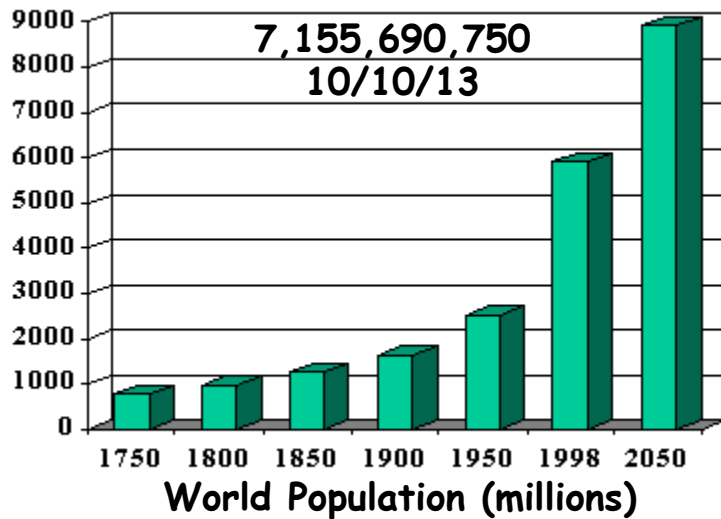


Using Genomics to Dissect Seed Development-Implications For Agriculture

Bob Goldberg
3/26/14



We Face Major Challenges in Agriculture



OVER THE NEXT 50 YEARS WE WILL NEED TO PRODUCE MORE FOOD THAN IN THE WHOLE OF HUMAN HISTORY

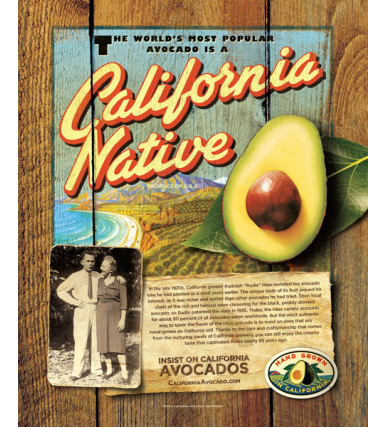
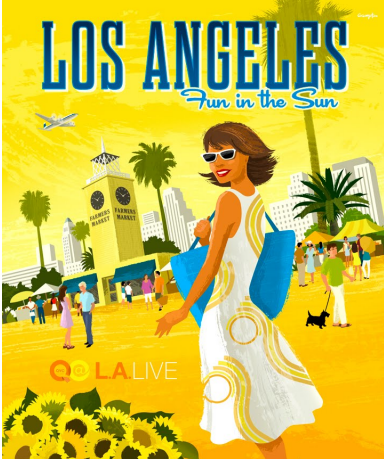
AND DO IT WITH FEWER INPUTS ON LESS ARABLE LAND!!!!

CROP YIELDS NEED TO BE INCREASED SIGNIFICANTLY!!

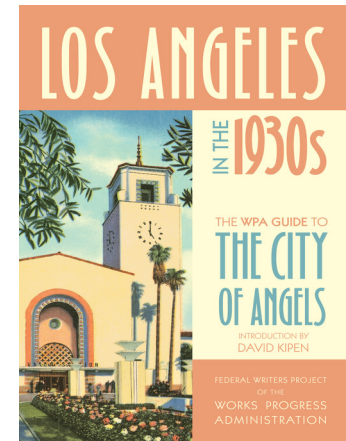
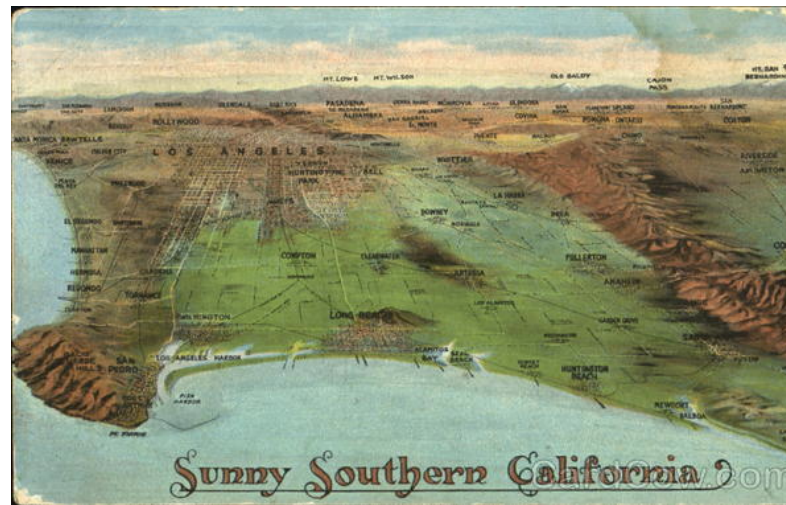
3,000 Acres/Day of Productive Farmland is Lost to Development Each Day in the United States



*"Major Land Uses Overview." USDA, Economic
Research Service, Web, April 3, 2013.*



An Example From Los Angeles History.....



Aerial Photograph of UCLA in 1929

*There Were 18,000 Farms in Los Angeles
County in 1930!!!*

*From 1901 to 1950 Los Angeles County Was the Largest
Agricultural Producing County in the US!!!*

*Bel-
Air*

Farms!!

*Beverly
Hills*

Sunset Blvd.

Hilgard Blvd.

Westwood Blvd.

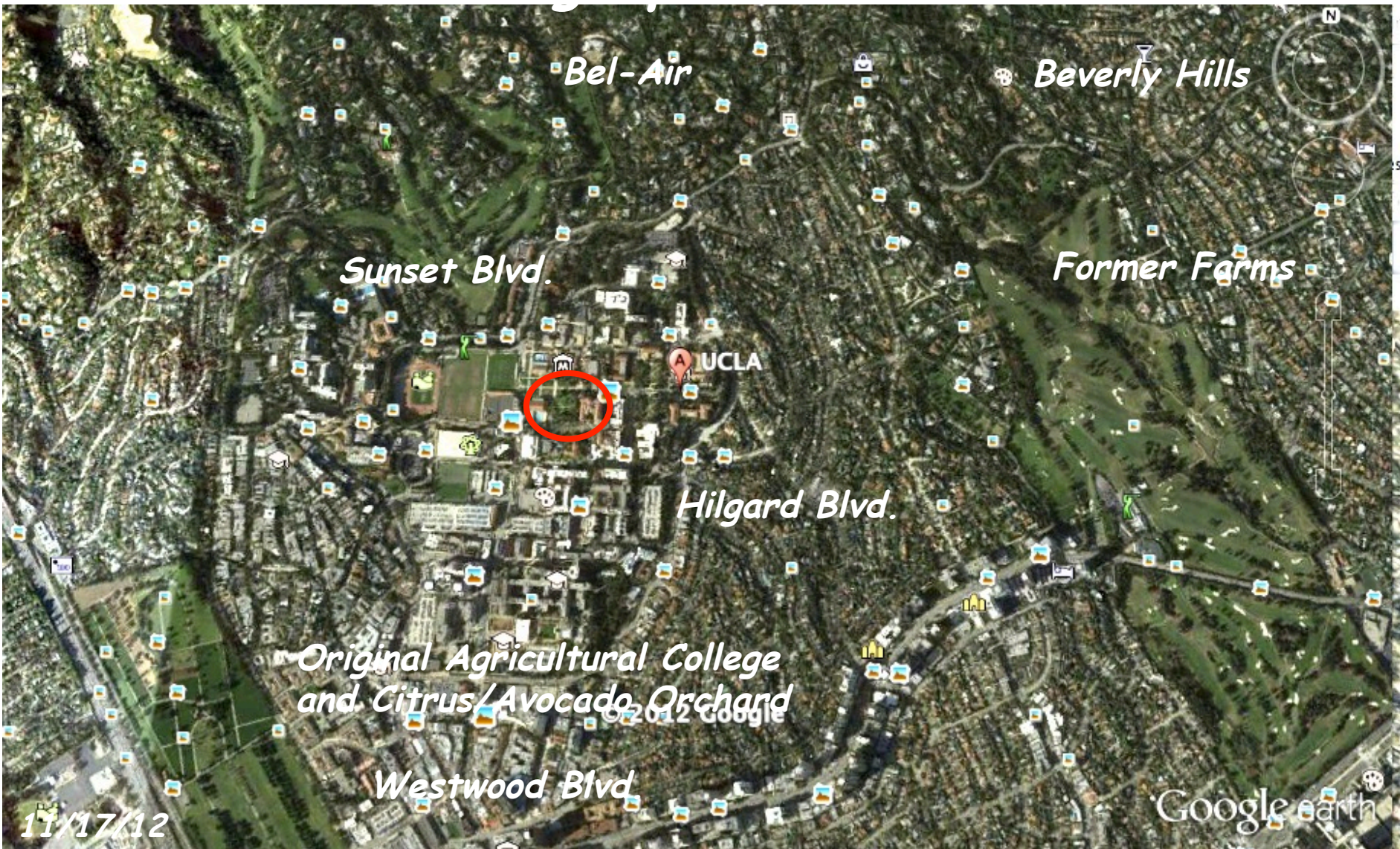
*Original Agricultural College
and Citrus/Avocado Orchard*

*Thelner Hoover
4/11/29*

Los Angeles Population = 1,238,000



Aerial Photograph of UCLA in 2014



Los Angeles Population = 3,893,000

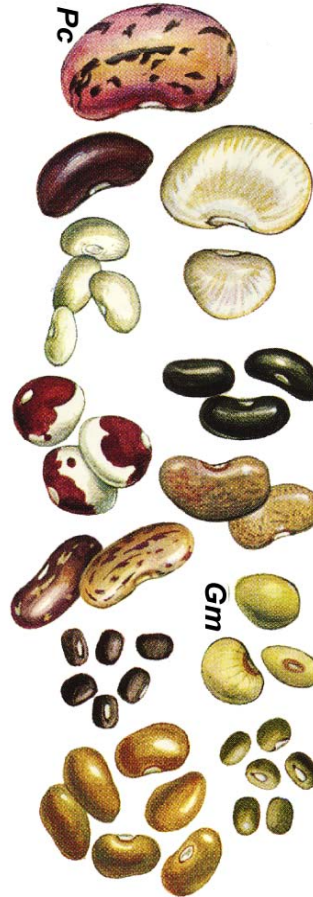
*How Will Crop Yields Be Increased
in the Future?*



...By Using a Variety of Approaches to Identify Genes and Processes That Will Help Increase Crop Yields and Food Production Significantly in the 21st Century....

Yield (Developmental Traits)

- *Seed Number*
- *Seed Size*
- *Growth Rate*
- *Organ Size (More Seeds)*
- *Plant Architecture*
- *Flowering Time*
- *Senescence*
- *Maturity*
- *Stature*



Yield (Stress Traits)

- *Nutrient Uptake*
- *Drought Resistance*
- *Heat Resistance*
- *Cold Tolerance*
- *Salt Tolerance*
- *Shade Tolerance*
- *Disease Resistance*

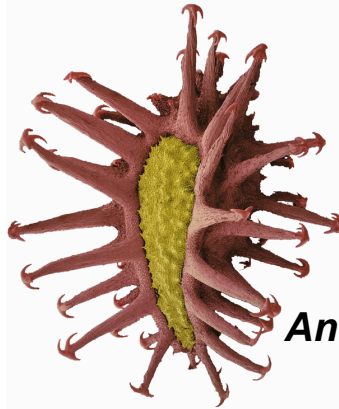


.....And by Using Genomics, Breeding, and Genetic Engineering to Introduce These “Yield” Genes Into Crops (One thing we can be sure of-we can’t predict what new technology will be the driver 10-25 years out!)

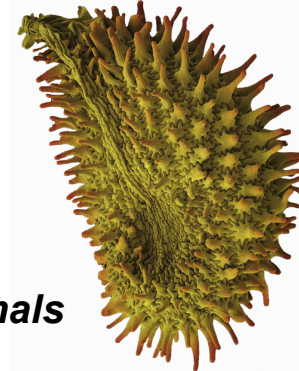
So.....Why Seeds??



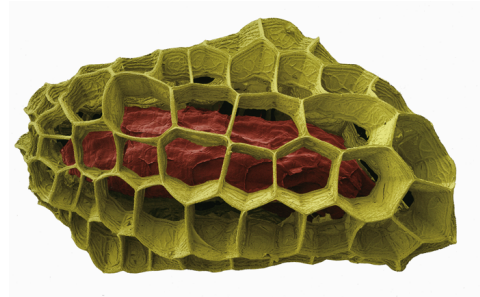
Water



Animals



Wind



*Seeds Protect and Disperse Plant Embryos
and Come in Many Shapes and Sizes!*

Most Importantly..... Our Food is Derived From Fourteen Crops & Over Half Produce Seeds For Human and Animal Consumption

Seed Crops



- *Wheat*
- *Rice*
- *Corn*
- *Barley*
- *Sorghum*
- *Soybean*
- *Common Bean*
- *Coconut*

Non-Seed Crops

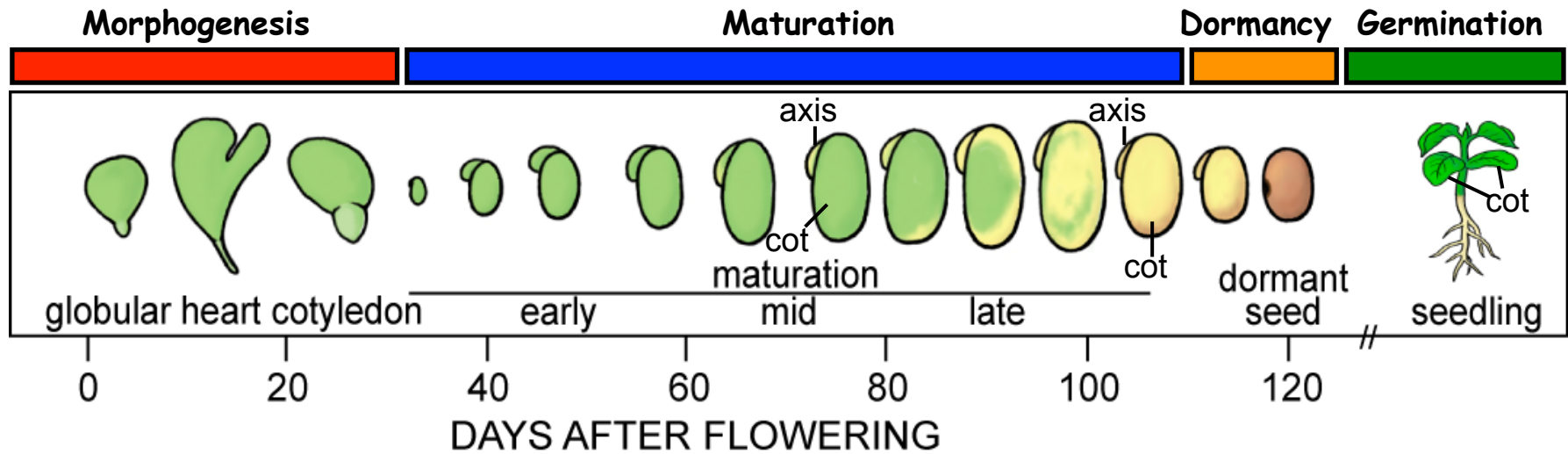
- *Potato*
- *Sweet Potato*
- *Cassava*
- *Sugar Beet*
- *Sugar Cane*
- *Banana*

In Some World Populations 75% of Calories Are Derived From Seeds!

How Is a Seed Formed?



Major Processes in Seed Development



**Differentiation of
Compartments, Tissues,
and Regions**

**Storage Protein
Deposition
Preparation or Dormancy**

**Mature Seed, Dormancy
and Germination**

Food Reserve Accumulation



In the Beginning....

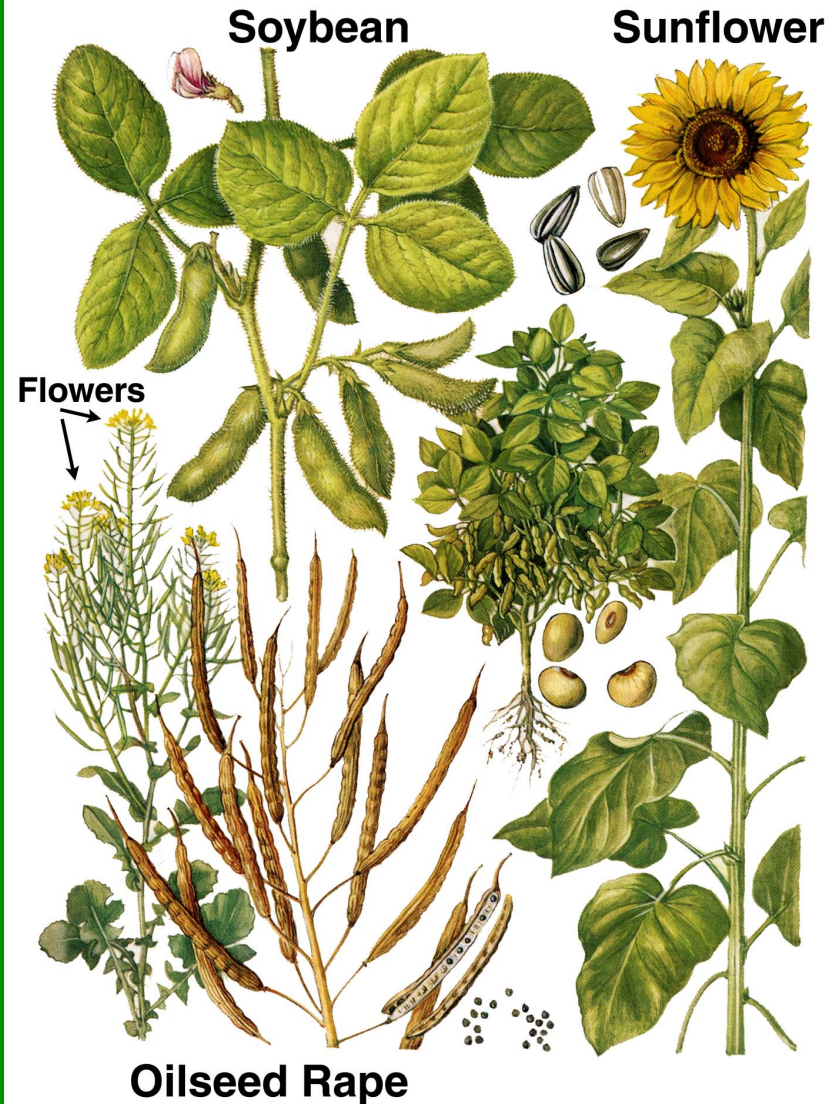


<http://estdb.biology.ucla.edu/seed/presentation>

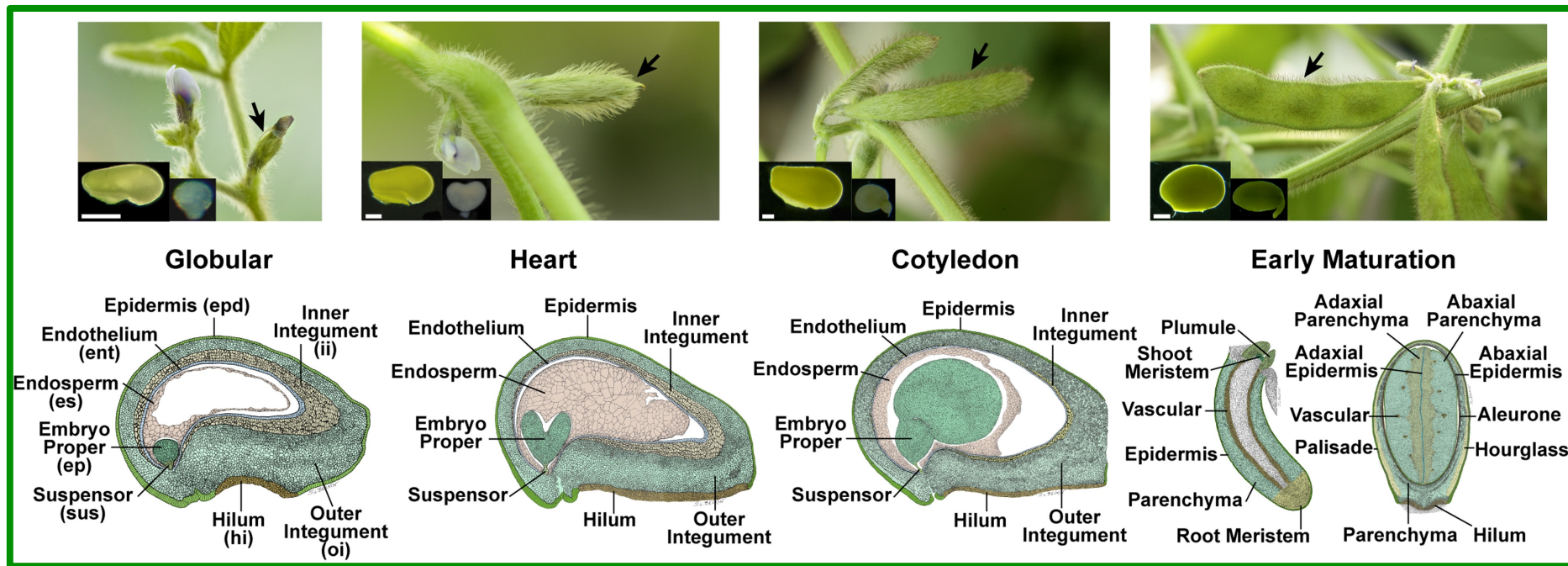
Why Soybean? - A Reminder

- **Second Major US Crop (72M Acres)**
- **Total Crop Value \$32Billion (50% Value Exported)**
- **Major Food Source**
- **Important Biofuel Source (Biodiesel~20% of US Soybean Oil Production)**
- **Excellent Model Plant (Transformation, Knockdowns, Genetics)**
- **Genome Sequenced**
- **Seed Gene Expression Data**

