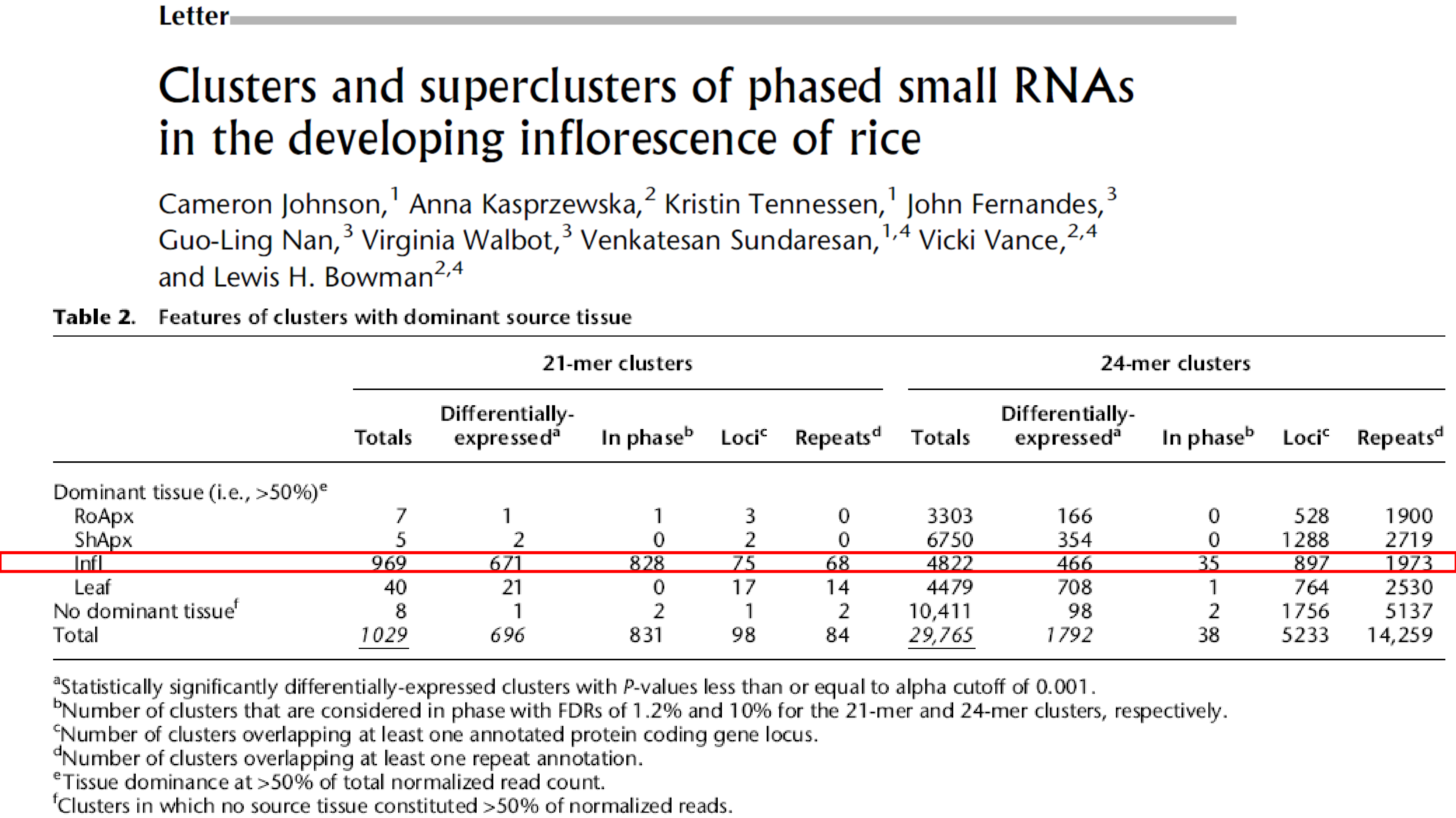






The transgenic plants containing *ARF3b* non-targeted of tasiR-ARFs show developmental defects mimic of *osdcl4-1* and *osrdr6-1.*

Song *et al*., 2012 (a) Plant Journal



Letter

Clusters and superclusters of phased small RNAs in the developing inflorescence of rice

Cameron Johnson,[1] Anna Kasprzewska,[2] Kristin Tennessen,[1] John Fernandes,[3] Guo-Ling Nan,[3] Virginia Walbot,[3] Venkatesan Sundaresan,[1,4] Vicki Vance,[2,4] and Lewis H. Bowman[2,4]

Table 2. Features of clusters with dominant source tissue

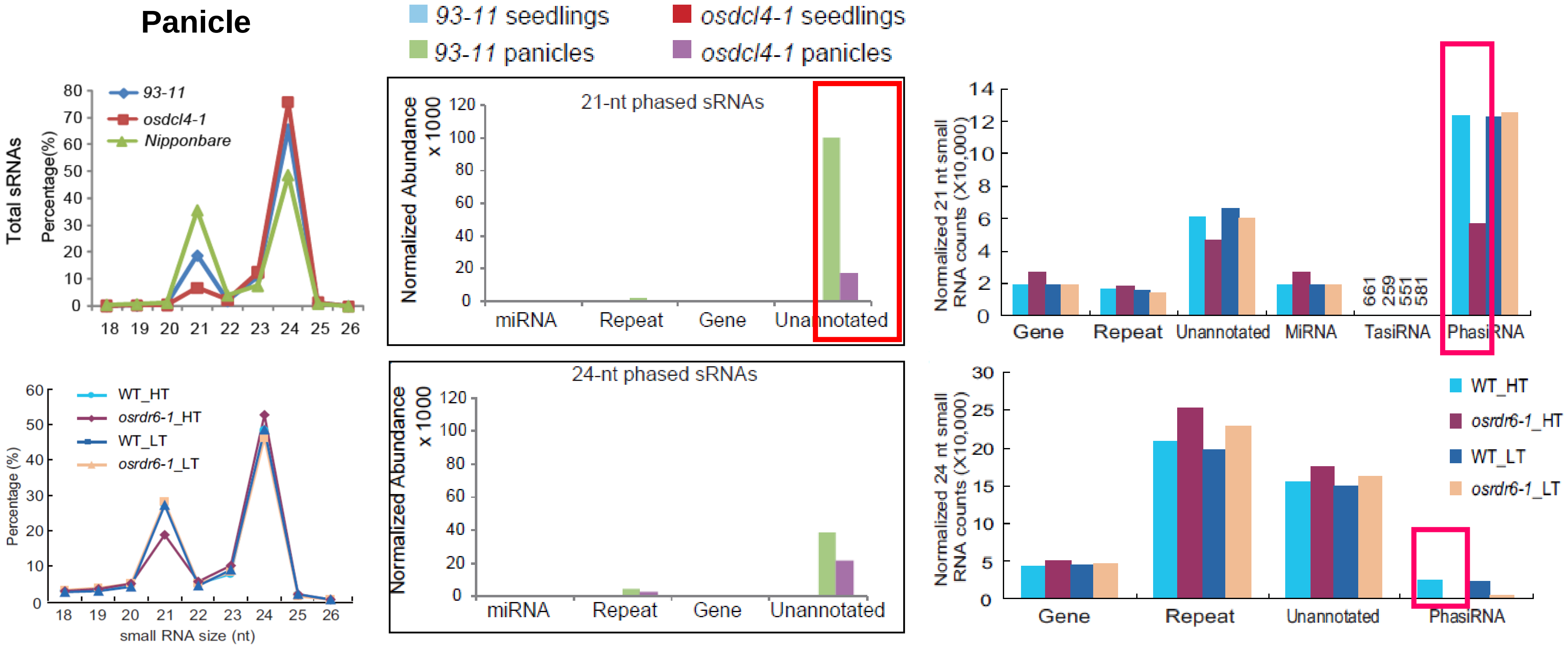
	21-mer clusters					24-mer clusters				
	Totals	Differentially-expressed[a]	In phase[b]	Loci[c]	Repeats[d]	Totals	Differentially-expressed[a]	In phase[b]	Loci[c]	Repeats[d]
Dominant tissue (i.e., >50%)[e]										
RoApx	7	1	1	3	0	3303	166	0	528	1900
ShApx	5	2	0	2	0	6750	354	0	1288	2719
Infl	969	671	828	75	68	4822	466	35	897	1973
Leaf	40	21	0	17	14	4479	708	1	764	2530
No dominant tissue[f]	8	1	2	1	2	10,411	98	2	1756	5137
Total	*1029*	*696*	831	98	84	*29,765*	*1792*	38	5233	14,259

[a] Statistically significantly differentially-expressed clusters with *P*-values less than or equal to alpha cutoff of 0.001.
[b] Number of clusters that are considered in phase with FDRs of 1.2% and 10% for the 21-mer and 24-mer clusters, respectively.
[c] Number of clusters overlapping at least one annotated protein coding gene locus.
[d] Number of clusters overlapping at least one repeat annotation.
[e] Tissue dominance at >50% of total normalized read count.
[f] Clusters in which no source tissue constituted >50% of normalized reads.

- Many 21- and 24-nt phased RNA (phasiRNA) were identified in rice panicle based on phased array like tasiRNAs.
- miR2118 and miR2275 are predicted to trigger 21- and 24-nt phasiRNAs.

Johnson *et al*., (2009) Genome Res

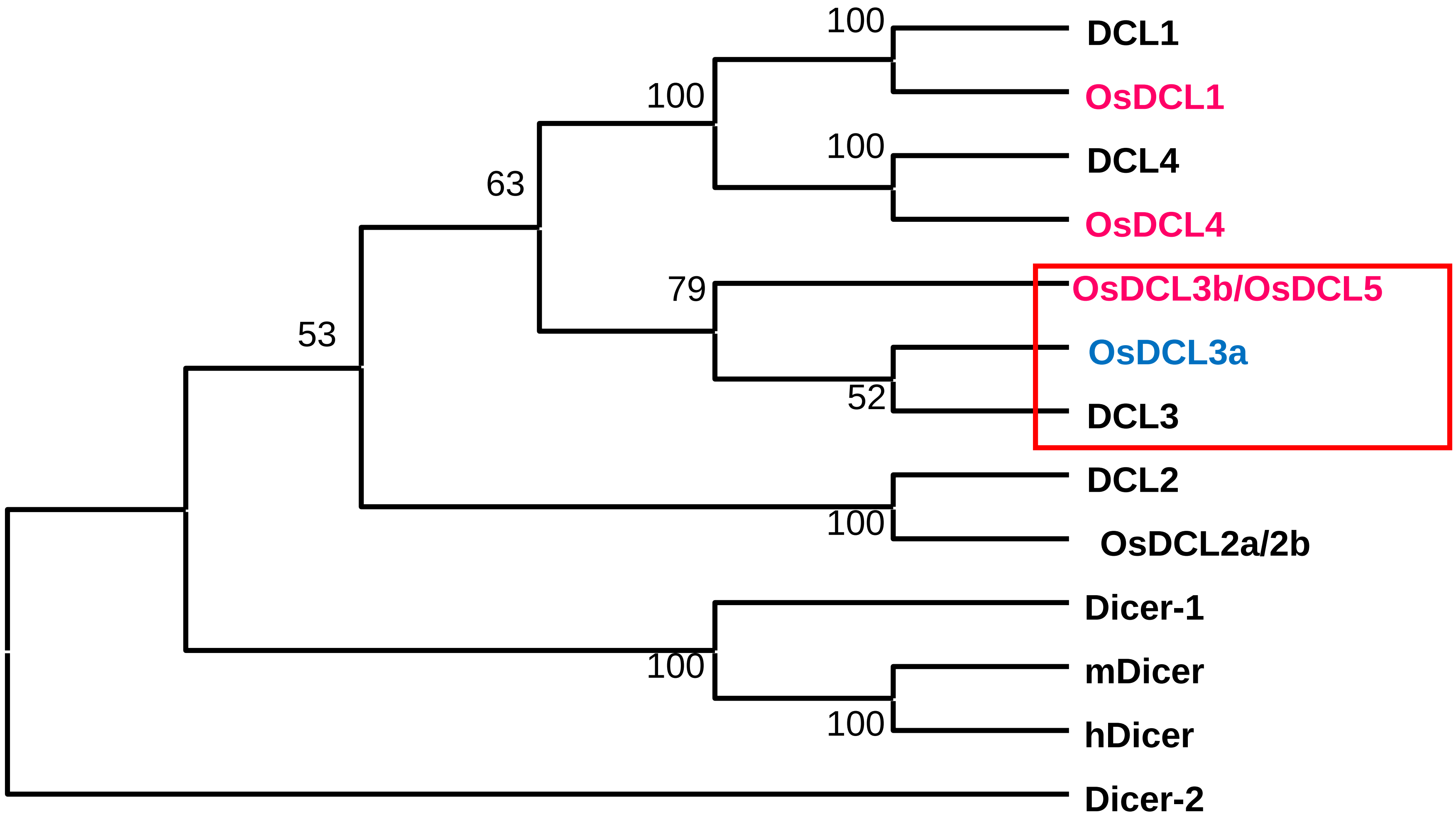
Differential effects of OsDCL4 on accumulations of 21- and 24nt phased small RNAs



