

MicroRNAs: Identification and Functional Analysis in Plants

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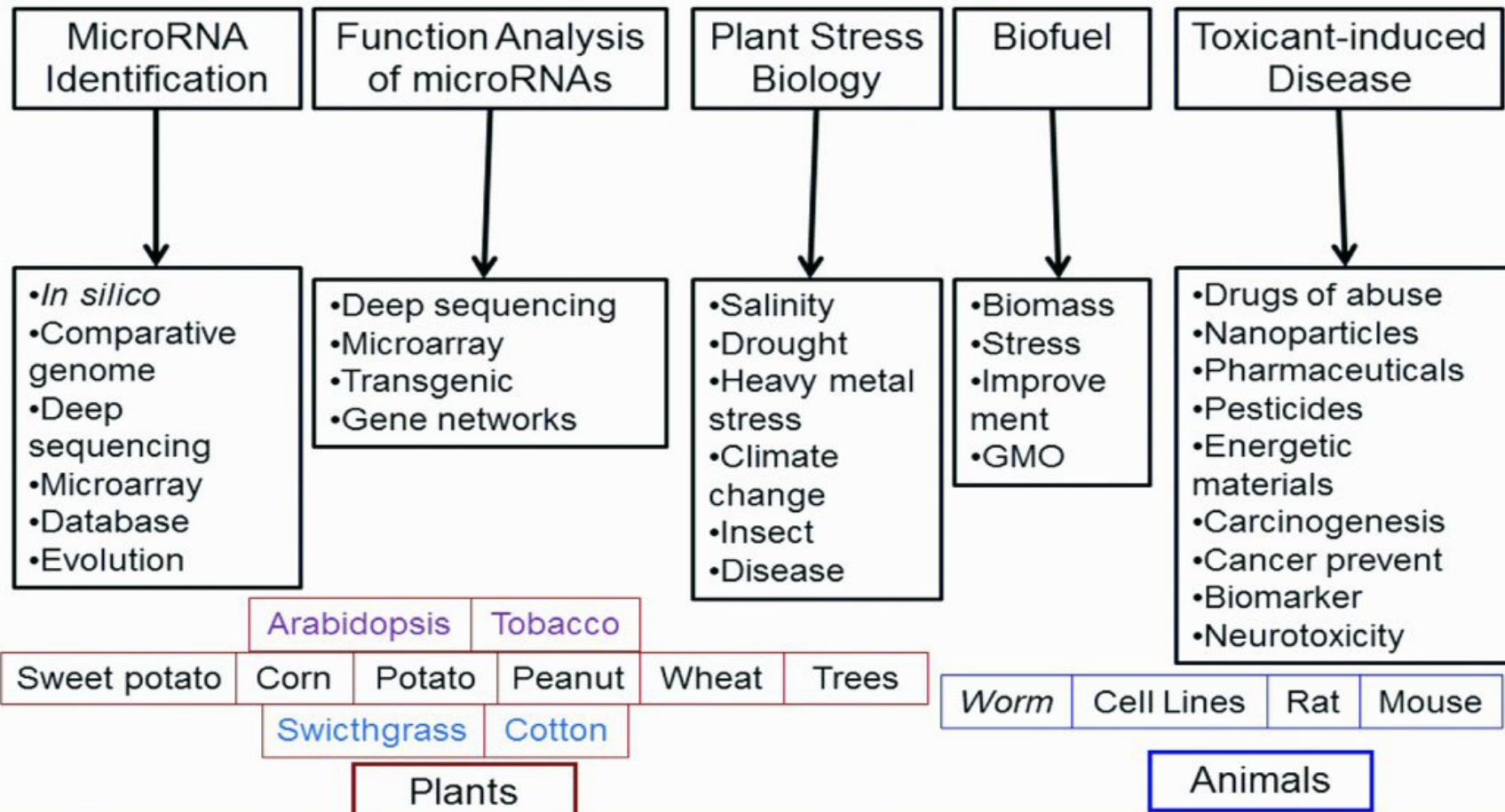
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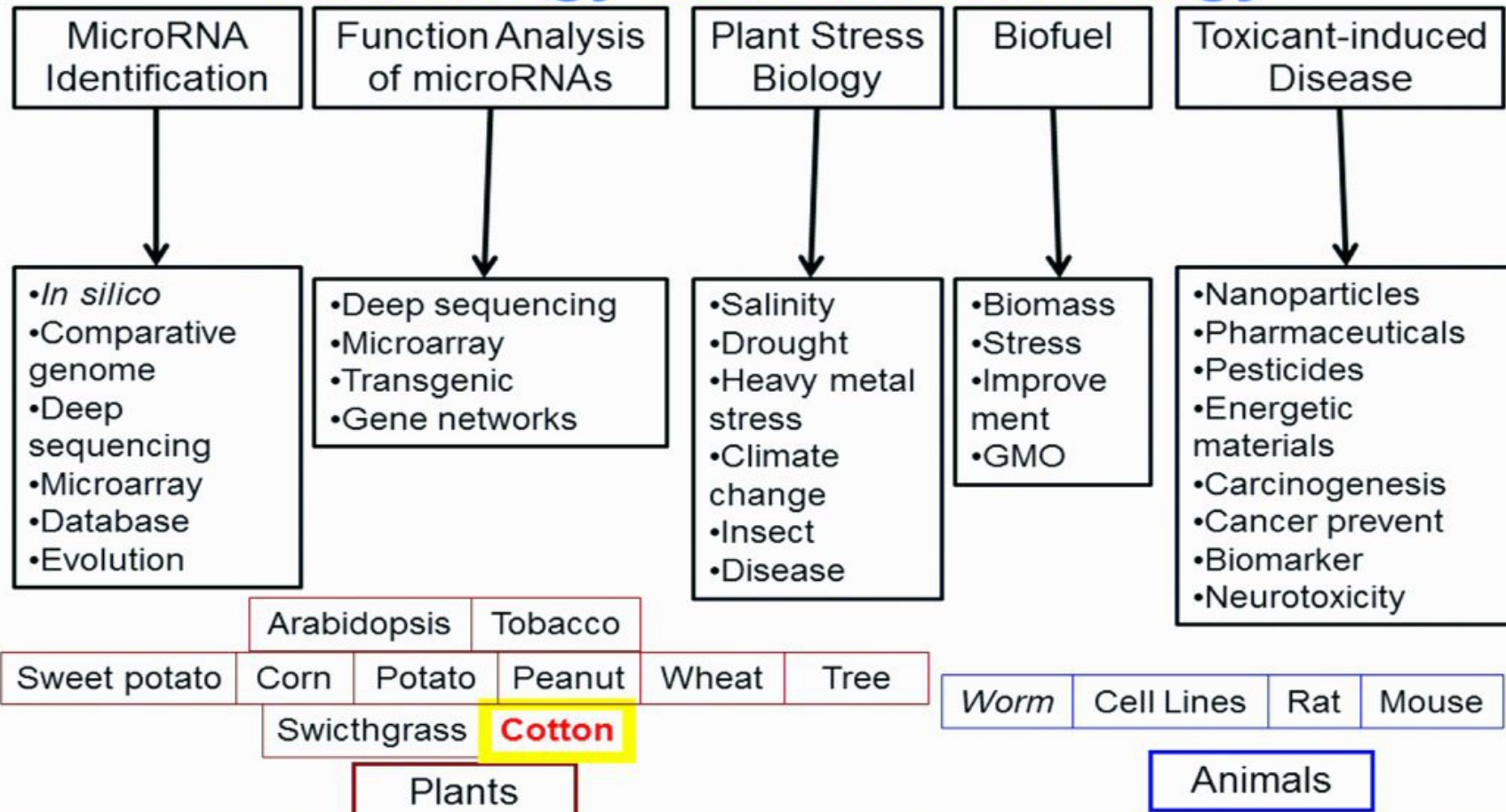
Dr. Zhang's Lab

Molecular Genetics, Environmental Toxicology and Biotechnology



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Cotton and microRNAs





Facts:

1. Genetic information: always from DNA to RNA to protein.
2. Every cell contains a complete set of genes.
3. Genes are not turned on in every cell or tissue; only a small set of genes is expressed.
4. Different genes are expressed at different times.

What controls gene expression?

microRNAs regulate the expression of more than 30% of protein-coding genes.

Outline

◆ Background on microRNAs (miRNAs)

What are miRNAs?

Why are miRNAs important?

The brief history of miRNAs

Biogenesis of miRNAs

Mechanism of miRNA-mediated gene regulation

◆ Our research on miRNAs in plants

miRNAs are different from other RNAs

Origin, diversity and conservation of miRNAs

Identifying miRNAs

miRNAs are differentially regulated in cotton

miRNA function in cotton fiber development

◆ Q & A

What is a microRNA (miRNA)?

microRNA is an abundant class of newly identified small non-coding regulatory RNAs.

Major characteristics of miRNAs:

- 18-26 nt in length with a majority of 21-23 nt
- non-coding RNA
- derived from a precursor with a long nt sequence
- this precursor can form a stem-loop 2nd hairpin structure
- the hairpin structure has low minimal free folding energy (MFE) and high MFE index



The history of miRNAs

1. Discovered *lin-4* in *C. elegans* in 1993 (Lee *et al.*, 1993)
2. Recognized miRNAs in animals in 2001 (Lee and Ambros, 2001)
3. First evidence that miRNAs are involved in cancer (Calin *et al.*, 2002)
4. First miRNA was identified in plant in 2002 (Reinhart *et al.*, 2002).
5. Lots of miRNAs and their targets discovered in model organisms during 2003 and 2004
6. Lots of studies indicate that almost all cancers relate to miRNA gene regulation (He *et al.*, 2005; Lu *et al.*, 2005; O'Donnell *et al.*, 2005)
7. miRNAs relate to a various diseases
8. Deep sequencing used in miRNA identification and expression analysis
9. 25,141 miRNAs deposited in miRBase miRNA database (08/2012). 5940 miRNAs from 67 plant species.

Functions of miRNAs

General functions in animals and plants

▲ Developmental timing

In animals: miR Lin 4: early stage L1 → L2

miR let 7: L2 → L3

In plants: miR 172: vegetative growth → reproductive growth

▲ Organ and tissue development

Brain, limb, nerve in animals

Leaf, flower, stem, root in plants

In animals: miR 196 → limb development

miR 1, 133 → muscle proliferation and differentiation

In plants: miR 172 → floral morphology

miR 165/166 → leaf morphology

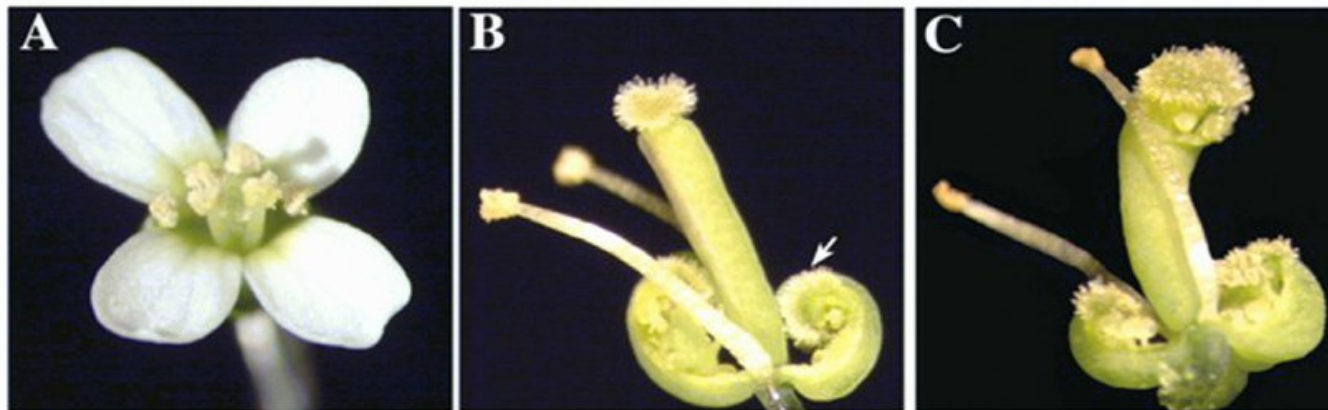
miR 164 → root and shoot development

▲ Signal transduction

Ex: In animals: miR 2, miR 7 and miR 11 → Notch signaling

In plants: miR 393 → auxin signaling → antibacterial resistance

miRNA 172 regulates floral development



Wild type

ap 2 loss-of-
function mutant

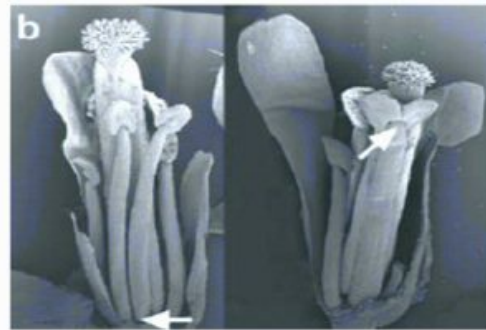
miR 172
overexpression

miRNA regulates plant development



miRNA 156

increasing leaf initiation,
decreasing apical dominance, and
forming bushier plant.



miRNA 164

stamens are fused together.



miRNA 172

sepal and petal disappeared.



miRNA 319

Leaf morphology

WT

miRNA

Functions of miRNAs

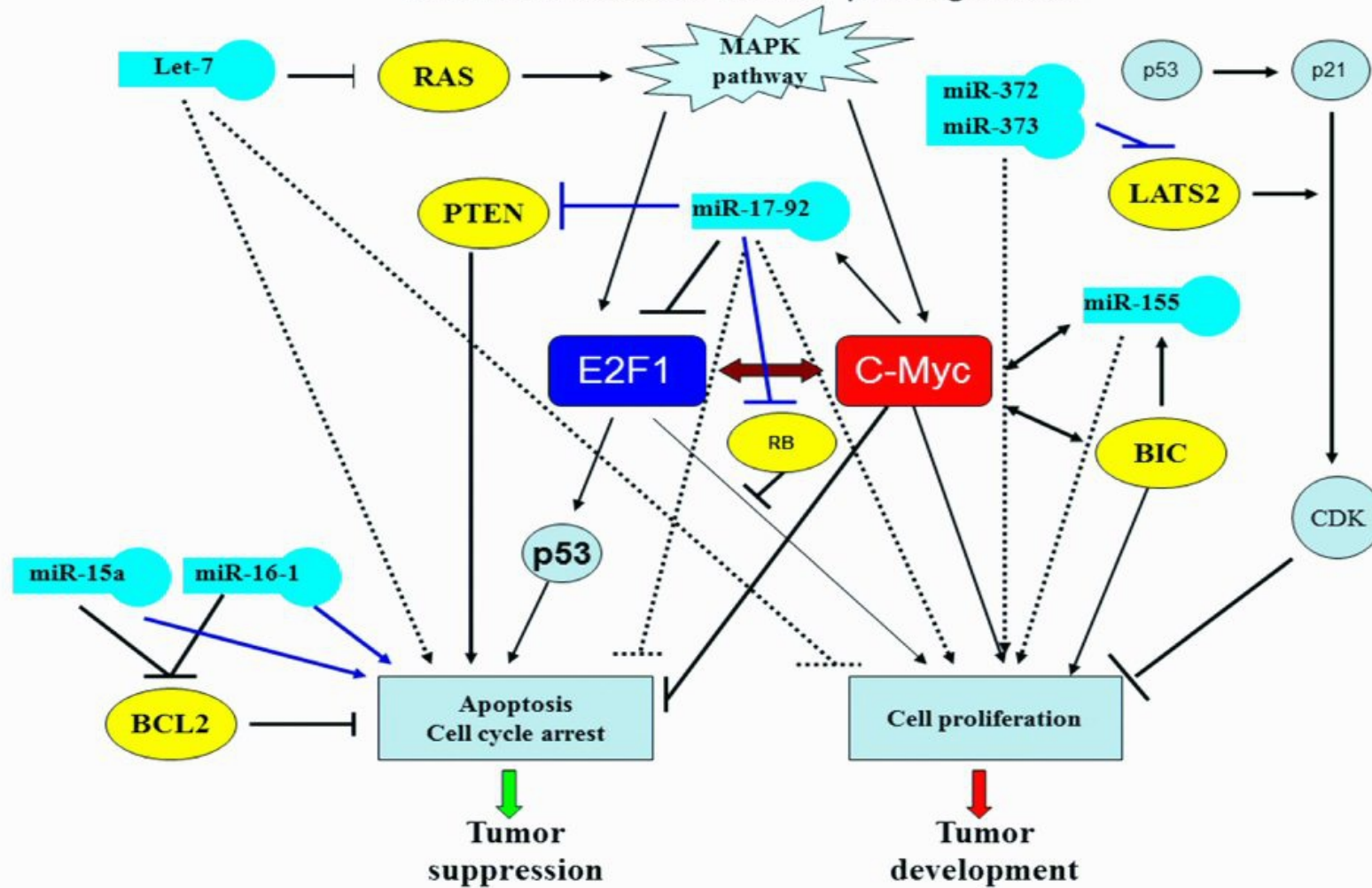
Specific functions in animals miRNA play critical roles in cancer pathogenesis