Diversity of Oil Seed Plants



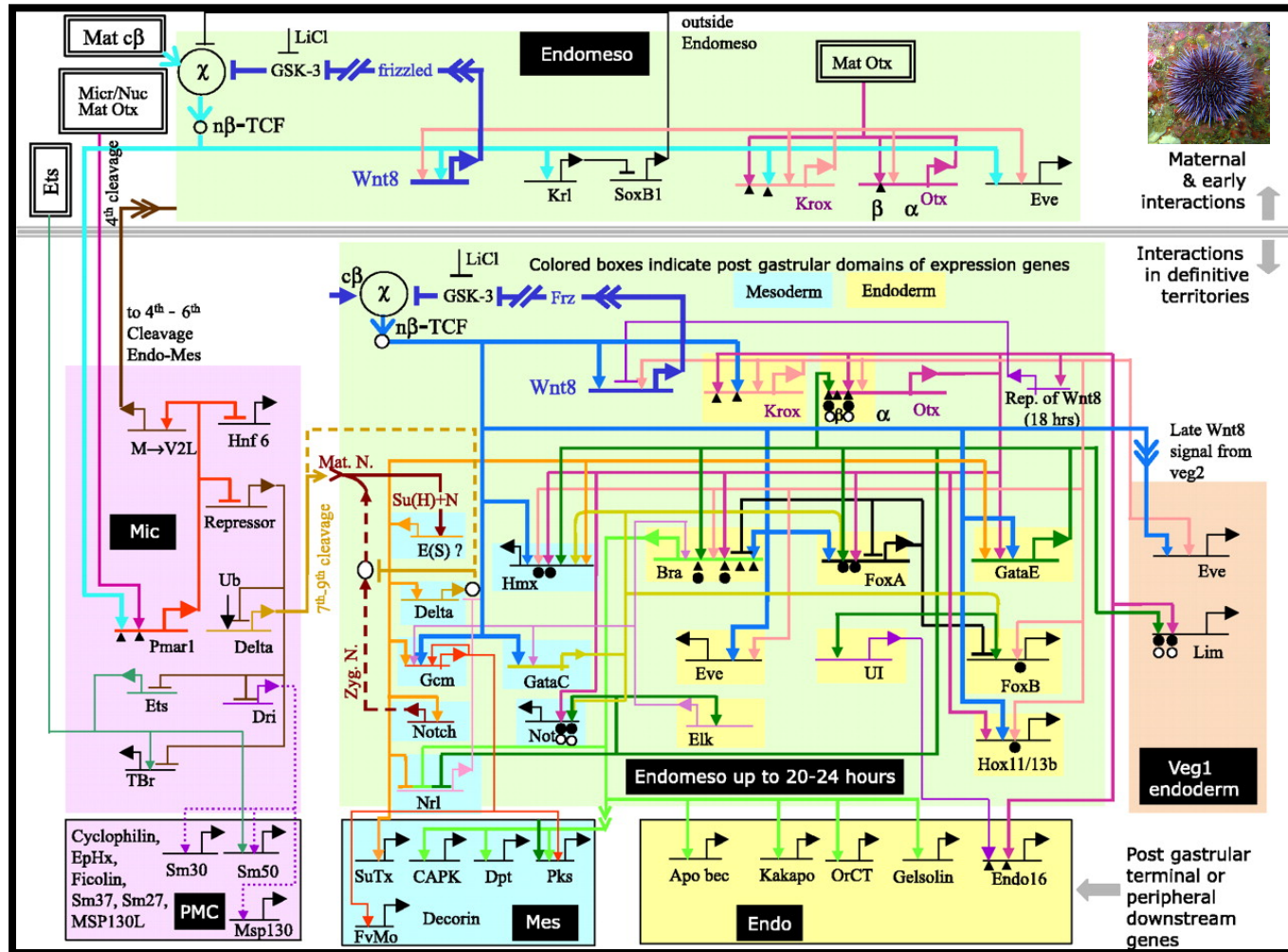
What Are the Questions?



- How Many Genes Program Seed Development?
- What Genes Are Active in Every Seed Compartment, Region, and Tissue Throughout Development?
- How Are Genes Regulated During Development
- What Are the DNA Sequences Controlling Seed Development?
- How Do Epigenetic Processes Influence the Development of Seeds?



Ultimate Goal.....To Uncover Regulatory Genes and Circuits Driving Seed Differentiation and Development Using Genomics

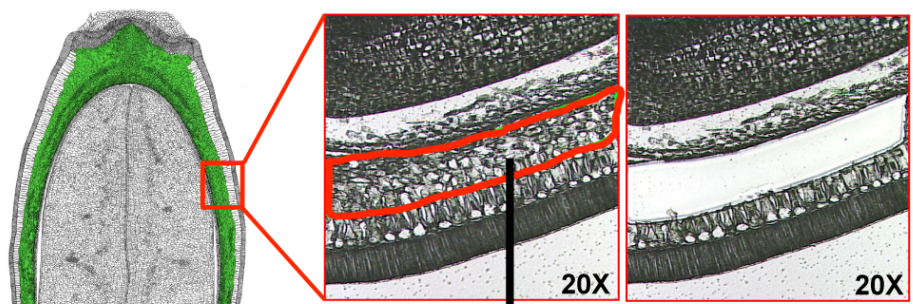
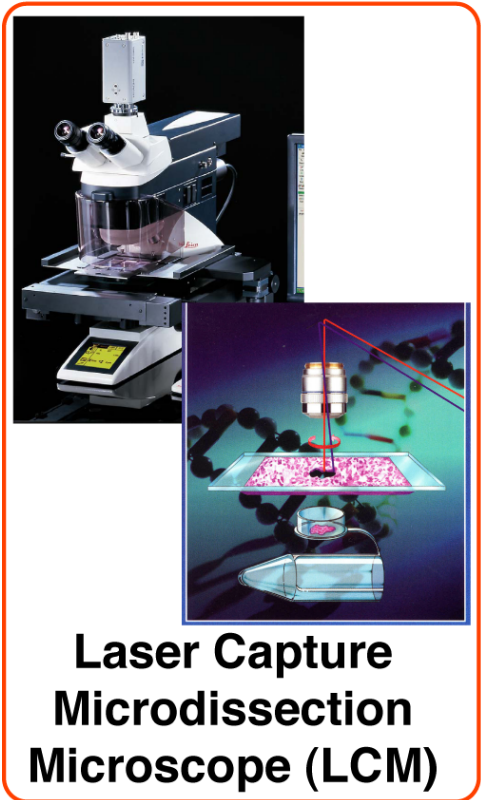


Learn How To Make a Seed!!



- Big Seeds
- More Seeds
- More Yield
- Increased Food and Fuel

A Genomics Strategy For Studying Seed Regulatory Processes



LCM Tissue

RNA Isolation

DNA Isolation

mRNA Selection

Small RNA Enrichment

Bisulfite Conversion

cDNA Sequencing Library

SmallRNA Sequencing Library

Bisulfite Converted Sequencing Library

Transcriptome

Methylome




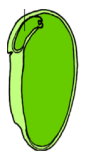
Data Analysis

Next Generation Sequencing

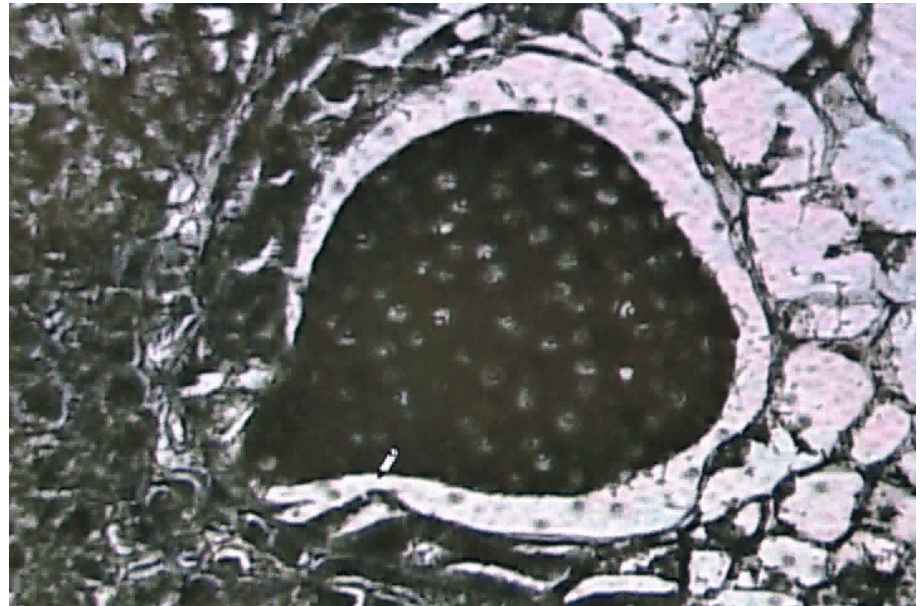


GCATCGCGATCGACGAT
CGCGATCGATCATCAGT
CGCGATCGATCGTCGAT
GCATCCGGATCAGTCGA
CGATCCCGATCCGATGA
CGGACGATCGATCGATCGA
CGAACAGTCGATGCGCT

How Many LCM Sections Were Captured and How Many Hours Were Spent for LCM Capturing?

Developmental Stage	Number of LCM Captures
 Globular	33,738
 Heart	26,341
 Cotyledon	21,301
 Early-Maturation	88,032
Total	169,412

Globular Stage Embryo Proper and Suspensor



Time for LCM
Captures
= ~ 2300 person hrs

How Many Genes Program Soybean Seed Development?



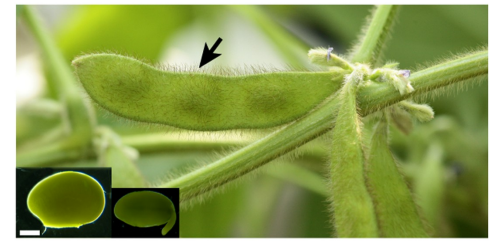
Globular



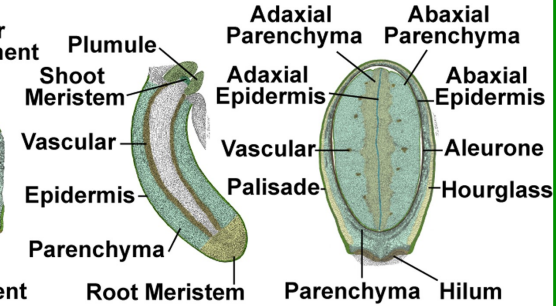
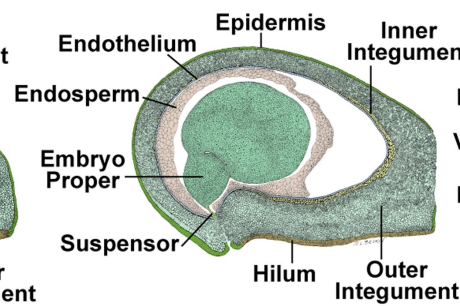
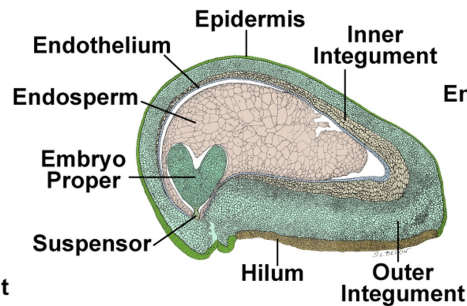
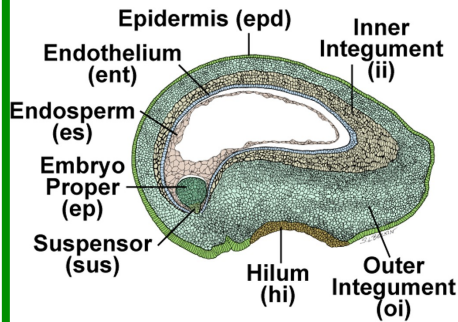
Heart



Cotyledon



Early Maturation



How Many Genes Are Active in Every Soybean Seed Region and Tissue throughout Development?

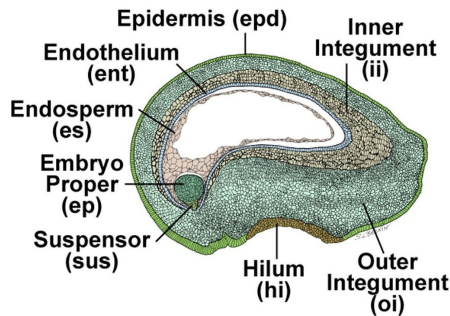
Differentiation & Morphogenesis

Maturation & Preparation for Dormancy

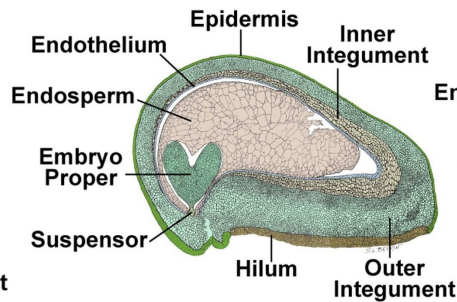
Early Development

Early Maturation

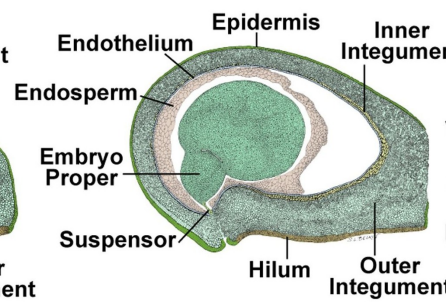
Globular



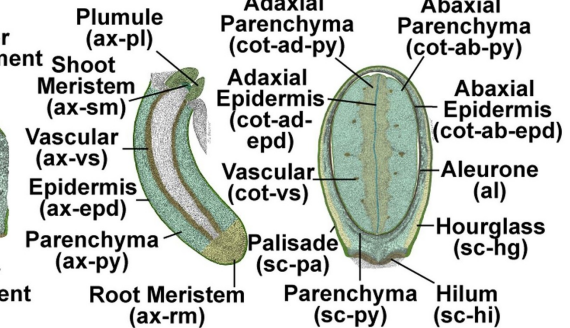
Heart



Cotyledon



Early-Maturation



1. Similar number of genes active in each seed tissue

2. Majority are shared but up-regulated

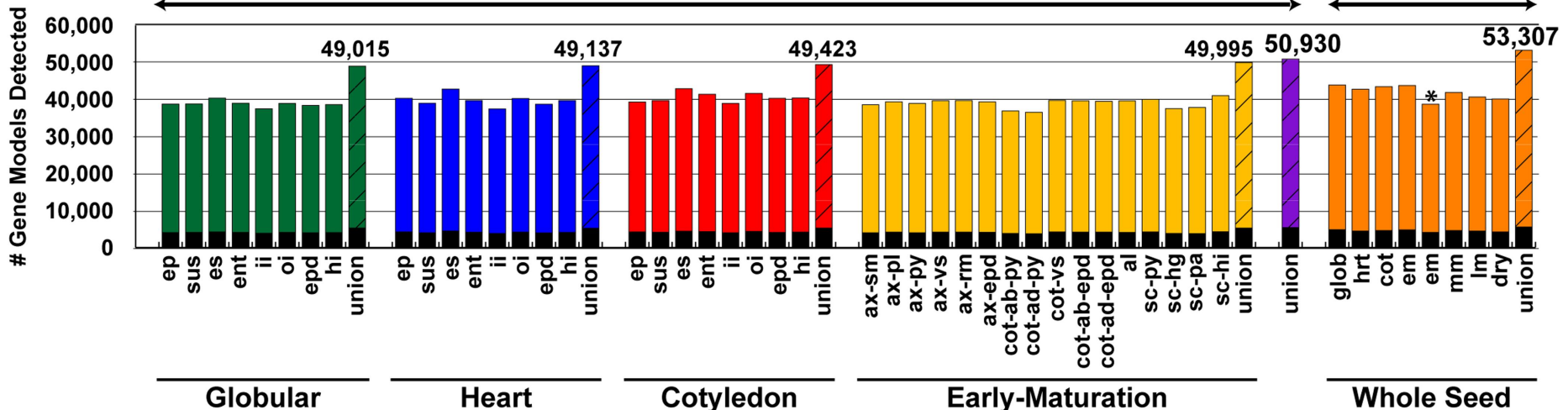
3. Small number of specifics

4. Majority TFs shared and small number of specifics

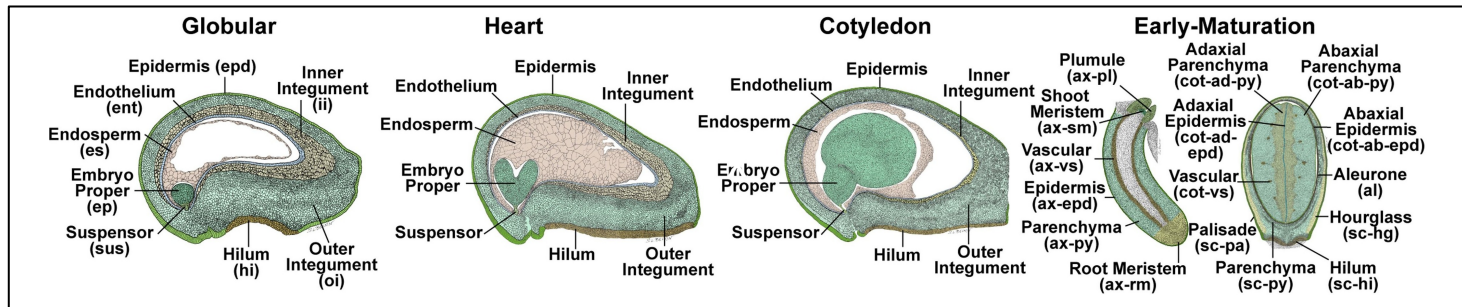
5. 53,000 genes are required to make a seed

Seed Regions, Subregions, and Tissues

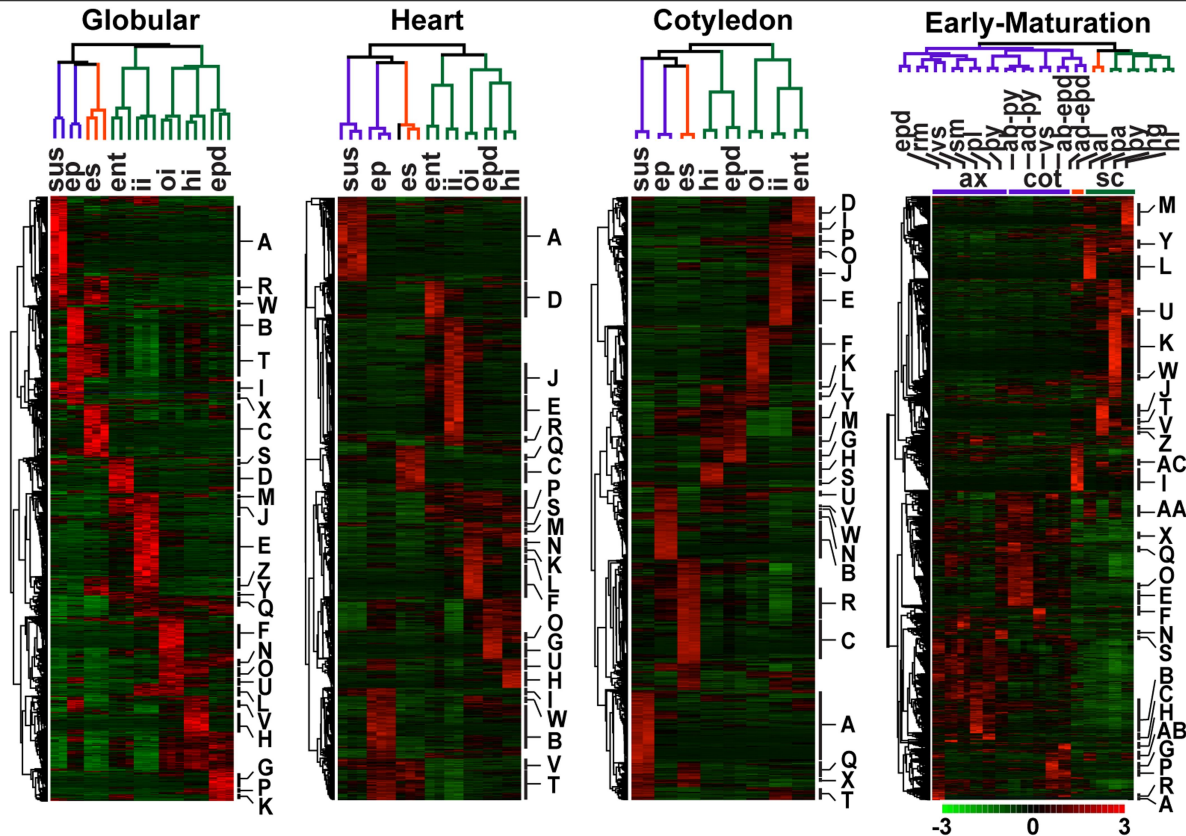
Whole Seed



How Are Genes Regulated Throughout Soybean Seed Development?