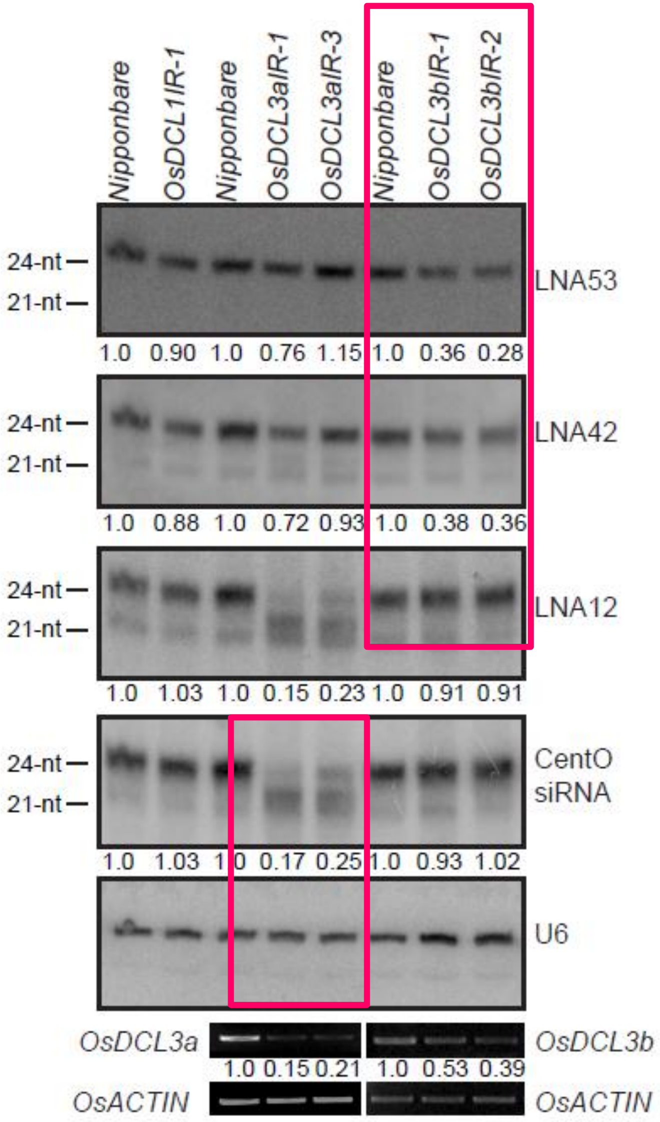
- Over one thousand 21nt phased RNA loci were largely dependent on OsDCL4
- The accumulation of 24nt phased RNAs was moderately affected by OsDCL4
- Both 21- and 24nt phased RNAs were RDR6 dependent

Which OsDCL is responsible for 24nt phased RNA biogenesis?

OsDCL3a and OsDCL3b



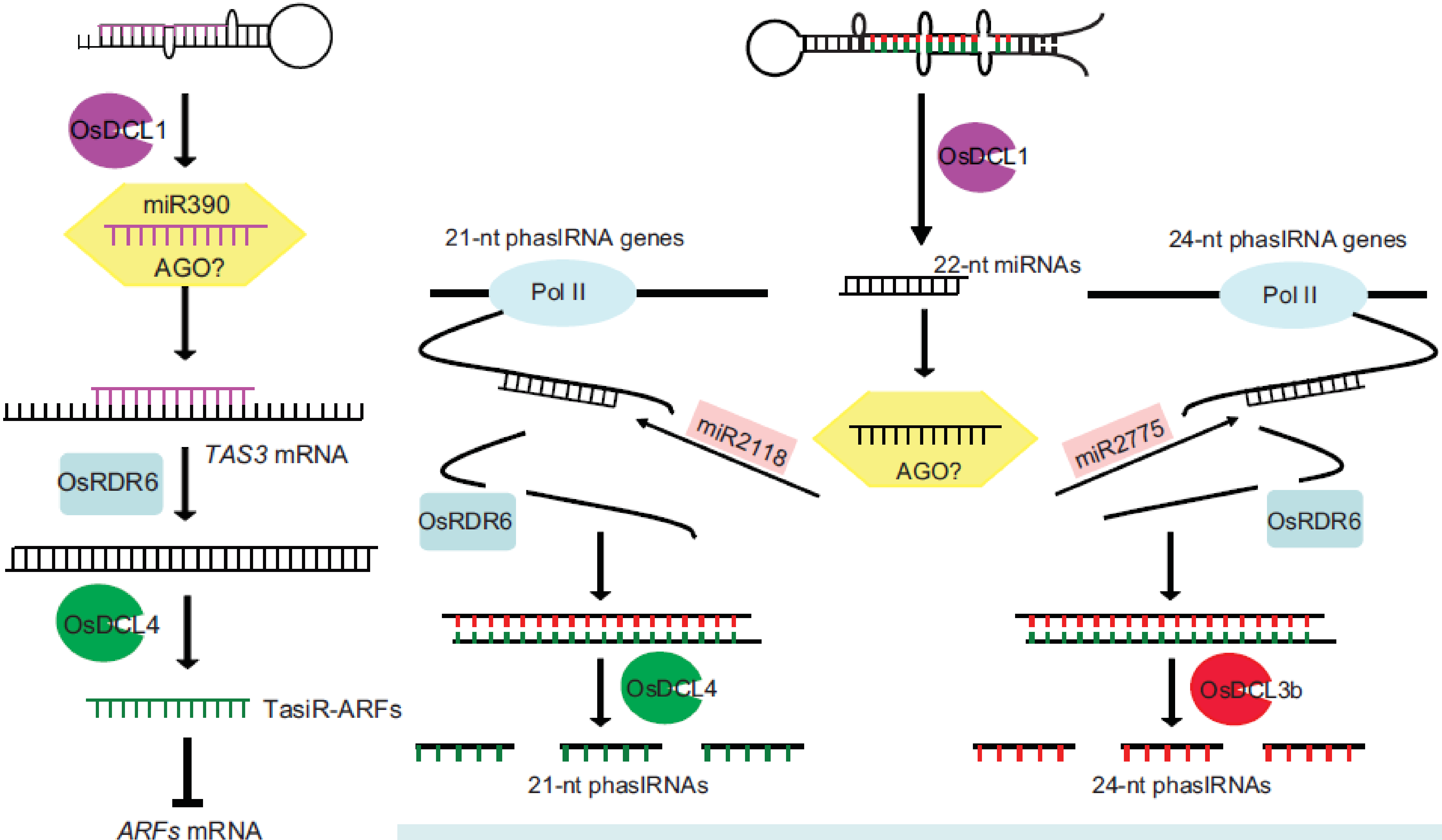
24nt phasiRNAs are processed by OsDCL3b



24nt phased RNAs

24nt unphased RNAs

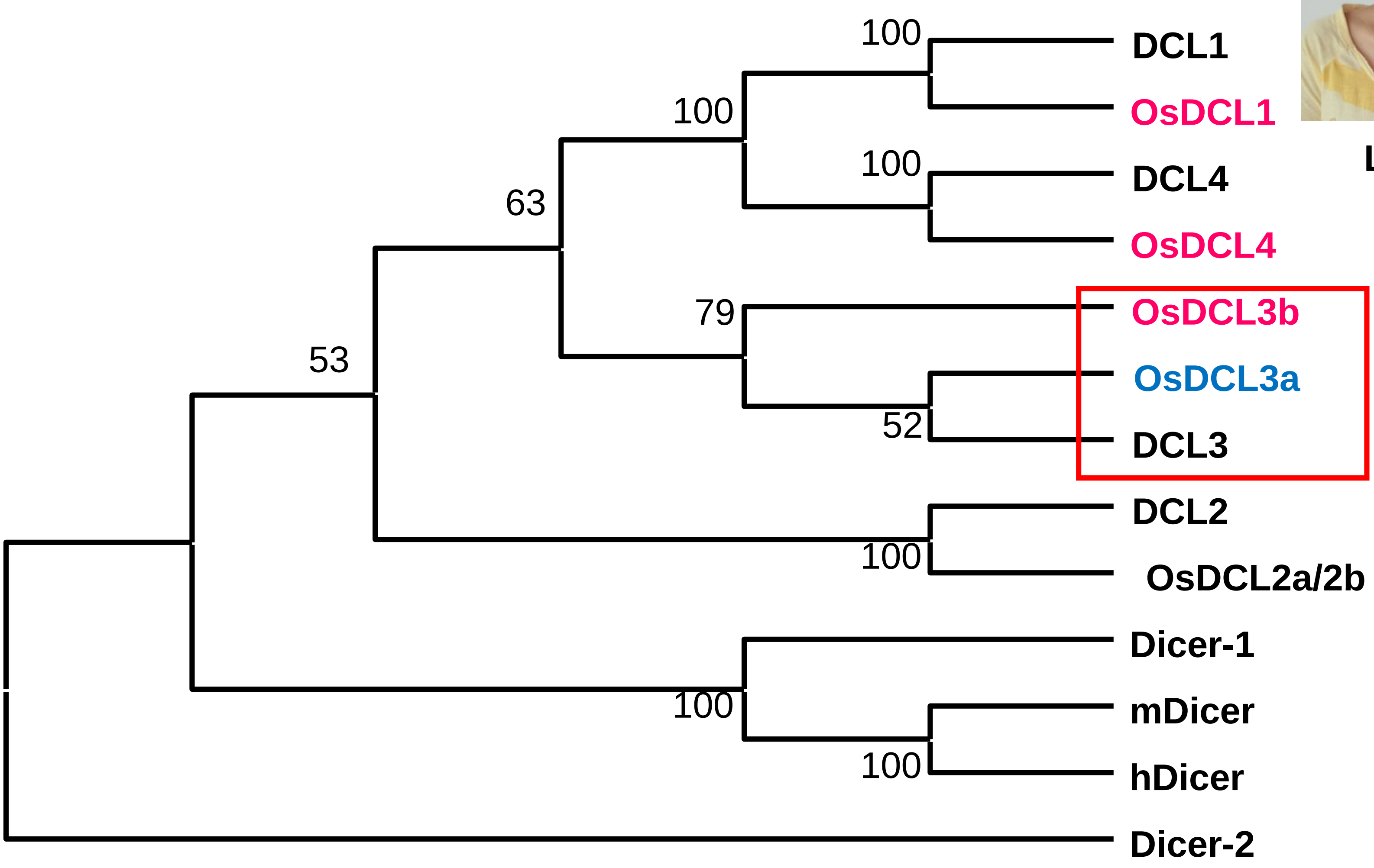
Conserved and non-conserved siRNA biogenesis pathways