Cancer	miRNAs involved *	References
Brain cancer (Glioblastoma)	miR-21↑, miR-221↑, miR-128↓, miR-181a,b,c↓	Ciafre et al., 2005; Chan et al., 2005
Breast cancer	miR-21↑, miR-146↑, miR-155↑, miR-10b↓, miR-17-5p↓, miR-125b↓, miR-145↓, miR-125b↓	Hossain et al., 2006; Iorio et al., 2005; Si et al., 2007; Volinia et al., 2006; Ma et al., 2007
Cholangiocarcinoma	miR-21↑, miR-141↑, miR-200b↑	Meng et al., 2006
Chronic lymphocytic leukemia	miR-15↓, miR-16↓	Calin et al., 2002; Calin et al., 2004; Cimmino et al., 2005
Colorectal neoplasia	miR-10a↑, miR-17-92↑, miR-20a↑, miR-31↑, miR-96↑, miR-183↑, let-7↓, miR-143↓, miR-145↓	Michael et al., 2003; Volinia et al., 2006; Johnson et al., 2005; Bandres et al., 2006; Lanza et al., 2007; Michael et al., 2003
Diffuse large B cell lymphoma	miR-21↑, miR-155↑, miR-221↑	Lawrie et al., 2007
Head and neck cancer	miR-21↑, miR-205↑	Tran et al., 2007
Hepatocellular carcinoma (HCC)	miR-18↑, miR-224↑, miR-199↓, miR-195↓, miR-200↓, miR-125↓	Murakami et al., 2006
Lung cancer	let-7↓, miR-17-92↑	Takamizawa et al., 2004; Johnson et al., 2005; Hayashita et al., 2005; O'Donnell et al., 2005; Yanaihara et al., 2006
Lymphomas	miR-155↑, miR-17-92↑	Eis et al., 2005; Metzler et al., 2004; He et al., 2005; Woods et al., 2007
Ovarian cancer	miR-200a,b,c↑, miR-141↑, miR-199a↓, miR-140↓, miR-145↓, miR-125b↓	Iorio et al., 2007
Pancreatic cancer	miR-221↑, miR-181a↑, miR-21↑, miR148a,b↓	Bloomston et al., 2007
Papillary thyroid carcinoma	miR-221↑, miR-222↑, miR-146↑, miR-181↑	He et al., 2005; Pallante et al., 2006
Pituitary adenomas	miR-212↑, miR-026a↑, miR-150↑, miR-152↑, miR-191↑, miR-192↑, miR-024-1↓, miR-098↓, miR-15a↓, miR-16-1↓	Bottoni et al., 2007; Bottoni et al., 2005
Prostate cancer	let-7↑, miR-195↑, miR-203↑, miR-128a↓	Volinia et al., 2006
Stomach cancer	miR-21↑, miR-103↑, miR223↑, miR-218↓	Volinia et al., 2006
Testicular germ cell tumors	miR-372↑, miR-373↑	Voorhoeve et al., 2006

* ↑ represents overexpression; ↓ represents downexpression.

Zhang et al. 2008. *Journal of cellular and Molecular Medicine.*

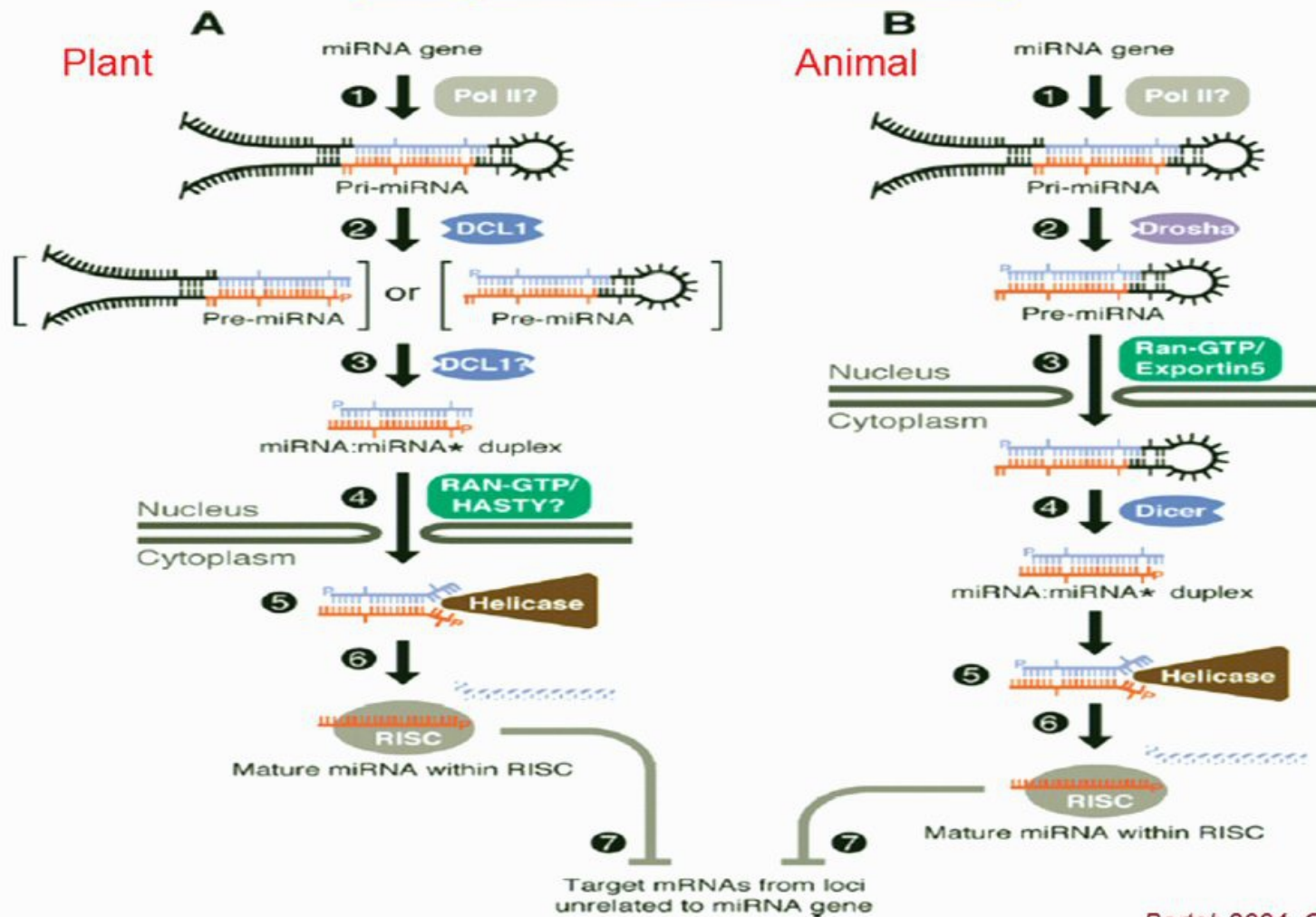
miRNA-mediated cancer pathogenesis



Let-7 tumor suppression gene; miR-17-92: oncogene.

Zhang et al. 2007. *Developmental Biology*.

Biogenesis of miRNAs

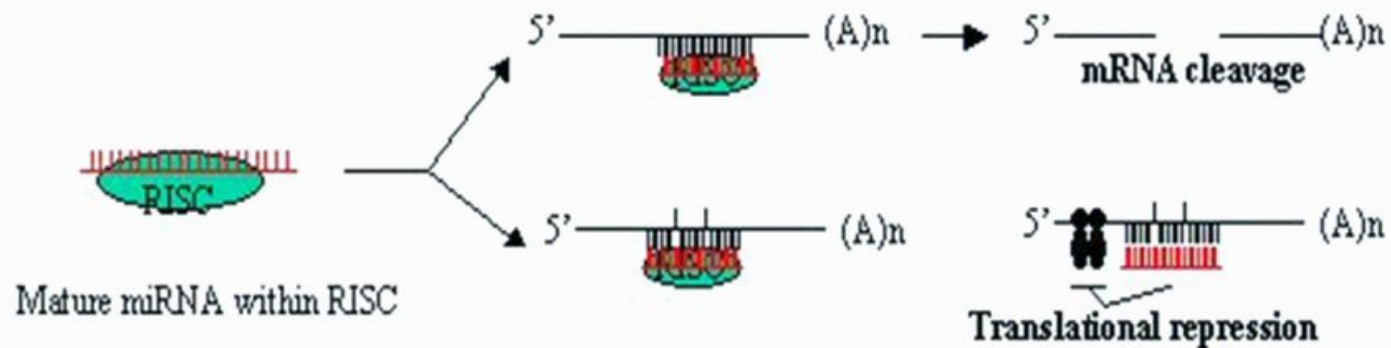


Bartel, 2004. Cell.

Mechanisms of miRNA-mediated gene regulation

Post-transcriptional gene regulation

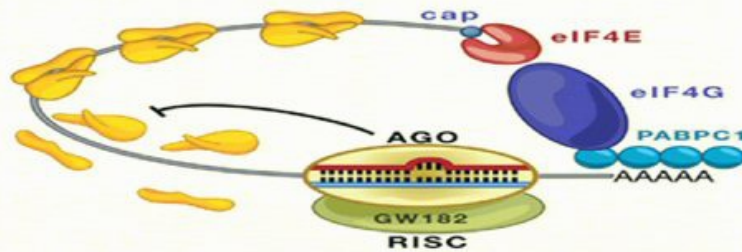
Two major molecular mechanisms



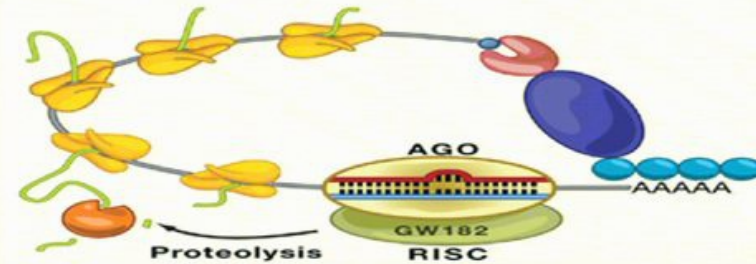
Zhang et al. 2006. Developmental Biology

Mechanisms of miRNA-mediated gene regulation

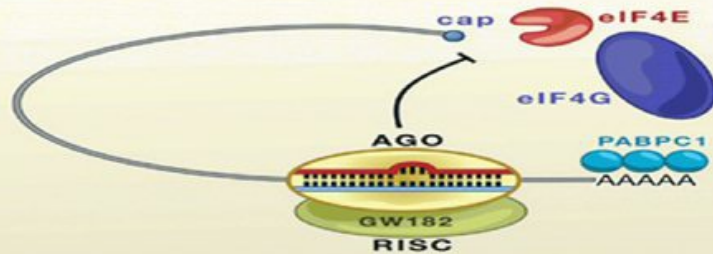
A Inhibition of translation elongation



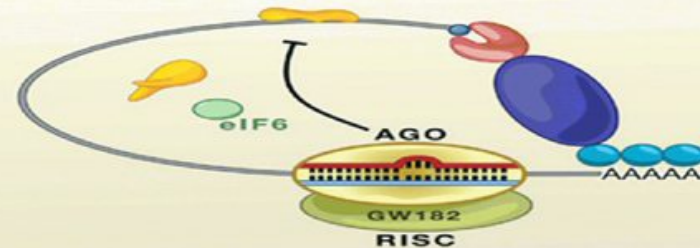
B Co-translational protein degradation



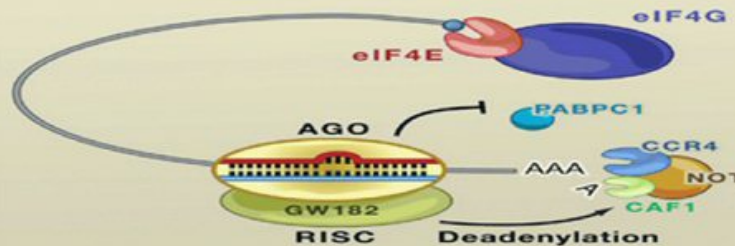
C Competition for the cap structure



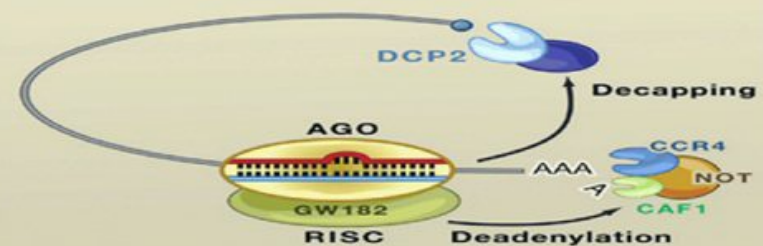
D Inhibition of ribosomal subunit joining



E Inhibition of mRNA circularization through deadenylation



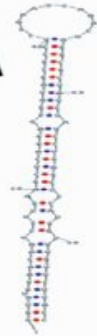
F Deadenylation and decapping



miRNAs are different from other RNAs: 2nd structure

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miRNA

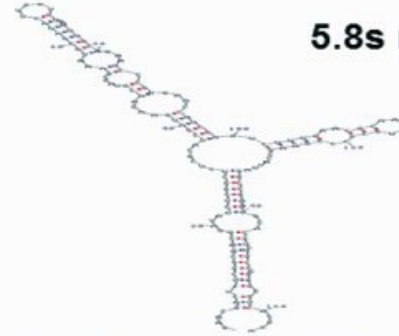


$\Delta G = -51.5$ (Initially -51.5) 05Jul19-20-46-04

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5.8s rRNA



$\Delta G = -49.01$ (Initially -50.01) 05Jul18-18-48-38

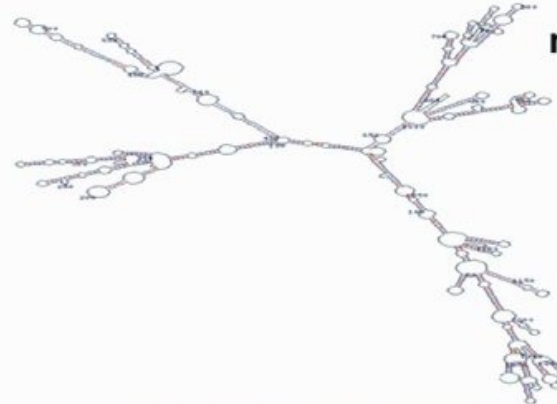
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tRNA



$\Delta G = -22.57$ (Initially -23.2) 05Jul18-14-49-20

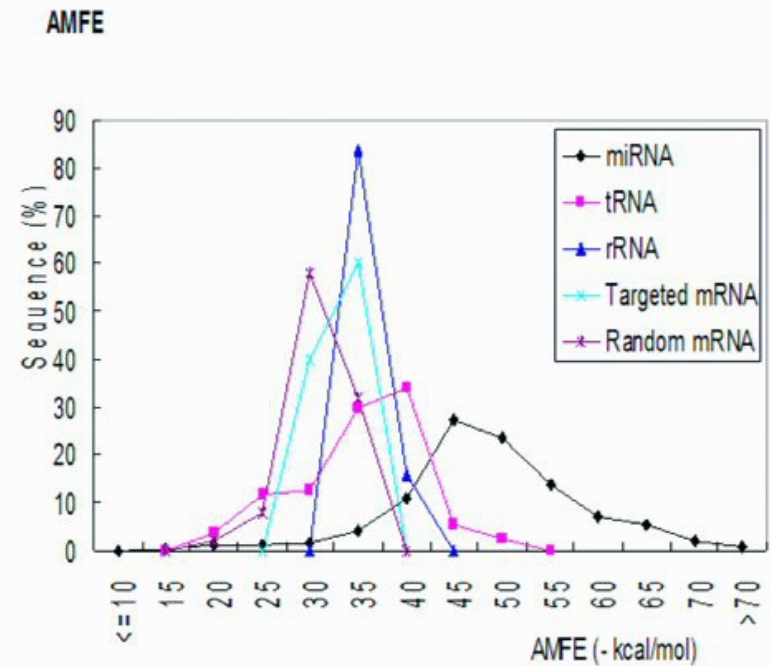
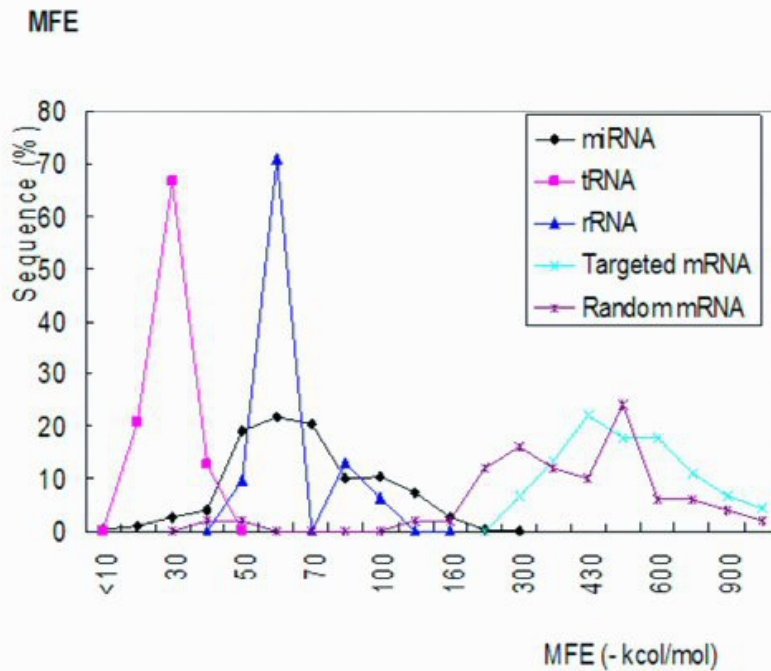
mRNA