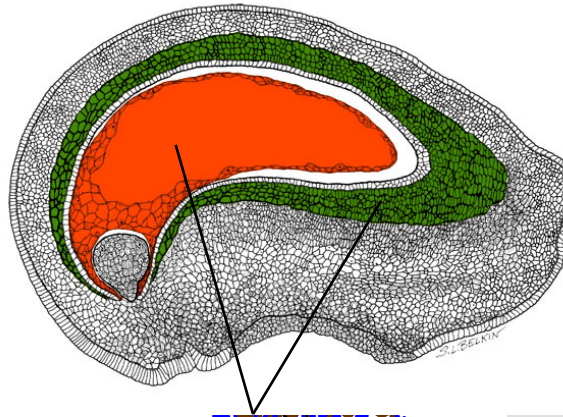
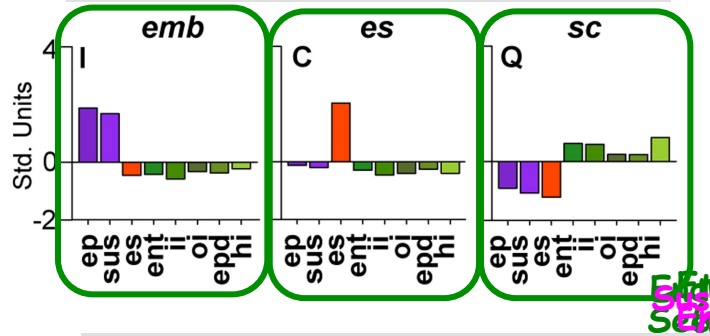


Hierarchical Clustering of Most Varying 4,000 mRNAs during Soybean Development

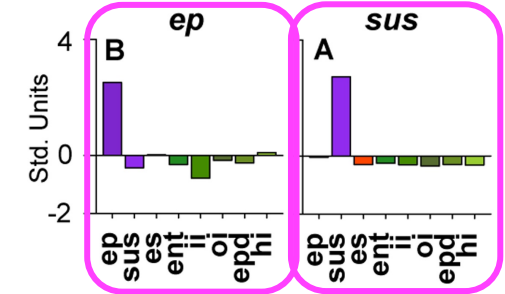


What Are the Major mRNA Accumulation Patterns in Different Seed Regions at Globular Stage?

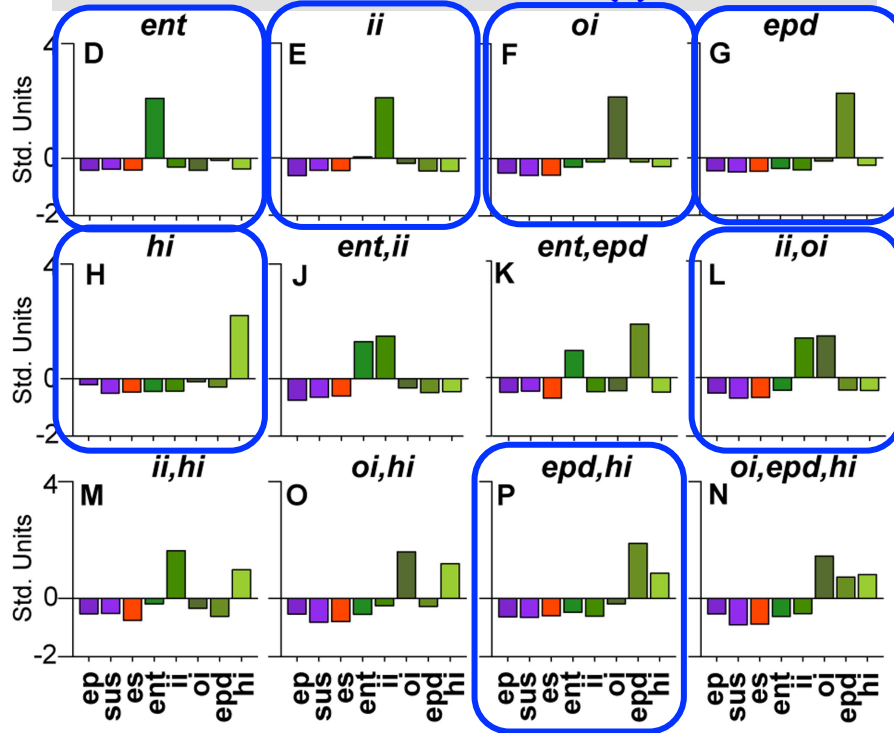
I. mRNAs Up-Regulated in Specific Seed Region



II. mRNAs Up-Regulated in Specific Embryo Subregion

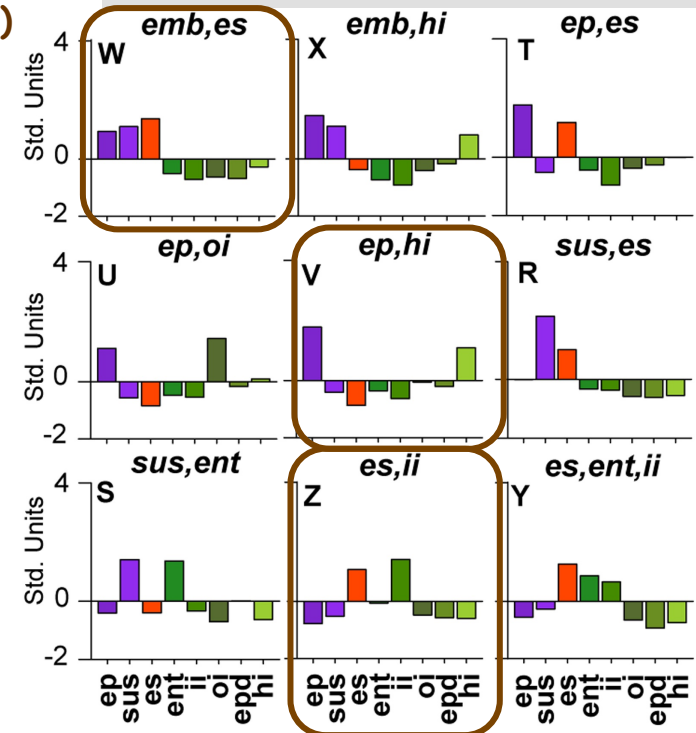


III. mRNAs Up-Regulated in Specific Seed Coat Tissue(s)



Endosperm & Embryo (ep & hi)

IV. mRNAs Up-Regulated in Multiple Seed Regions



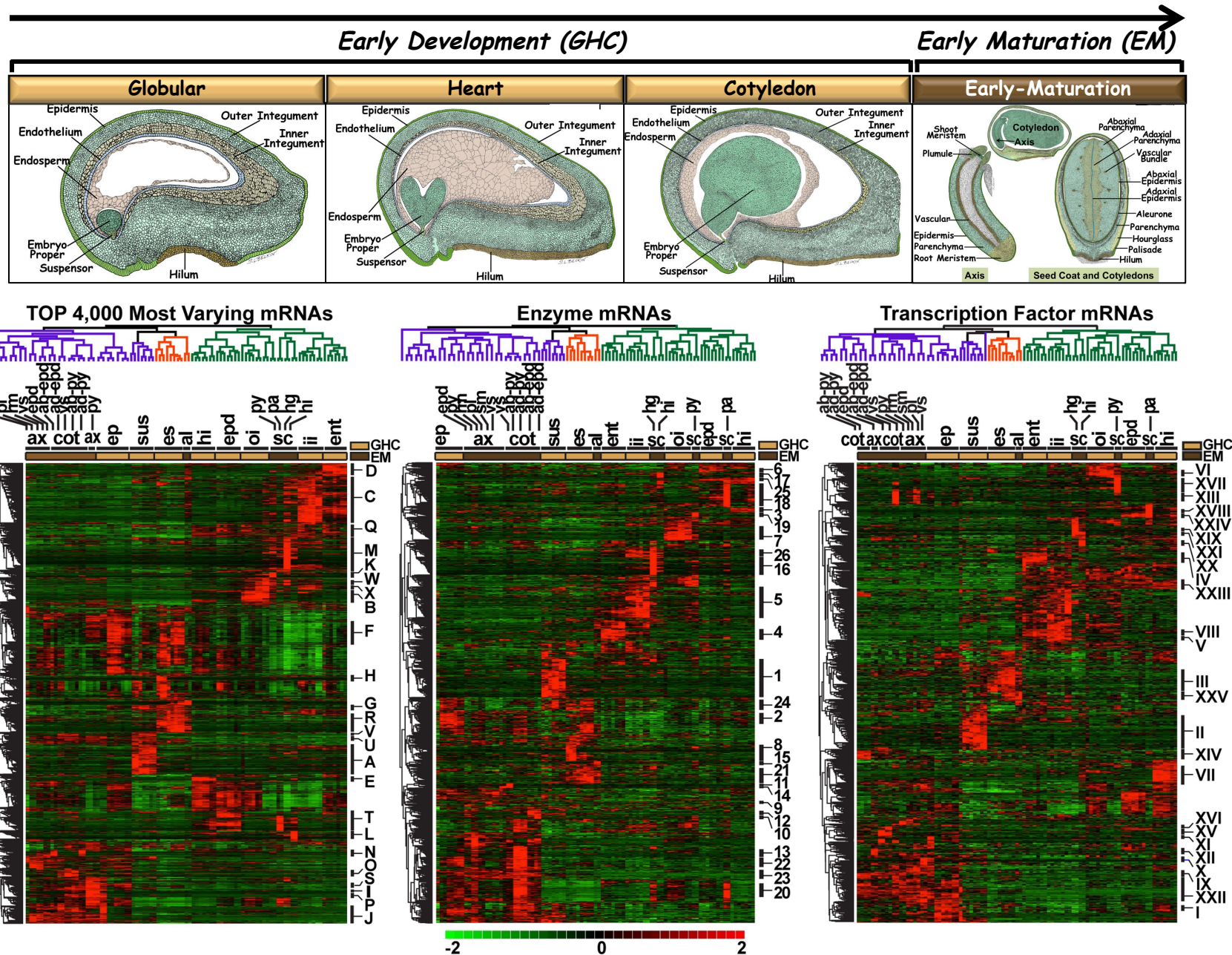
Metabolic Pathways Are Prevalent in Different Regions during Soybean Development?

Globular Stage

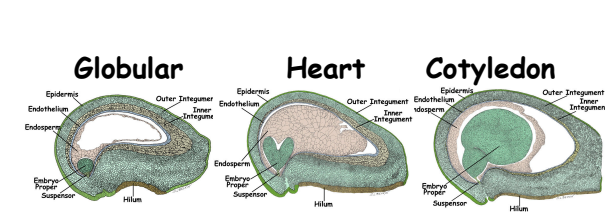
Heart Stage

egradation
tion &
port

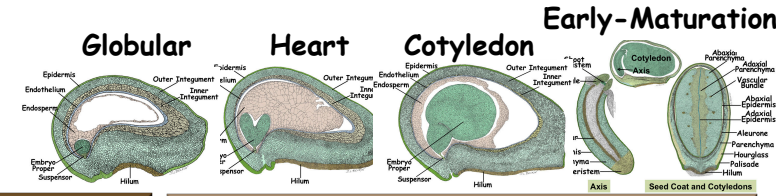
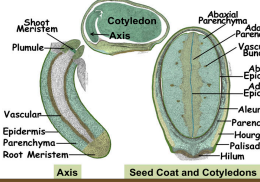
How Are Genes Temporally Regulated in Different Seed Regions Throughout Soybean Development?



What Are the Major Temporal and Spatial mRNA Accumulation Patterns throughout Seed Development?

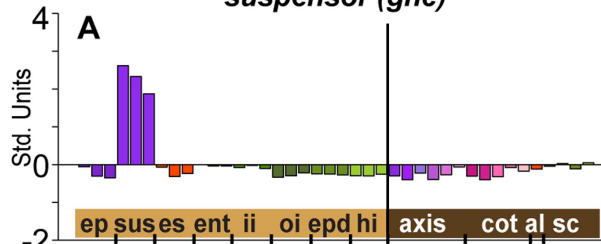


Early-Maturation

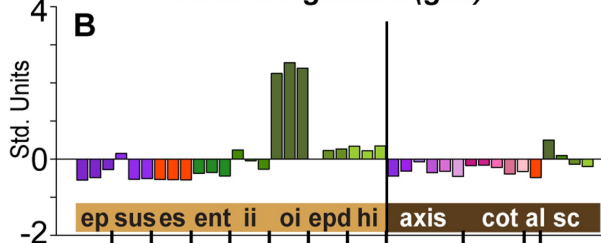


I. mRNAs Spatially Up-Regulated during Early Development

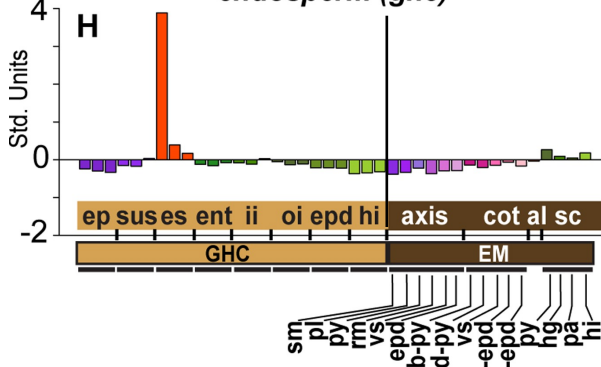
suspensor (ghc)



outer integument (ghc)

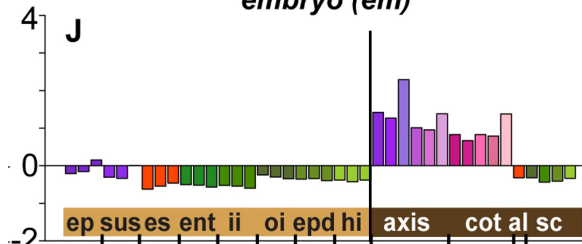


endosperm (ghc)

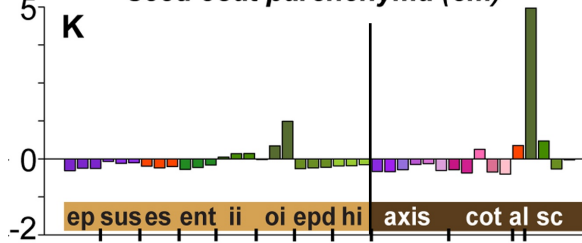


II. mRNAs Spatially Up-Regulated during Early Maturation

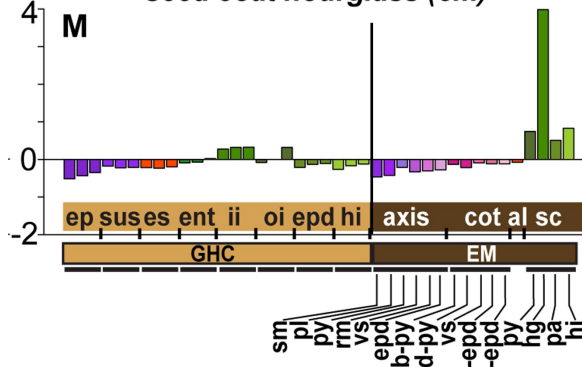
embryo (em)



seed coat parenchyma (em)

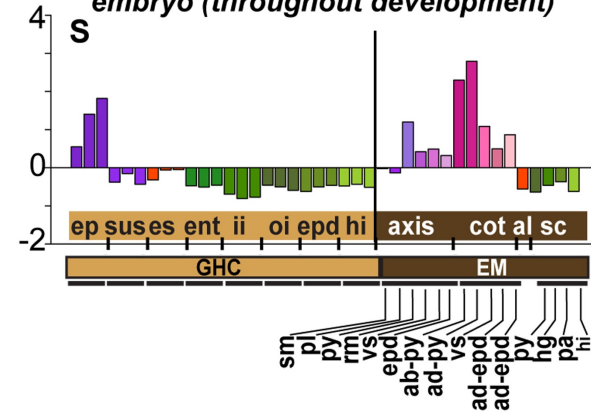


seed coat hourglass (em)



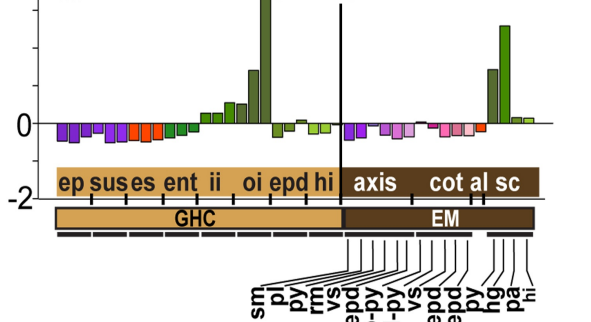
III. mRNAs Up-Regulated throughout Development

embryo (throughout development)

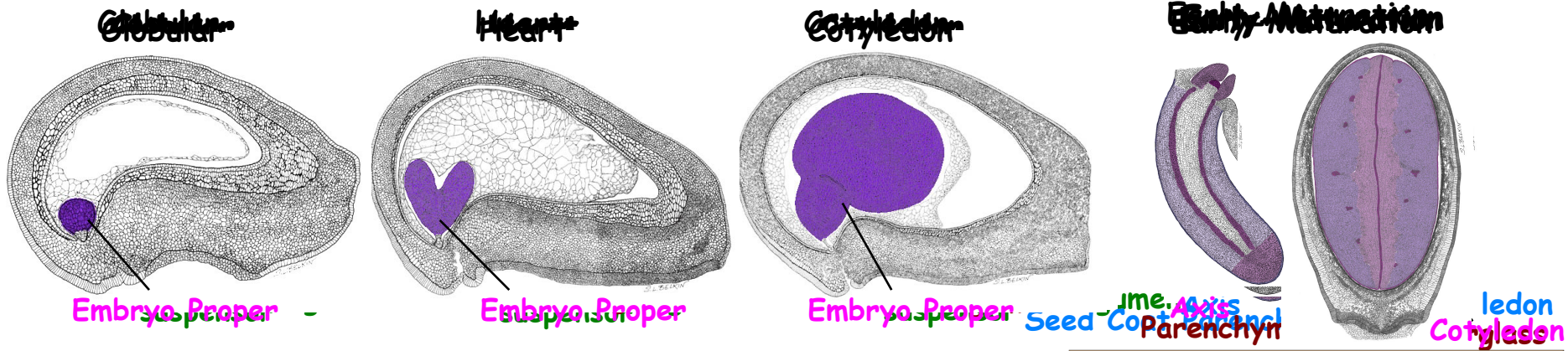


IV. mRNAs Up-Regulated in Different Tissues at Multiple Stages

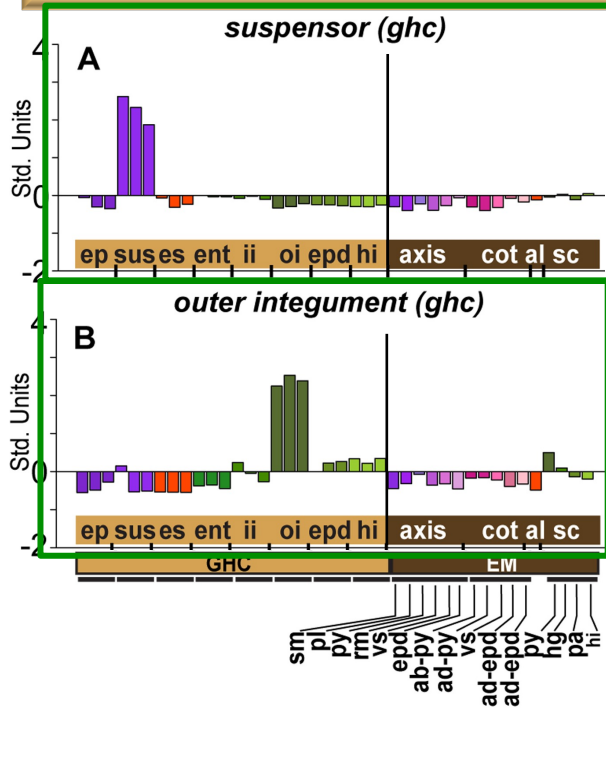
outer integument parenchyma/hourglass (hcem)



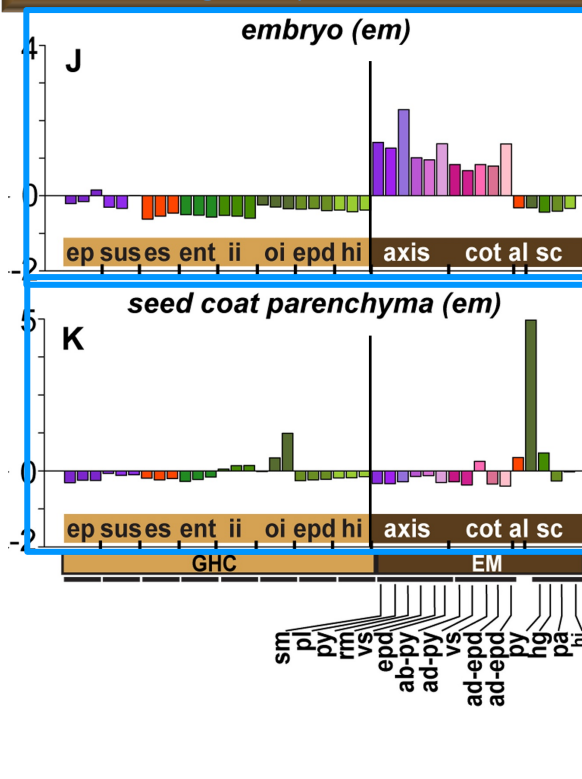
Examples of Major Temporal and Spatial mRNA Accumulation Patterns throughout Seed Development?