24nt phased RNAs are processed by OsDCL3b, but not OsDCL3a

Song *et al*., 2012 (a) Plant Journal
Song *et al*., 2012 (b) Plant Journal

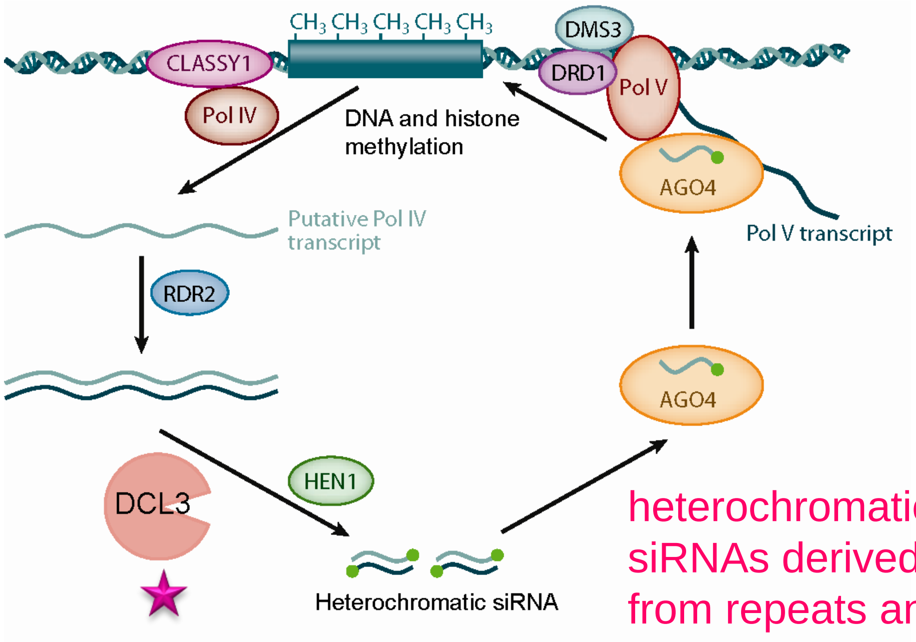
OsDCL3a



Liya Wei

Wei *et al*., (2014) PNAS

RNA-directed DNA methylation (RdDM) pathway in Arabidopsis



heterochromatic siRNAs derived from repeats and TEs

- ➢**DCL3 is responsible for 24-nt siRNA heterochromatic siRNAs biogenesis, which tend to be derived from repeats and transposable elements.**
- ➢**Loss of function DCL3 line displays no obvious development phenotypes.**

Gasciolli, V., et al (2005) Curr Biol 15, 1494-1500.
Henderson, I.R., et al (2006) Nat Genet 38, 721-725.
Chen, X.M, (2009), Annu Rev Cell Dev Bi 25, 21-44.

Molecular Cell
Article

DNA Methylation Mediated by a MicroRNA Pathway

Liang Wu,[1,2,3] Huanyu Zhou,[1,3] Qingqing Zhang,[1] Jianguang Zhang,[1] Fangrui Ni,[1] Chang Liu,[1] and Yijun Qi[1,*]
[1]National Institute of Biological Sciences, Zhongguancun Life Science Park, Beijing 102206, China
[2]College of Life Sciences, Beijing Normal University, Beijing 100875, China
[3]These authors contributed equally to this work
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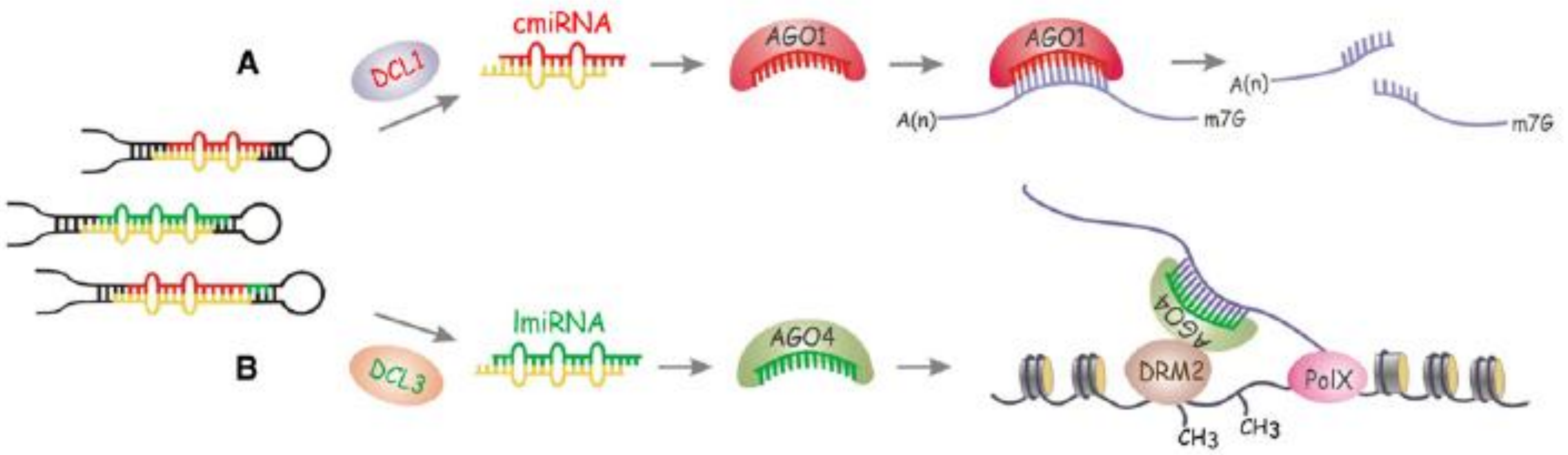
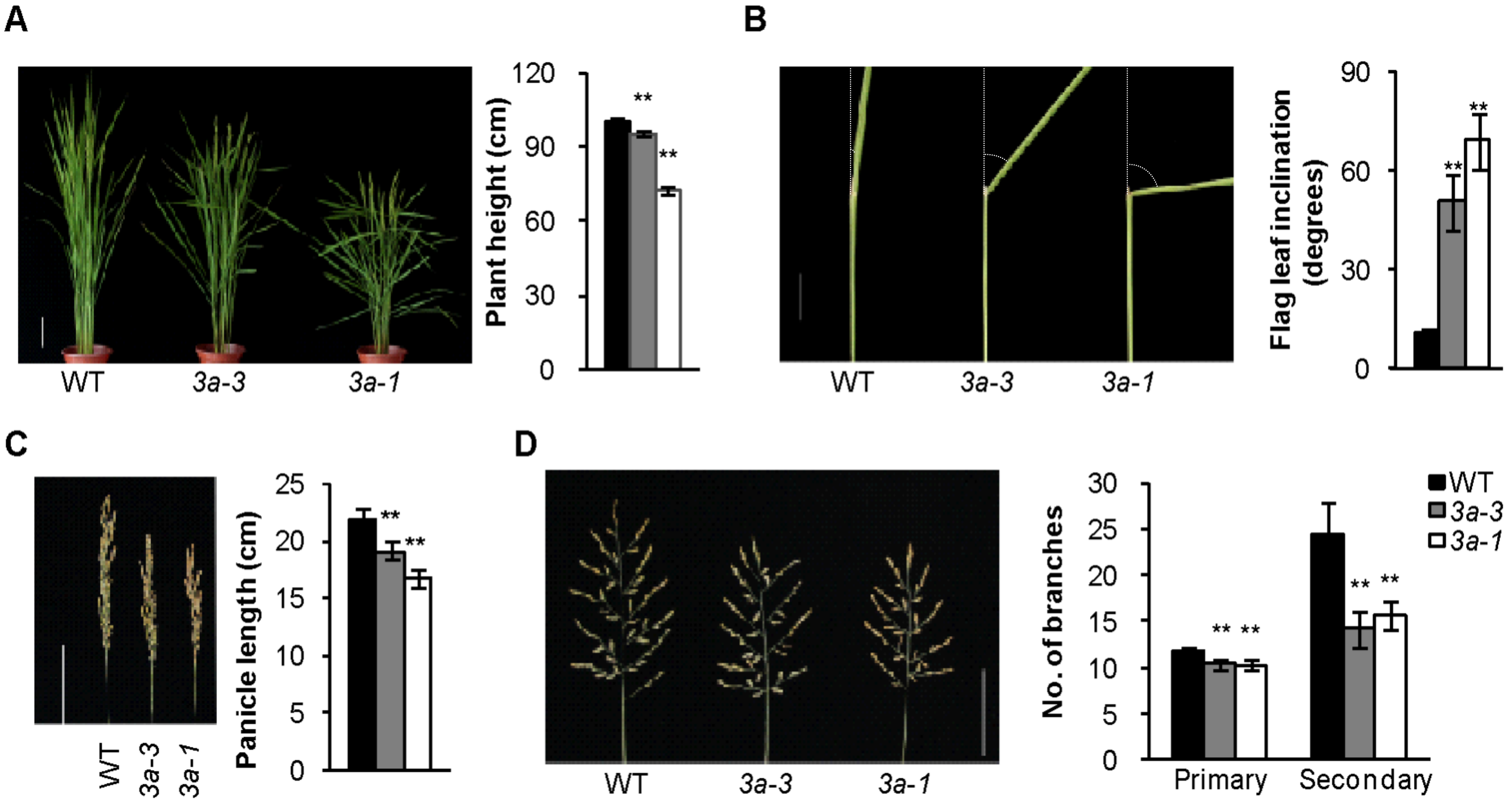
DOI 10.1016/j.molcel.2010.03.008

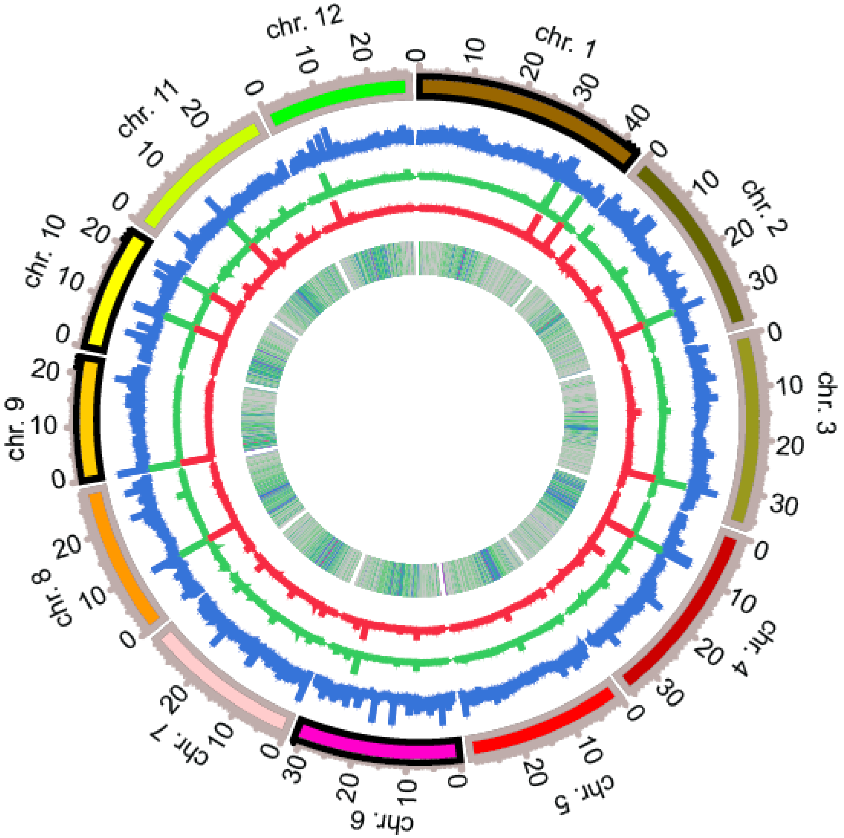


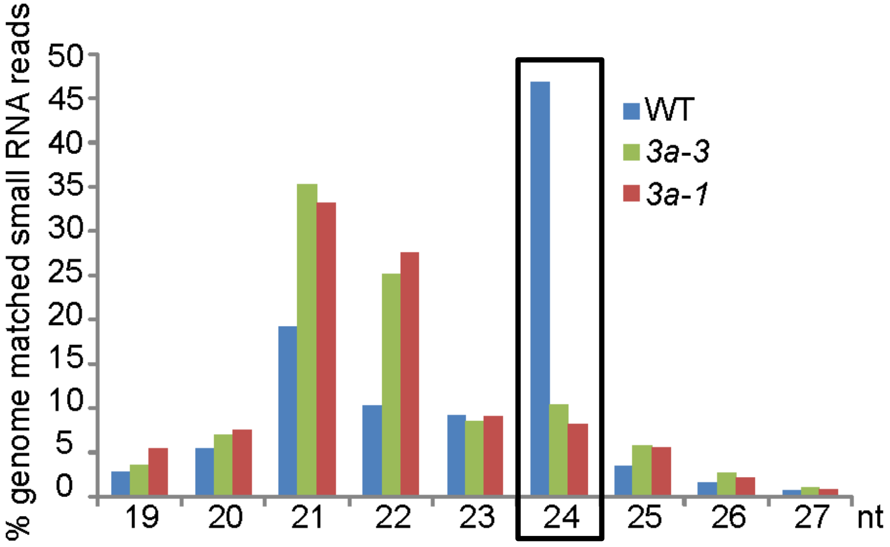
Wu *et al*., 2010 Molecular Cell (Qi's lab)