$\Delta G = -390.53$ (Initially -426.9) 05Jul17-14-23-11

Zhang et al. 2006. Cellular and Molecular Life Sciences

miRNAs are different from other RNAs: Minimal free folding energy

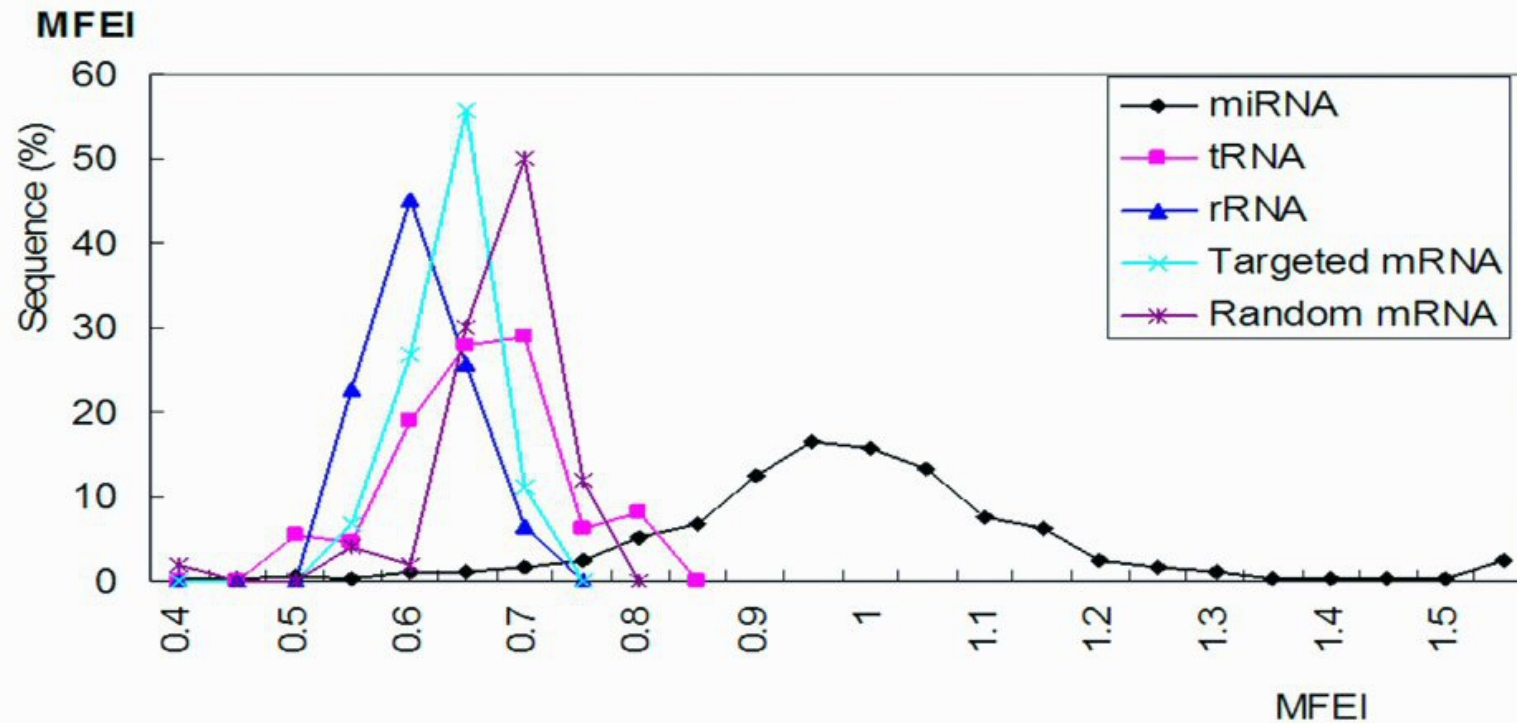


$$\text{AMFE} = \frac{-\text{MFE}}{\text{length of RNA sequence}} \times 100$$

Conclusion: MFE and AMFE are not sufficient criterion to distinguish miRNAs from other RNAs.

Zhang et al. 2006. Cellular and Molecular Life Sciences

miRNAs are different from other RNAs:
High minimal free folding energy index



$MFEI = AMFE / (G+C \text{ content } \%)$

Conclusion: MFEI is better criterion to distinguish miRNAs from other RNAs.

Zhang et al. 2006. Cellular and Molecular Life Sciences

Conservation and diversity

mir-156 family

AtMiR156a	TGACAGAAGA AC GAGCA	19
AtMiR156b	TGACAGAAGA AC GAGCA	19
AtMiR156c	TGACAGAAGA AC GAGCA	19
AtMiR156d	TGACAGAAGA AC GAGCA	19
AtMiR156e	TGACAGAAGA AC GAGCA	19
AtMiR156f	TGACAGAAGA AC GAGCA	19
OsMiR156a	TGACAGAAGA AC GAGCA	19
OsMiR156b	TGACAGAAGA AC GAGCA	19
OsMiR156c	TGACAGAAGA AC GAGCA	19
OsMiR156d	TGACAGAAGA AC GAGCA	19
OsMiR156e	TGACAGAAGA AC GAGCA	19
OsMiR156f	TGACAGAAGA AC GAGCA	19
OsMiR156g	TGACAGAAGA AC GAGCA	19
OsMiR156h	TGACAGAAGA AC GAGCA	19
OsMiR156i	TGACAGAAGA AC GAGCA	19
OsMiR156j	TGACAGAAGA AC GAGCA	19
AtMiR157a	TGACAGAAGA IAGAGAGCA	20
AtMiR157b	TGACAGAAGA IAGAGAGCA	20
AtMiR157c	TGACAGAAGA IAGAGAGCA	20
AtMiR157d	TGACAGAAGA IAGAGAGCA	19
Consensus	tgacagaaga ag gagca	

mir-158 family

AtMiR158	TCCCAAATGTAGACAAAGCA	20
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mir-159 family

AtMiR159a	TTGGAT TGAAGGGAGCTCT	20
AtMiR159b	TTGGAT TGAAGGGAGCTCT	20
AtMiR319a	TTGGAT TGAAGGGAGCTCC	19
AtMiR319b	TTGGAT TGAAGGGAGCTCC	19
Consensus	ttgga tgaagggagctc	

mir-160 family

AtMiR160a	TGCCTGGCTCCCTGTATGCC	20
AtMiR160b	TGCCTGGCTCCCTGTATGCC	20
AtMiR160c	TGCCTGGCTCCCTGTATGCC	20
OsMiR160a	TGCCTGGCTCCCTGTATGCC	20
OsMiR160b	TGCCTGGCTCCCTGTATGCC	20
OsMiR160c	TGCCTGGCTCCCTGTATGCC	20
OsMiR160d	TGCCTGGCTCCCTGTATGCC	20
Consensus	tgcttggctccctgtatgcc	

mir-161 family

AtMiR161	TTGAAAGTGACT...ACATCGCG	20
AtMiR163	TTGAAAGTGACTTGGAACTTCGAT	24
Consensus	ttgaa gact tggaa ac tccgat	

mir-162 family

AtMiR162a	TCGATAAACCTCTGCATCCA	20
AtMiR162b	TCGATAAACCTCTGCATCCA	20
OsMiR162	TCGATAAACCTCTGCATCCA	20
Consensus	tcgataaaacctctgcatcca	

mir-164 family

AtMiR164a	TGGAGAAGCAGGGCAGGTGC	20
AtMiR164b	TGGAGAAGCAGGGCAGGTGC	20
OsMiR164a	TGGAGAAGCAGGGCAGGTGC	20
OsMiR164b	TGGAGAAGCAGGGCAGGTGC	20
Consensus	tggagaagcagggcaggtgc	

mir-165 family

AtMiR165a	CGGACCAGGCTTCAT CC	20
AtMiR165b	CGGACCAGGCTTCAT CC	20
AtMiR166a	CGGACCAGGCTTCAT CC	20
AtMiR166b	CGGACCAGGCTTCAT CC	20
AtMiR166c	CGGACCAGGCTTCAT CC	20
AtMiR166d	CGGACCAGGCTTCAT CC	20
AtMiR166e	CGGACCAGGCTTCAT CC	20
AtMiR166f	CGGACCAGGCTTCAT CC	20
AtMiR166g	CGGACCAGGCTTCAT CC	20
OsMiR166a	CGGACCAGGCTTCAT CC	20
OsMiR166b	CGGACCAGGCTTCAT CC	20
OsMiR166c	CGGACCAGGCTTCAT CC	20
OsMiR166d	CGGACCAGGCTTCAT CC	20
OsMiR166e	CGGACCAGGCTTCAT CC	20
OsMiR166f	CGGACCAGGCTTCAT CC	20
NsMiR166	CGGACCAGGCTTCAT CC	18
ZmMiR166a	CGGACCAGGCTTCAT CC	20
ZmMiR166b	CGGACCAGGCTTCAT CC	20
ZmMiR166c	CGGACCAGGCTTCAT CC	20
ZmMiR166d	CGGACCAGGCTTCAT CC	20
Consensus	cggaccaggcttcat cc	

mir-167 family

AtMiR167a	TGAAGCTGCCA CGATGATCTA...	21
AtMiR167b	TGAAGCTGCCA CGATGATCTA...	21
OsMiR167a	TGAAGCTGCCA CGATGATCTA...	21
OsMiR167b	TGAAGCTGCCA CGATGATCTA...	21
OsMiR167c	TGAAGCTGCCA CGATGATCTA...	21
AtMiR169CAGCCAAGCATGACTTGCCG	20
OsMiR169CAGCCAAGCATGACTTGCCG	20
Consensus	c gcc a g atg a t	

mir-168 family

AtMiR168a	TCGCTTGGTGCAGGTCGGGA	20
AtMiR168b	TCGCTTGGTGCAGGTCGGGA	20
Consensus	tcgcttgggtgcaggctcggga	

mir-170 family

AtMiR170	TGATTGAGCCGTGTCAATATC	21
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mir-172 family

AtMiR172a	AGAATCTTGATGATGCTGCA	20
AtMiR172b	AGAATCTTGATGATGCTGCA	20
Consensus	agaatctt gat gat gct gca	

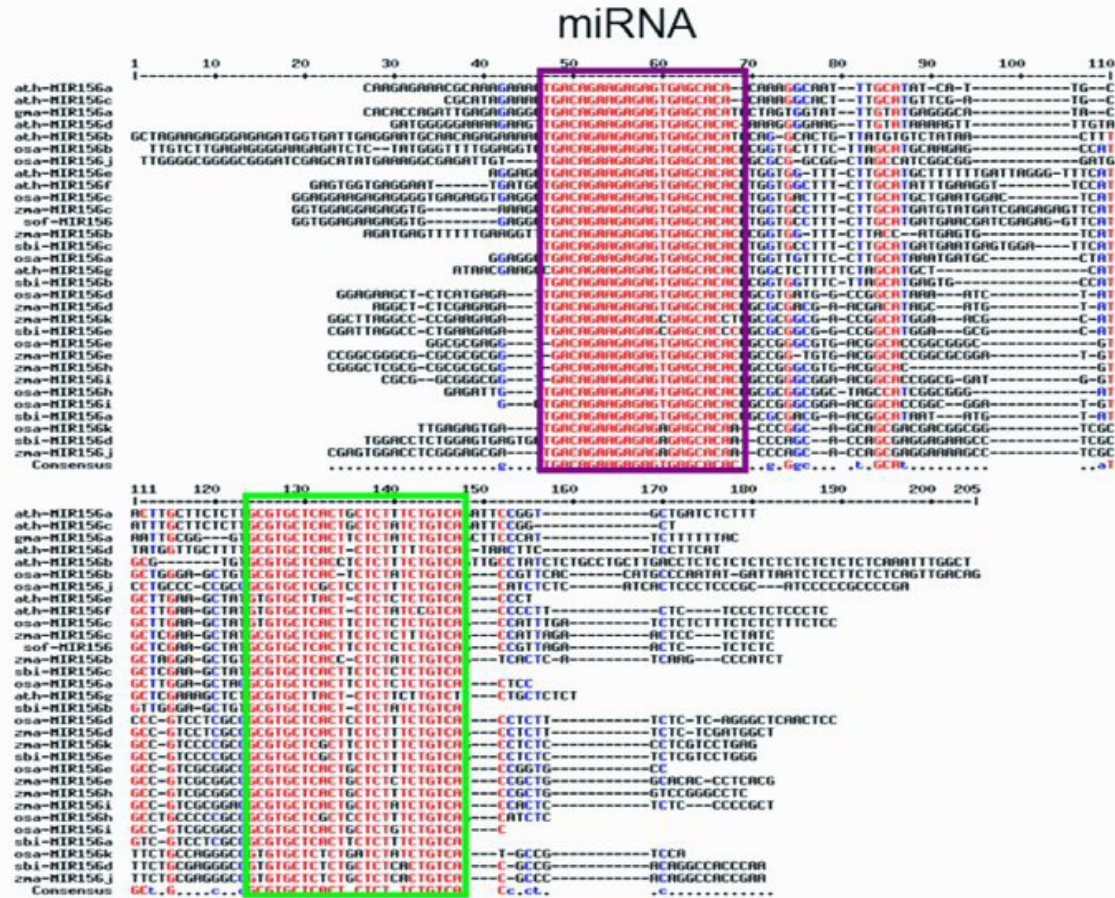
mir-173 family

AtMiR173	TTCGCTTGCAGAGAGAAATCAC	22
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mir-175 family

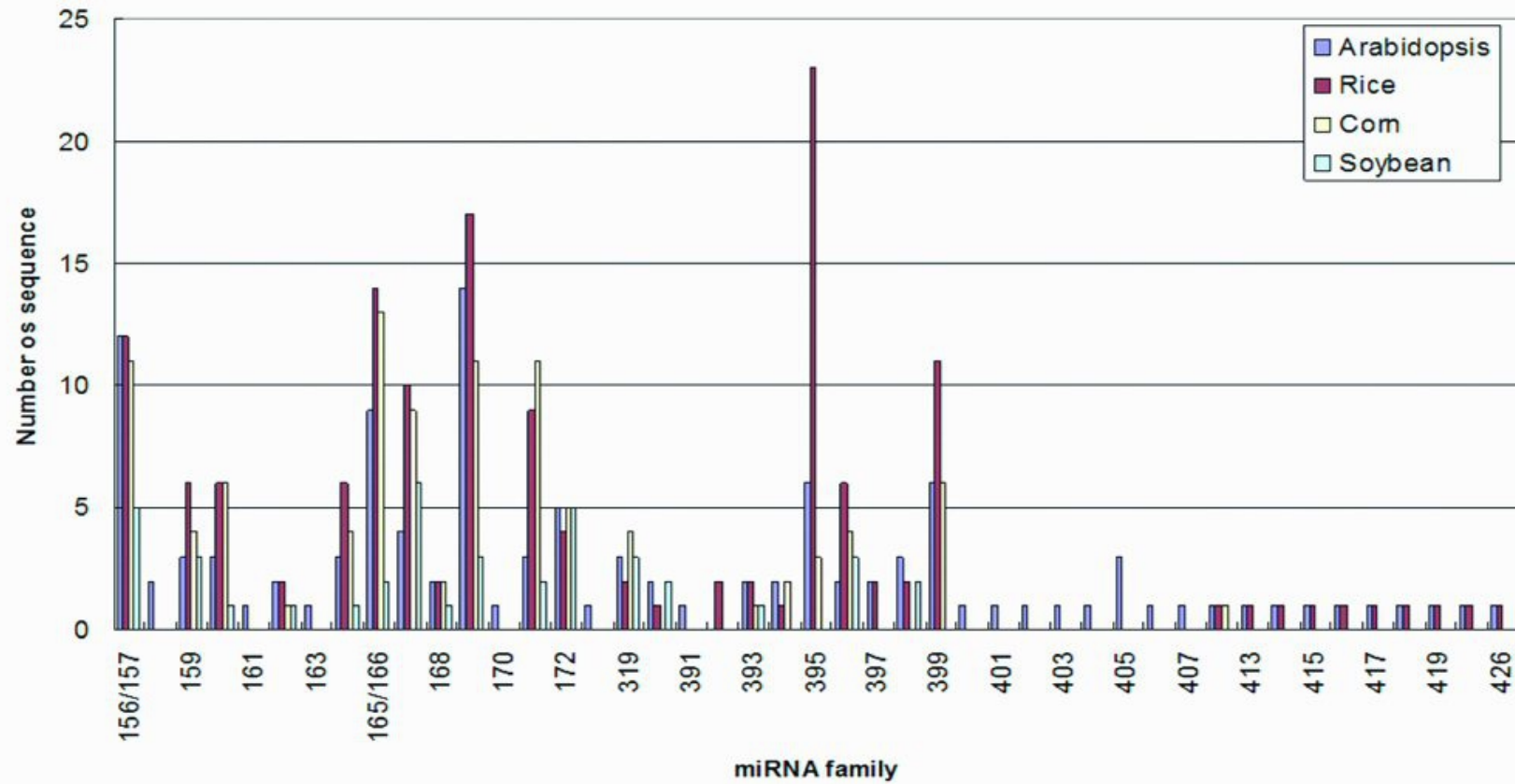
AtMiR175a	AAGTAGATGATGTAATTTGAGG	23
AtMiR175b	AAGTAGATGATGTAATTTGAGG	23
AtMiR175c	AAGTAGATGATGTAATTTGAGG	23
AtMiR175d	AAGTAGATGATGTAATTTGAGG	23
Consensus	aagtagatgatgtaat t t gagg	

