

The Small RNA Landscape of Maize Meiosis

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Overview

Our lab focuses on plant meiosis and meiotic recombination, and our approaches include Illumina sequencing on isolated meiocytes during zygotene (an early stage during prophase I).

Previously, we examined the meiotic transcriptome in maize, focusing on genes that are up-regulated in male meiocytes (cells undergoing meiosis) compared with somatic seedling tissue¹.

When genes for small RNA pathways were up-regulated in meiocytes, it was usually even more pronounced in whole anthers, with only a few exceptions (Table 1).

Whole-genome sequencing of small RNAs in maize meiocytes, anthers and seedlings yielded ~36-82 million reads of which 39-54% aligned to the genome. Analysis indicated that there is a small subset of 21nt-long sRNAs prevalent in meiocytes (Figure 1).

	meiocytes	anthers	seedlings	
Dcl1	2199.9	3295.3	2245.6	Dicer-like (dsRNA chopping)
Dcl2	868.1	795.4	790.4	
Dcl3a	0.0	0.0	0.0	
Dcl3b	2240.2	8371.9	25.6	
Dcl4	539.4	311.5	575.4	
Rdr1	1916.8	791.3	354.6	RNA-dependent RNA polymerases
Mop1	1531.2	1263.9	546.7	
Rdr3	4.1	0.5	0.0	
Rdr4	266.9	540.4	413.0	
Rdr5	0.0	0.0	0.0	
Ago1a	916.1	1782.7	2579.7	Argonaute proteins (slicing activity)
Ago1b	448.7	834.5	925.5	
Ago1c	2137.6	3200.3	5115.0	
Ago1d	1728.2	3662.1	14.3	
Ago1e	1273.9	2344.2	1566.4	
Ago2	2565.1	2050.4	693.3	
Ago4a	#N/A	#N/A	#N/A	
Ago4d	2447.1	4579.4	2394.0	
Ago5a	1324.6	698.1	1429.7	
Ago5b	86.6	118.8	10.9	
Ago5c	556.7	1296.3	152.3	
Ago7	81.5	178.1	43.6	
Ago10a	894.3	1479.4	2091.1	
Ago10b	85.3	123.9	150.5	
Ago10c	#N/A	#N/A	#N/A	
Ago18a	3557.2	1080.7	67.5	
Ago18b/c	10389.6	10863.2	42.9	

Table 1: Normalized gene expression of components of small RNA pathways

Gene IDs as identified by Qian et al. (2009)¹; expression data from Dukowic-Schulze et al. (2014)²