OsDCL3a knockdown plants display pleiotropic phenotypes affecting important agricultural traits

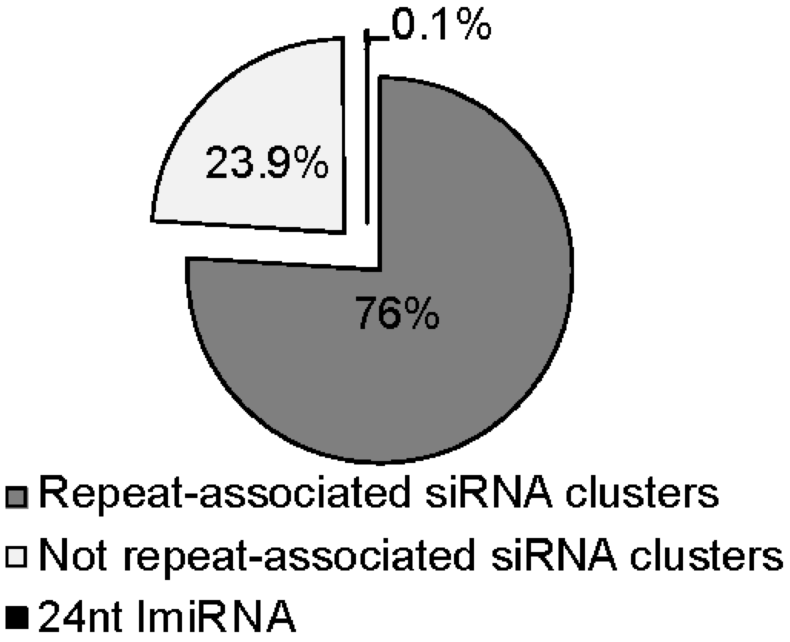


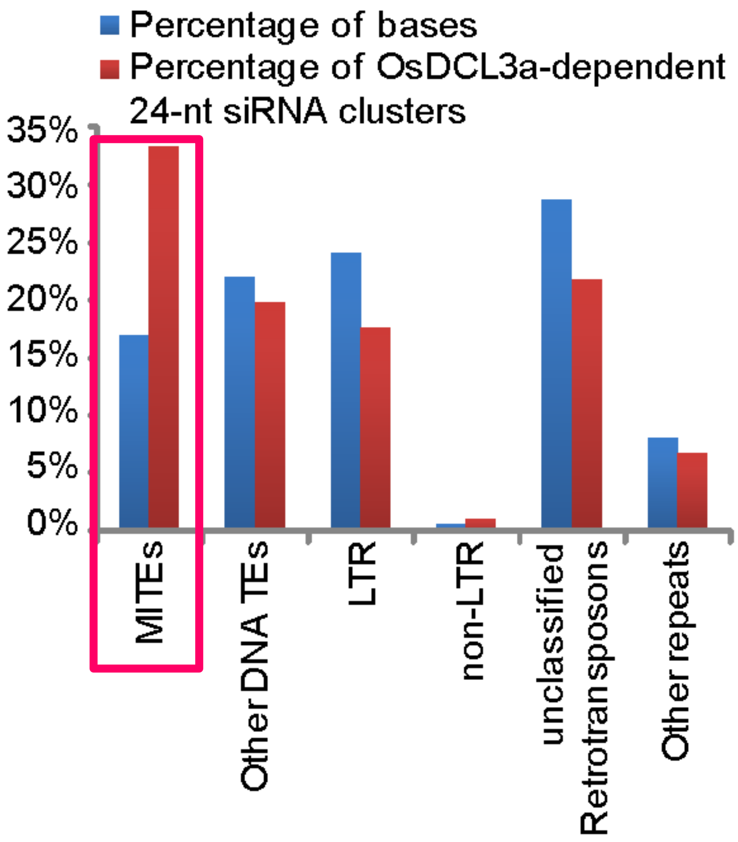
OsDCL3 processes genome-wide 24nt siRNAs





OsDCL3-dependent siRNAs significantly enriched in MITE repeats





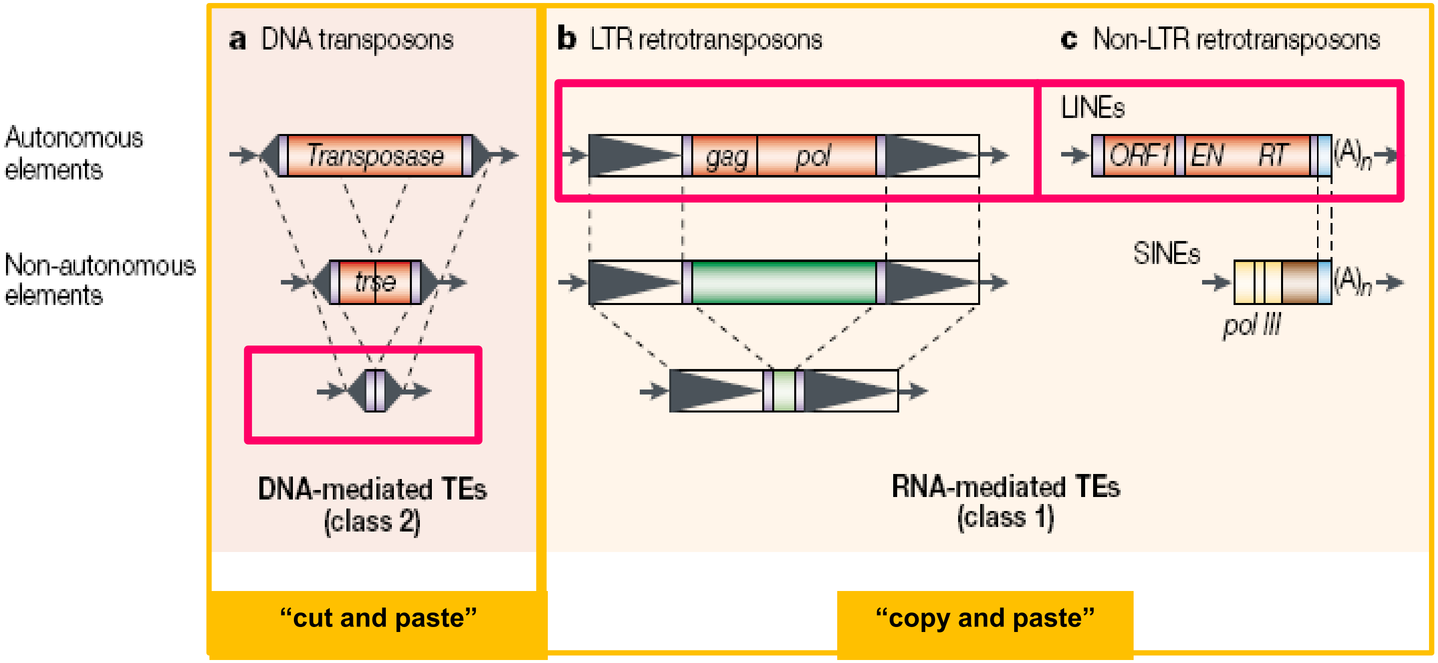
Transposable element (TE)

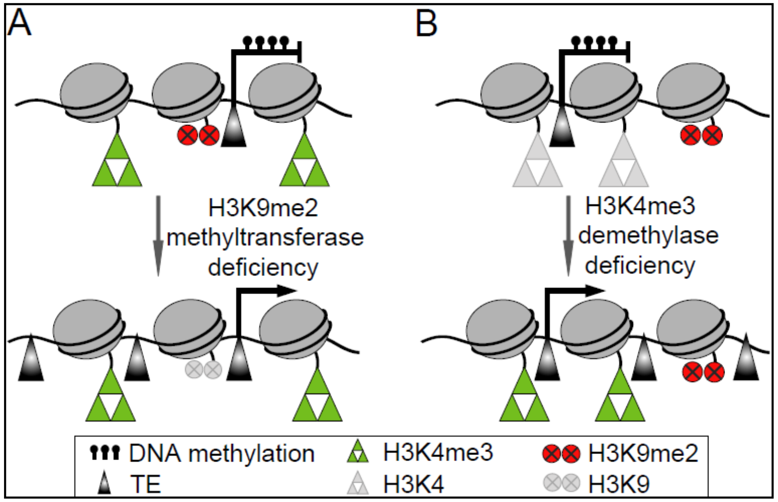
Barbara McClintock: Winner of the Nobel Prize in 1983

- First identified as regulatory elements in maize by McClintock (1950’s)
- Ubiquitously present with high abundant in plant and animal genomes
- Transposition of TEs is a major driving force for genome evolution
- Host genomes have evolved diverse mechanisms to limit harmful mobilization
- Epigenetic regulation has been implicated to control TE activities