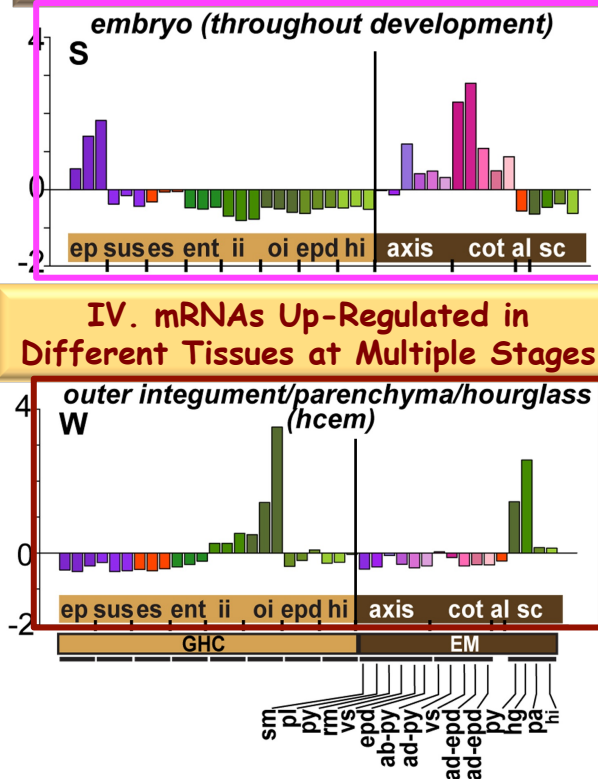
I. mRNAs Spatially Up-Regulated during Early Development



II. mRNAs Spatially Up-Regulated during Early Maturation

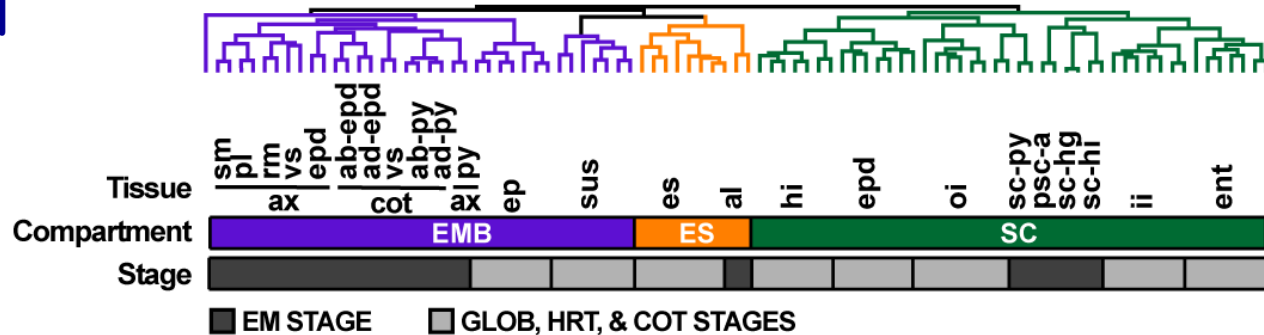


III. mRNAs Up-Regulated throughout Development

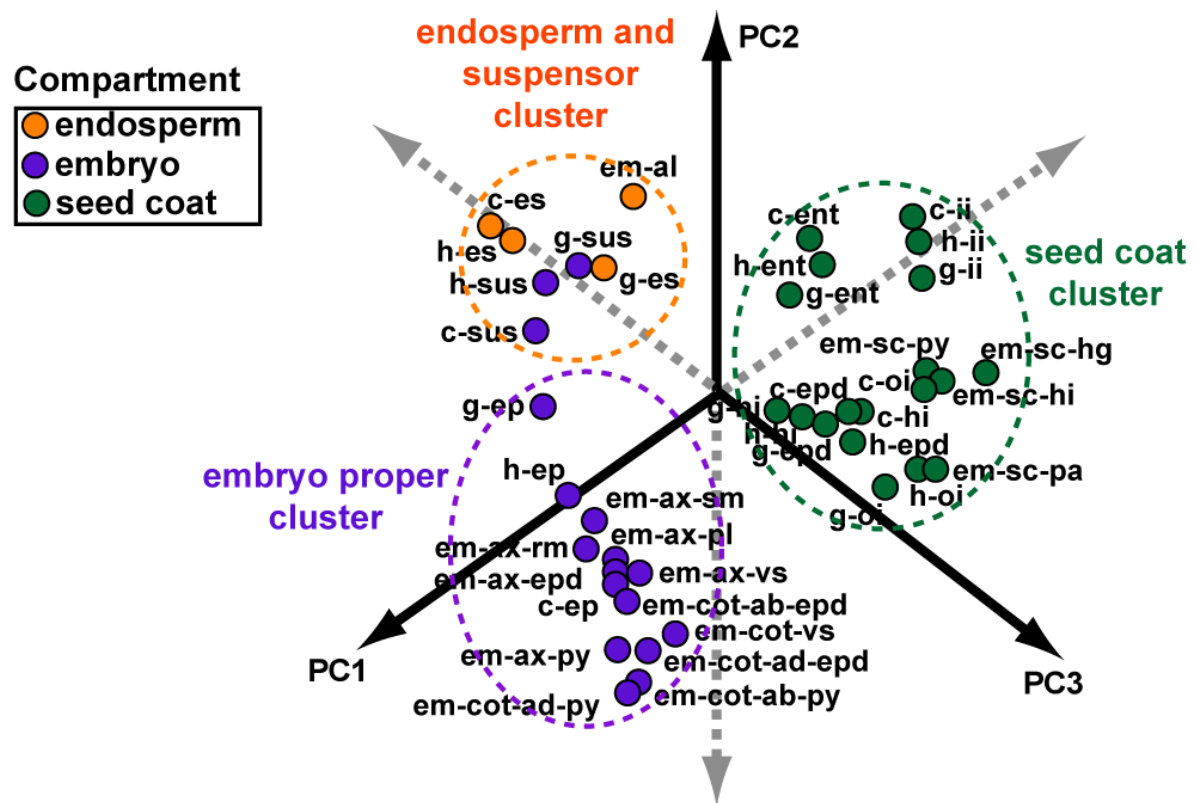


What Are the Biological Relationships Between 40 Seed Compartments, Regions, and Tissues Throughout Development?

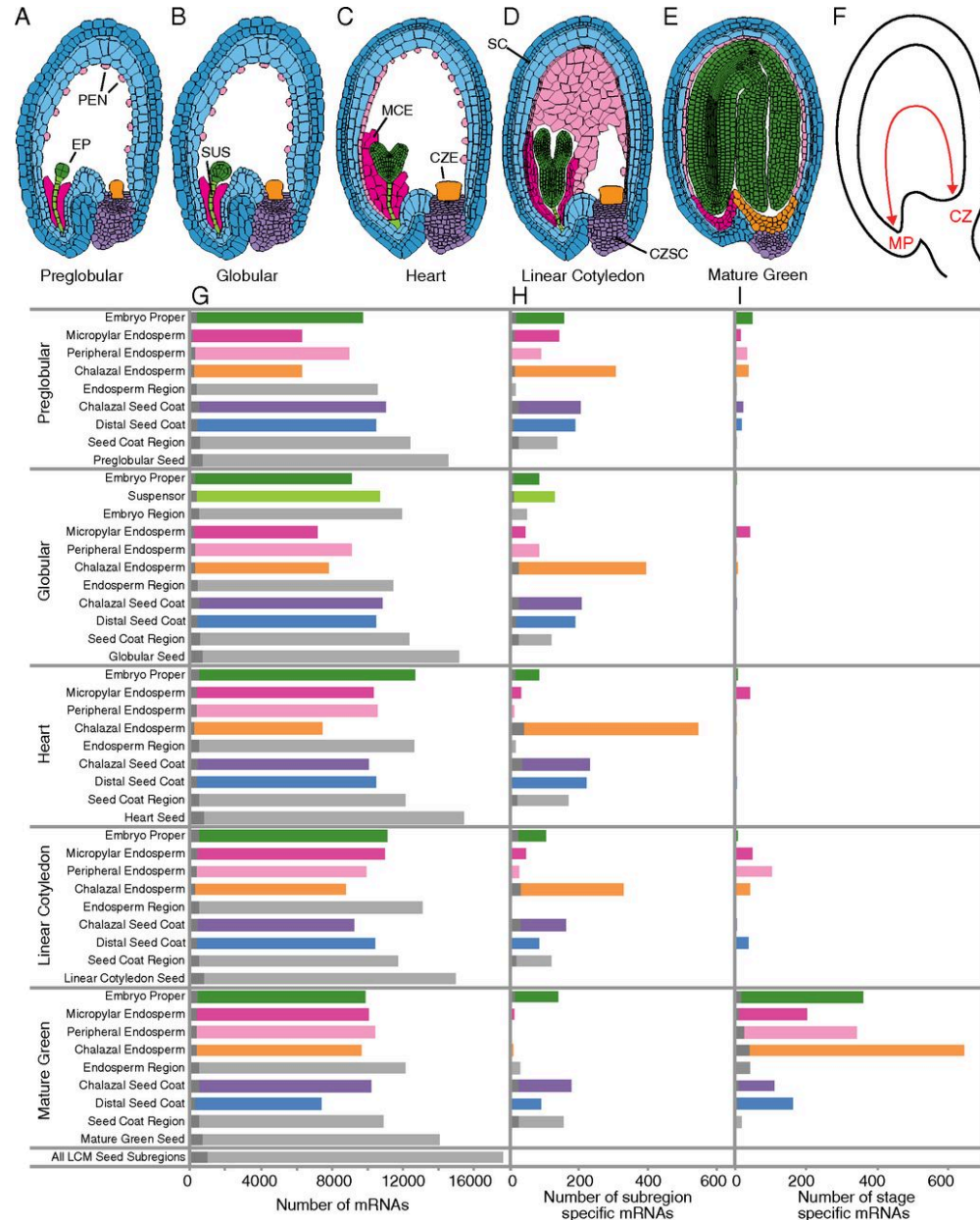
Hierarchical Clustering



Principal Component Analysis

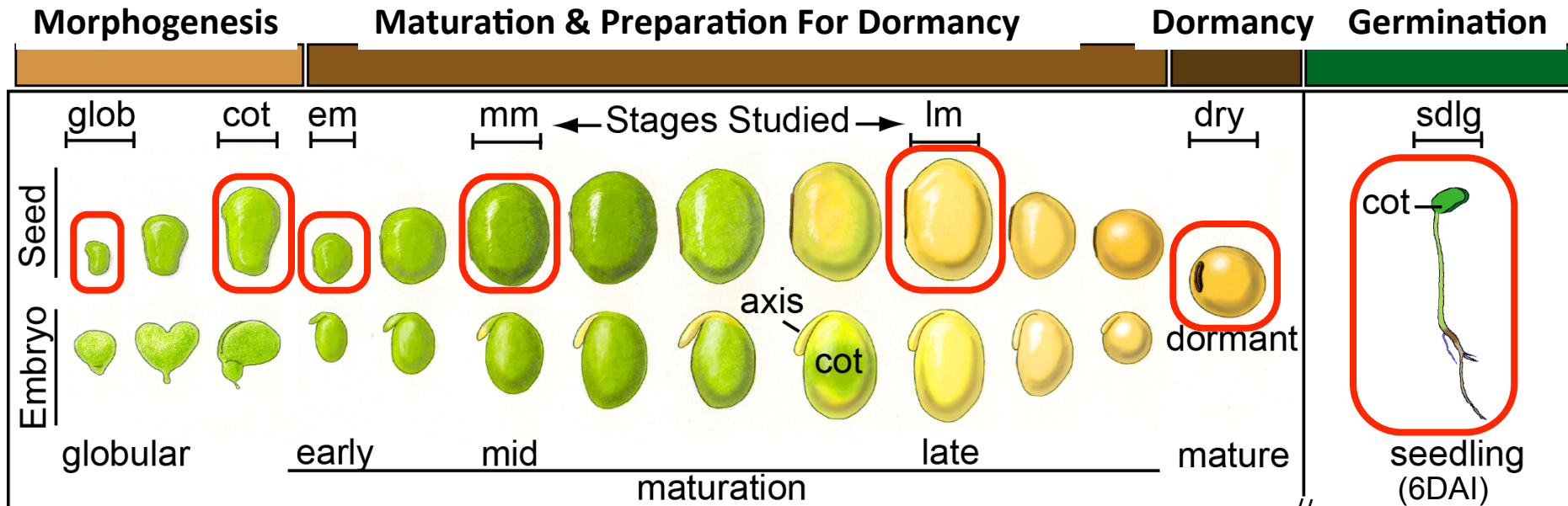


Arabidopsis Seed Gene Expression Patterns Are the Same as in Soybean !!



**Seed Gene
Regulatory Circuits
Are Conserved**

Do Whole-Genome DNA Methylation Changes Occur During Seed Development?

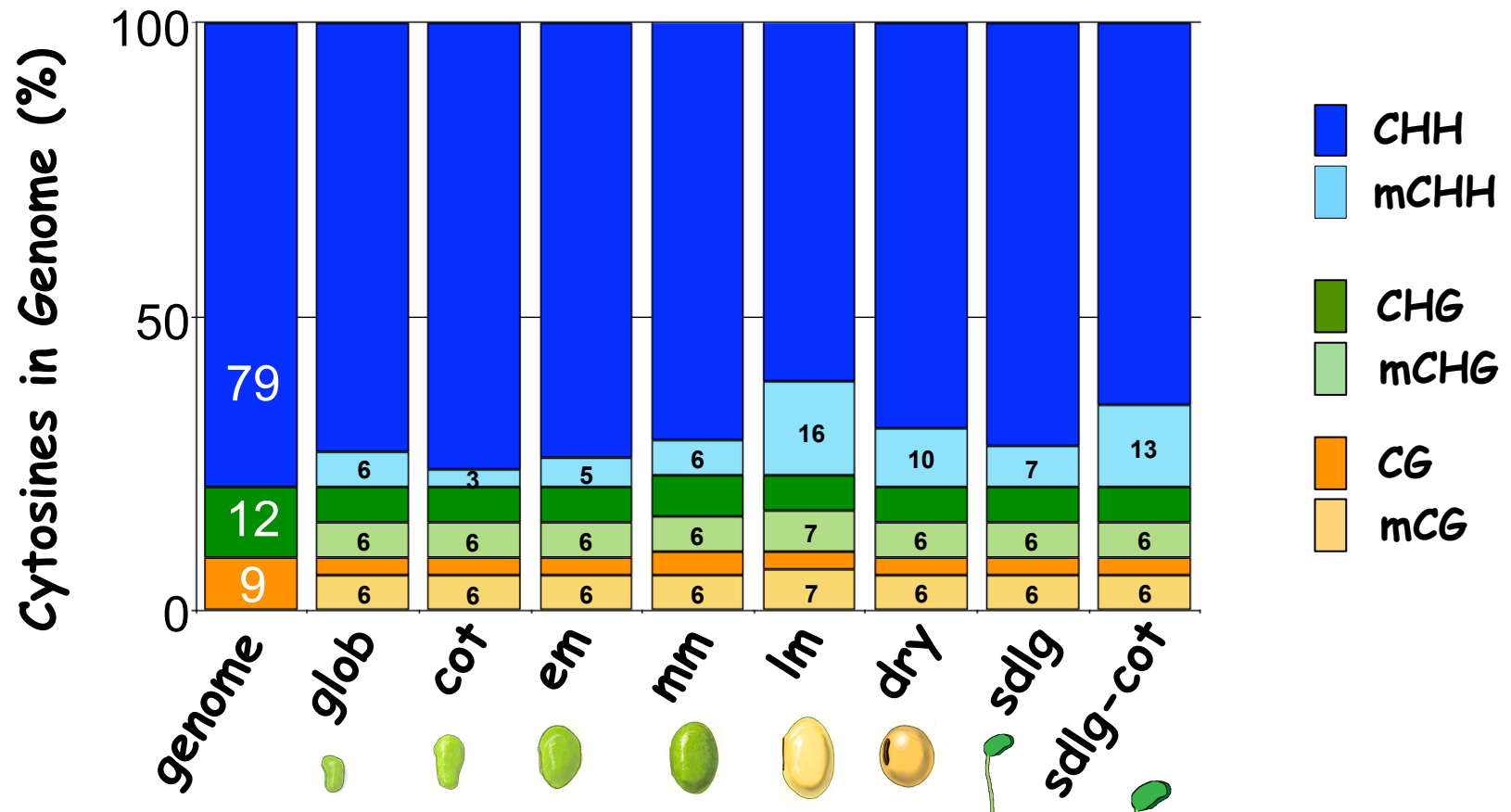


To Date, We Have Generated ~10 Billion
BS-DNA-Seq Reads (>1000 Gigabases)

Developmental Stages Studied

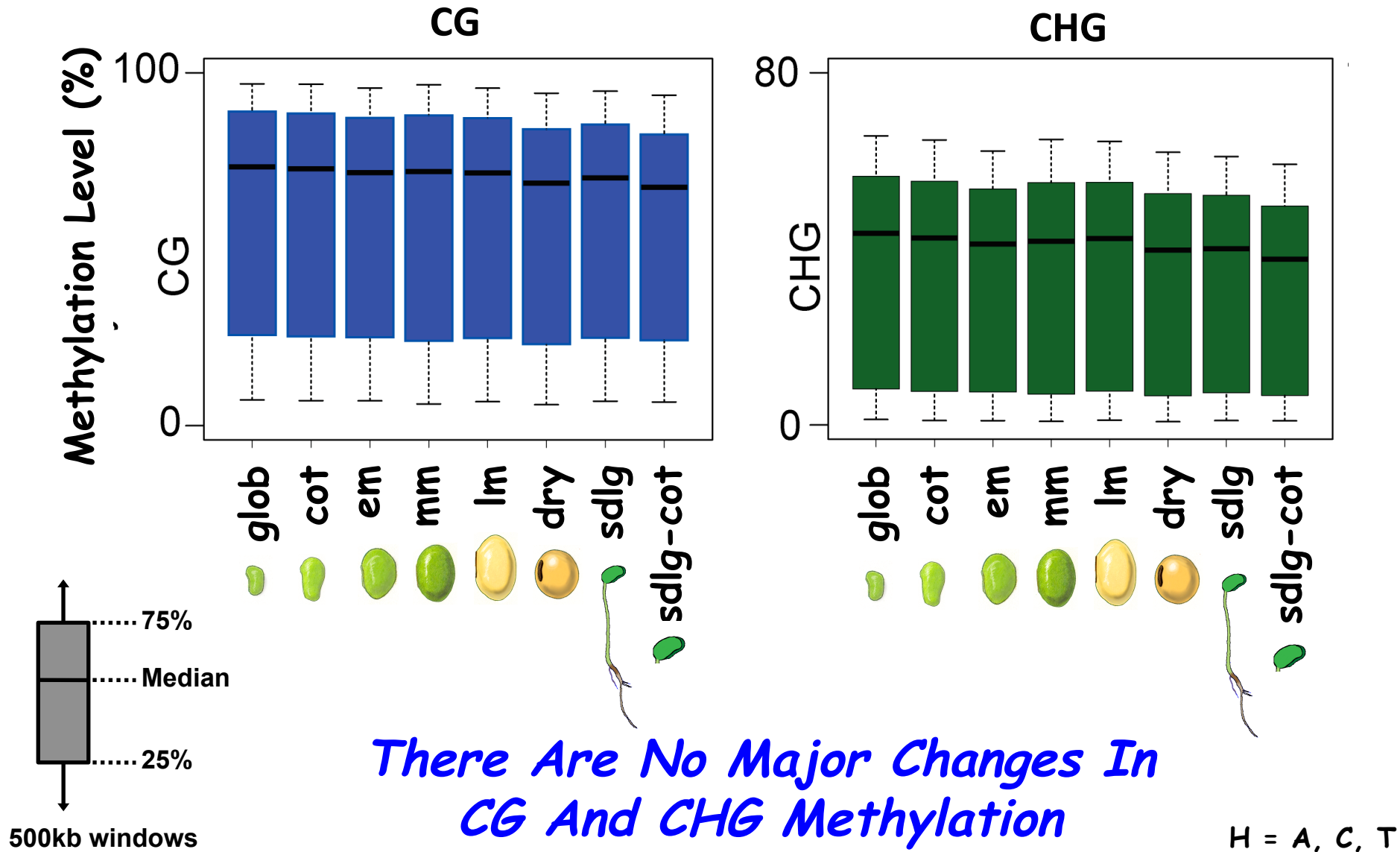
DAI = Days After Imbibition

How Many Cytosines Are Methylated in the Soybean Genome?

