Transcriptomic characterization of male sexual reproduction in maize

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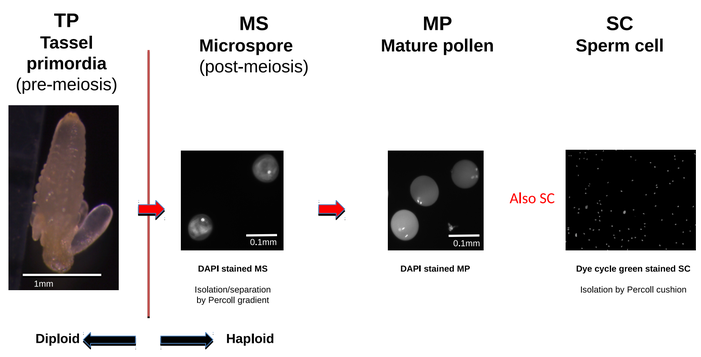
INTRODUCTION

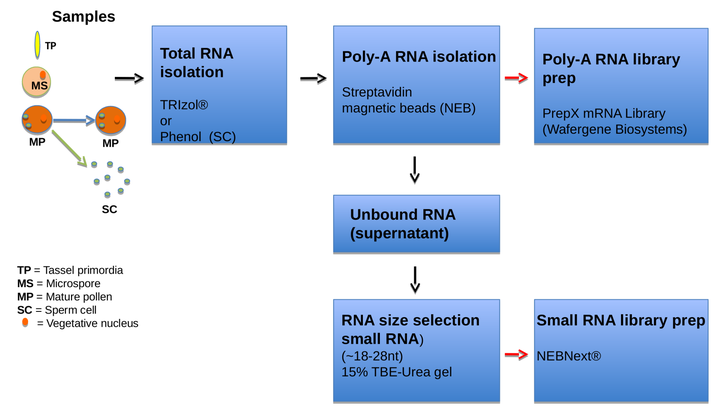
Sexual reproduction in plants involves developmental regulation of key cellular processes (e.g., pollen tube growth, cell-cell signaling, fertilization). Recent results demonstrate that plant genomes undergo large scale alteration of gene expression and epigenetic modifications as plants undergo meiosis to produce haploid gametophytes for the next generation. **We have undertaken a transcriptomic study of male sexual development in maize to investigate these regulatory events.**

REPRODUCTIVE TISSUES



We developed methods to isolate and sequence paired samples of mRNA and small RNA from each of four biological replicates at four developmental stages.

RNA SEQUENCING

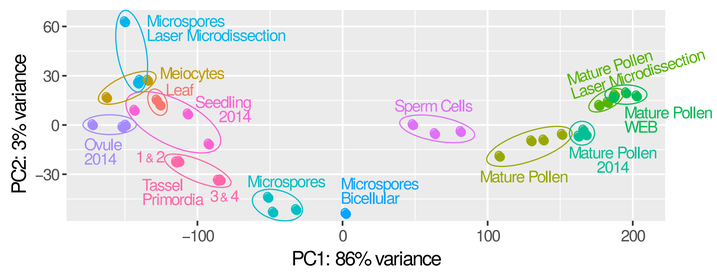


Mature pollen (MP) and sperm cell (SC) samples were isolated from the same pollen sample that was a pool of pollen from 3 plants. Part of each pool was used for MP RNA isolation and part was used for SC RNA isolation. All of the samples were used for both poly-A RNA and small RNA sequencing.

PCA analysis

PCA analysis of the datasets indicate that replicates from each tissue have low variance, enabling statistical confirmation of differential expression of genes or transposable elements observed between tissues.

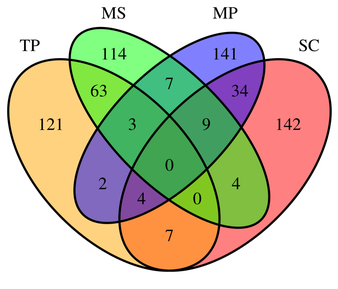
Our mature pollen (MP) sequencing datasets resemble previously sequenced high-quality datasets.



PCA plots show the quality of our reproducible RNA-seq datasets when assaying non-TE gene expression. Note that the vast majority of variance is on the X-axis. Also note the clustering of our Mature Pollen samples with those already published in the field: '* 2014' = Chettoor, et al. (2014); 'WEB' = Walley, et al. (2016); 'Laser Microdissection' = NCBI BioProject 306885 (2015).

Highly-expressed genes

Top 200 FPKM genes per tissue

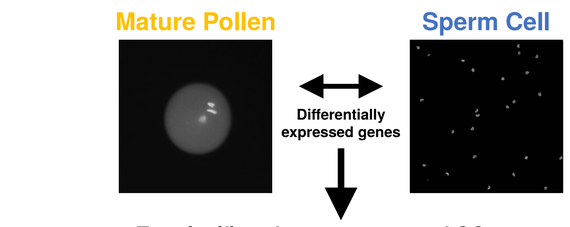


High expression levels are associated with developmental specificity: approximately 2/3 of the genes associated with the highest FPKM values in each of the four sample types are highly expressed in only that sample type.