Conservation of miRNAs

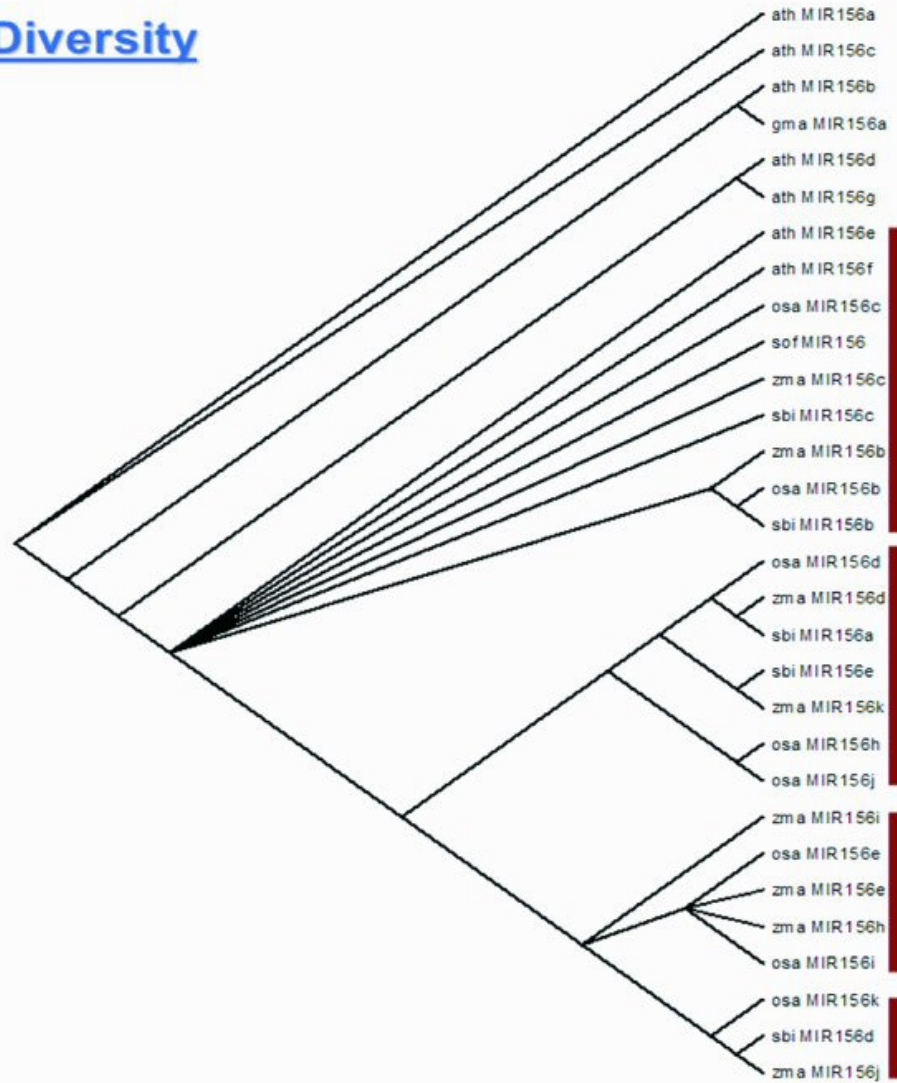


Zhang et al. 2006. *the Plant Journal*

Plant miRNA families

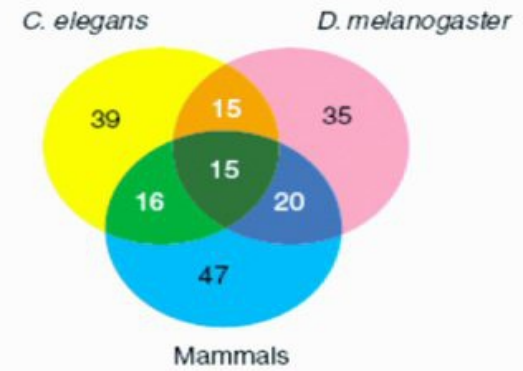
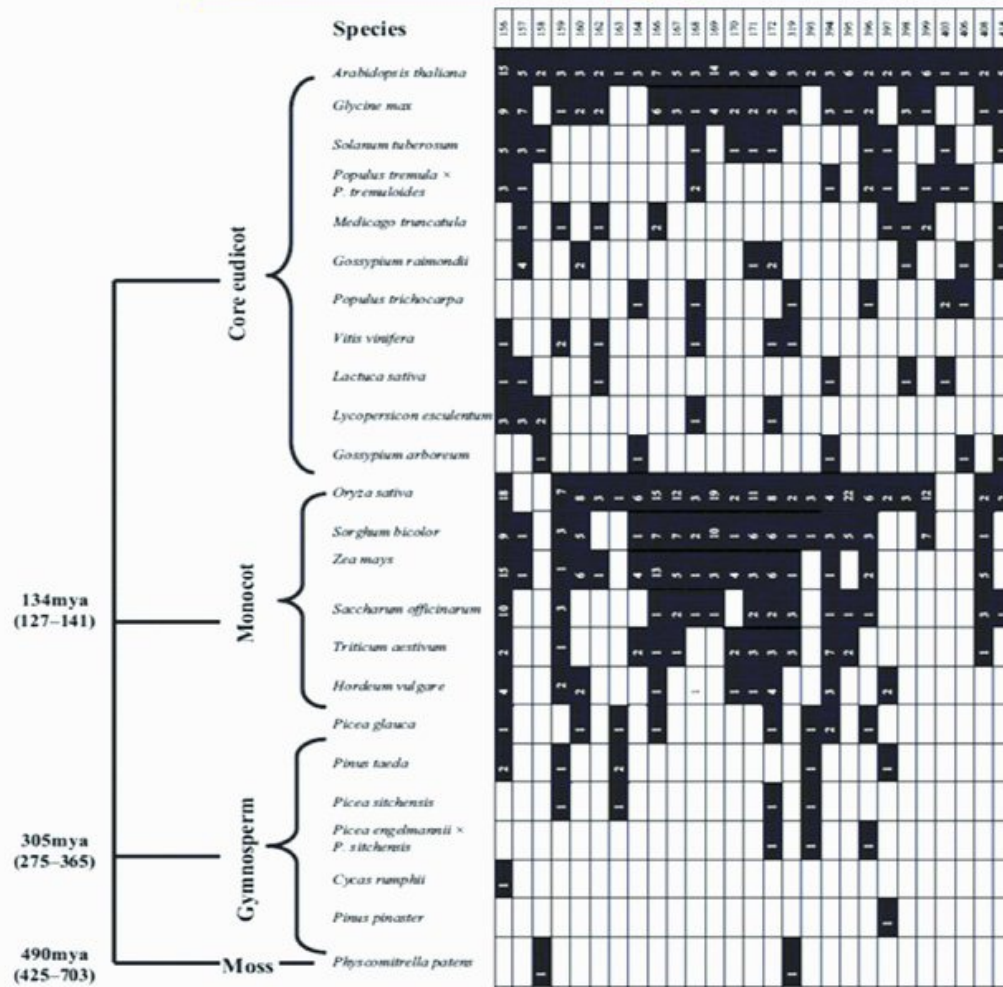


Diversity



Zhang et al. 2006. the Plant Journal

miRNAs are highly evolutionarily conserved



Zhang et al. 2006. the Plant Journal

Approaches for identifying miRNAs

	Genetic screening	Direct cloning after isolation of small RNAs	High throughput sequencing	Computational method	EST analysis
Specific software	No	No	Yes	Yes	No
Require genome sequence	No	No	No	Yes	No
Cost	High	High, but less than genetic screening	Highest	Low	Low
Efficiency	Low	High	Highest	Low	High
False positive possibility	Low	Low	Low	High	Moderate
Need experimental confirmation	No	No	No	Yes	Moderate
Possibility for new miRNAs	High	High	High	High	Low
Suitable to wide variety of species	Yes	Yes	Yes	No	Yes
Comprehensive	Yes	Yes	Yes	Moderate	Yes
miRNA quantitative information	No	Yes	Yes	No	Somewhat

Approach: EST analysis

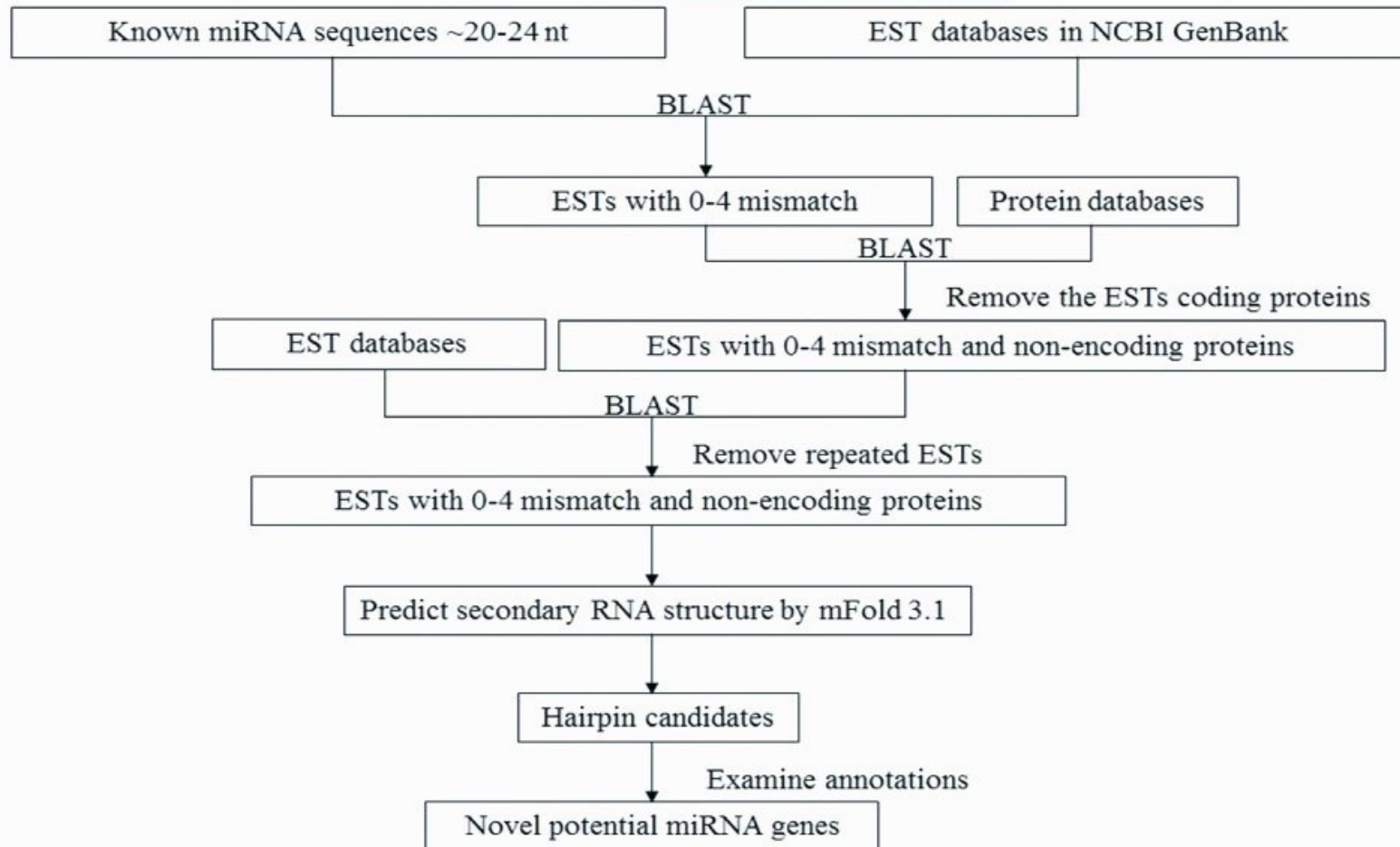


Figure Schematic representation of the miRNA gene search procedure used to identify homology of known *Arabidopsis* miRNA genes.

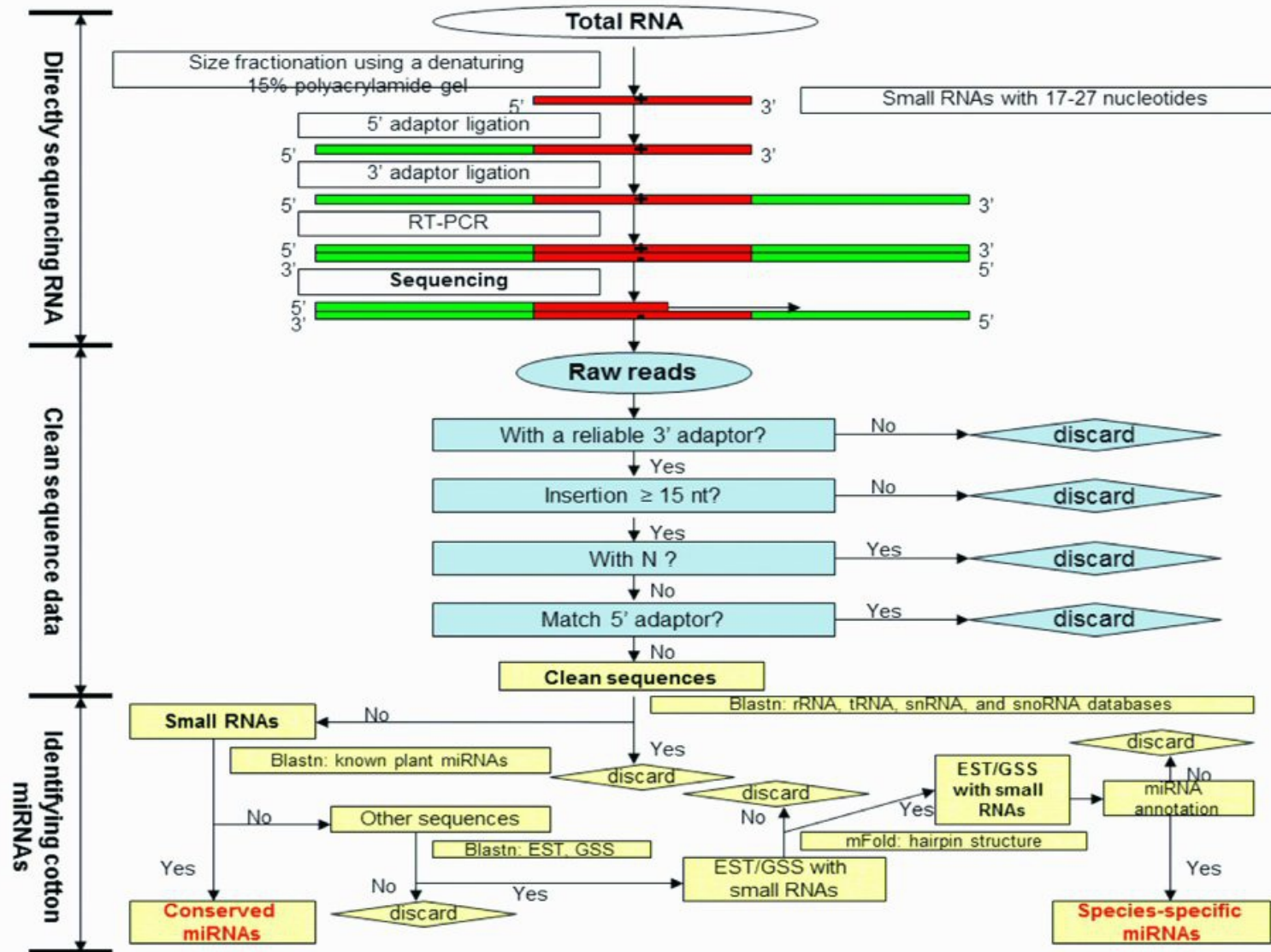
Zhang et al. 2005. Cell Research. 2006. FEBS Letters.