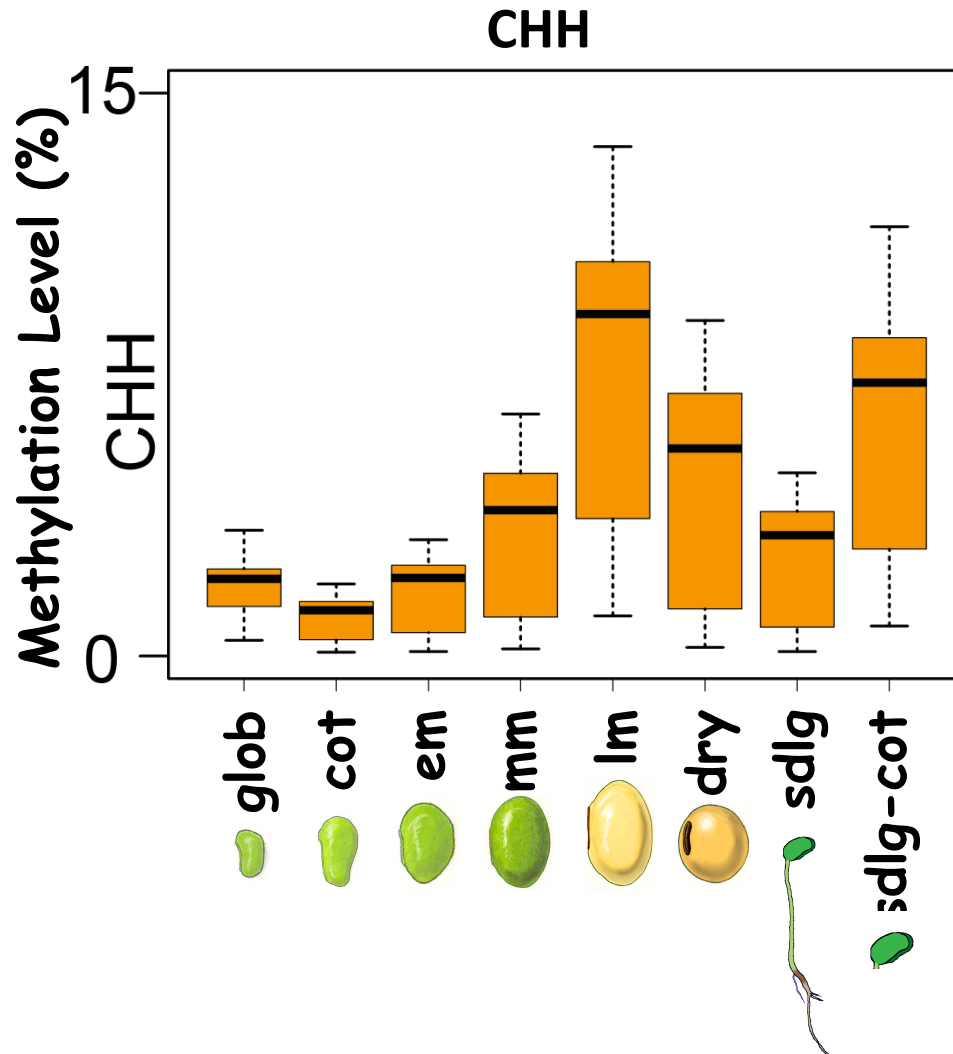


18%~26% Cytosines in the Soybean Genome are Methylated

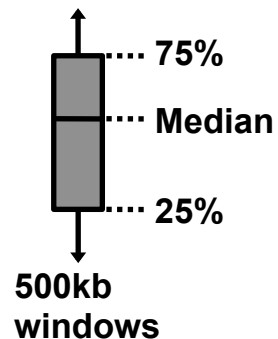
Are There DNA Methylation Changes During Seed Development?



Are There DNA Methylation Changes During Seed Development?



- *CHH methylation increases during maturation towards dormancy*
- *CHH methylation decreases after dormancy during post-germination*



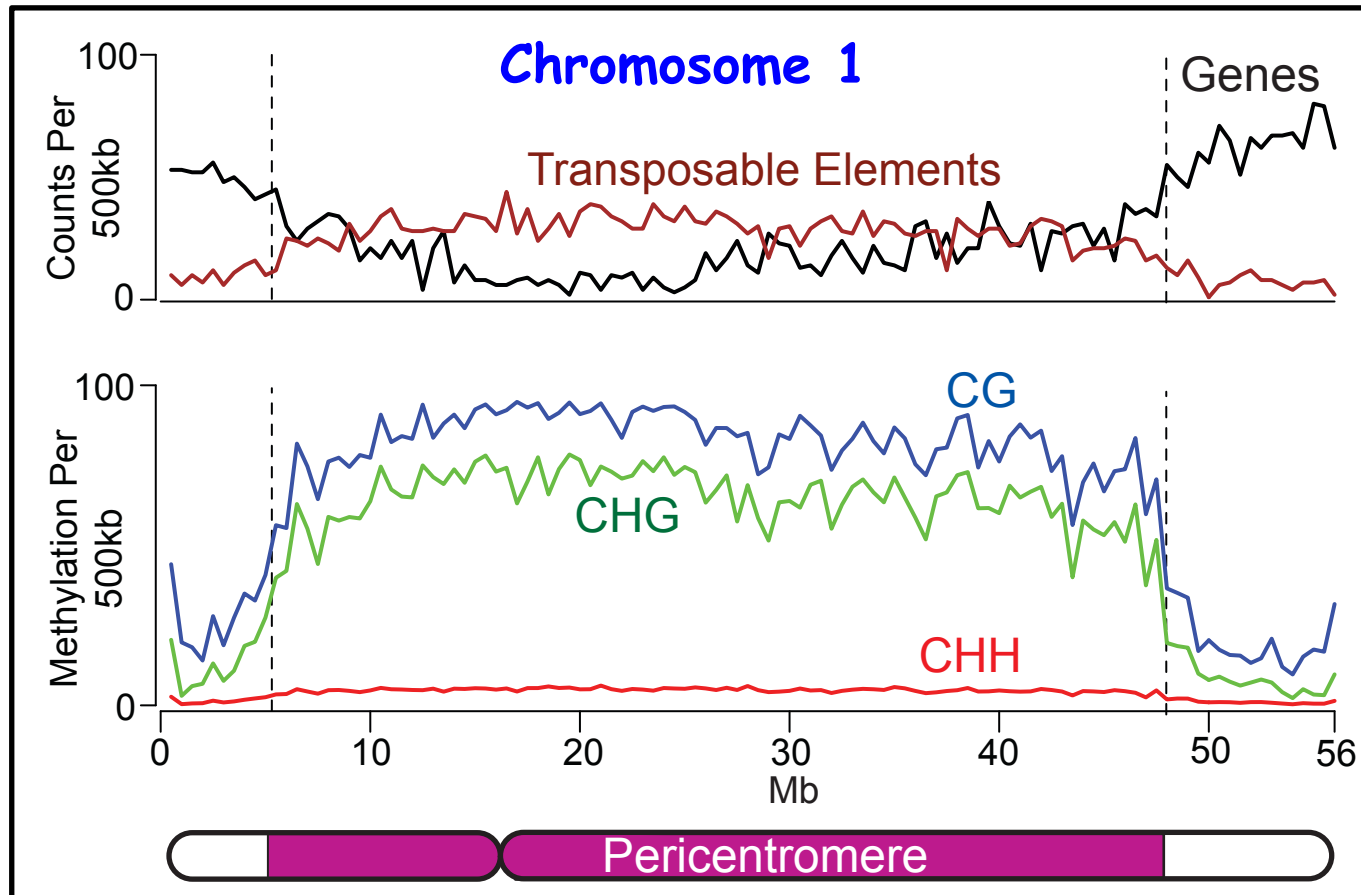


mm-stage

Where are the the DNA Methylation Changes Located Within the Soybean Genome?



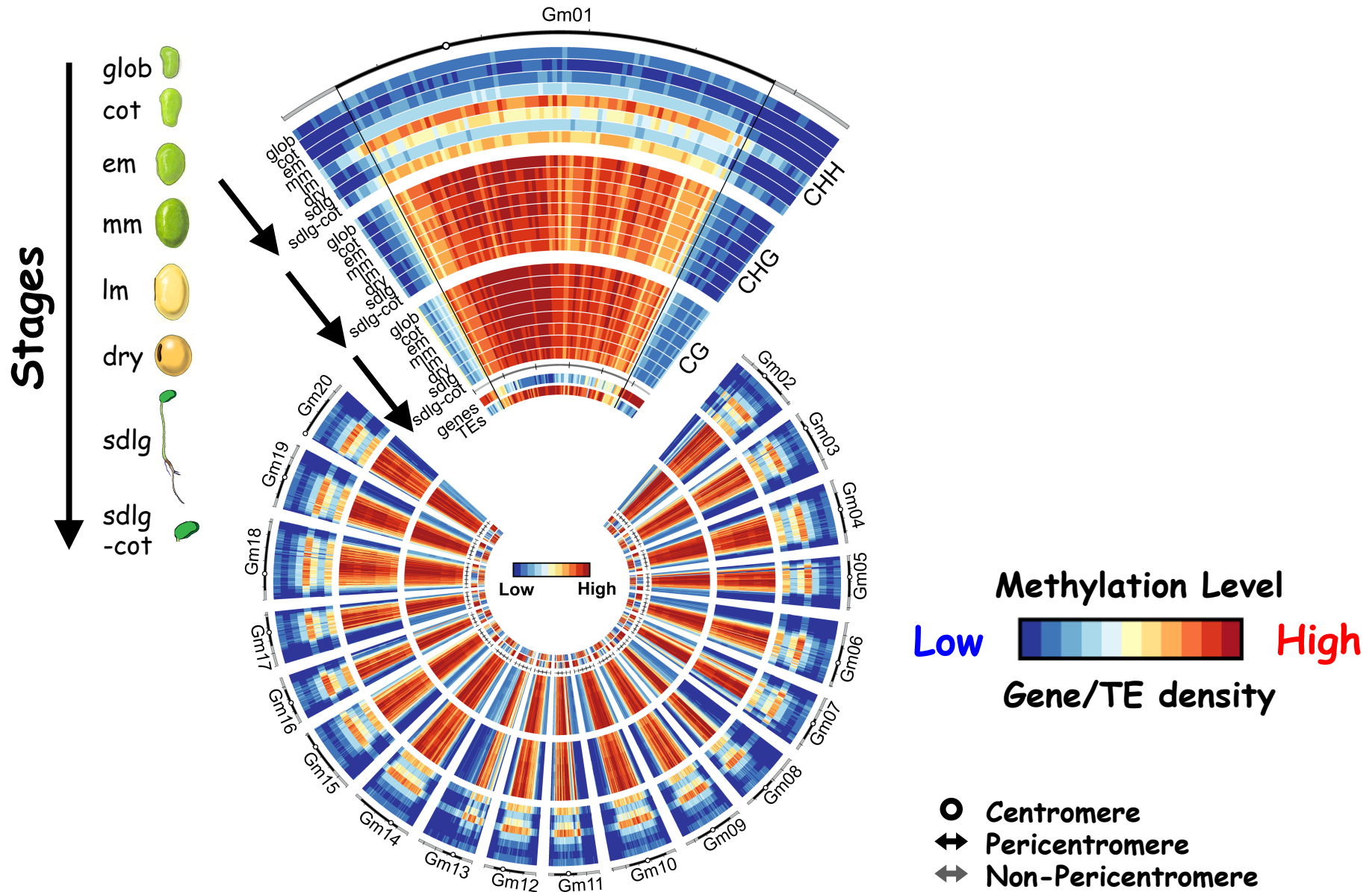
mm-stage



*High Methylation
Around TE-Dense
Pericentromeric
Regions*

*Low Methylation
Around Gene-
Dense
Chromosome Arms*

Developmental CHH-Context DNA Methylation Changes Occur Within TE-Dense Regions of The Genome in All 20 Chromosomes



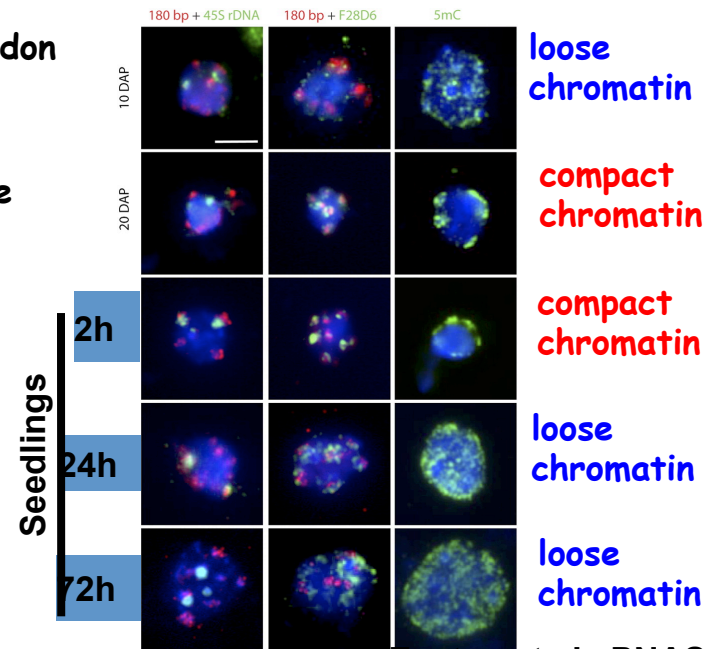
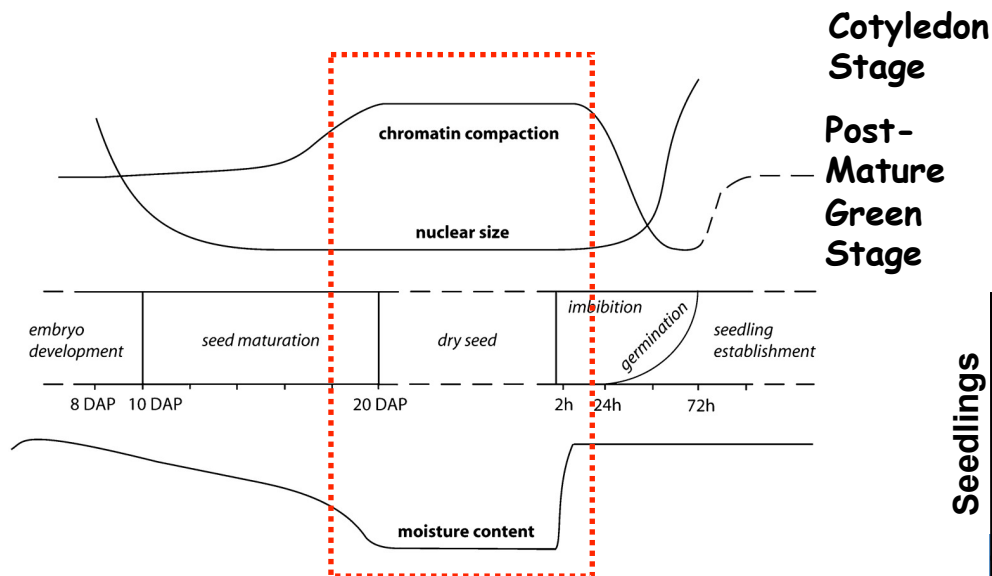
What Role Might Increases in CHH Methylation Play During Seed Development?



Increased methylation during bud setting and decreased methylation during bud bursting

Castanea sativa Bud bursting and Bud Setting (dormancy)

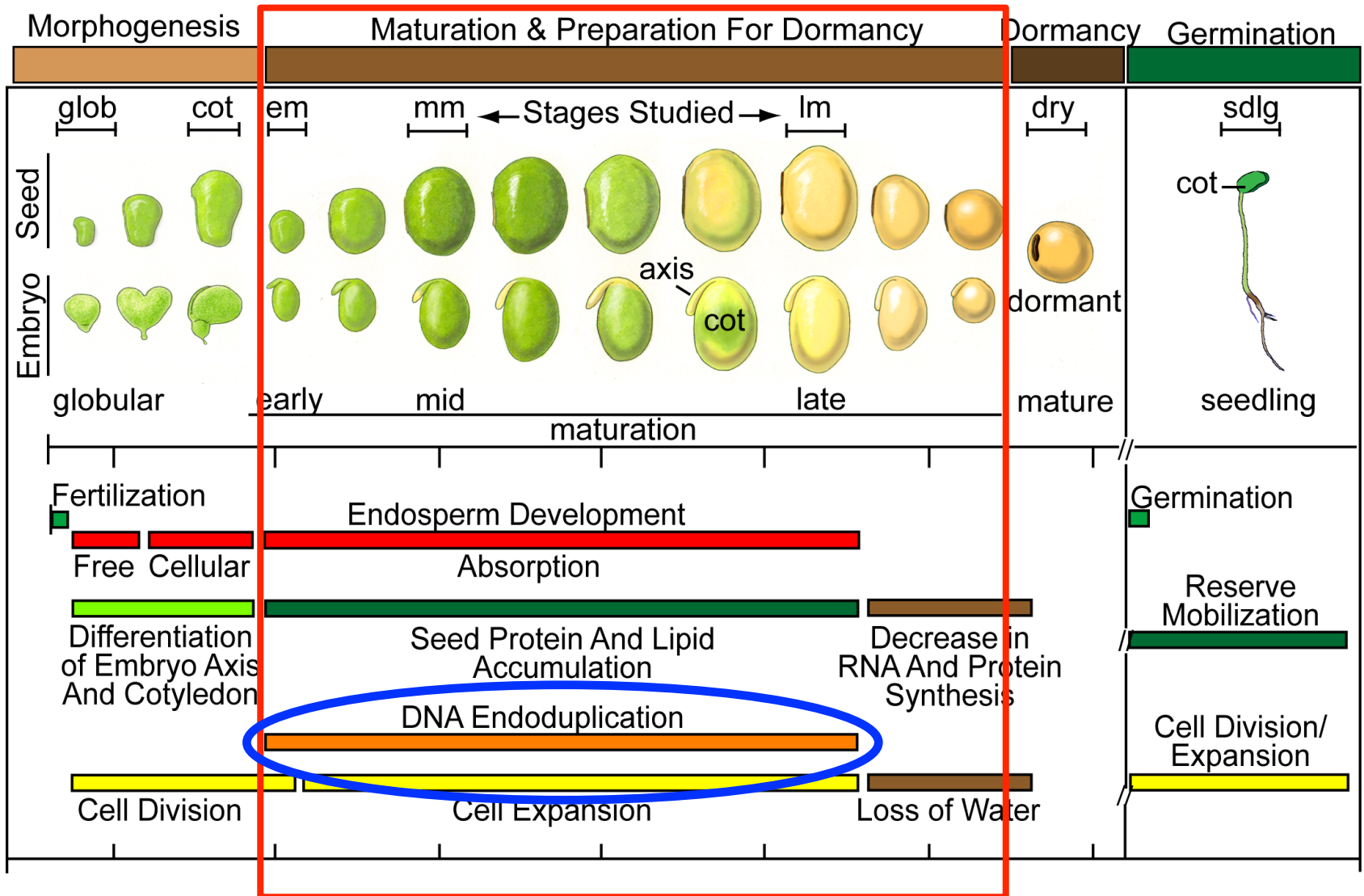
Santamaría et al., J. Plant Physiol 2009