Transposable elements

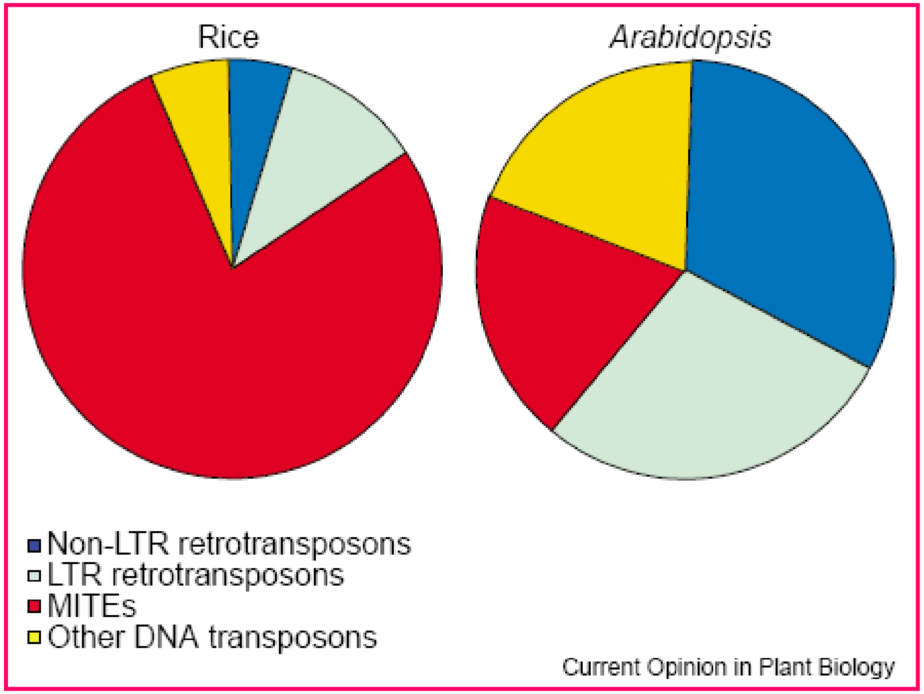
MITE **_Tos 17_** **_Karma_**



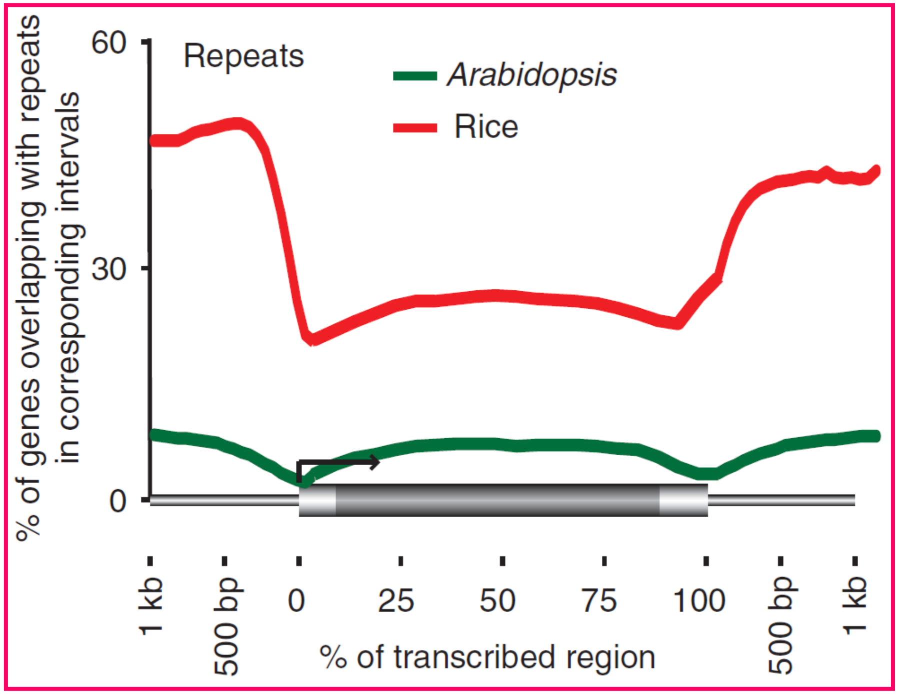


Ding *et al.*, (2007) Plant Cell
Cui *et al.*, (2013) PNAS
Feschotte *et al.*, (2002) Nat. Rev. Genet.

MITEs are the highest-copy-number TEs in rice



Current Opinion in Plant Biology

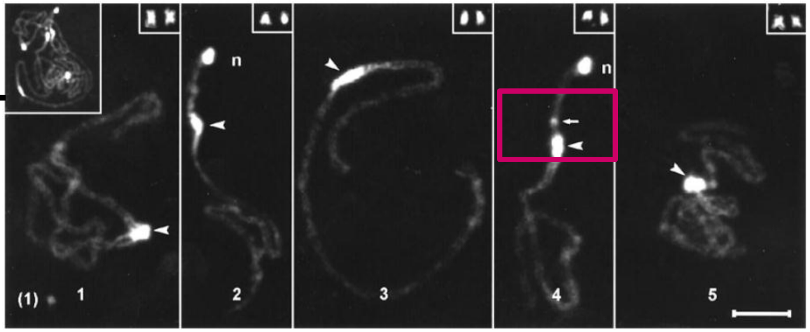


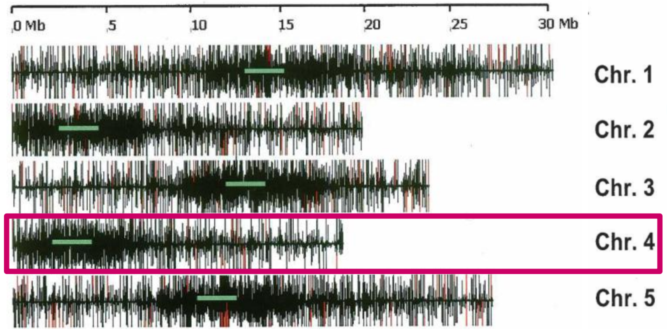
Mainly distributed in gene-rich regions, especially in upstream, downstream and introns of genes.

Jiang, N *et al.*, (2004) Curr Opin Plant Biol
Zhang, X. (2008) Science

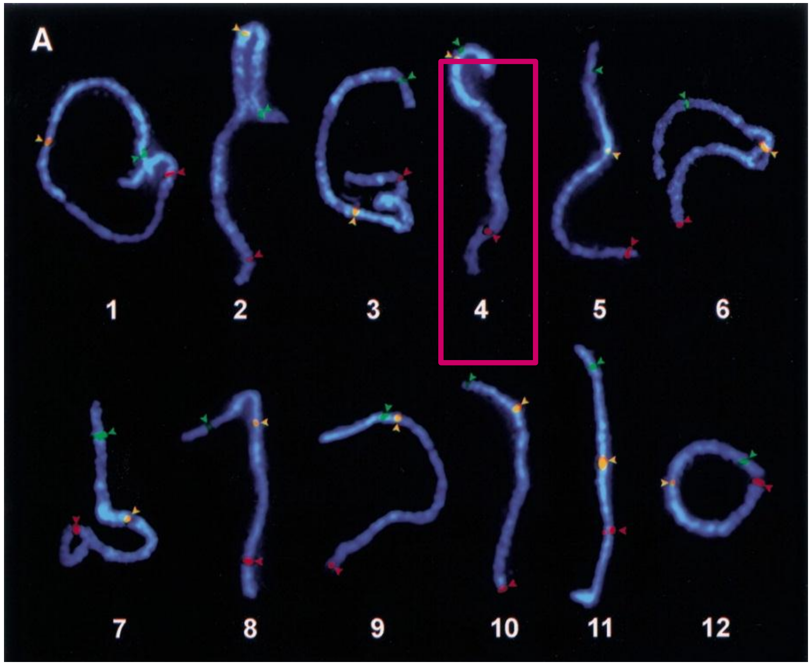
Distinct heterochromatin and small RNA distribution in Arabidopsis and rice

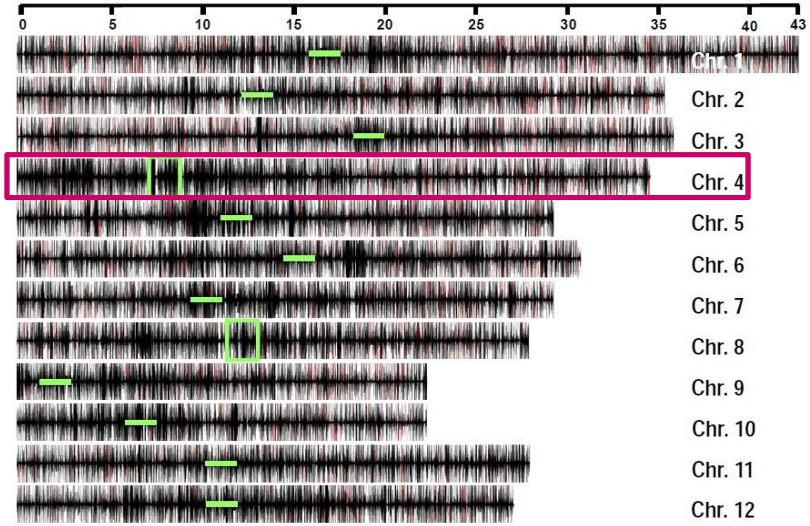
Arabidopsis





rice



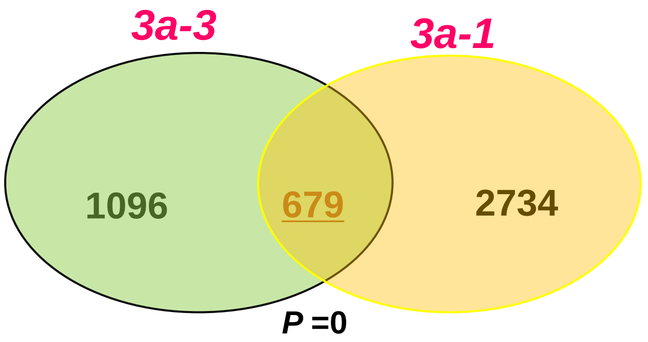


Rice small RNAs more widely distributed on chromosomes

Fransz *et al*. (1998). Plant J
Cheng *et al*. (2001). Genome Res

Nobuta *et al*., (2007), Nat Biotech
Lu, *et al*., (2006), Genome Res.

MITEs correlate with 24nt siRNAs distribution in up-regulated genes



3a-3

3a-1

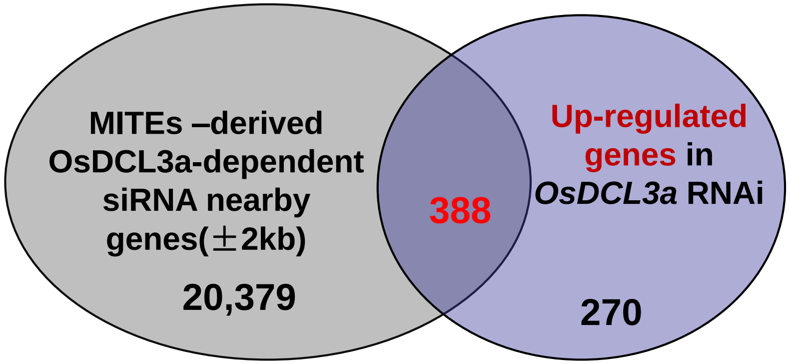
1096

679

2734

P =0

Up-regulated genes in *OsDCL3a* RNAi lines



MITEs –derived OsDCL3a-dependent siRNA nearby genes(±2kb)