Identification of miRNAs using EST Analysis

- Cotton: 30 (Zhang et al., 2007)
- Corn: 188 (Zhang et al., 2006)
- Soybean: 69 (Zhang et al., 2008)
- Tobacco: 197 (Frazie et al., 2009)
- Switchgrass: 121 (Xie et al., 2007)
- Other plant species: ~600

Other labs adopting this method:

Apple, tomato, potato, grapes, wheat, grass etc.



miRDeepFinder: a miRNA analysis tool for deep sequencing of plant small RNAs

Fuliang Xie · Peng Xiao · Dongliang Chen ·
Lei Xu · Baohong Zhang

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Abstract miRDeepFinder is a software package developed to identify and functionally analyze plant microRNAs. The software package was also incorporated into miRDeepFinder for miRNA target identification using degradome

BIOINFORMATICS APPLICATIONS NOTE

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doi:10.1093/bioinformatics/btq568

Data and text mining

Advance Access publication October 7, 2010

Target-align: a tool for plant microRNA target identification

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Department of Biology, East Carolina University, Greenville, NC 27858, USA

Associate Editor: Jonathan Wren

ABSTRACT

Motivation: MicroRNAs (miRNAs) are important regulatory molecules. A critical step in elucidating miRNA function is identifying potential miRNA targets. However, few reliable tools have been developed for identifying miRNA targets in plants.

cleave targets by perfectly or near-perfectly binding to their target (Rhoades *et al.*, 2002; Schwab *et al.*, 2005). Thus, all the three currently available tools predict plant miRNA targets based on very strictly limited criteria. However, recent studies show that some miRNAs may inhibit translation by non-perfectly binding to

Identification of miRNAs using Solexa Deep Sequencing

- A powerful tool to identify miRNAs and their expression profiles.

Ongoing projects:

Chinese yew: 58 conserved and 2 novel miRNAs (Qiu et al., 2009).

Peanut: 75 conserved and 14 novel miRNAs (Zhao et al., 2010).

Barley: 31 conserved and 3 new miRNAs (Ozhuner et al., 2013, PLOS One).

Cotton: 121 conserved miRNA families and 328 potential novel miRNAs in cotton fiber project.

Identification of miRNAs using Solexa Deep Sequencing

- A powerful tool to identify miRNAs and their expression profiles.

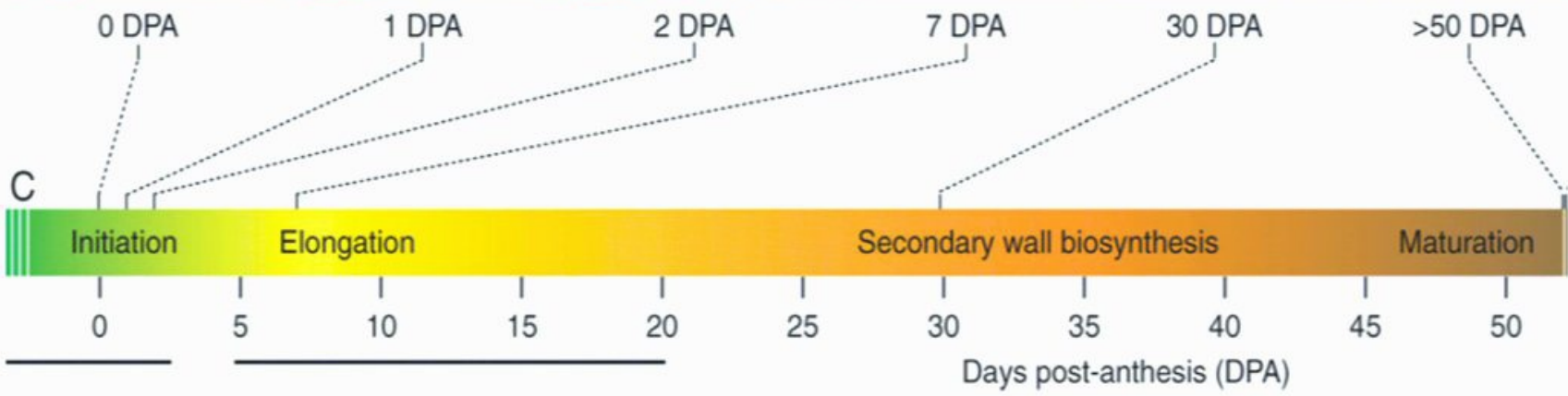
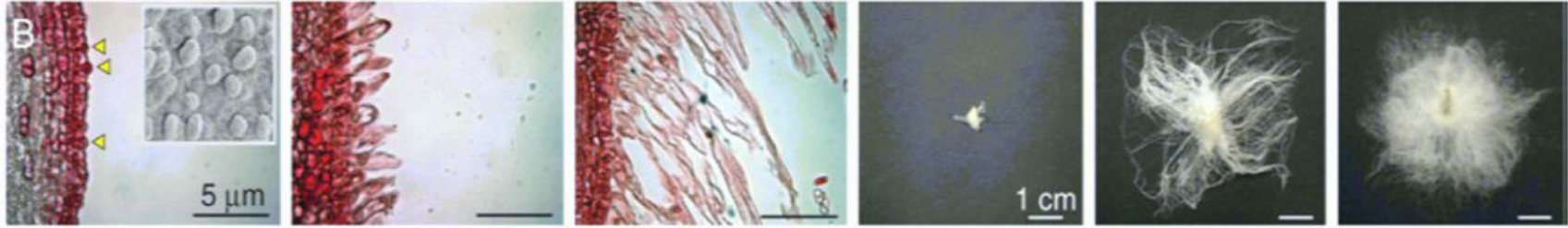
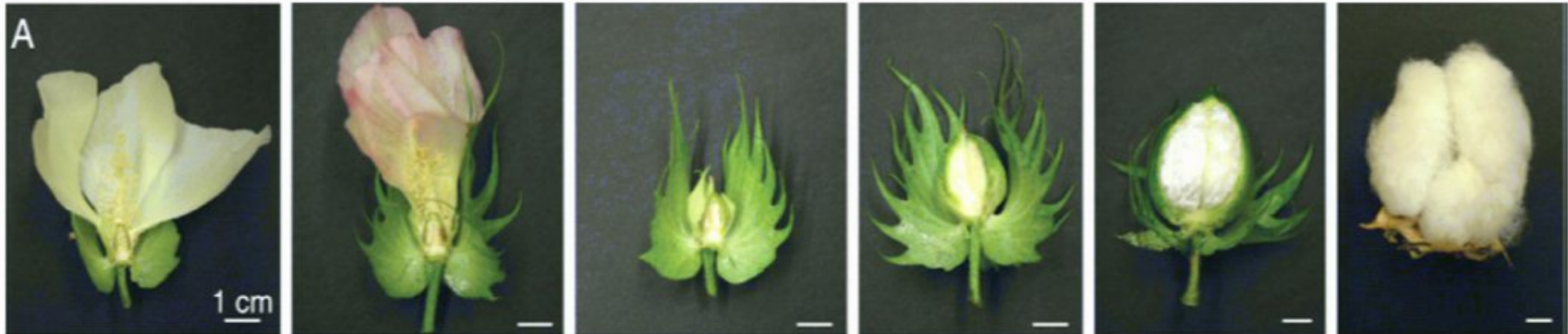
Ongoing projects:

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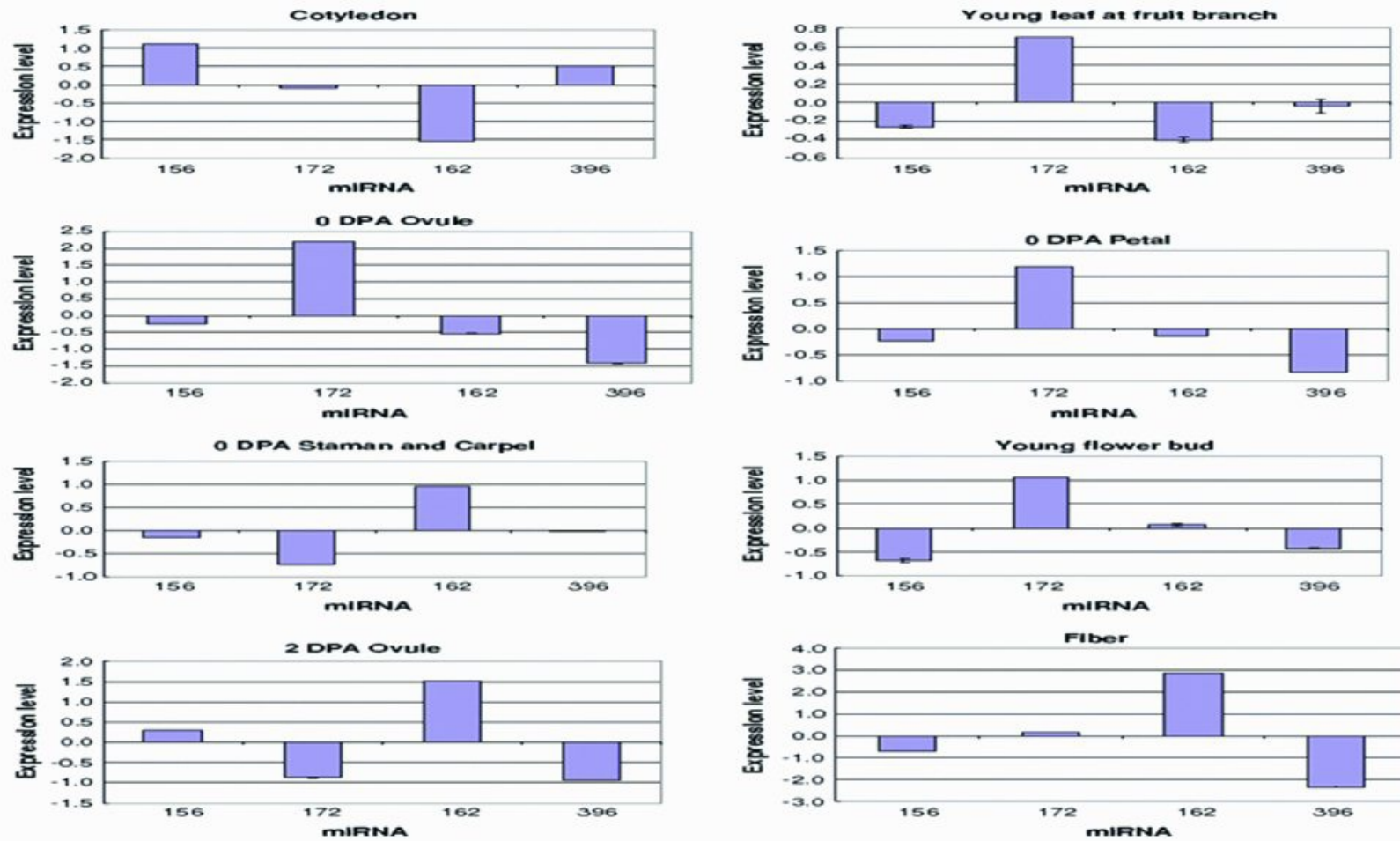
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Cotton: 121 conserved miRNA families and 328 potential novel miRNAs in cotton fiber project.

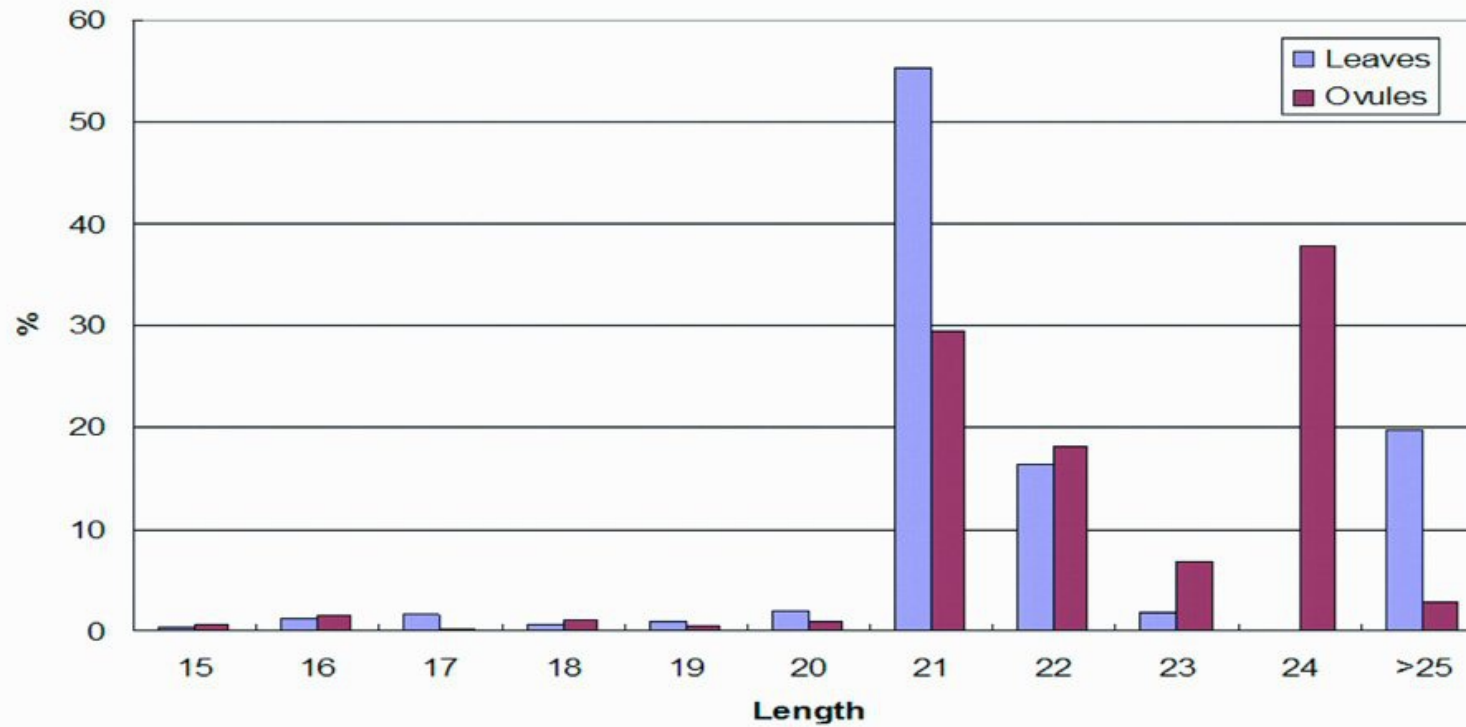


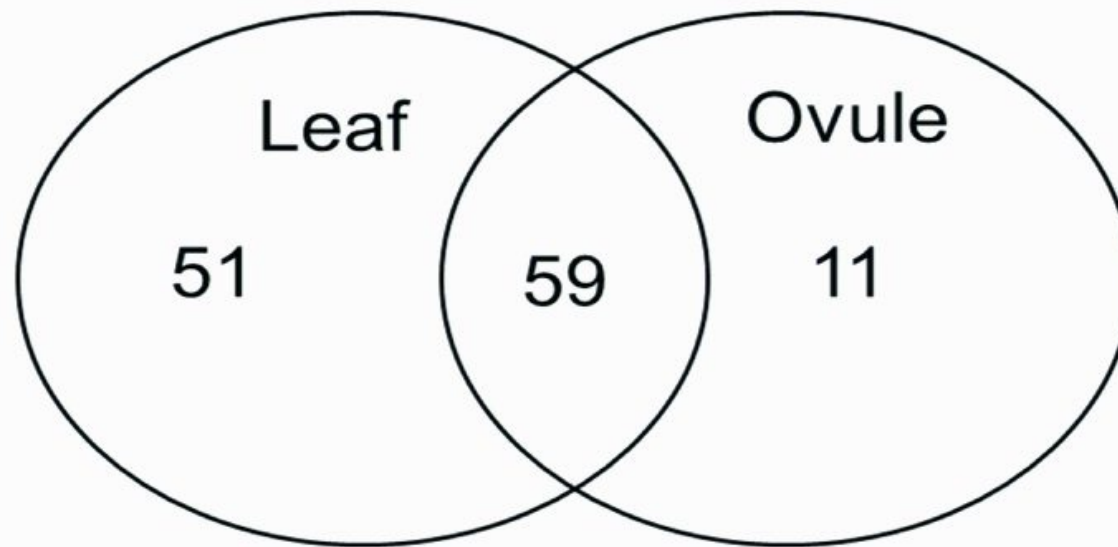
Differential Expression of miRNAs in Cotton