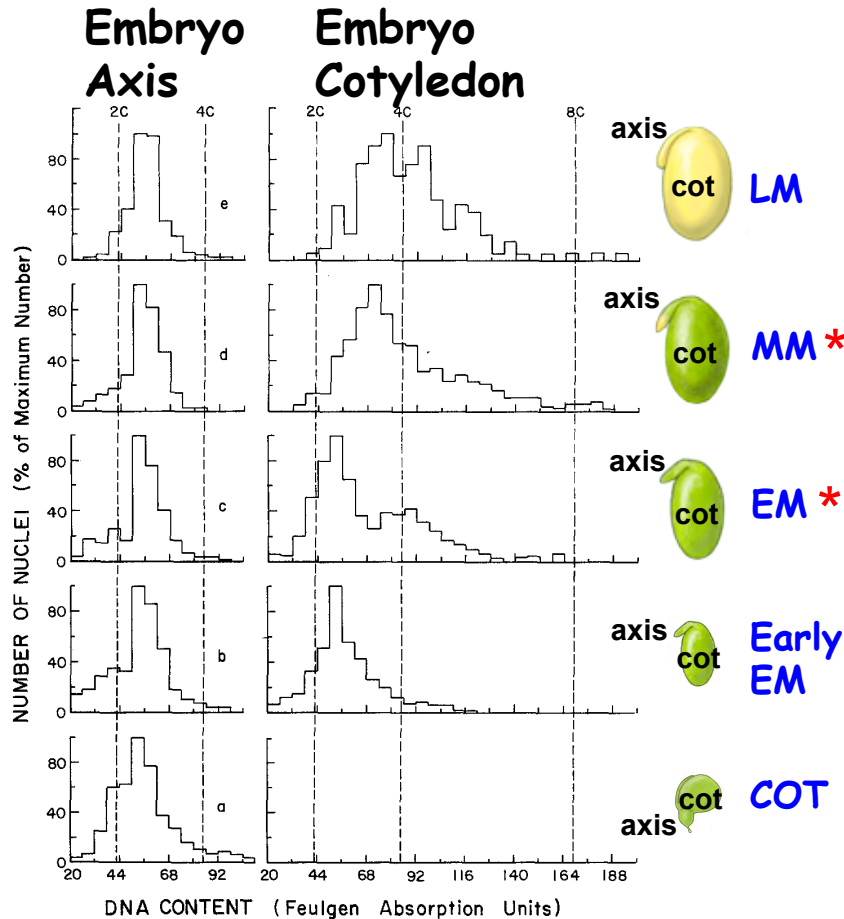
van Zanten et al., PNAS 2011

- I. Chromatin compaction and condensation
- II. Silence TEs to maintain genome integrity

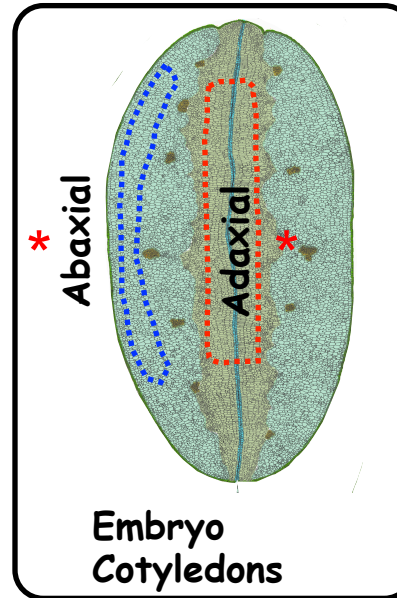
Is the Genome Methylation Landscape Maintained During Seed Endoreduplication?



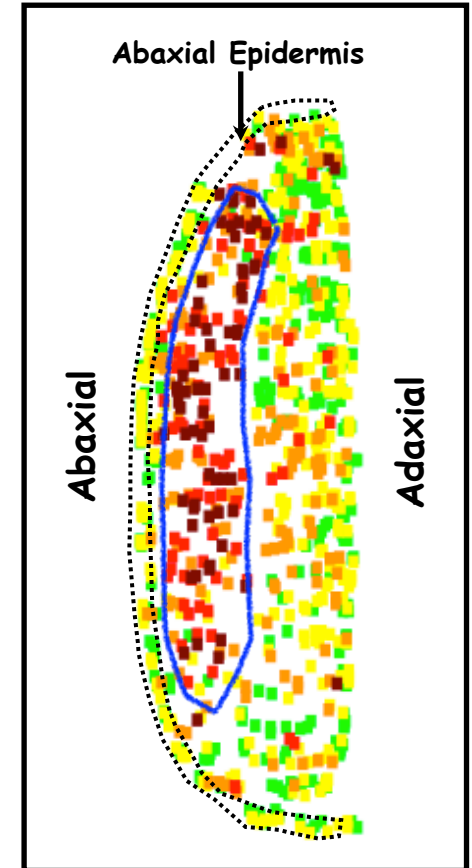
When & Where Does Endoreduplication Occur Within the Seed?



Dhillon SS, Miksche JP (1983)
Histochem. J. 15:21-37.

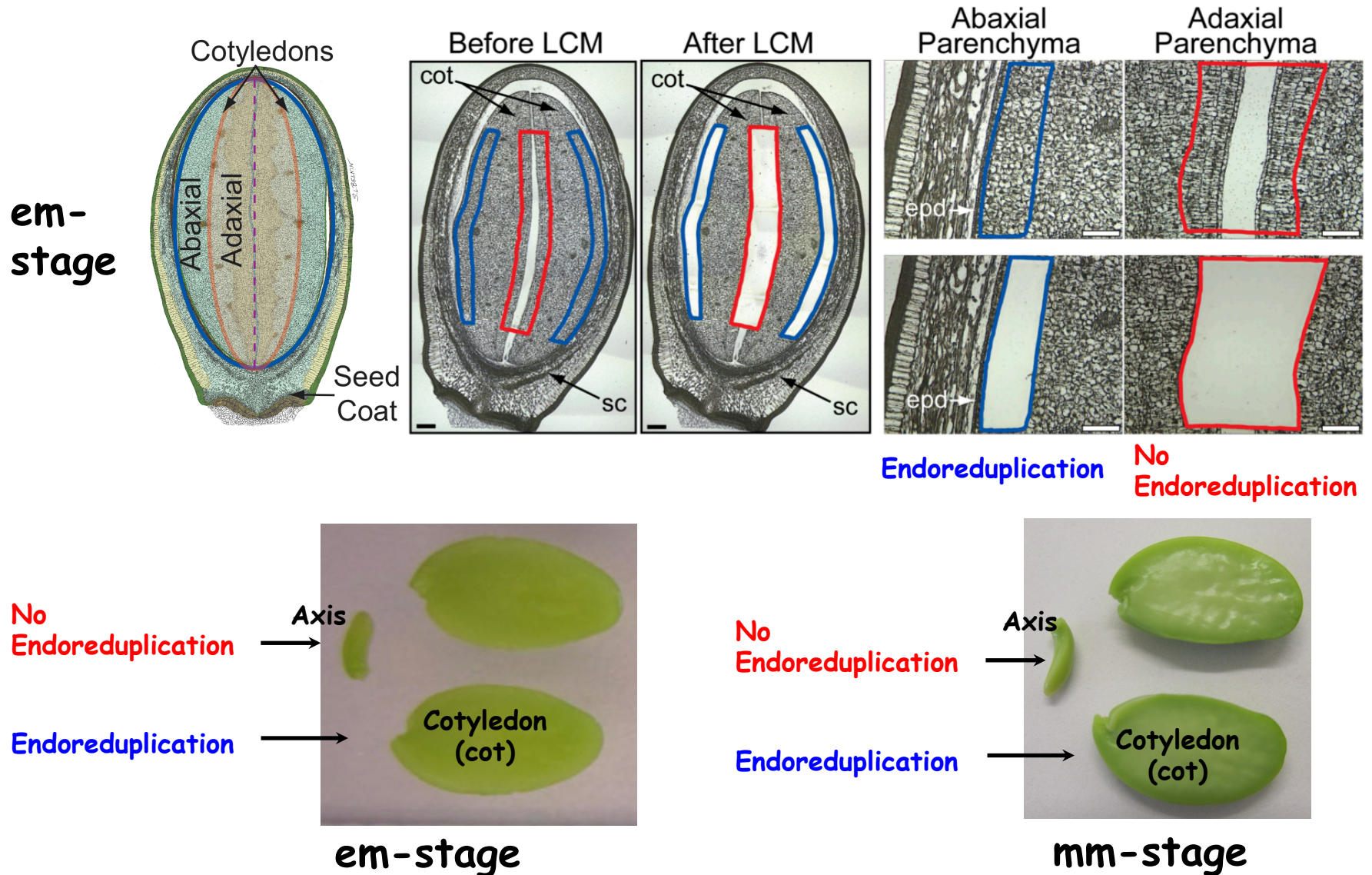


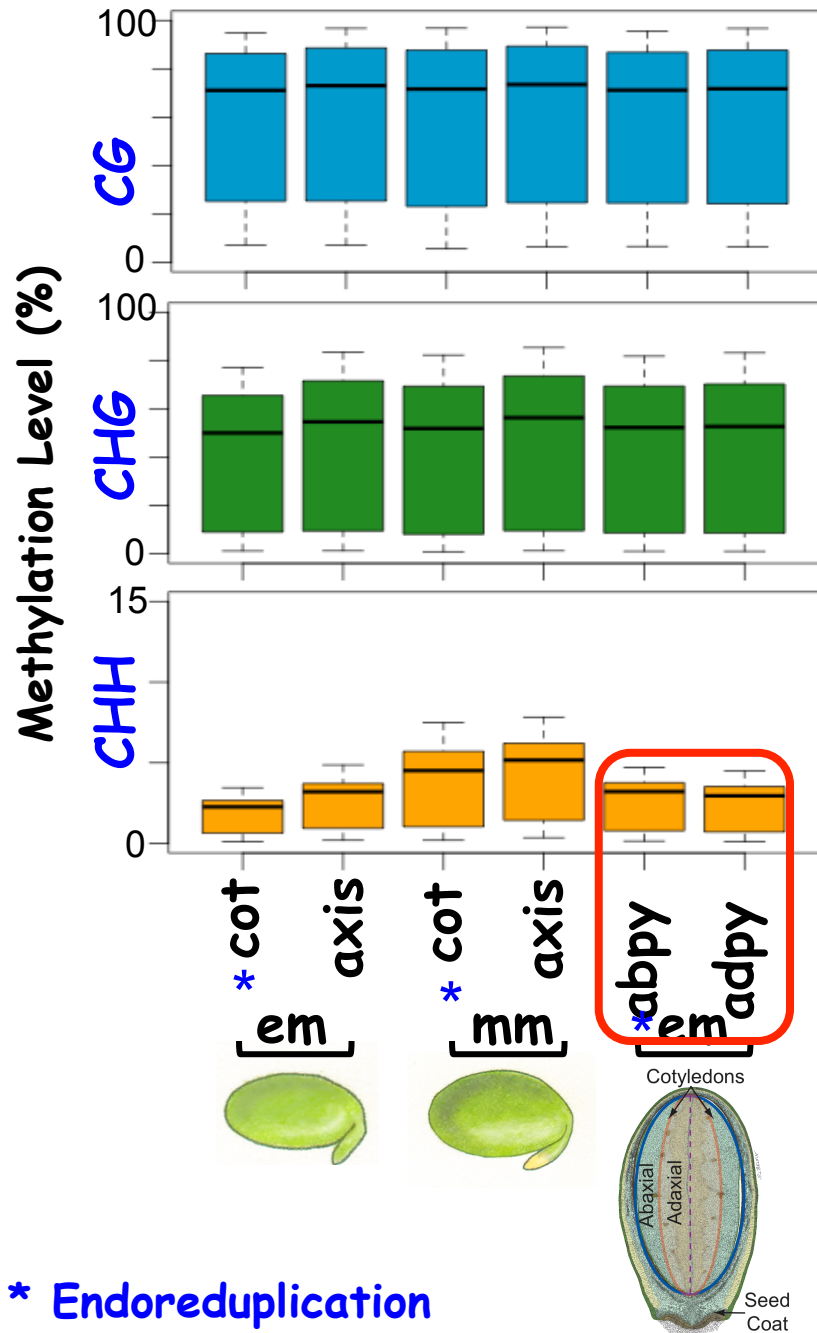
- DNA < 2C
- 2C < DNA < 3C
- 3C < DNA < 4C
- 4C < DNA < 5C
- DNA > 5C



Li S, Nielsen NC (2004)
Thesis. Purdue University

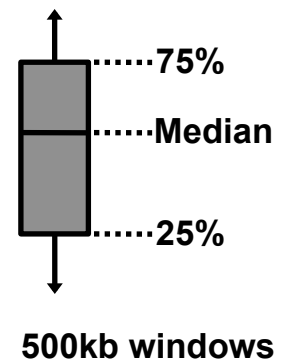
Using LCM For Investigating the Genome Methylation Landscape During Seed Endoreduplication



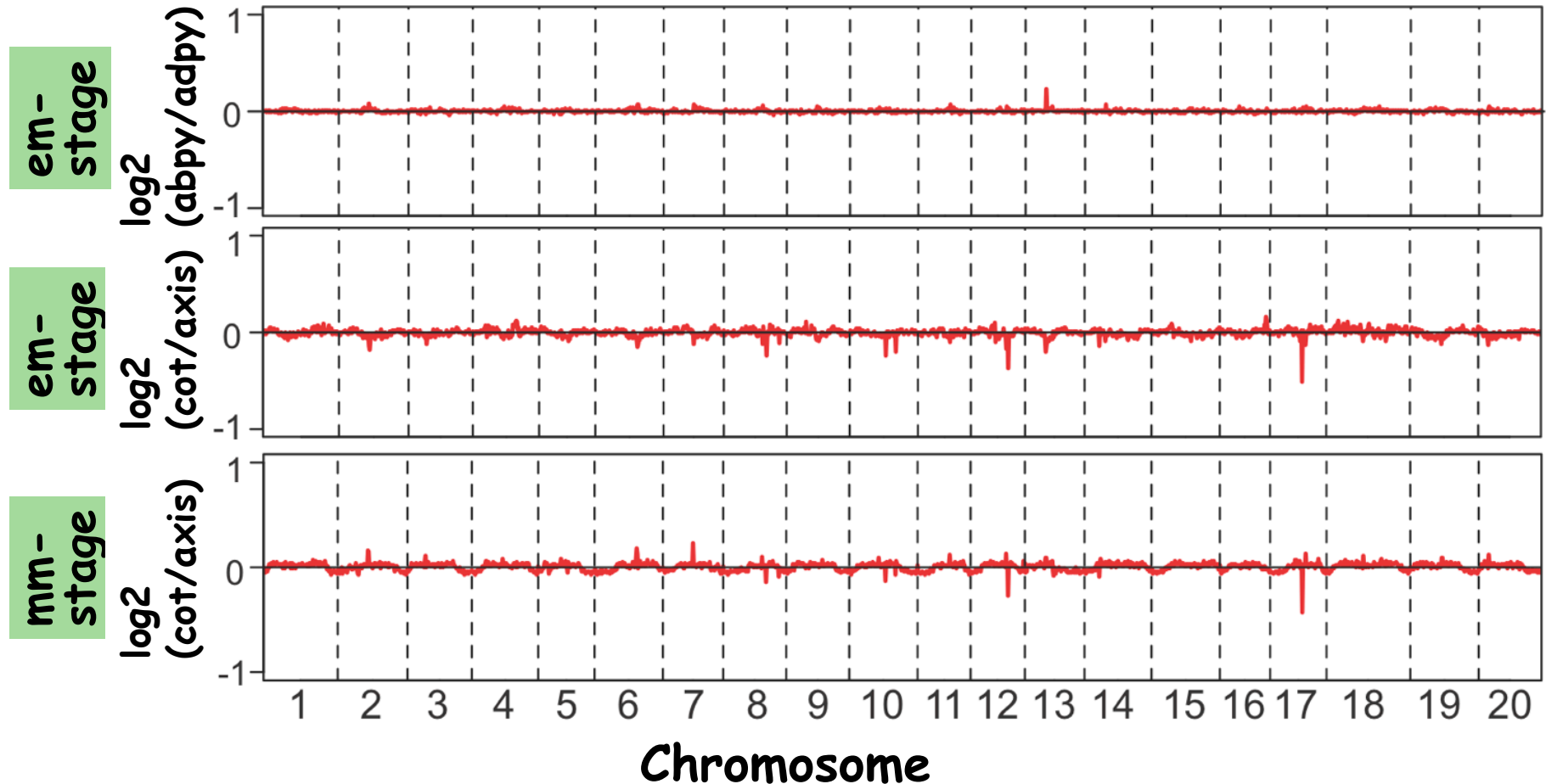


- No Major Differences in Endoreduplicating Versus Non-Endoreduplicating Cells*

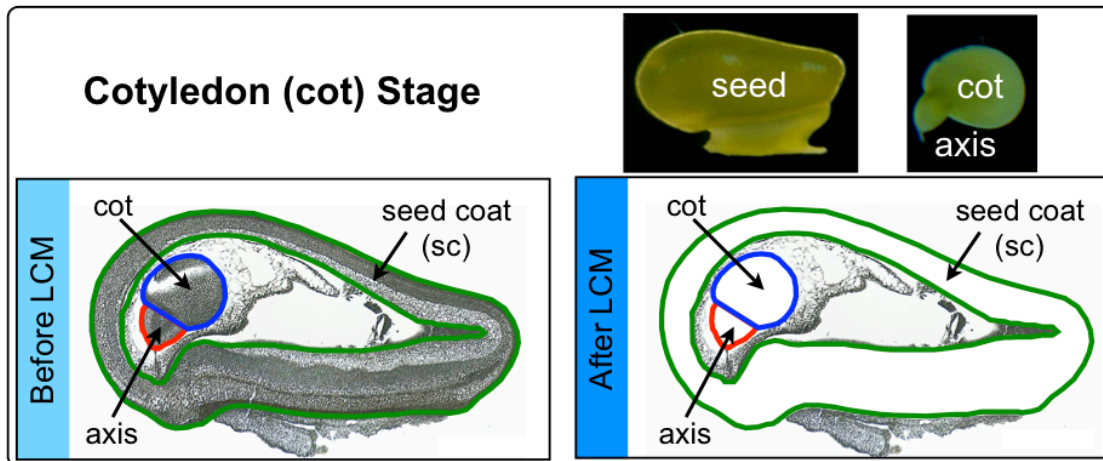
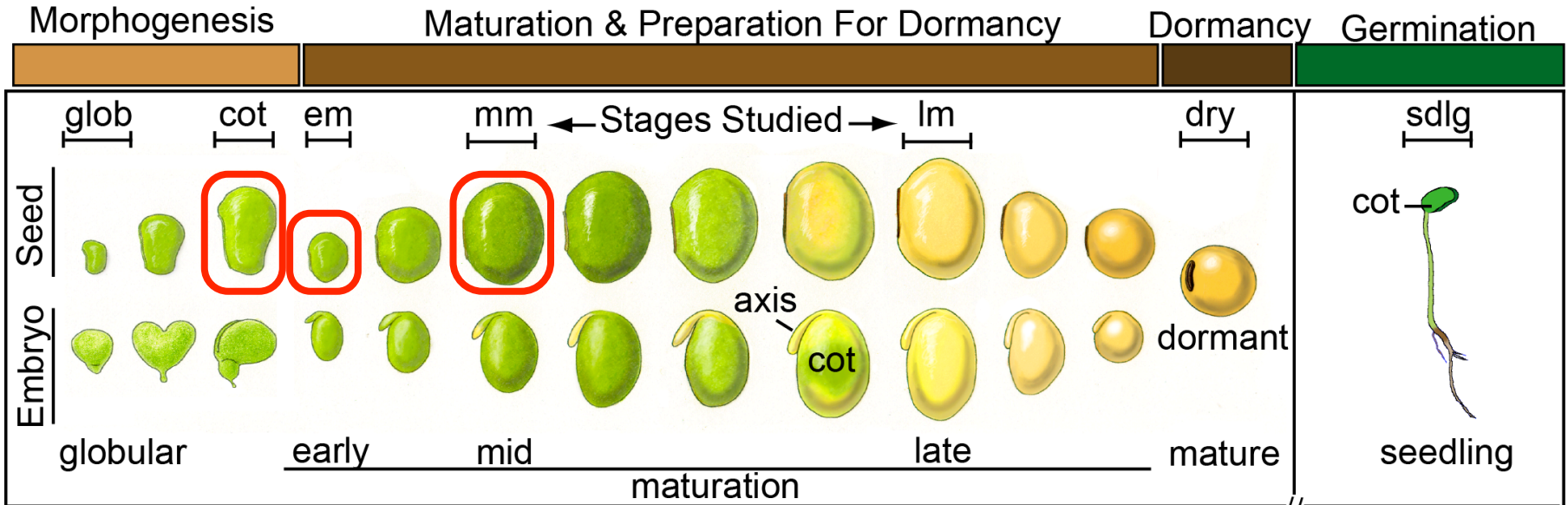
- DNA Methylation Is Maintained During Endoreduplication!!*



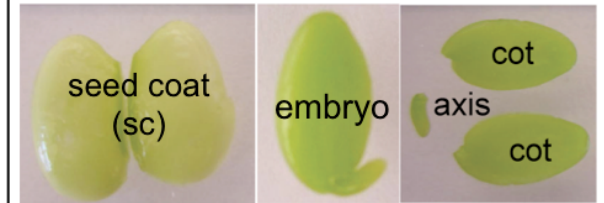
Endoreduplication Occurs Evenly Across the Soybean Genome - No Selective Region Amplification



Are There Methylation Differences Between Specific Seed Parts?