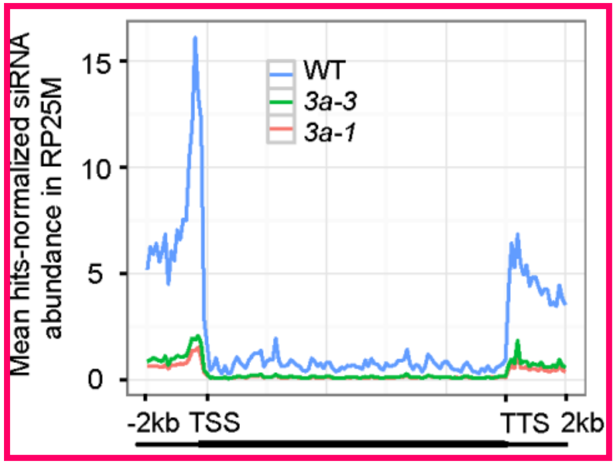
20,379

388

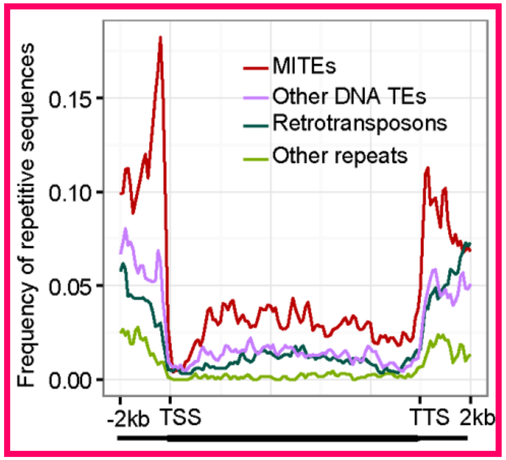
Up-regulated genes in *OsDCL3a* RNAi

270

$P = 1.70 \times 10^{-31}$



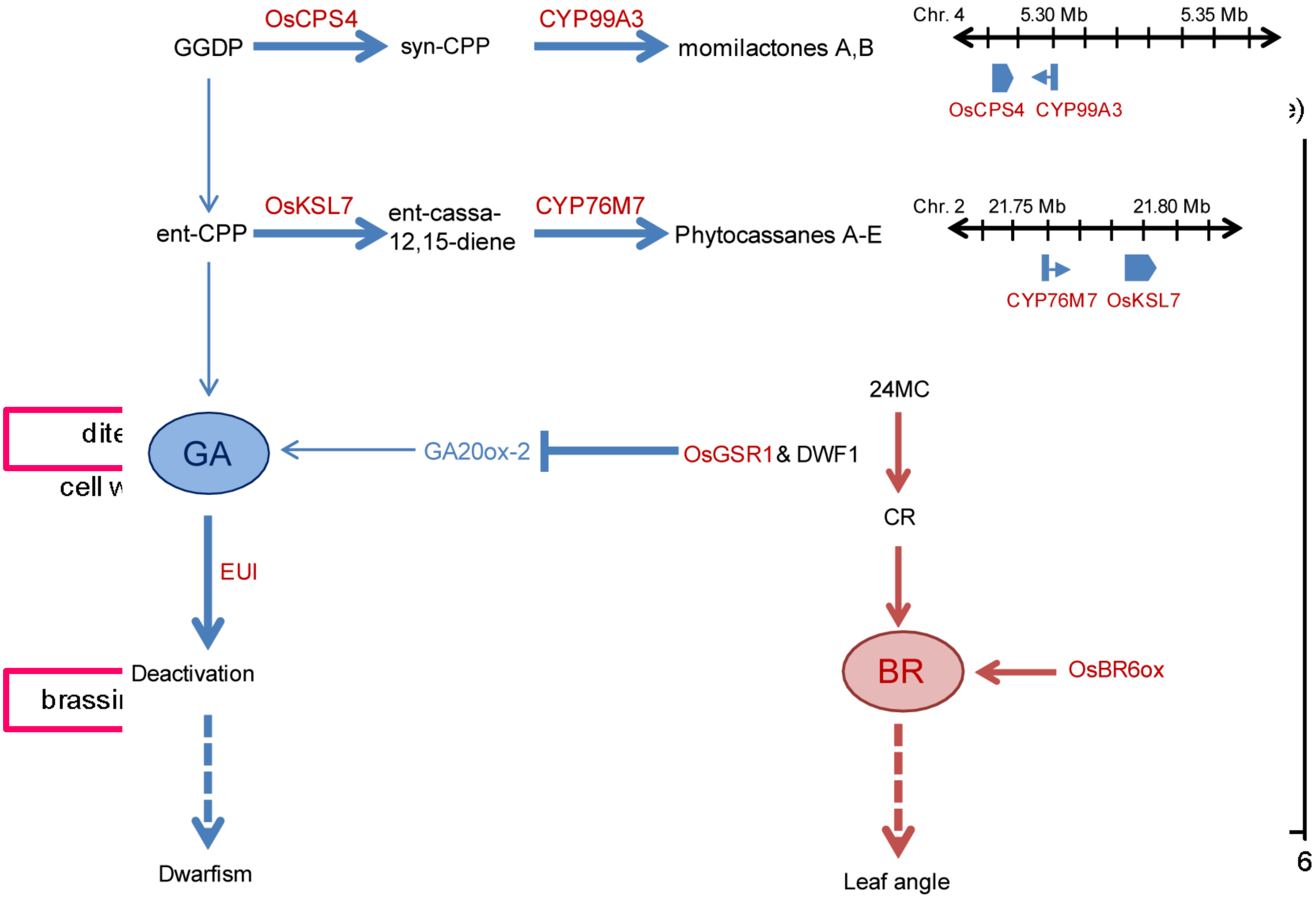
sRNA



TEs and Other repeats

679 Up-regulated genes, P-value <0.01 and Fold change >1.5

Up-regulated genes involved in many biological processes including GA and BR homeostasis



GGDP → OsCPS4 → syn-CPP → CYP99A3 → momilactones A,B

Chr. 4 5.30 Mb 5.35 Mb

OsCPS4 CYP99A3

ent-CPP → OsKSL7 → ent-cassa-12,15-diene → CYP76M7 → Phytocassanes A-E

Chr. 2 21.75 Mb 21.80 Mb

CYP76M7 OsKSL7

GA

GA20ox-2

OsGSR1 & DWF1

24MC

CR

BR

OsBR6ox

EUI

Deactivation

Dwarfism

Leaf angle

dite

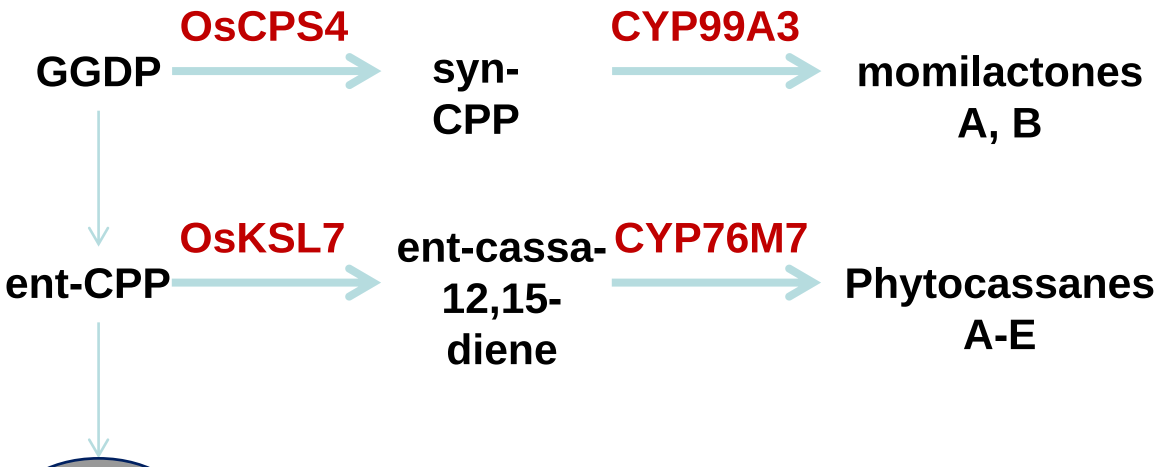
cell v

brassi

e)

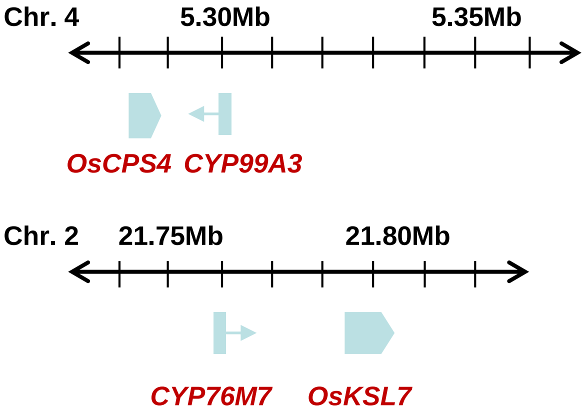
6

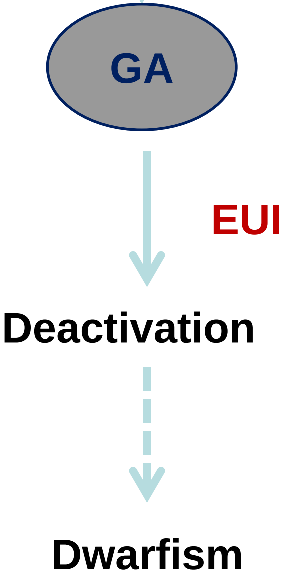
The dwarf phenotype of OsDCL3a RNAi lines may due to GA deficiency



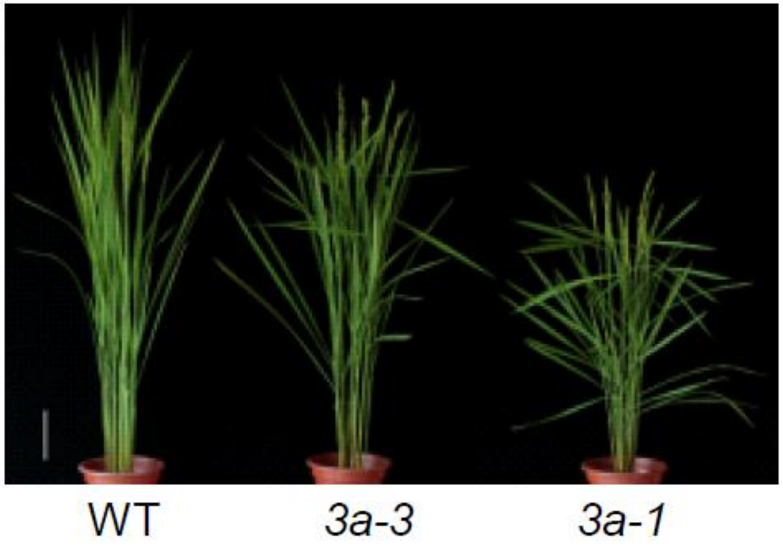
GGDP
OsCPS4
syn-CPP
CYP99A3
momilactones A, B