Zhang and Pan, 2009

Distribution of Small RNAs in Cotton Leaves and Ovules





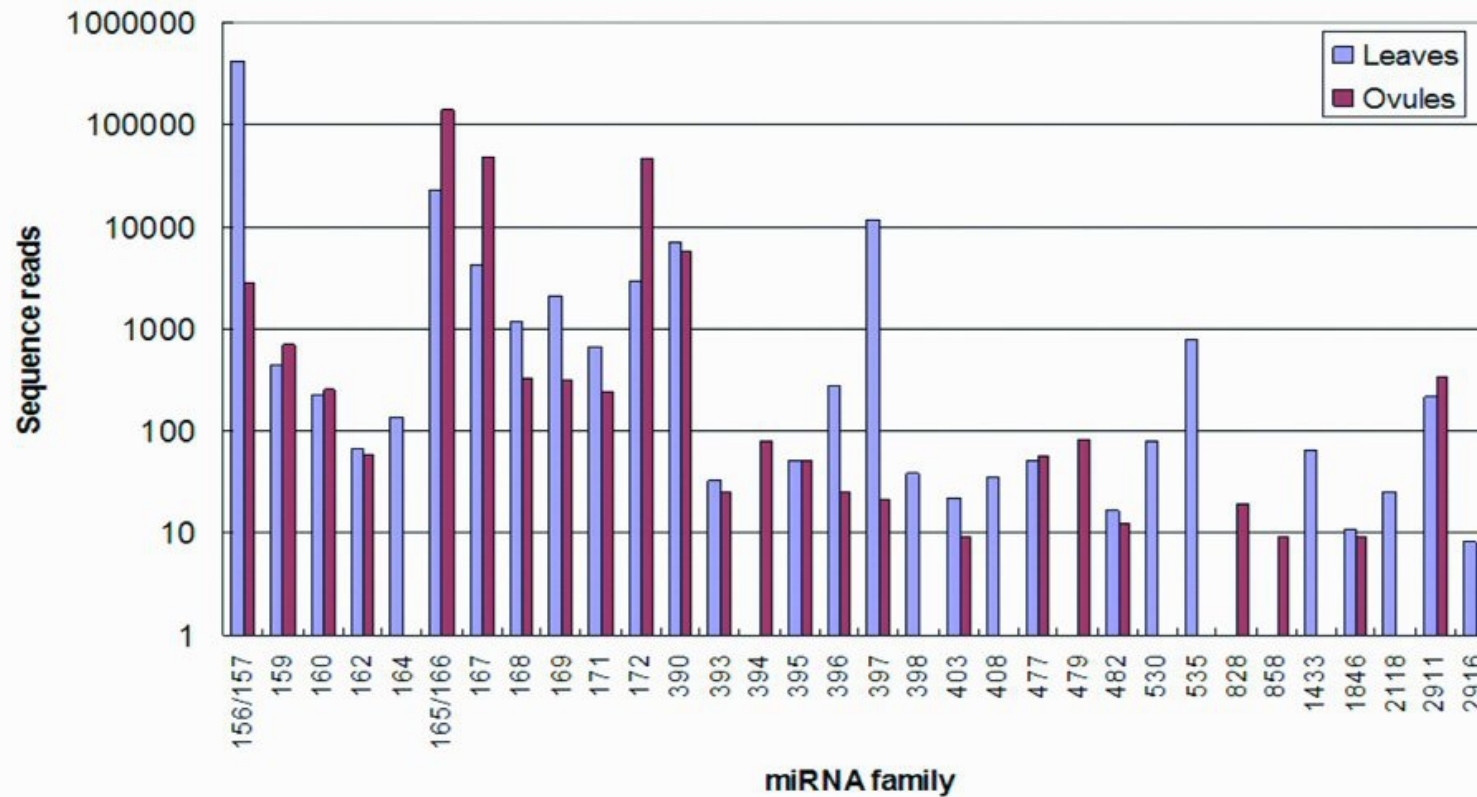
A total of 121 conserved miRNAs were identified in cotton leaves and ovules

Conserved miRNAs identified in cotton

miR family	Sequence Reads (per millions)			Significance	Number of Plant Species
	Leaves	Ovules	Ratio (ovule/leaf)		
156/157	405737	2786	0.007	**	9
159	447	693	1.552		9
160	222	258	1.163		9
162	67	59	0.887		7
164	139	0	0.000	**	10
165/166	22447	138448	6.168	**	9
167	4217	47687	11.309	**	10
168	1154	333	0.288		9
169	2114	317	0.150	*	9
171	671	246	0.366		10
172	2891	47302	16.363	**	8
390	6986	5724	0.819		8
393	33	25	0.747		9
394	0	78		**	6
395	53	53	1.003		7
396	277	25	0.090	**	8
397	11657	22	0.002	**	6
398	39	0	0.000	**	6
403	22	9	0.420		3
408	36	0	0.000	**	7
477	53	56	1.062		2
479	0	81		**	1
482	17	12	0.747		3
530	80	0	0.000	**	2
535	799	0	0.000	**	2
828	0	19		**	2
858	0	9		**	1
1433	64	0	0.000	**	1
1846	11	9	0.841		1
2118	25	0	0.000	**	2
2911	216	342	1.580		1
2916	8	0	0.000	**	1

A total of 32 miRNA families were identified in cotton leaves and ovules.

Conserved miRNAs identified in cotton



Novel miRNAs identified in cotton

A total of 328 novel potential miRNAs were identified in cotton leaves and ovules.

19 existed in both leaves and ovules

76 unique to leaves

252 unique to ovules

Novel miRNAs identified in cotton

Novel-257

```

AA  U  A      ACU      C      C--|  U
   AGC CGU GUUGG  UAGGGGUGGGU GGCCGGU  CGCC C
   UCG GUA CGGCC  AUCCUGCUCG CUGGCCA  GUGG A
CC  C  G      GUC      U      CGA^  C
  
```

Novel-313

```

U  UUA      A  -----|  UU
GUU AUG  UAUUCCAUCUCUUGCACACUGG CU  AGCCAGC \
UAG UAC  AUAAGGUGGGGGACGUGUGGU GA  UCGGUUG U
U  UAC      G  CUUCGACU^  UU
  
```

Novel-304

```

UG A      A-|  GC      AUGA      UUC
GGGAGU G GGUGUGGGAG  GUUGG  AAGAAUU  CCUUUG \
CCUUUA U CCAUACCCUC  CAACC  UUCUUAA  GGAAAC  A
GU  C      CC^  A-      CGG-      CUA
  
```

Novel-269

```

A  C  G  -  G      A  UGUUU  -----|  G
AG AAGCU GUGG AUGGGU GAG GGGUAAGAC AUGGAC  CAGUCUUU  UG A
UC UUUGG CACC UACCCA CUC UCCGUUCUG UAUUUG  GUCGGAAG  AC A
C  U  G  C  A      -  -----  AAGAAAUAAG^  A
  
```

Novel-70

```

CUUGAA---| GA  C  U  U  U  A-  UU  A  CA
      CG  CGAUG UCG GC CUGU AUG  CCUC  CGU GCU \
      GC  GCUGC AGC CG GACG UAC  GGAG  GUA CGG C
CGUAAGGAG^ --  -  -  U  -  CG  UU  -  GG
  
```

Red color: miRNA. Green color: miRNA*

Diversity of Conserved miRNA Families in Cotton Leaves and Ovules

miRNA family	Numbers of <u>Isoforms</u>			
	Total	Leaves	Ovules	Same ones
156/157	668	668	30	30
159	7	2	7	2
160	8	4	5	1
162	2	2	1	1
164	3	3	0	0
165/166	662	135	658	129
167	313	38	311	32
168	8	8	3	3
169	31	27	6	1
171	8	6	6	4
172	362	30	346	14
390	90	52	70	32
393	4	2	2	0
394	2	0	2	0
395	1	1	1	1
396	6	6	1	1
397	76	76	1	1
398	2	2	0	0
535	10	10	0	0

Cotton MicroRNA examples

156

```

u      -|  a      a      acu  uu      cuuga
gacaga agag gugagcac cagggg  uuc  gcaug  \
cugucu ucuc cacucgug gucucg  aag  cguac   g
a      a^  c      c      ---  uu      uuucc
  
```

169

```

      a      c      -  g  ---|  g
uagccaag auga uugccug ca cu  caugg a
aucgguuc uacu aacggac gu gg  guacc a
      c      -      a  g  uuu^  c
  
```

172

```

-  ag gc au      a      cu-      aa aa      gacc  .-aaaaacauaaaaauua  aaa
cu au u aa uggugc uggg  agcua uc gaucucau  uc      caag  \
ga ua g uu accacg accu  ucgau ag uuggggua  ag      guuc  a
ca aa -- --      -  ucu  aa cc      aa--      (447 nt loop)  agg
  
```

395

```

      ug u      cug      -  u  g      -----|  u
gaguucccc a cacuua  ggguuau gggg cu cucuu      cucu c
cucaagggg u gugaagu  uccagua ccuc gg gggaa      gaga u
      gu u      ca-      g      u  a      aauguucc^  c
  
```

779

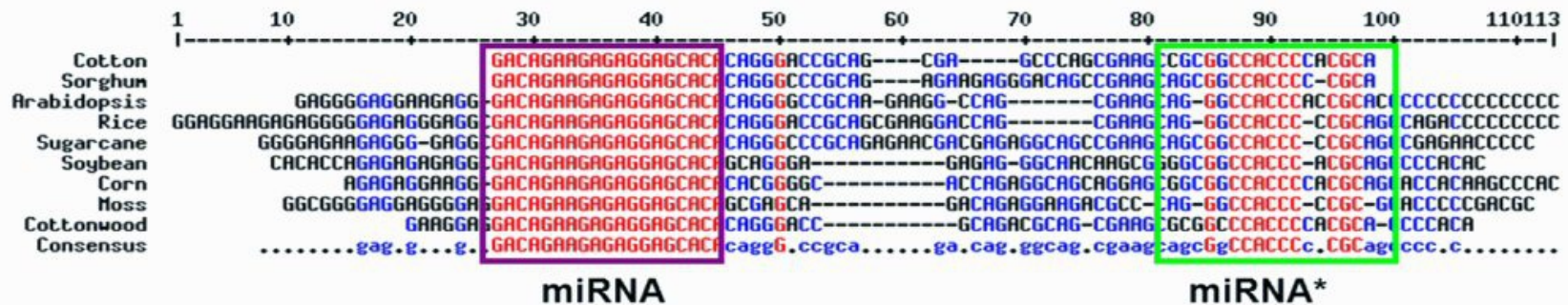
```

-      -|  u      g
aug gcagc acgug gaga a
uac cgucg uguac uucu u
u      u^  -  g
  
```

Comparison of miRNAs from different species

miR156

Species	Common name		miRNA 156
<i>Gossypium hirsutum</i>	Cotton	Eudicots	UGACAGAAGAGAGUGAGCAC
<i>Arabidopsis thaliana</i>		Eudicots	UGACAGAAGAGAGUGAGCAC
<i>Glycine max</i>	Soybean	Eudicots	UGACAGAAGAGAGUGAGCAC
<i>Populus trichocarpa</i>	Black cottonwood	Eudicots	UGACAGAAGAGAGUGAGCAC
<i>Oryza sativa</i>	Rice	Monocots	UGACAGAAGAGAGUGAGCAC
<i>Sorghum bicolor</i>	Sorghum	Monocots	UGACAGAAGAGAGUGAGCAC
<i>Saccharum officinarum</i>	Sugarcane	Monocots	UGACAGAAGAGAGUGAGCAC
<i>Zea mays</i>	Corn	Monocots	UGACAGAAGAGAGUGAGCAC
<i>Physcomitrella patens</i>		Mosses	UGACAGAAGAGAGUGAGCAC



Comparison of miRNAs from different species

miRNA 156

A. Cotton

```

u - | a a acu uu cuuga
gacaga agag gugagcac cagggg uuc gcaug \
cugucu ucuc cacucgug gucucg aag cguac g
a a^ c c --- uu uuucc
    
```

B. *Arabidopsis thaliana*

```

- - a --- uu ua
ugacagaa gag agugagcac caa aggcaa ugca u
acugucuu cuc ucacucgug guu uucguu acgu c
u g c cuc -c ua
    
```

C. Soybean

```

- - au agug u a
ugacaga agaga gugagcac gcu gua uuguau g
acugucu ucucu cacucgug ugg cgu aacauac g
a u cg ---g u g
    
```

D. Black cottonwood

```

- a a acu uu ac
ugacaga agag gug gcac cagggg uuc gcaug g
acugucu ucuc cac cgug guuucg aag cguac u
a c c c --- uu u
    
```

E. Rice

```

- - gacu uu cu aa
ugacaga agaga gugagcacacau ggu uuc gcaug g u
acugucu ucucu cacucguguguaucg aag cguac c g
c u ---- uu -u ag
    
```

F. Sugarcane

```

- a gccu uu ugaacg
ugacaga agaga gugagcac cauggu uuc gcauga auc
acugucu ucucu cacucgug guaucg aag cguacu ugç
c u ---- cu -----
    
```

G. Sorghum

```

- a gccu uu ugaau g
ugacaga agaga gugagcac cauggu uuc gcauga a
acugucu ucucu cacucgug guaucg aag cguacu g
c u ---- cu uaggu
    
```

H. *Zea mays*

```

- a gguu c ag
ugacaga agag gugagcac cacggg ucuua caug u
acugucu ucuc cacucgug gugucg aggau guac u
a c c ---- c u
    
```