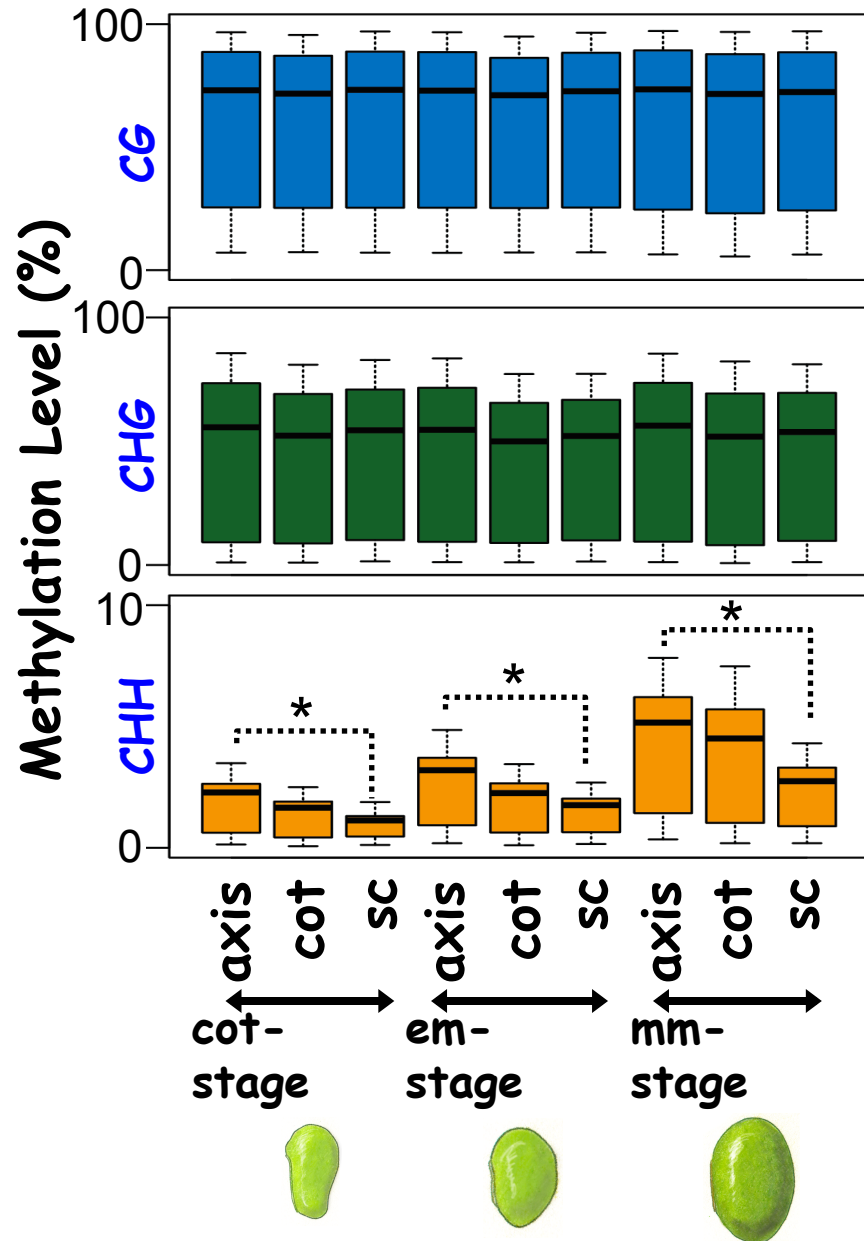


Early-Maturation (em) Stage



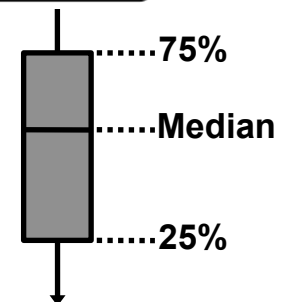
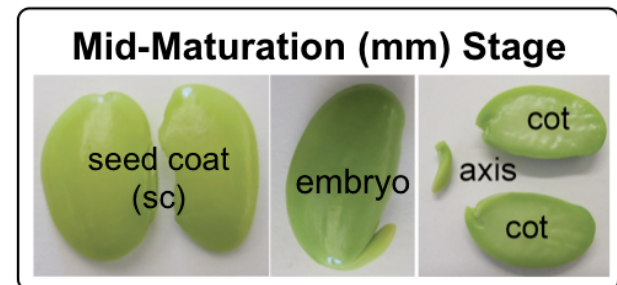
Mid-Maturation (mm) Stage





- *CHH Methylation Increases During Seed Development in All Seed Parts*

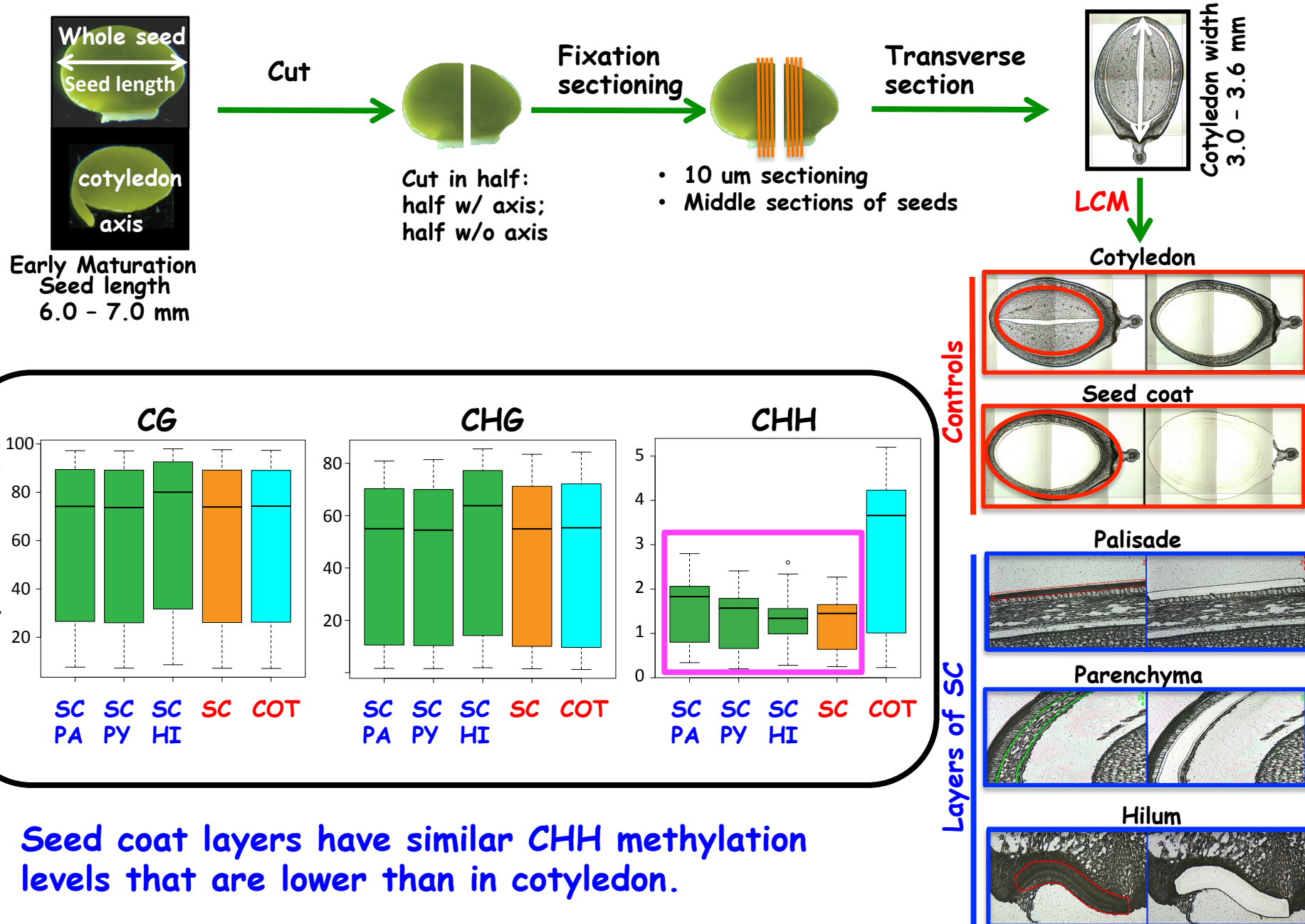
- *CHH Methylation Hypomethylated in the Seed Coat Compared To The Embryo*



* (t-test p-value ≤ 0.001 , mean ratio ≥ 1.5)

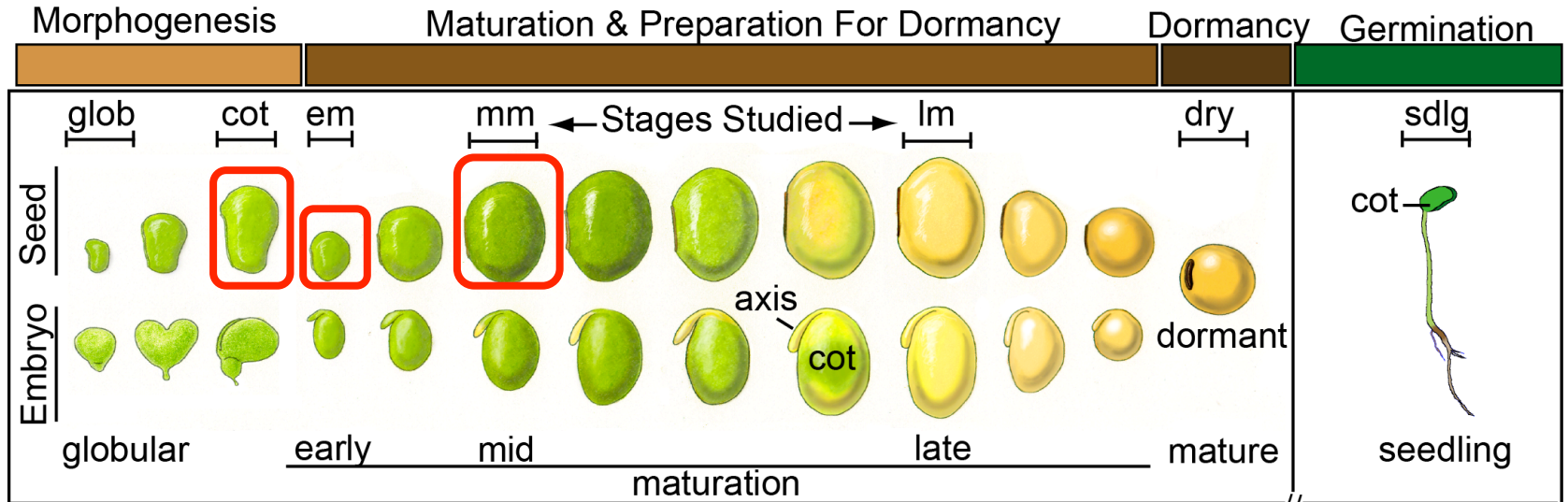
500kb windows

Are Methylation Levels In Seed Coat Layers Similar With Each Other?

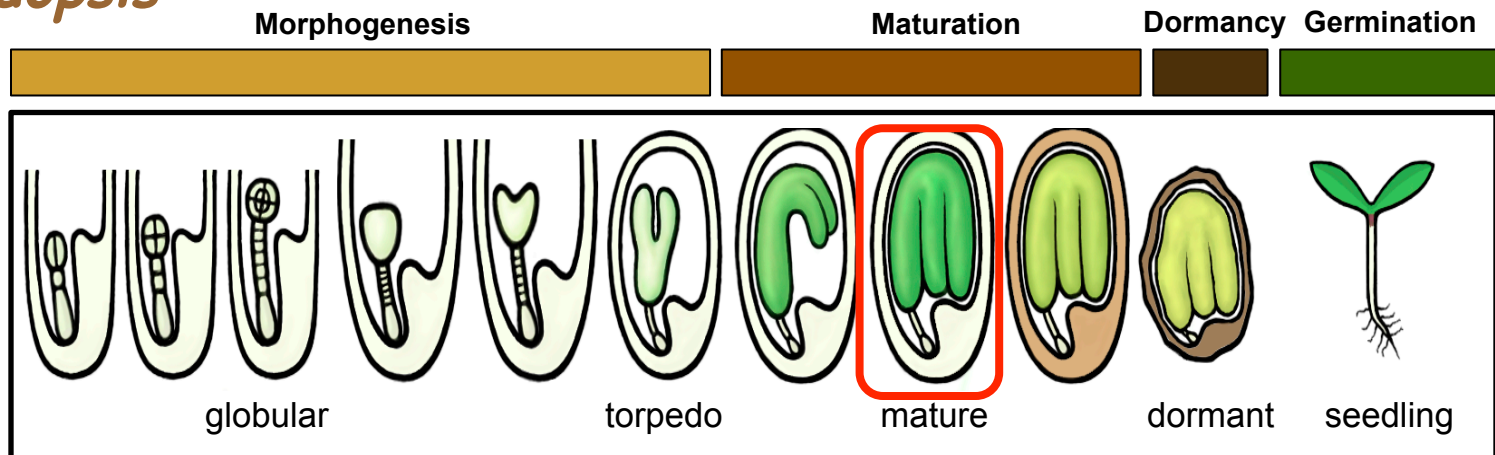


Does Seed Coat Hypomethylation Occur In Other Plants?

Soybean

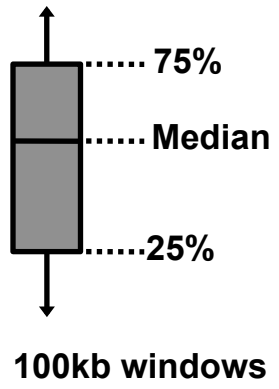
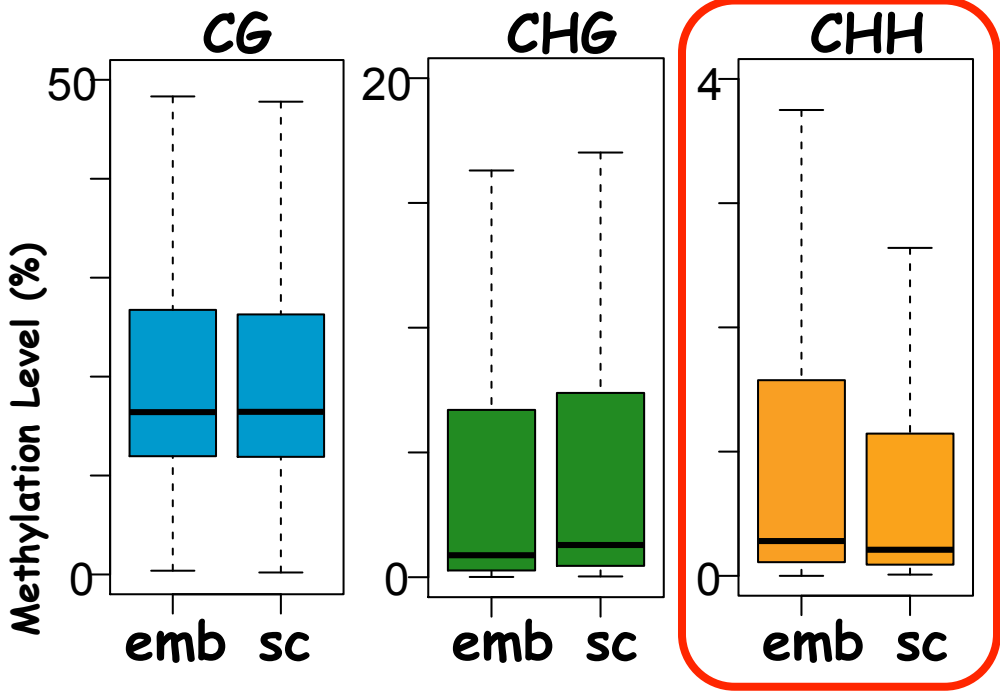


Arabidopsis

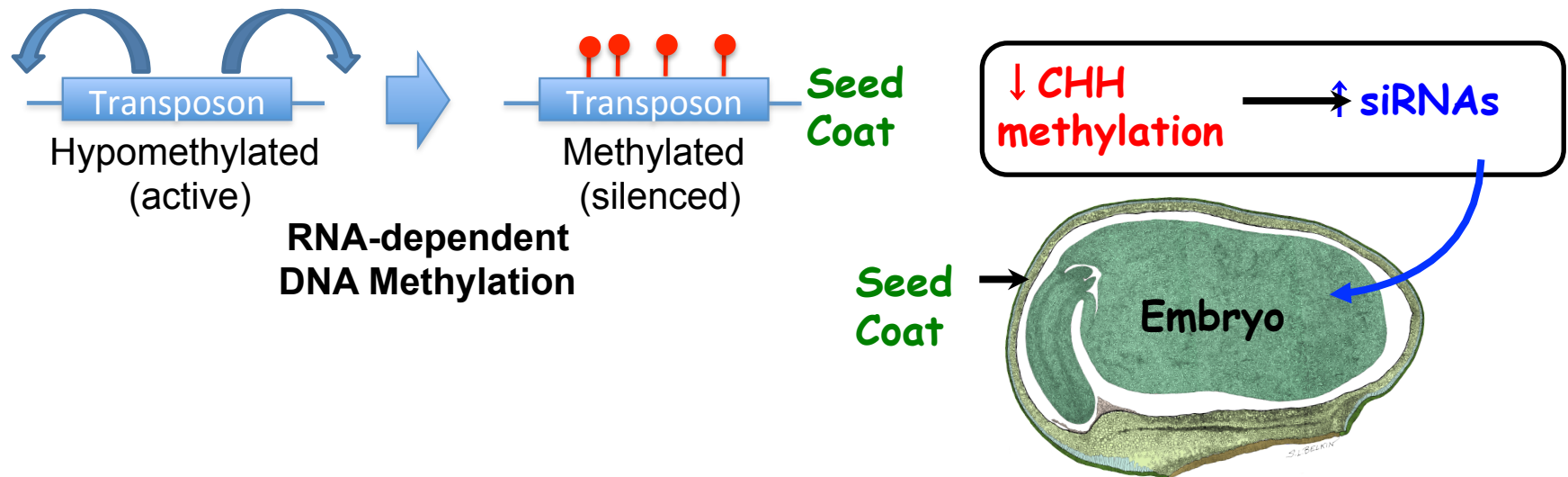


Seed Coat CHH Hypomethylation Occurs in Arabidopsis!