Consistent with the PCA analysis, overlaps in highly-expressed genes suggests that mature pollen (MP) and sperm cells (SC) are more similar, and distinct from the tassel primordia (TP) and microspore (MS) pairing.

GO enrichment

Gene ontology (GO) analysis of mRNA transcripts highlights contrasting cellular processes in mature pollen (containing sperm cells as well as the vegetative cell) and sperm cells alone, consistent with the distinct roles of the sperm cells and the vegetative cell in reproduction.



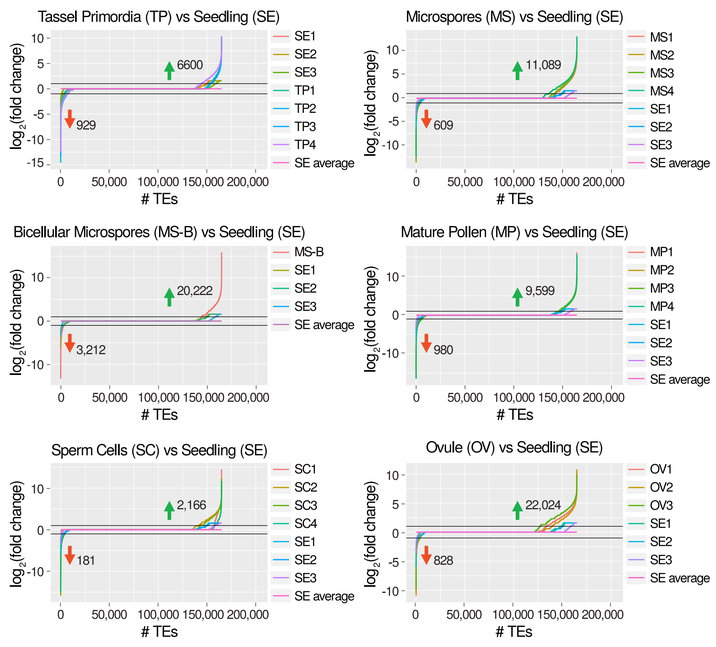
Top significantly overrepresented GO terms

GO Term	Description	p-value
GO:0043687	Post-translational protein modification	3.50E-17
GO:0046686	Response to cadmium ion	5.70E-16
GO:0051788	Response to misfolded protein	1.40E-15
GO:0044710	Single-organism metabolic process	5.20E-15
GO:0080129	Proteasome core complex assembly	3.40E-14
GO:0061025	Membrane fusion	1.50E-12
GO:0033121	Regulation of purine nucleotide catabolism	1.50E-11
GO:0009118	Regulation of nucleoside metabolic processes	1.50E-11
GO:0006511	Ubiquitin-dependent protein catabolic processes	1.90E-11
GO:0006886	Intracellular protein transport	2.90E-11
GO:0035295	Tube development	8.80E-11
GO:0009853	Photorespiration	1.30E-10
GO:0006487	Protein N-linked glycosylation	3.80E-10
GO:0009860	Pollen tube growth	1.70E-09
GO:0016926	Protein desumoylation	3.60E-09
GO:0006623	Protein targeting to vacuole	5.20E-09
GO:0050665	Hydrogen peroxide biosynthetic process	5.40E-09
GO:0006897	Endocytosis	6.30E-09

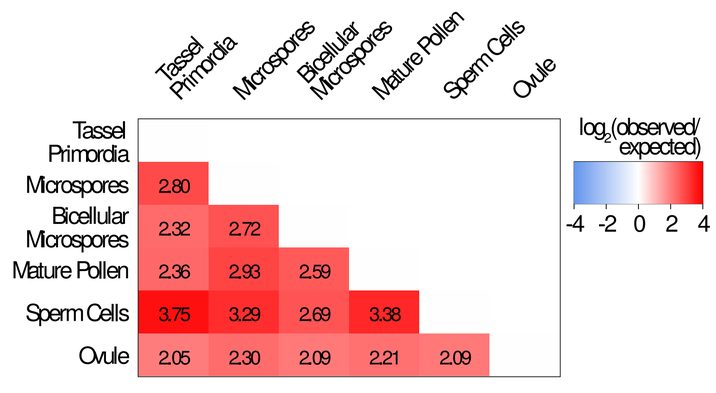
GO Term	Description	p-value
GO:0007062	Sister chromatid cohesion	1.30E-14
GO:0009154	Purine ribonucleotide catabolic process	5.70E-14
GO:0042138	Meiotic DNA double-strand break formation	4.70E-09
GO:0000278	Mitotic cell cycle	5.00E-09
GO:0031048	Chromatin silencing by small RNA	1.60E-08
GO:0000911	Cytokinesis by cell plate formation	1.80E-08
GO:0009630	Gravitropism	2.90E-08
GO:0035196	Production of miRNAs involved in gene silencing	5.50E-08
GO:0006396	RNA processing	9.80E-08
GO:0010228	Vegetative to reproductive phase transition	1.00E-07
GO:0006635	Fatty acid beta-oxidation	1.60E-07
GO:0000724	Double-strand break repair	2.40E-07
GO:0045132	Meiotic chromosome segregation	2.80E-07
GO:0010267	Production of ta-siRNAs involved in RNA interference	4.40E-07
GO:0019563	Glycerol catabolic process	2.20E-06
GO:0031338	Regulation of vesicle fusion	2.30E-06
GO:0010564	Regulation of cell cycle process	4.10E-06
GO:0000398	mRNA splicing, via spliceosome	5.80E-06