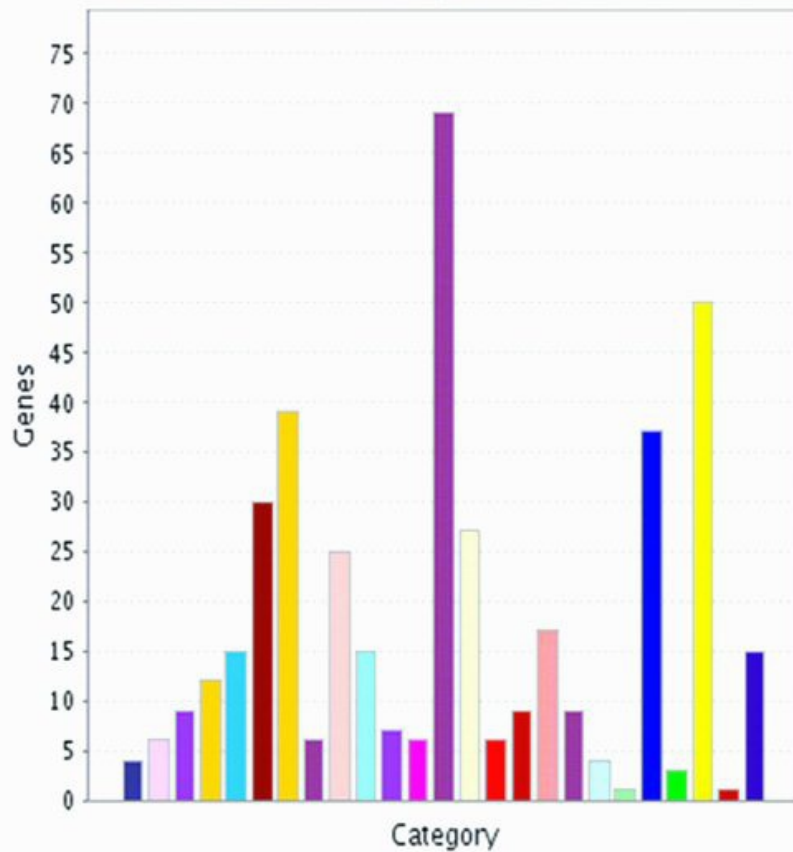
I. *Physcomitrella patens*

```

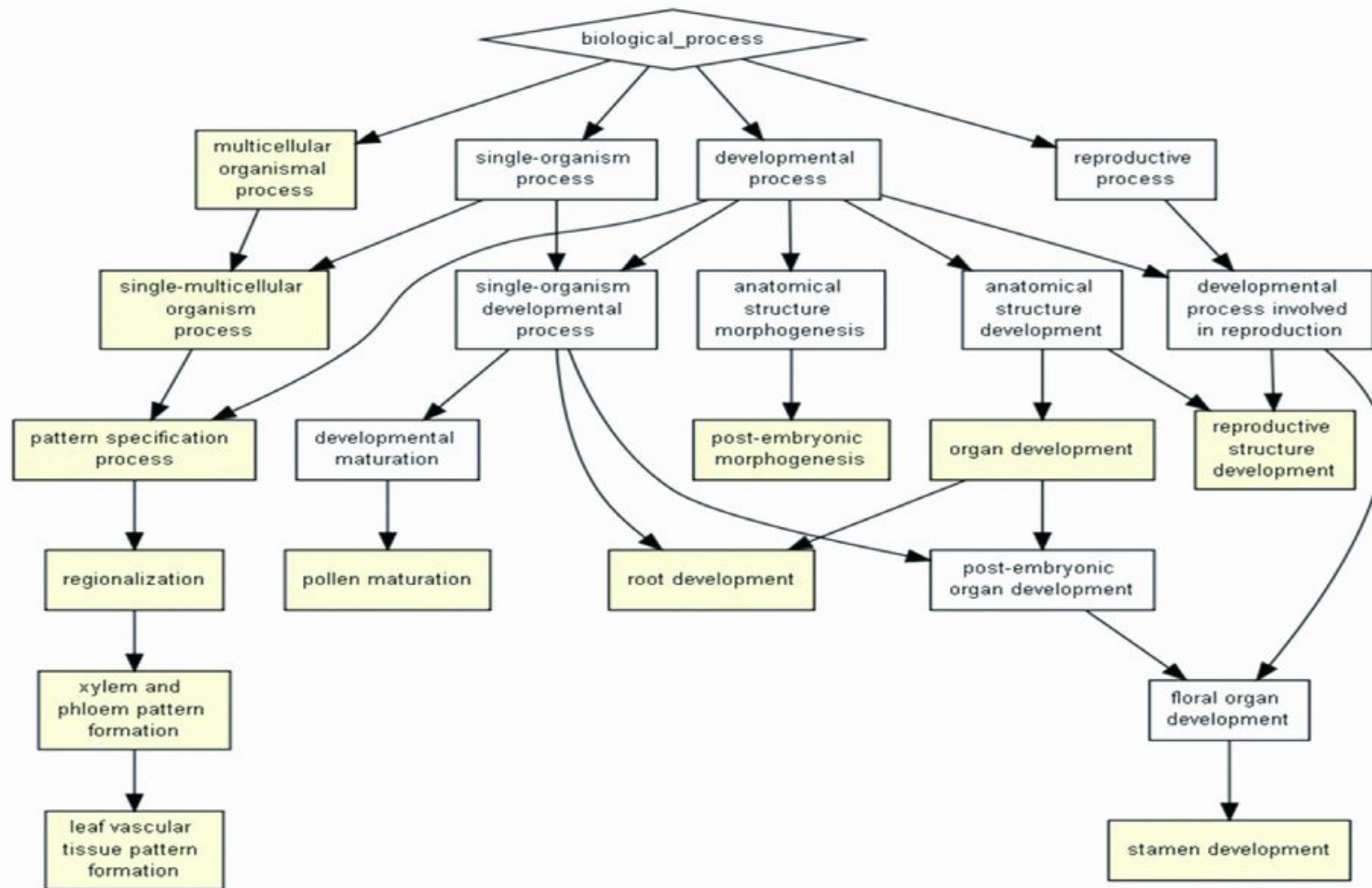
- gc a u aug
ugacag aagagagugagcac a ug gca guac a
gcuguc uuucucuacucgugu ac cgu caug g
c gu c u aag
    
```

Function type	Number of miRNA	Number of target
Apoptosis	7	2
Cell cycle	3	2
Cell migration	2	2
Chromatin remodeling	1	1
Circadian clock	1	2
Development	7	3
Fiber development	22	45
Hormone	8	5
Metabolism	42	60
signal transduction	23	13
Stress response	13	22
Transcription factor	82	99
Total	93	256

Target classification



- calcium-binding protein (PC00060)
- cell adhesion molecule (PC00069)
- chaperone (PC00072)
- cytoskeletal protein (PC00085)
- defense/immunity protein (PC00090)
- enzyme modulator (PC00095) 7.1%
- hydrolase (PC00121) 9.2%
- isomerase (PC00135)
- kinase (PC00137)
- ligase (PC00142)
- lyase (PC00144)
- membrane traffic protein (PC00150)
- nucleic acid binding (PC00171) 16.4%
- oxidoreductase (PC00176)
- phosphatase (PC00181)
- protease (PC00190)
- receptor (PC00197)
- signaling molecule (PC00207)
- storage protein (PC00210)
- structural protein (PC00211)
- transcription factor (PC00218) 8.8%
- transfer/carrier protein (PC00219)
- transferase (PC00220) 11.8%
- transmembrane receptor regulatory/adaptor protein (PC00226)
- transporter (PC00227)



miRNA Targets in Cotton

SBP (AY779340)	5'	<u>592</u>	<u>acgcuccucucucuguca</u>	601	3'
miR-156	3'				5'
Heat-shock protein (AY819767, 5'-3')	659	<u>cuangcuaucgcgccugcua</u>	678		
miR-391 (3'-5')			•		
TC39605 (Katanin)	1851	<u>guguucaagaaaguuuuaca</u>	1871		
miR-396 (3'-5')			• • •		
TC39605 (Katanin)			<u>uucaguucuuucg auagugu</u>		
AF085717 (callose synthase)	2834	<u>caguucaggaugcuuuuug</u>	2854		
miR-396 (3'-5')			• • • •		
			<u>uucaguucuuucg auagugu</u>		
TC34607 (fiber protein Fb23)	81	<u>auucggagauguugaggauca</u>	101		
miR-414 (3'-5')			• •		
			<u>uaagcuacuacuucucuacu</u>		
TC37120 (Fiber quinone-oxidoreductase)	1037	<u>aagagcauugaaggccaugc</u>	1056		
miR-782 (3'-5')			• •		
			<u>uucuuguagguuccgguacg</u>		
Unknown mRNA from fiber(TC41261)	5'	15	<u>tggccaaucuuguucaaaa</u>	35	3'
Novel miR-96	3'				5'
			<u>agccguuuaggucaaguuuu</u>		

miRNA Targets in Cotton

SBP (AY779340)	5'	592	<u>acgcuccucucucuguca</u>	601	3'
miR-156	3'				5'
Heat-shock protein (AY819767, 5'-3')	659	<u>cuavgcuavgcgccugcua</u>	678		
miR-391 (3'-5')			•		
			<u>gauacgauagagaggacggu</u>		
TC39605 (Katanin)	1851	<u>guguucaagaaaguuuuaca</u>	1871		
miR-396 (3'-5')			• • •		
			<u>uucaaguucuuucgauagugu</u>		
AF085717 (callose synthase)	2834	<u>caguucaggaugcuuuuug</u>	2854		
miR-396 (3'-5')			• • • •		
			<u>uucaaguucuuucgauagugu</u>		
TC34607 (fiber protein Fb23)	81	<u>auucggagauugaggauca</u>	101		
miR-414 (3'-5')			• •		
			<u>uaagcuacuacuucuacu</u>		
TC37120 (Fiber quinone-oxidoreductase)	1037	<u>aagagcauugaaggccaugc</u>	1056		
miR-782 (3'-5')			• •		
			<u>uucuuguagguuccgguacg</u>		
Unknown mRNA from fiber(TC41261)	15	<u>tcggccaaucuuguucaaaa</u>	35	3'	
Novel miR-96	3'				5'
			<u>agccguuuaggucaaguuuu</u>		

Fiber development

miRNA Targets in Cotton

SBP (AY779340)	5'	592	<u>acgcuccucucucuguca</u>	601	3'
miR-156	3'				5'
Heat-shock protein (AY819767, 5'-3')	659	<u>cuavgcuaucgcgccugcua</u>	678		
miR-391 (3'-5')			•		
			<u>gauacgauagagaggacggu</u>		
TC39605 (Katanin)	1851	<u>guguucaagaaaguuuuaca</u>	1871		
miR-396 (3'-5')			• • •		
			<u>uucaaguucuuucgauagugu</u>		
AF085717 (callose synthase)	2834	<u>caguucaggaugcuuuuug</u>	2854		
miR-396 (3'-5')			• • • •		
			<u>uucaaguucuuucgauagugu</u>		
TC34607 (fiber protein Fb23)	81	<u>auucggagauugaggauca</u>	101		
miR-414 (3'-5')			• •		
			<u>uaagcuacuacuucucuacu</u>		
TC37120 (Fiber quinone-oxidoreductase)	1037	<u>aagagcauugaaggccaugc</u>	1056		
miR-782 (3'-5')			• •		
			<u>uucuuguagguuccgguacg</u>		
Unknown mRNA from fiber(TC41261)	15	<u>tcggccaaucuuguucaaaa</u>	35	3'	
Novel miR-96	3'				5'
			<u>agccguuuaggucaaguuuu</u>		

Fiber development



miRNAs target MYB

miR6158a: 3' ACCUU-AUGAGUAAACUCGUUCU 5'
||||| | |||||:|:| |
MYB4 (AF034132.1): 5' UGGAACU-CUCAUUUGAGUAGAA 3'

ghr-n6: AGUUAGAGGAGGUUUGGAAGG
||| |||||:|||||
MYB5 (20264): CCAAGCUCCCAAUCUUCU

miR164e/166a,166b/393c: 3' UACGU-CG-UAGUAGUUCUAAGA 5'
||||| || |::||| |||||
MYB6 (AF034134.1): 5' AUGCACGCCAUUGUCAAA-AUUCU 3'

miR6158a ACCUUAUGAGUAAACUCGUUCU
||||| |||||:||||| |
MYB23 (18473): UGGAACACUCAUUUGAGCAAAA

miR6158a: 3' ACCUUAUGAGUAAACUCGUUCU 5'
||||| || | |||||:| |
MYB38 (AF336285.1): 5' UGGAACACAAAUUGAGCAAAA 3'

miR447a: 3' GGCAAGCAGGUUUGAGC 5'
:|||| |:||| ||||
MYB73 (11235): 5' UCGUU-GUUCAA-CUCG 3'

miR482b: 3' CUGCGAAGUGAGUUGAAC 5'
||| | |: |||||
MYB85 (19772): 5' GACCCUCGAUCAACUUG 3'

miR447a: 3' AUAUAUUCAAAGAAGG 5'
|| ||||: || ||||:
MYB124 (15689): 5' UAAUAUAG-UUGCUUCU 3'

miR394b: 3' UUCUAGUACGACCGUCGAAGU 5'
||||| : ||||| ||:||||
MYB124 (15689): 5' AAGAUGG-GCUGG-AGUUUCA 3'

ghr-n4: 3' AUAUAUUC-CAAAGAAGG 5'
| || ||| |||||:| |
MybPA2 (421): 5' UCUU-UAAAGUUUCUUUC 3'

miR4370: 3' ACCUUAUGAGUAAACUCGUUCU 5'
||||| || | |||||:| |
MybPA2 (421): 5' UGGAACACAAAUUGAGCAAAA 3'

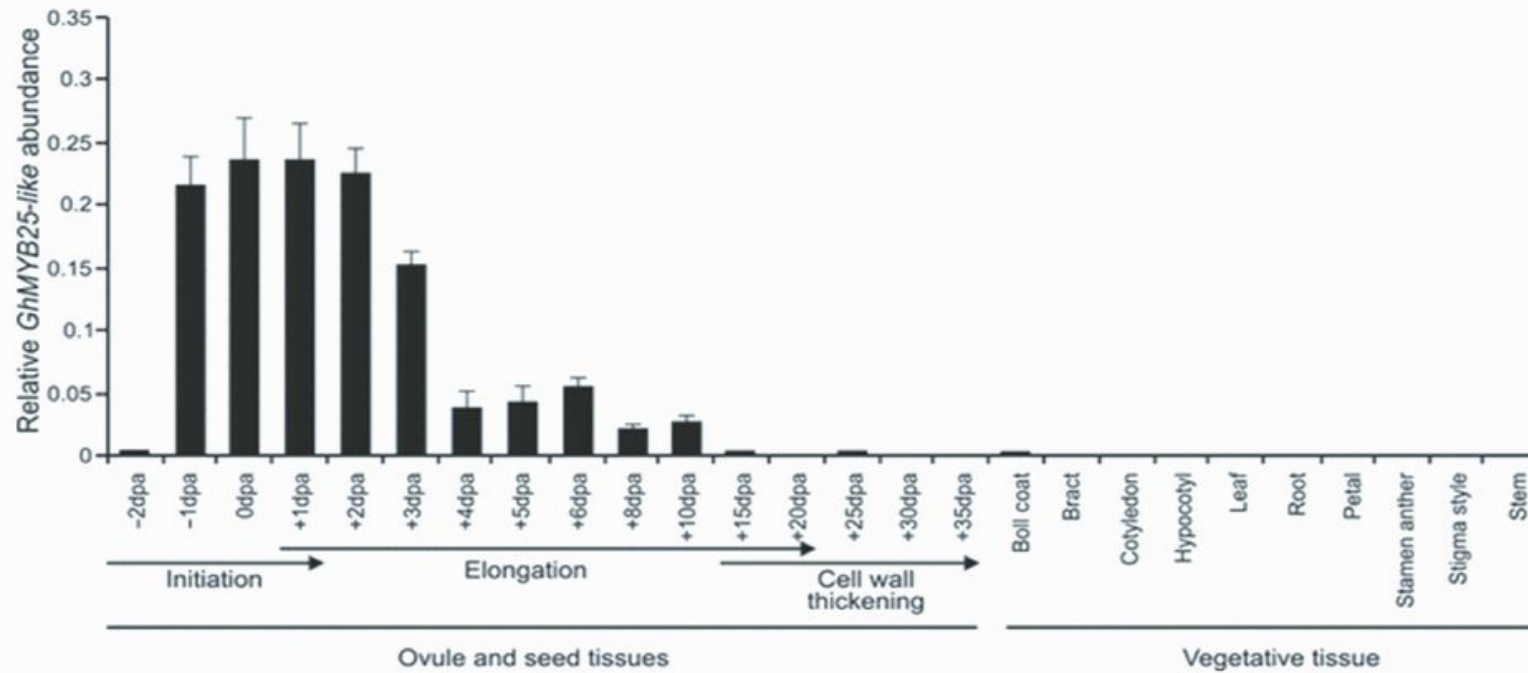
miR172c: AUAUAUUCAAAGAAGG
||| |||||:| |
MYB APL-like (6397) AAUUUUAAGUUUCUUUC

miR172c CUGC-GAAGUGAGUUGAAC
||||| : |||||:| |
MYB APL-like (6397) GACGCCGUCACUCAACUUG

miRNAs target MYB

```
miR172g/6158b:          3' GGCAAGCAGGUUUGAGC      GGCAAGCAGGUUUGAGC 5'  
                        :|||  |||||:| |      :|||  |||||:| |  
MYB25 (AY464054.1): 559 UCGUGAGUCCAAGCUAG ..... UCGUGAGUCCAAGCUAG 657
```

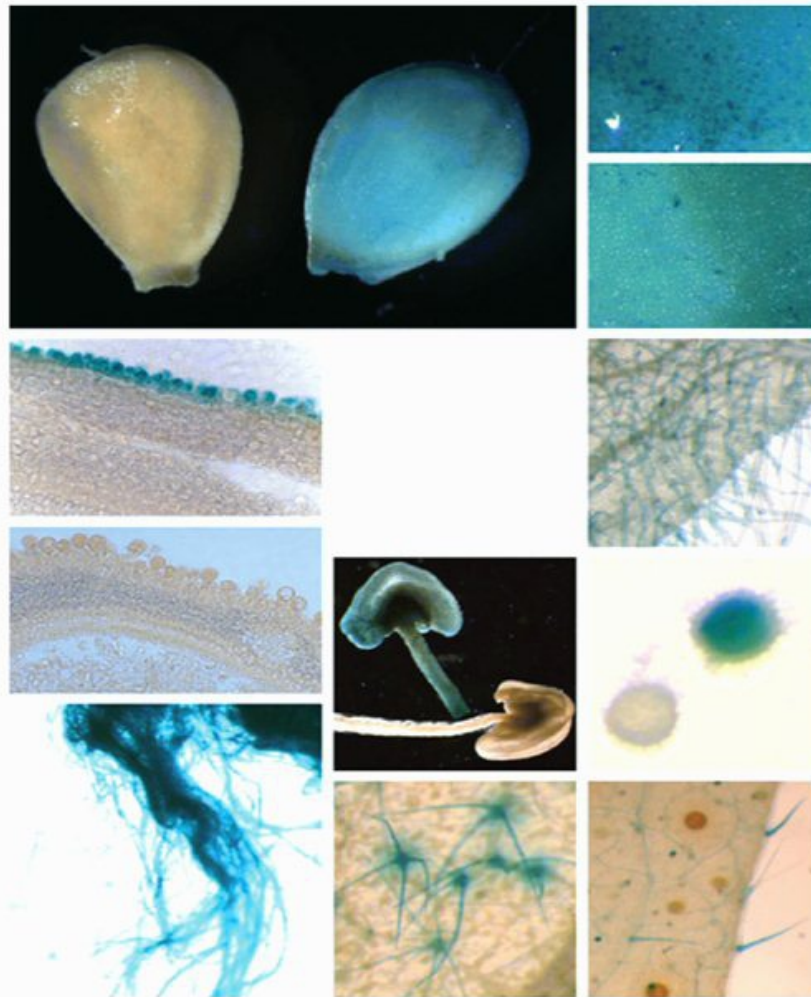
```
miR172f:                3' GUUUCAGC-CUGAUCUCA 5'  
                        ||||  :| |:| ||||  
MYB25-like (HM134084.1): 5' CAAAGAUGUGGCAAGAGU 3'
```