ent-CPP
OsKSL7
ent-cassa-12,15-diene
CYP76M7
Phytocassanes A-E

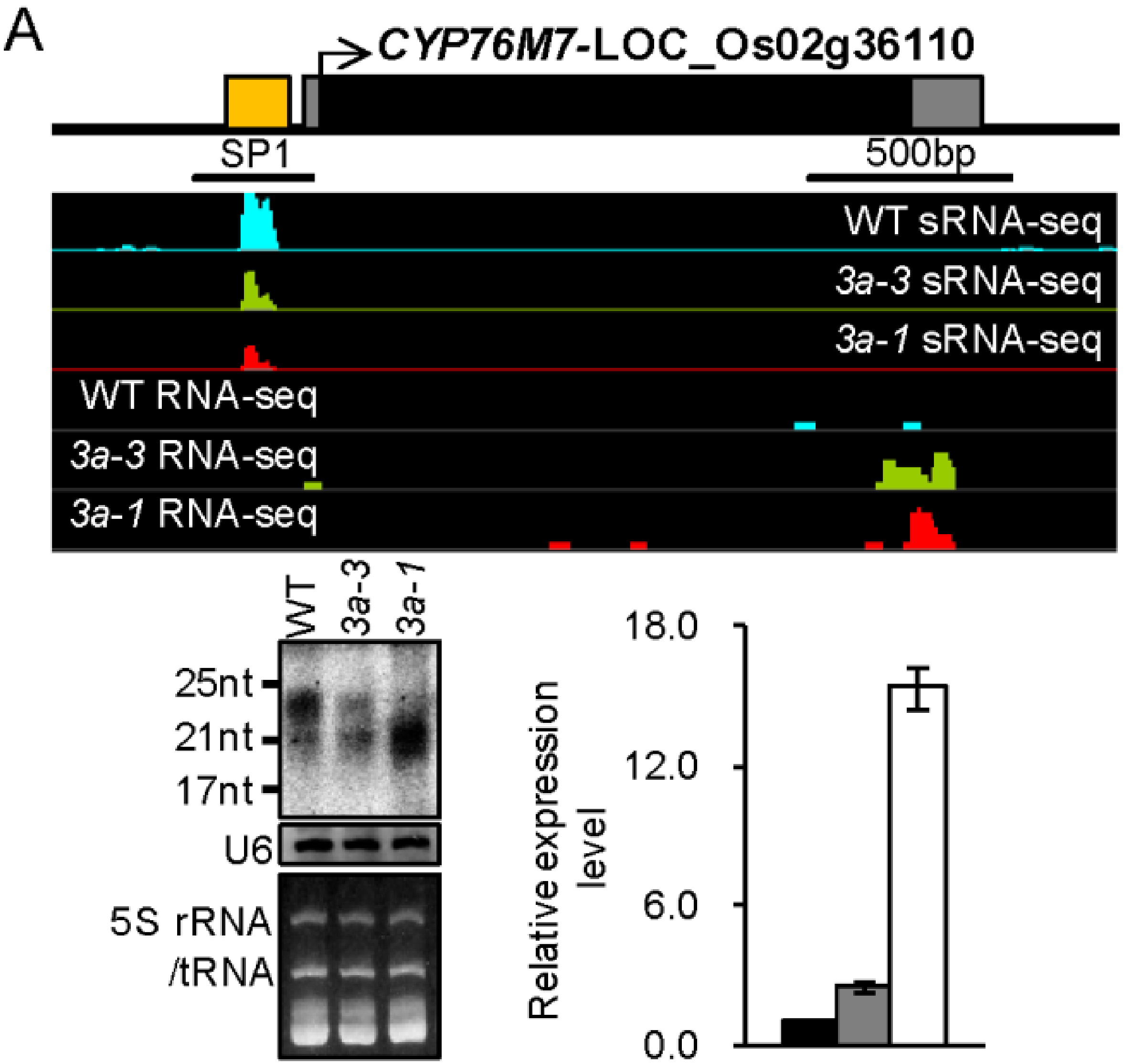




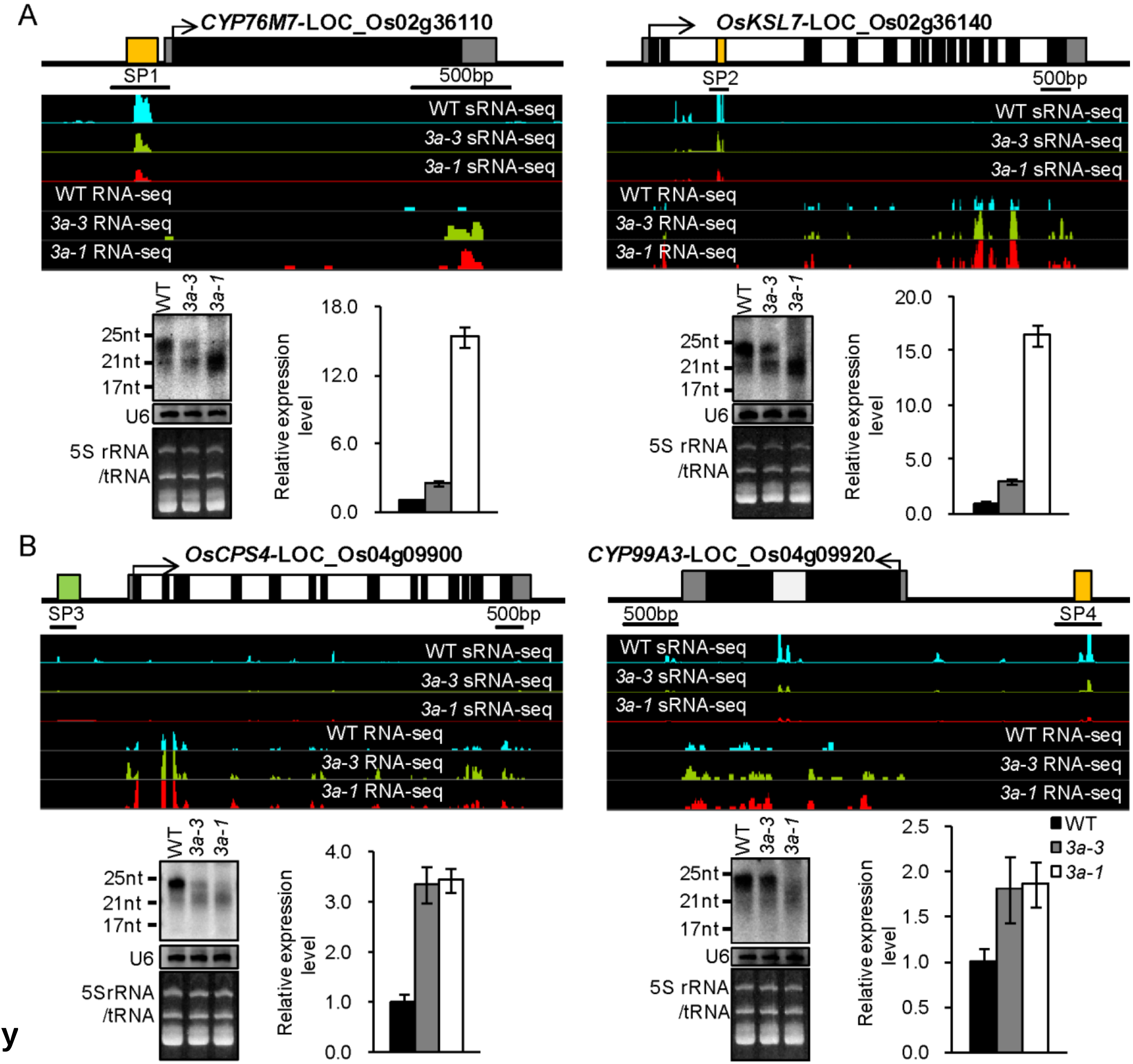
GA
EUI
Deactivation
Dwarfism



Up-regulated genes reduced MITE associated 24nt siRNAs

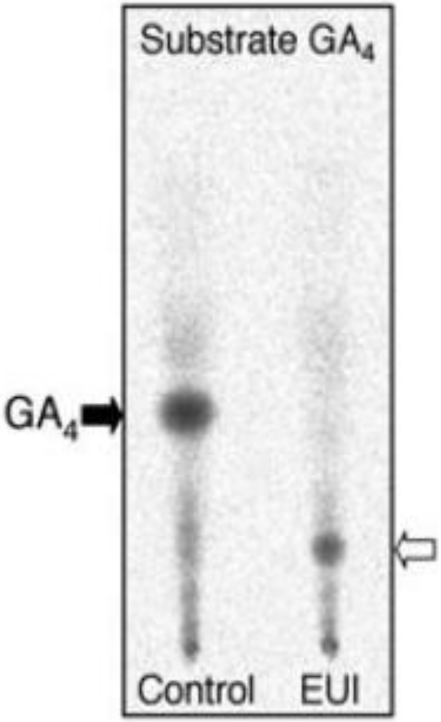


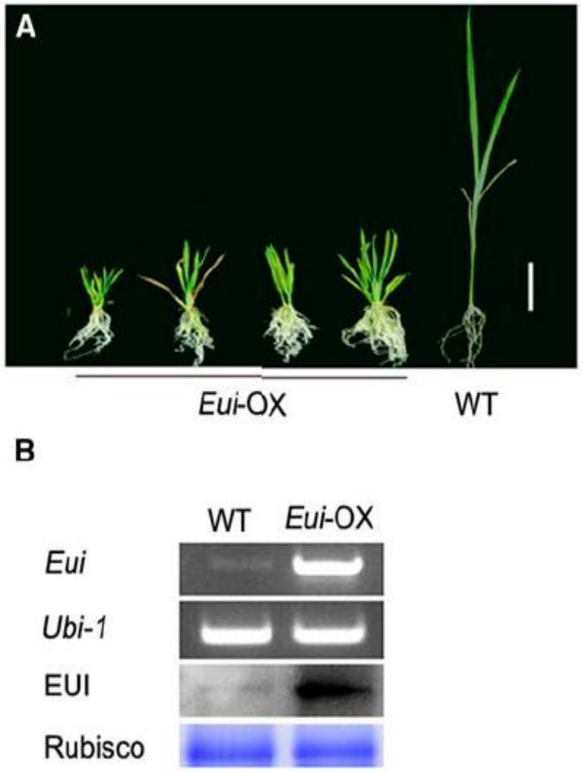
- **GA biosynthesis is reduced.**

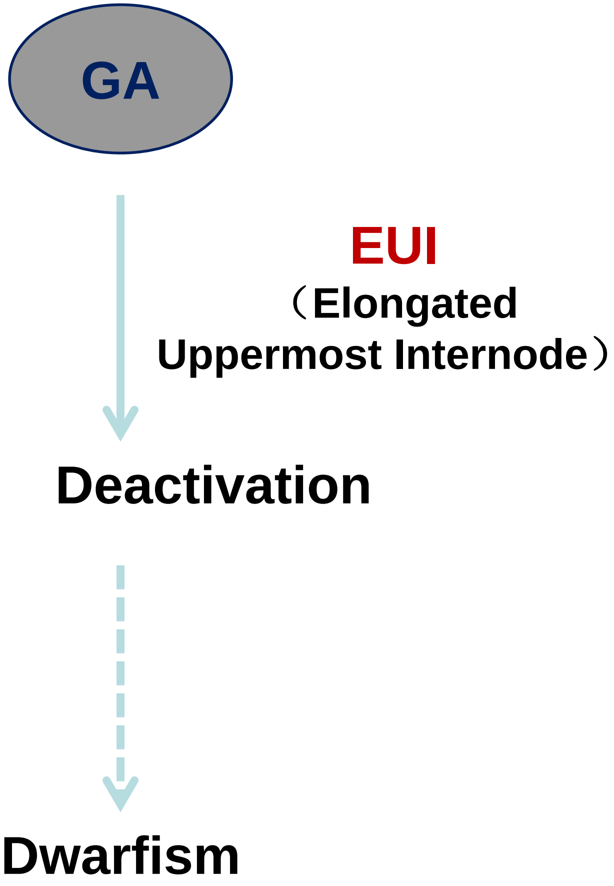

A
CYP76M7-LOC_Os02g36110
SP1
500bp
WT sRNA-seq
3a-3 sRNA-seq
3a-1 sRNA-seq
WT RNA-seq
3a-3 RNA-seq
3a-1 RNA-seq
WT
3a-3
3a-1
25nt
21nt
17nt
U6
5S rRNA
/tRNA
Relative expression level
18.0
12.0
6.0
0.0
OsKSL7-LOC_Os02g36140
SP2
500bp
WT sRNA-seq
3a-3 sRNA-seq
3a-1 sRNA-seq
WT RNA-seq
3a-3 RNA-seq
3a-1 RNA-seq
WT
3a-3
3a-1
25nt
21nt
17nt
U6
5S rRNA
/tRNA
Relative expression level
20.0
15.0
10.0
5.0
0.0
B
OsCPS4-LOC_Os04g09900
SP3
500bp
WT sRNA-seq
3a-3 sRNA-seq
3a-1 sRNA-seq
WT RNA-seq
3a-3 RNA-seq
3a-1 RNA-seq
WT
3a-3
3a-1
25nt
21nt
17nt
U6
5SrRNA
/tRNA
Relative expression level
4.0
3.0
2.0
1.0
0.0
CYP99A3-LOC_Os04g09920
500bp
SP4
WT sRNA-seq
3a-3 sRNA-seq
3a-1 sRNA-seq
WT RNA-seq
3a-3 RNA-seq
3a-1 RNA-seq
WT
3a-3
3a-1
25nt
21nt
17nt
U6
5S rRNA
/tRNA
Relative expression level
2.5
2.0
1.5
1.0
0.5
0.0
■ WT
■ 3a-3
□ 3a-1