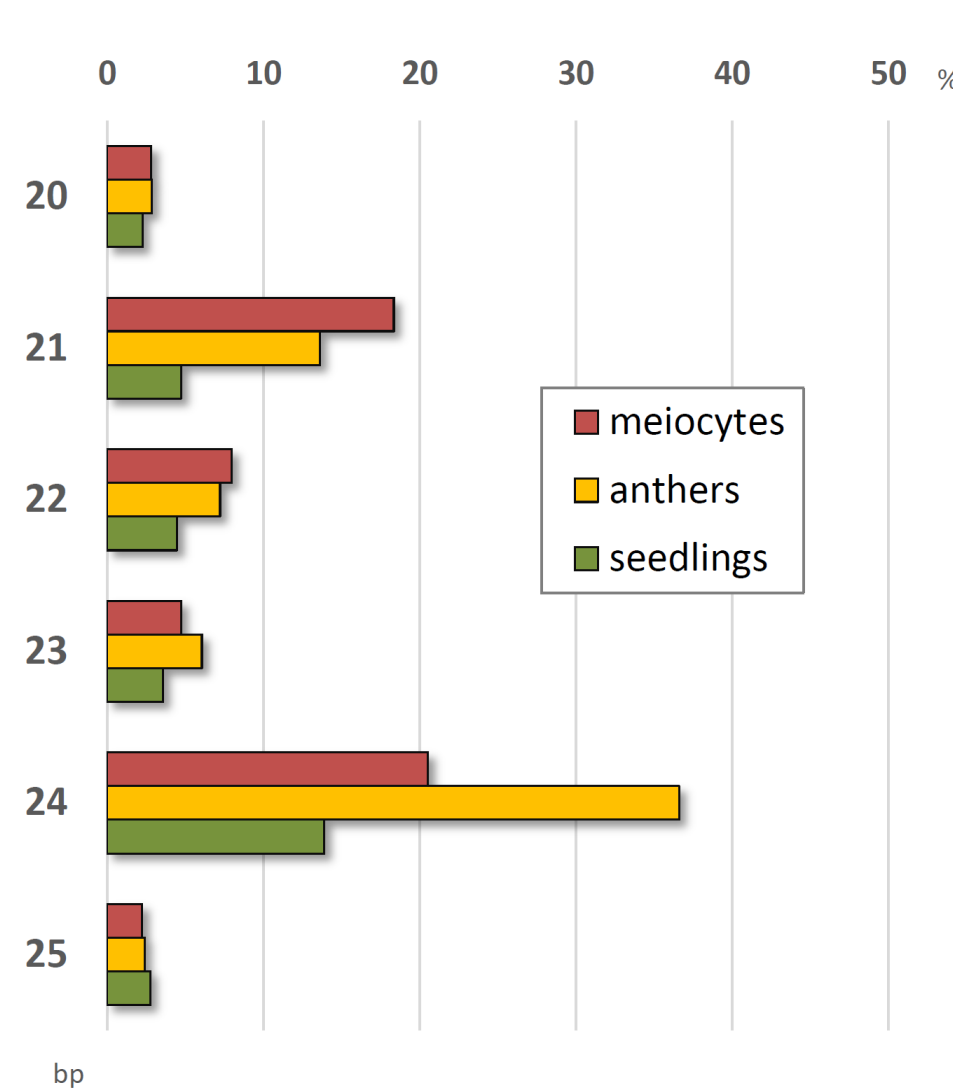


Figure 1: Percentage of detected reads per nucleotide length miRNAs ("micro RNAs") are typically 21 or 22nts long, siRNAs ("small interfering RNAs") ~21 or 24 nts long. The reads at 20, 23 and 25 might thus be interpreted as background level.

Conclusions

- The small RNA landscape of **meiocytes and anthers** is **highly similar** and shows pronounced differences from seedlings.
- Small RNA populations **possibly specific to meiocytes** might be mainly of 21 nt length and/or at non-clustered loci.

miRNAs

Known miRNAs that were most up-regulated in meiocytes included miR169 and 159, and members of the phased RNA-initiating miR families 2275 and 218 (Table 2). However, predicted target genes³ were up-regulated instead of down-regulated in our mRNAseq data¹ when compared to seedlings, thus suggesting fine-tuning expression by the miRNAs rather than abolishing it completely.

Table 2: Normalized expression of miRNAs up-regulated in meiocytes

For most miRNA families, multiple members are up-regulated in meiocytes, but only a few higher than in whole anthers. In rice inflorescences, miR2275 and 218 have been shown by Johnson et al. (2009)⁴ to trigger secondary phased sRNAs, whose functions remain unknown. Table sorted for p-value (<0.1 above line).

miRNA	meiocytes	anthers	seedlings	Mvs.S	Mvs.A
MIR2275b	156716.4	180928.1	78.2	2003.3	0.9
MIR2275c	63281.4	106643.0	38.3	1650.9	0.6
MIR2275a	24030.8	29790.0	21.5	1117.1	0.8
MIR2118g	7515.5	6479.2	6.6	1130.3	1.2
MIR2118d	4987.5	4365.5	10.6	472.3	1.1
MIR2118e	1141.1	734.9	0.4	2917.3	1.6
MIR2118a	2945.6	3654.9	5.9	502.1	0.8
MIR2118c	2122.0	2514.1	3.5	602.8	0.8
MIR2118f	855.1	239.2	0.8	1093.1	3.6
MIR2118b	6689.0	4580.4	62.2	107.6	1.5
MIR399b	514.8	602.3	3.1	164.5	0.9
MIR169b	680.6	1788.7	7.8	87.0	0.4
MIR159b	1804.5	623.3	36.8	49.1	2.9
MIR159k	566.2	429.8	11.7	48.3	1.3
MIR529	49008.2	65000.4	7484.0	6.5	0.8
MIR169k	2385.1	5074.0	168.2	14.2	0.5
MIR167g	592.0	7185.1	72.0	8.2	0.1
MIR2275d	17.2	2.9	0.8	21.9	5.9
MIR827	5819.7	4424.6	1448.8	4.0	1.3
MIR398a	2267.8	1736.5	874.2	2.6	1.3
MIR171l	80.1	49.3	13.3	6.0	1.6
MIR169b	117.3	113.1	25.8	4.5	1.0
MIR172c	68.6	35.5	15.6	4.4	1.9
MIR167h	17.2	9.4	8.2	2.1	1.8