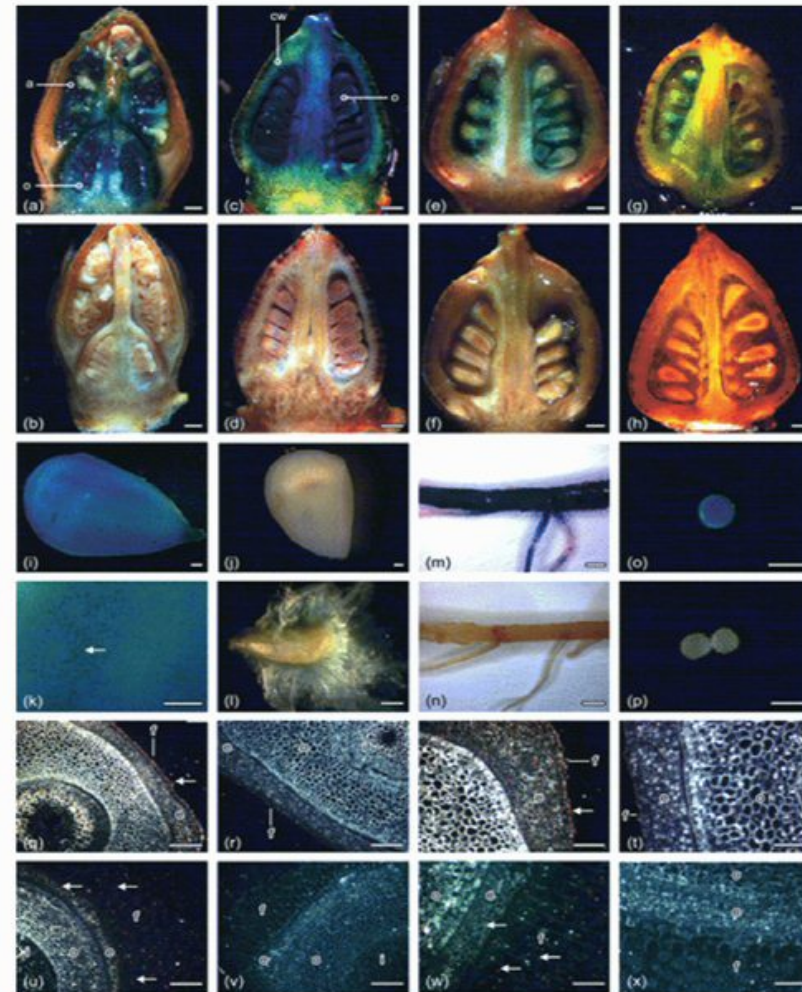
Quantitative real-time RT-PCR measurements of *GhMYB25-like* in cotton ovules, seeds and vegetative tissues. Expression was normalized against the expression of the cotton ubiquitin gene

Walford et al., 2011. Plant Journal

MYB 25

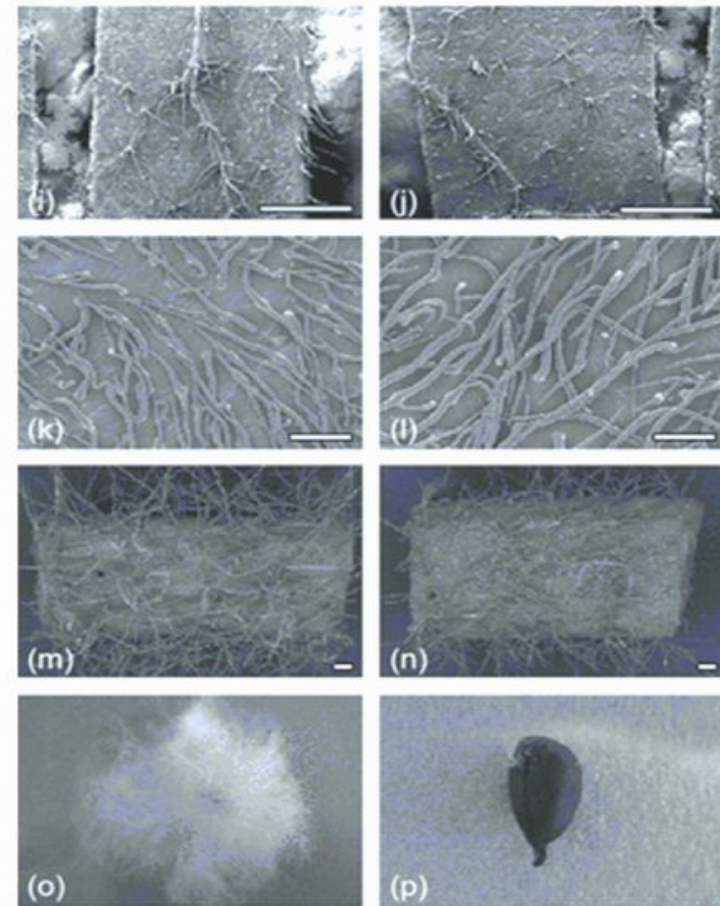
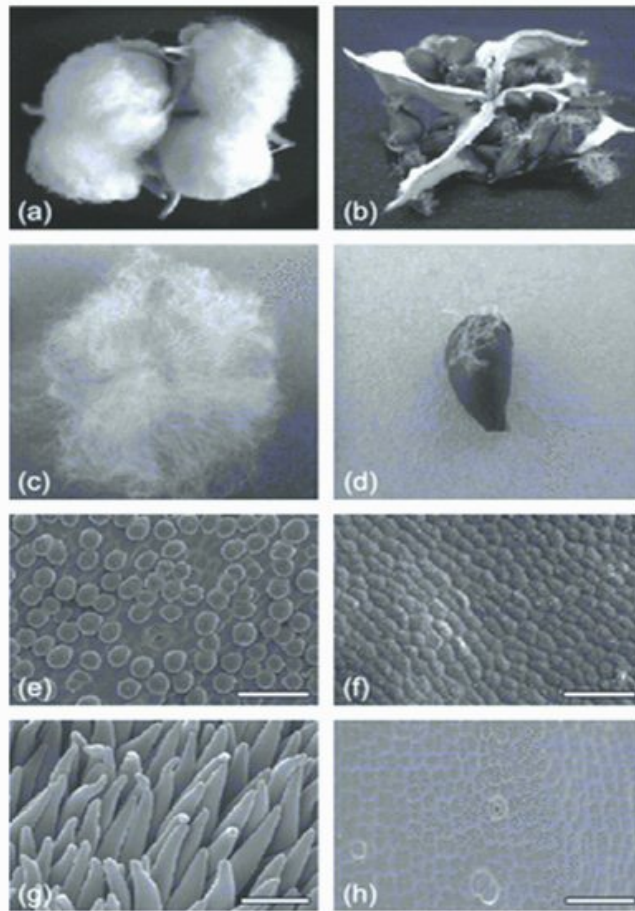


MYB 25-like



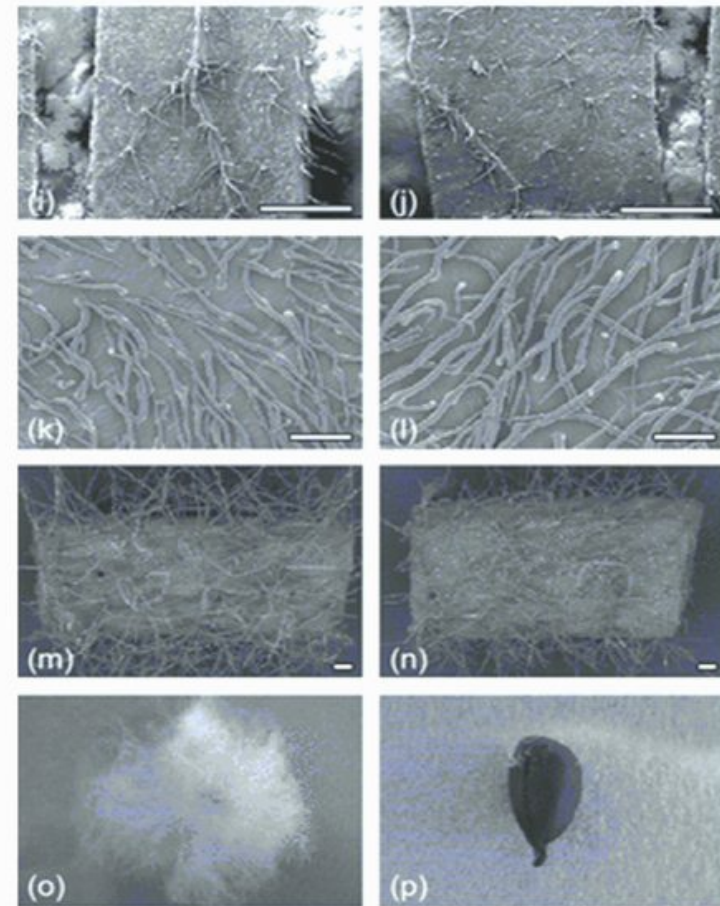
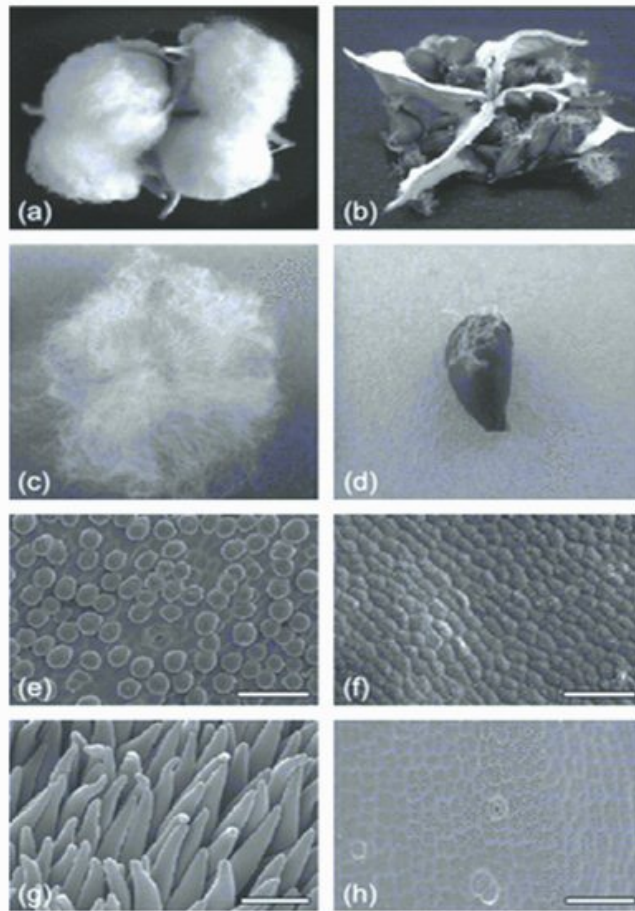
Walford et al., 2011. Plant Journal; Machado et al., 2009

Knockdown MYB25-like inhibit fiber differentiation but not on trichomes



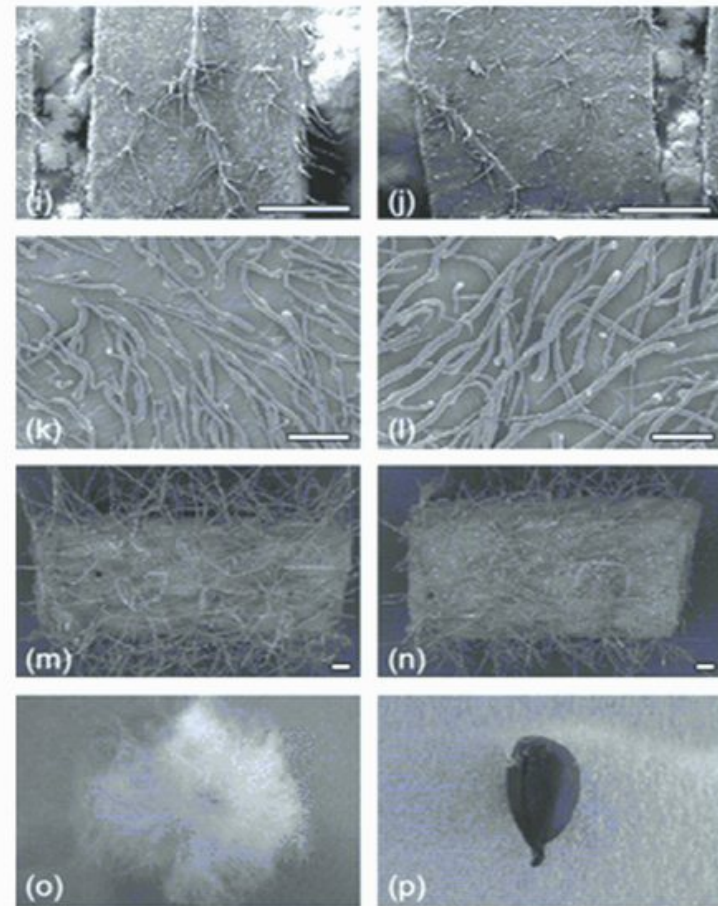
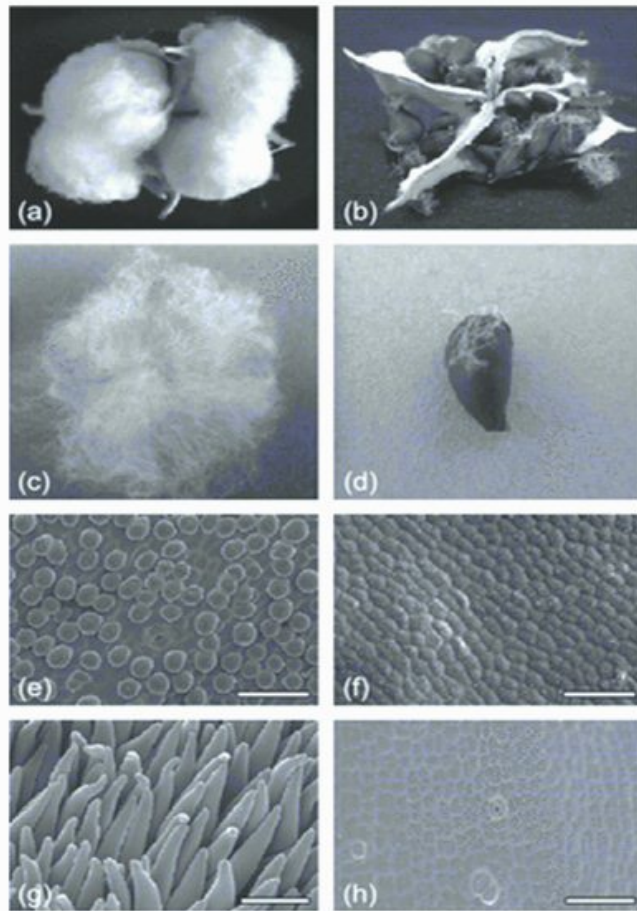
Walford et al., 2011. Plant Journal

Knockdown MYB25-like inhibit fiber differentiation but not on trichomes



Walford et al., 2011. Plant Journal

Knockdown MYB25-like inhibit fiber differentiation but not on trichomes



Walford et al., 2011. Plant Journal

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China:

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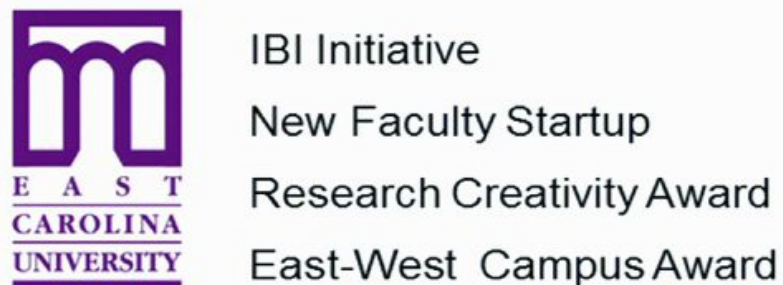
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Questions?

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Comments?

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Thanks!