- **GA biosy**

EUI functions as a GA deactivating enzyme in rice







GA

EUI

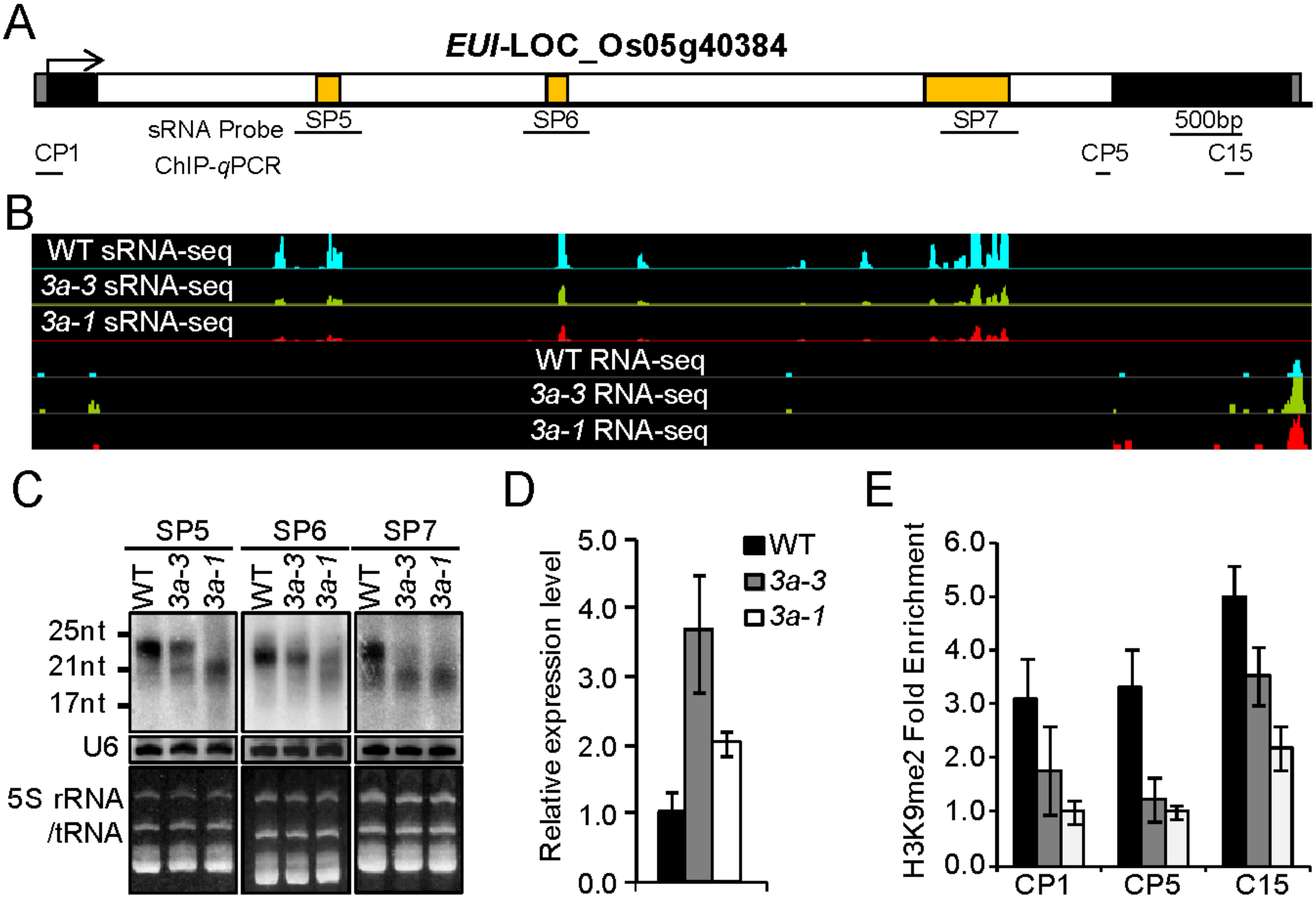
（Elongated Uppermost Internode）

Deactivation

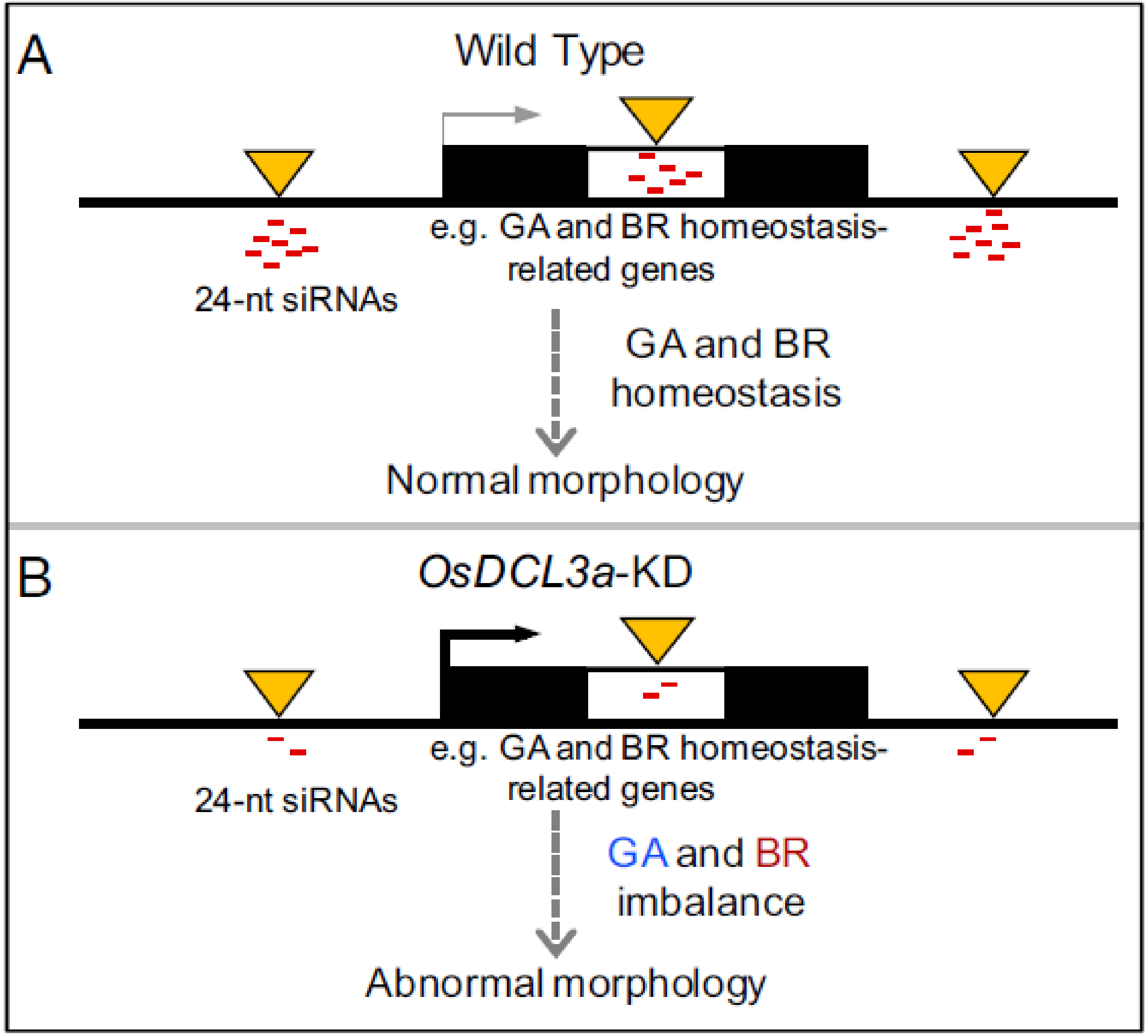
Dwarfism

Zhu, Y *et al.,* (2006) Plant Cell 18, 442-456. Luo, A *et al.,* (2006) Plant Cell Physiol 47, 181-191.

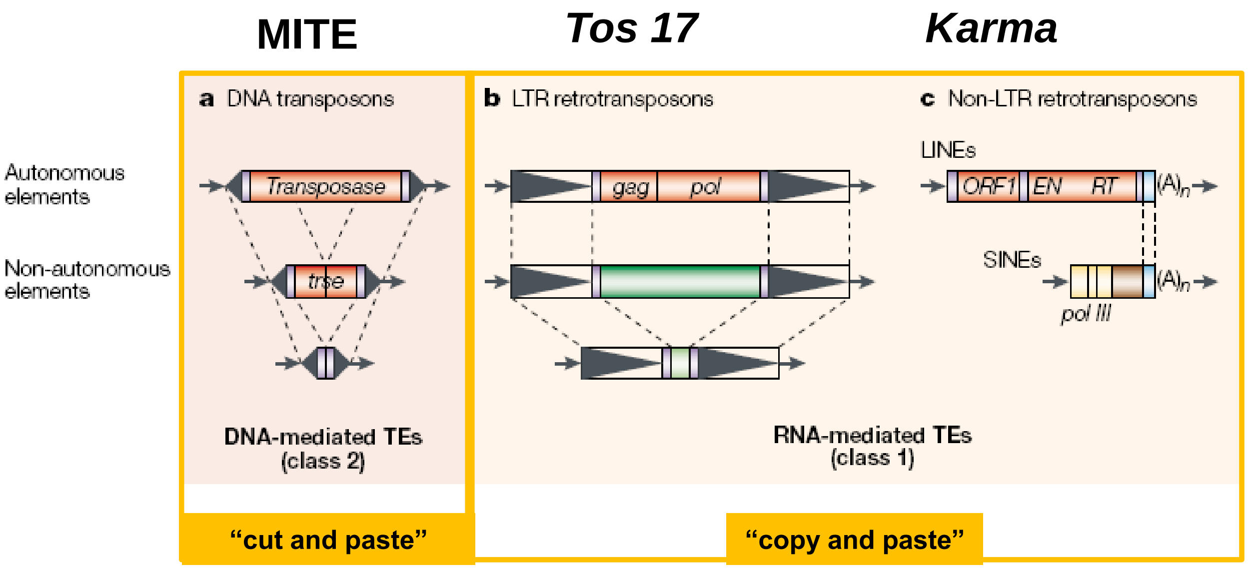
GA catabolism gene (*EUI*) are epigenetically regulated by MITE-derived 24nt siRNAs

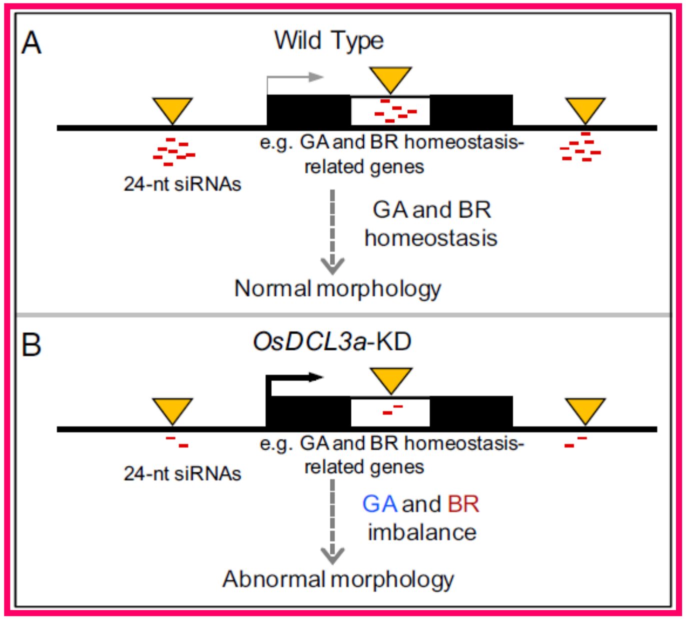


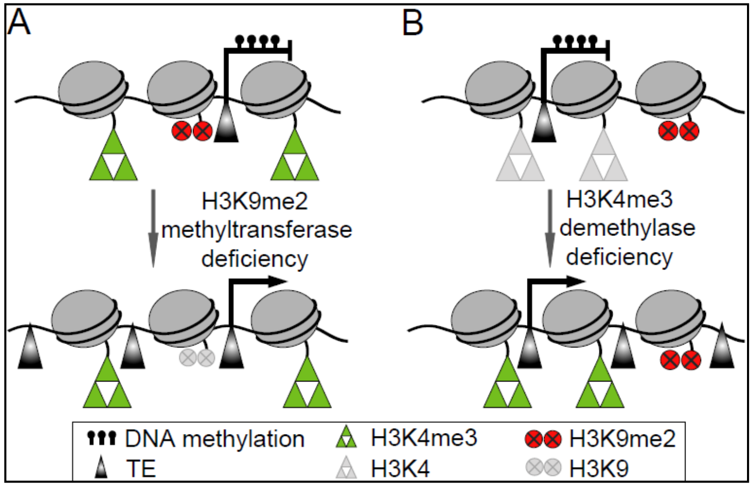
Hc-siRNAs associated with MITE impact adjacent genes controlling GA and BR homeostasis



Transposable elements of distinct chromosomal niche in rice







Modified figure in Feschotte *et al.*, (2002) *Nat. Rev. Genet.*

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