MIR2275 and MIR2118 families characterized in rice inflorescences

MIR159 regulates Arabidopsis anther development and has 17 predicted target genes in maize, especially MYB transcription factors

Highly enriched sRNA loci

We focused on 21, 22 and 24nt long sRNAs of substantial expression, defined as ≥ 2 RPM (reads per million reads). The distribution of 21 and 24nt sRNA was very similar in meiocytes and anthers, but distinct from seedlings (Figure 2).

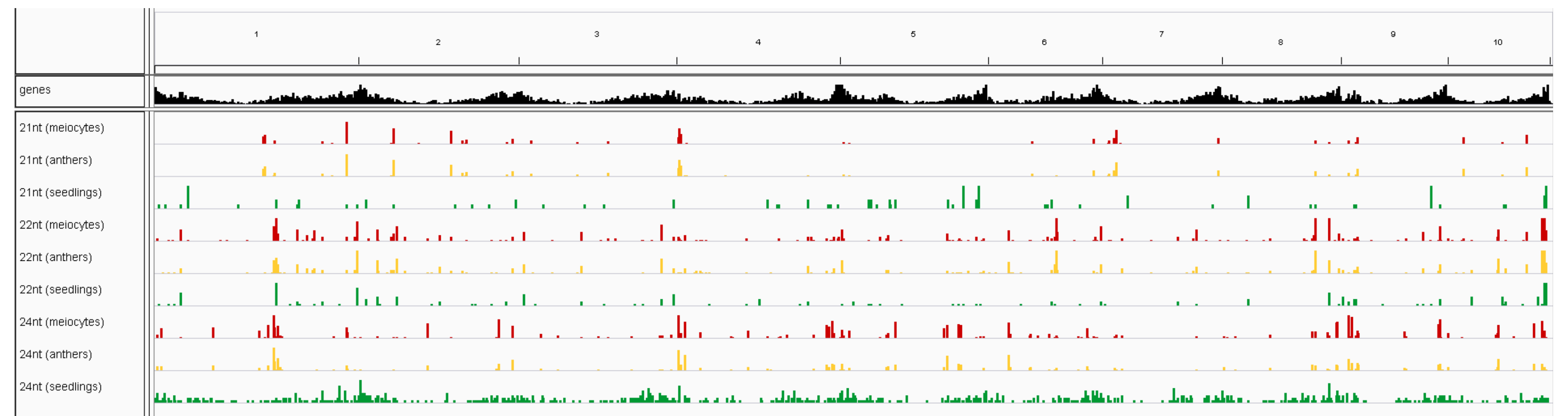


Figure 2: Overview of sRNA loci

Displayed with IGV (Integrated Genome Viewer). Meiocytes in red, anthers in yellow, seedlings in green.

Only around 10% of the loci with ≥ 2 RPM overlapped a TE (transposable element). Figure 3 shows the contributing superfamilies.

Although few sRNA loci (<5%) overlapped with annotated genes, intronless genes seemed to be over-represented (Figure 4).

Examining sRNA loci in anthers and meiocytes more closely revealed many clustered loci (which include the previously identified phased siRNAs⁴). These clustered loci can usually be found in both meiocytes and anthers while there are more specific non-clustered loci for both samples (Figure 5).