GO annotations were assigned to highly differentially expressed genes ($q < 0.01$) using the R package `topGO` (Alexa, 2016) in combination with maize-GAMER, a recent maize gene functional annotation (Wimalanathan, 2017). GO annotations associated with genes highly-expressed in mature pollen are shown in yellow, while those associated with genes highly-expressed in sperm cells are shown in blue.

Dynamic TE regulation



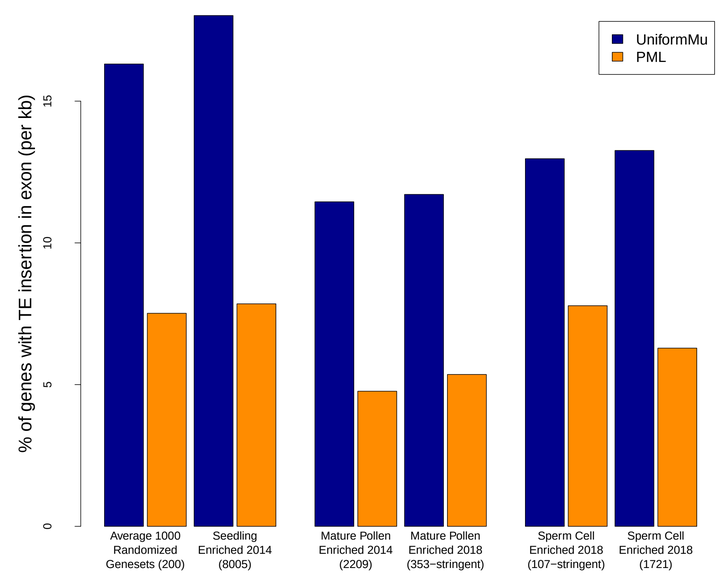
Compared to the baseline TE expression in seedlings, TEs are up-regulated in Tassel Primordia through Mature Pollen development. Most of these are not expressed in Sperm Cells. On the female side, we see many TEs up-regulated in ovules as well. We are only considering TEs farther than 2kb from genes and hence, read-through transcription from developmental changes in gene expression should not be an underlying cause for the observed dynamic behavior of TEs.



There is significant overlap between the sets of TEs that are up-regulated at specific stages of maize reproductive development. This demonstrates that there are a class of TEs that are dynamically and specifically up-regulated on both the male and female side during maize reproduction.

Mu insertions

To better assess dynamic transposable element regulation in maize male reproductive tissues, we developed a bioinformatic tool to easily visualize and identify differentially-expressed transposable elements in these samples. Intriguingly, genes that are highly-expressed in either mature pollen or sperm cells are associated with fewer transposable element insertions in exons in sequenced populations (UniformMu, Photosynthetic Mutant Library), relative to highly-expressed seedling genes. This is consistent with the idea that deleterious mutations in genes important for either pollen or sperm cell function are subject to relatively higher purifying selection, likely due to the haploid nature of these stages.



In two large populations (UniformMu and Photosynthetic Mutant Library), gene sets based on transcript enrichment in mature pollen and sperm cells are associated with reduced likelihood of Mu insertion sites in exons, relative to comparators. This is consistent with the hypothesis that these genesets are enriched for genes important during the haploid phase, as selection would reduce recovery of deleterious gametophyte mutations. Gene sets are based on data from Chettoor, et al. (2014) and this study (marked 2018); number in parenthesis is the number of genes in each set. Comparators are either seedling-enriched genes, or an average of percentages from 1000 randomized 200-member genesets.

CONCLUSIONS

Maize provides a useful model for the regulation of transposon expression in a model system in which many transposon classes are still active. Analysis of a time course of male reproductive developmental using paired RNA-seq and sRNA-seq reveals changes in the regulation of transposon expression during the transition from the sporophytic phase to the gametophytic phase to the germ cells. This analysis also provides a list of genes and pathways that are important to the whole male gametophyte and the sperm cells specifically.

Further analysis of how regulation of expression of the transposon landscape is coordinated with expression of the functional genes during gametophyte development is an important area for future research.

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