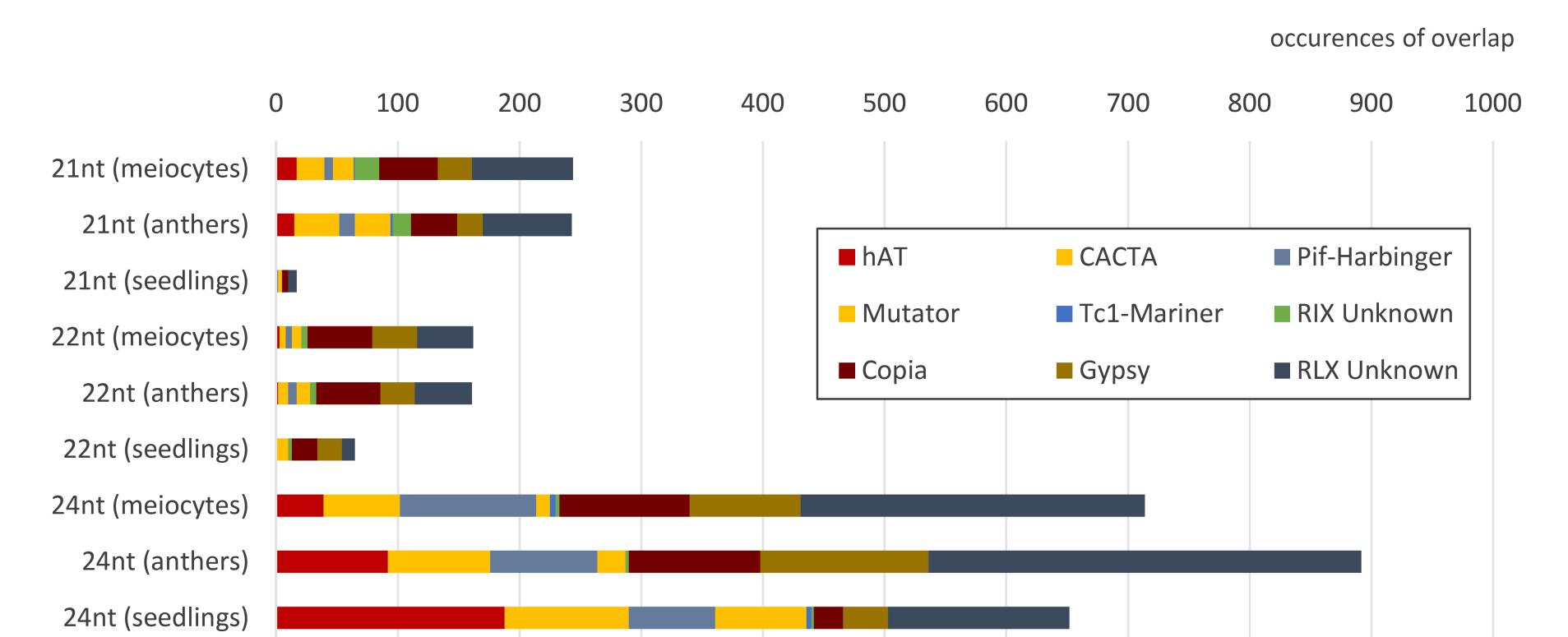


Figure 3: sRNAs hits per TE superfamily

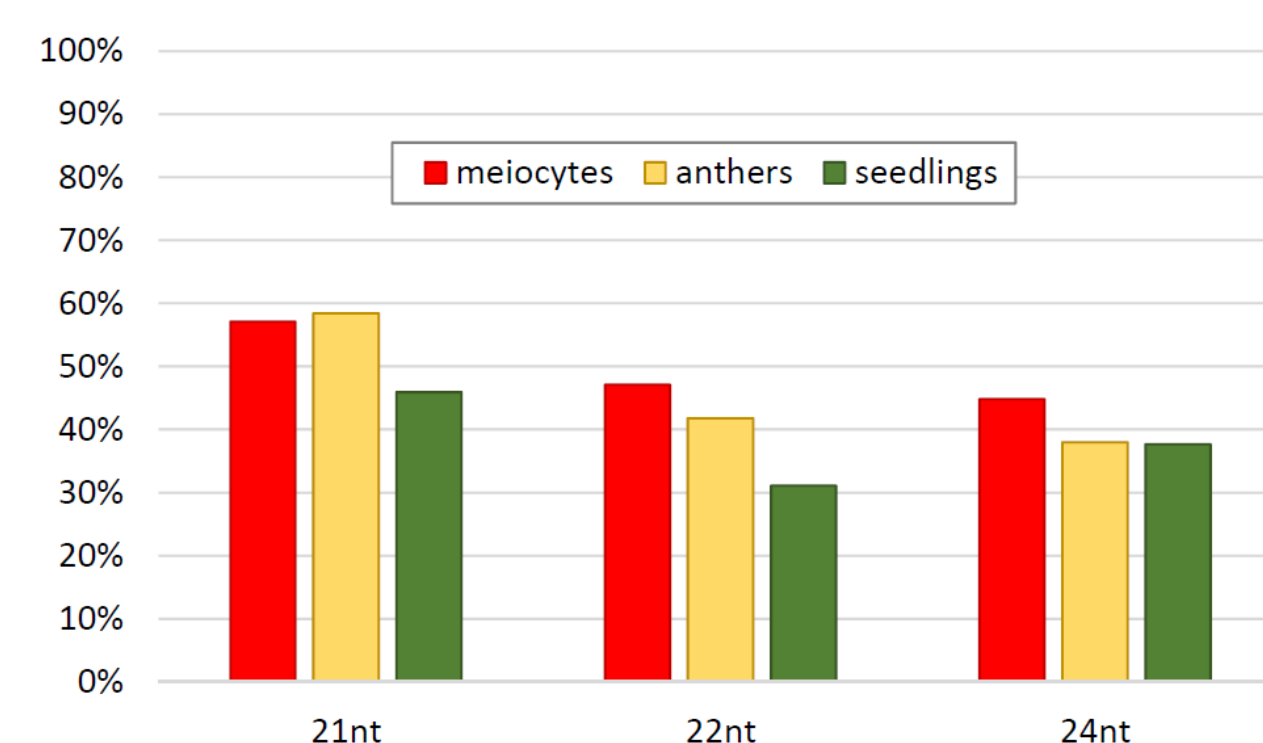
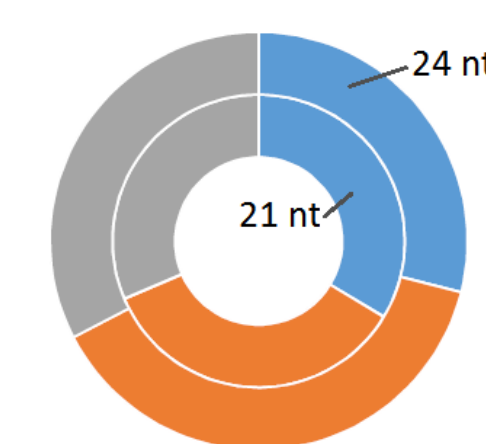


Figure 4: Percentage of intronless genes in all genes overlapping sRNA loci

The theoretical genome-wide average is ~37% (14,623 intronless, 39,656 total genes)

Non-clustered loci



Clustered loci

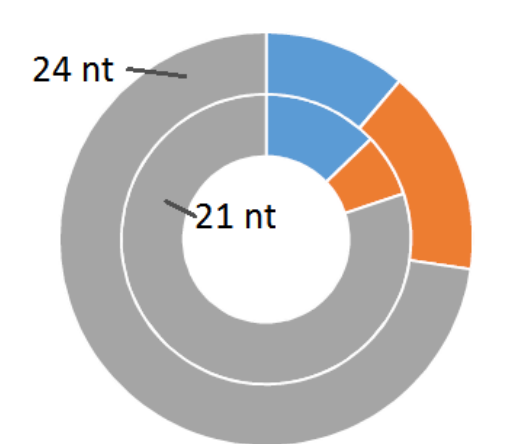


Figure 5: Proportions of loci specific or common for meiocytes and anthers

Literature resources:

- Dukowic-Schulze et al. (2014). The transcriptome landscape of early maize meiosis. BMC Plant Biology 14(1): 118
- Qian et al. (2011). Identification and characterization of Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in maize. Plant Cell Reports 30(7): 1347-1363
- Zhang et al. (2009). A genome-wide characterization of microRNA genes in maize. PLoS Genetics 5(11): e1000716
- Johnson et al. (2009). Clusters and superclusters of phased small RNAs in the developing inflorescence of rice. Genome Research 19(8): 1429-1440



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