Mature Green (mg) Stage

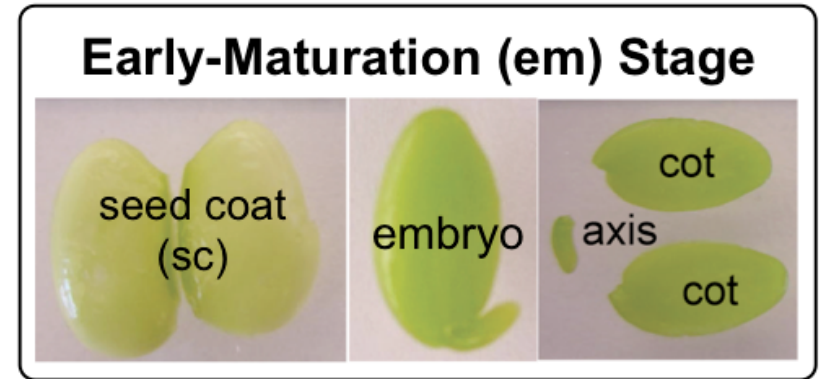
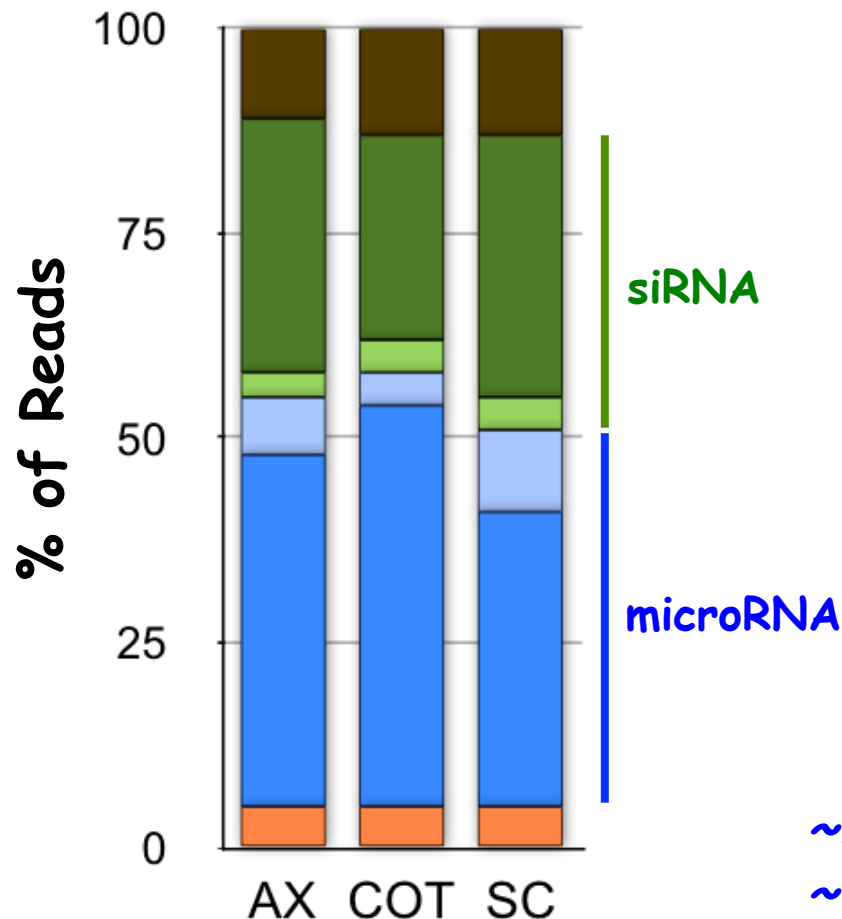


What Does the Reduced CHH Methylation Level in the Seed Coat Layer Mean - A Hypothesis



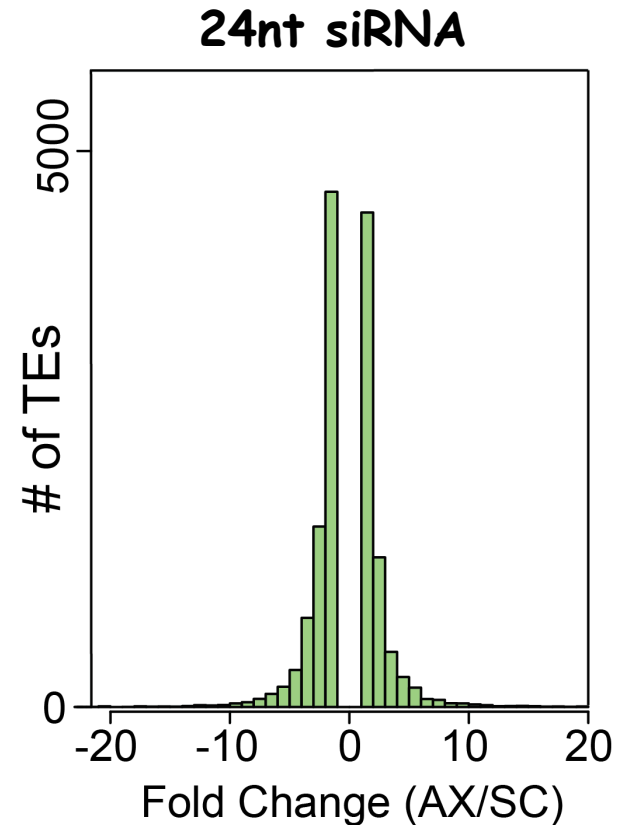
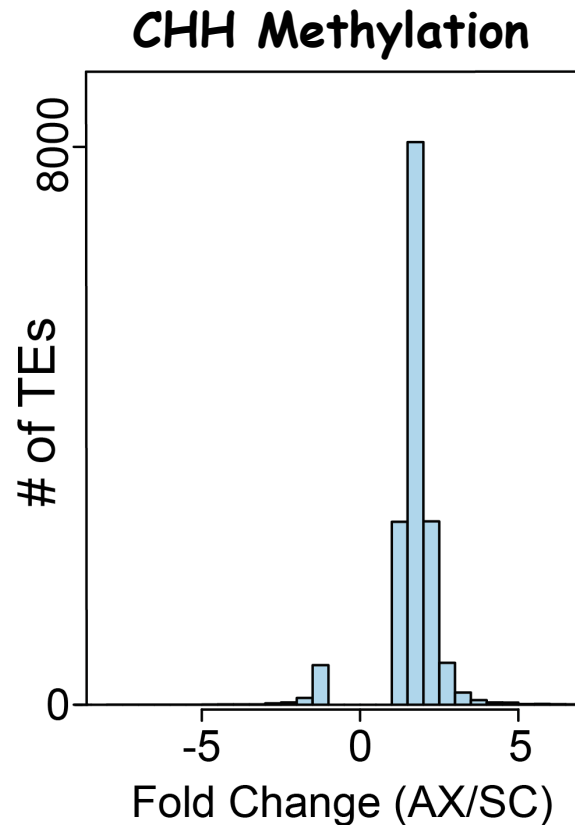
*Reinforce Suppression Of TEs
in Part of the Embryo/Seed
That Becomes the Plant
Following Germination*

Is There a Correlation Between DNA Methylation and smRNA Accumulation in the Early Maturation Stage Seed Parts?



*~50% of reads are 21nt in length.
~30% of reads are 24nt in length.*

Is There a Correlation Between Methylation Levels & TE 24nt siRNA Accumulation in Axis and Seed Coat?

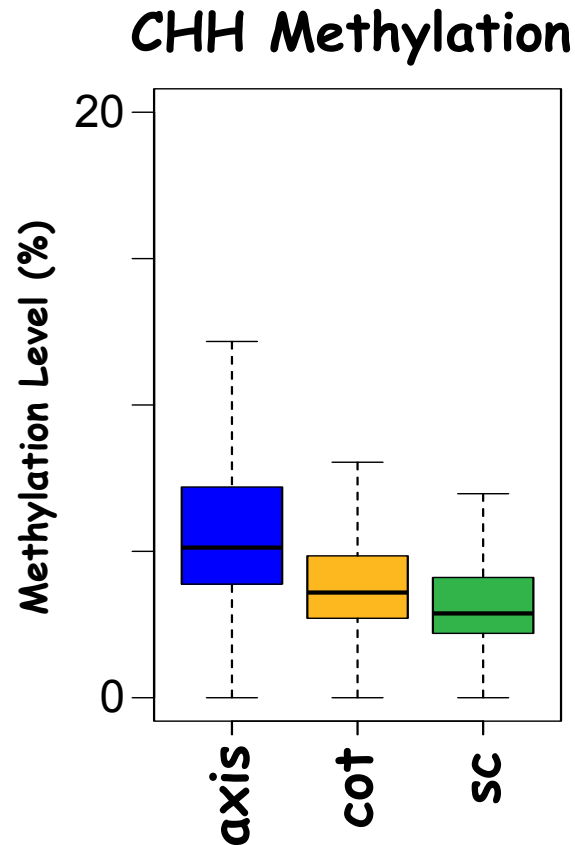


NO!!

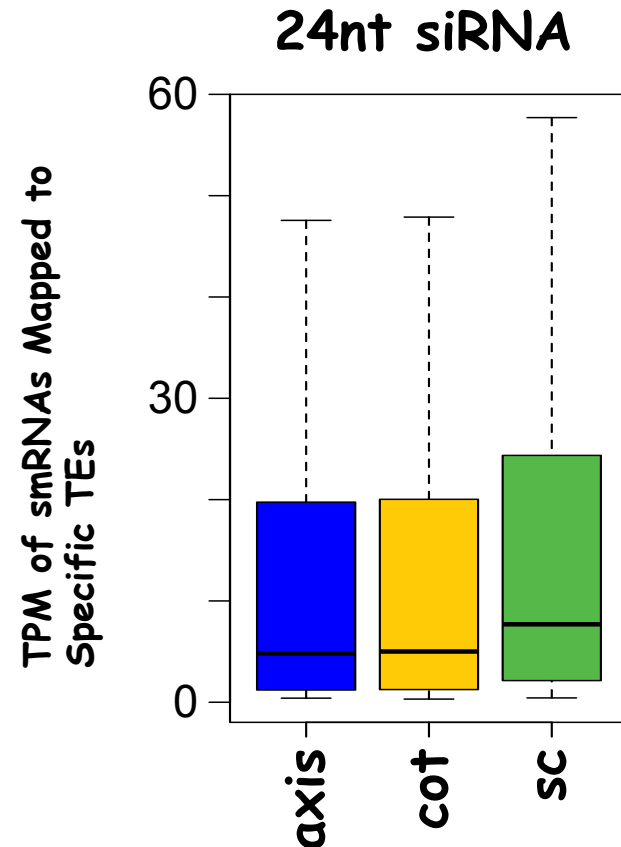
Early-Maturation (em) Stage



siRNA Levels are Higher in Seed Coat Layer Relative to Rest of Seed and Correlate With Reduced CHH-Methylation Level

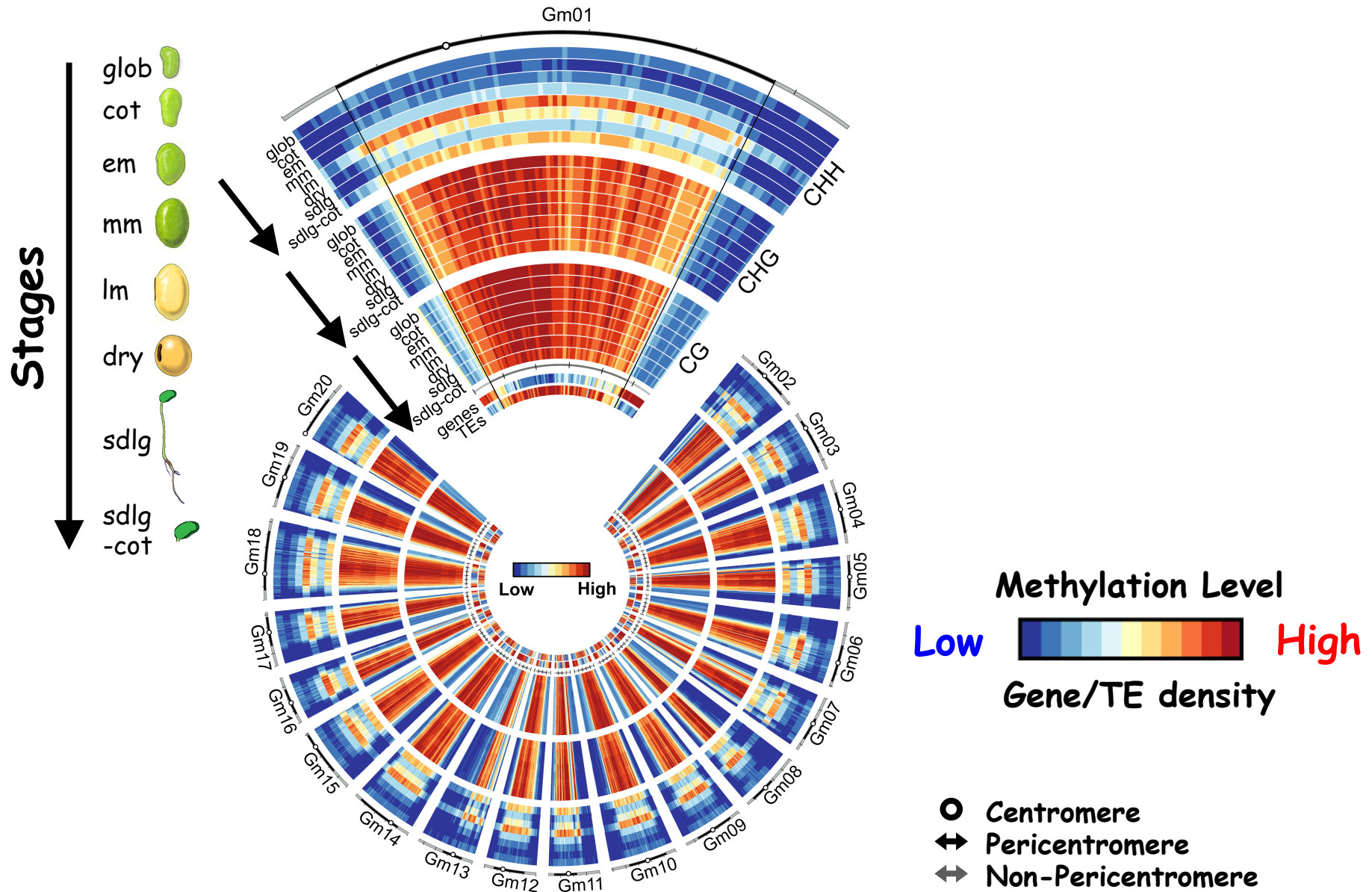


TEs in the seed coat are hypomethylated

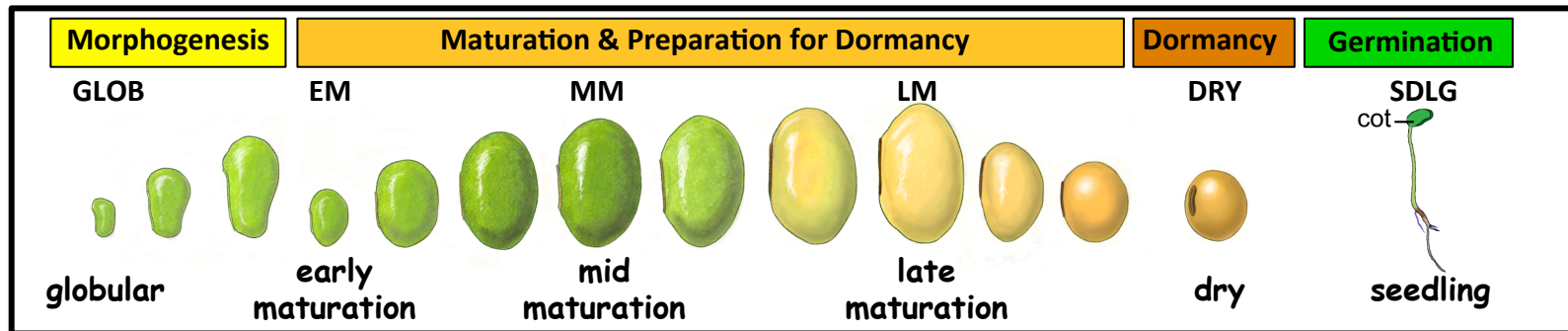


TEs in the seed coat are enriched for 24nt smRNAs

Developmental CHH-Context DNA Methylation Changes Occur Within TE-Dense Regions of The Genome in All 20 Chromosomes



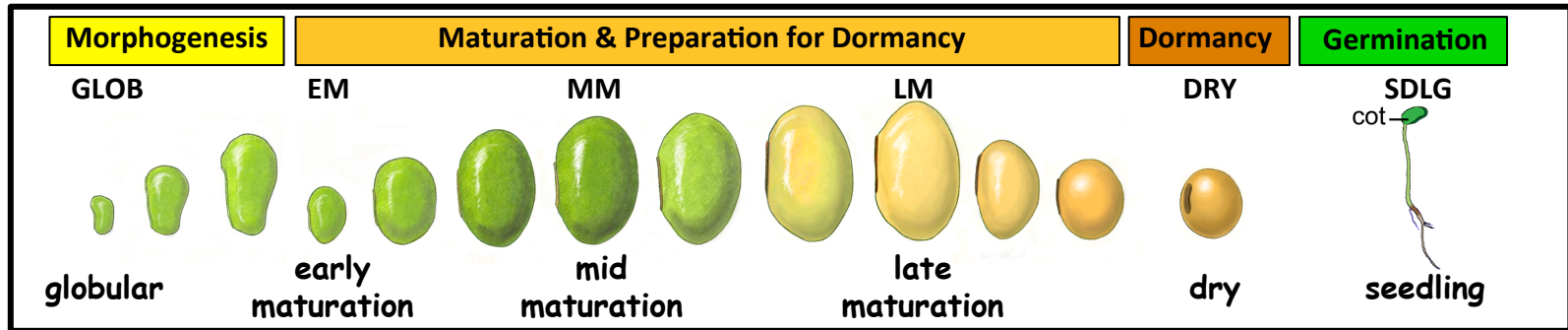
What Approach Did We Use To Examine If There Are Any Local Methylation Changes During Seed Development?



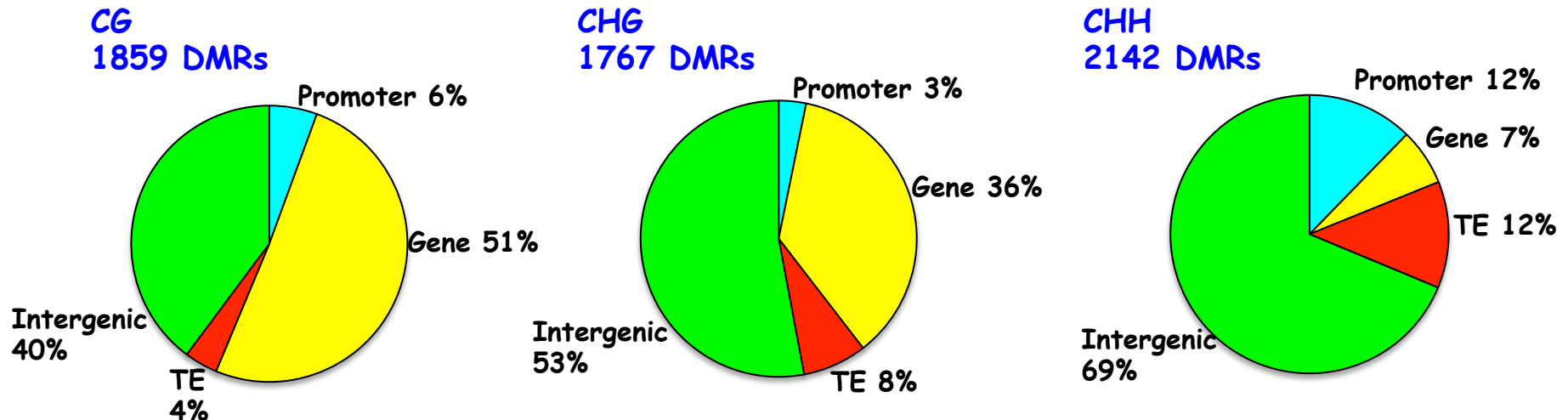
- The criteria for differential methylated regions (DMRs)
 - 200 bp window; 100 bp step (9,500,678 windows)
 - Common Cs per window ≥ 5
 - Coverage for common C site $\geq 5x$
 - $|Z \text{ score}| \geq 10$ ($p < 7 \times 10^{-23}$) for CG, CHG;
 ≥ 16 for CHH ($p < 1 \times 10^{-56}$)
 - The variation of methylation levels between two samples $\geq 50\%$
- Testing false positive
 - Divide dry seed data equally
 - Half DRY data vs. the other half DRY data
 - False positive rate of DMRs
 - CG: $1.1 \times 10^{-4} \%$ (11 out of 9.5 M windows)
 - CHG: $4.2 \times 10^{-5} \%$ (4 out of 9.5 M windows)
 - CHH: $3.2 \times 10^{-5} \%$ (3 out of 9.5 M windows)

We use very stringent criteria to get DMRs with very low false positive rate.

What Criteria Did We Use To Examine Local Methylation Changes During Seed Development?



- Pairwise comparison between stages

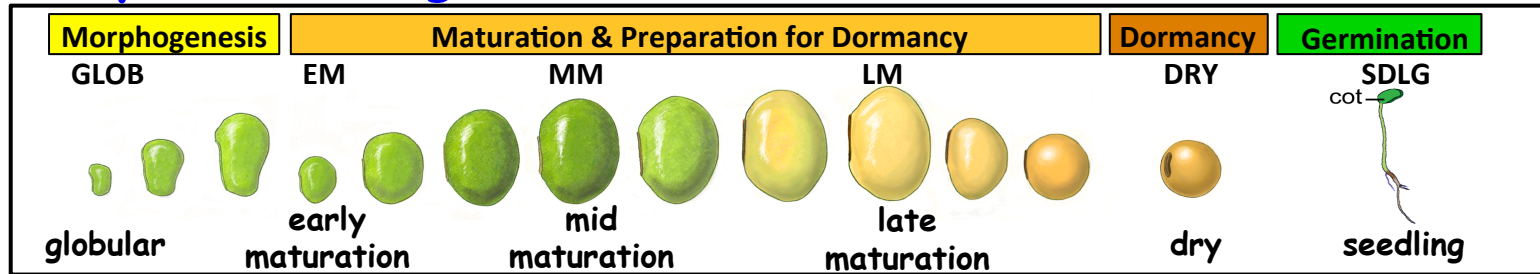


The majority DMRs

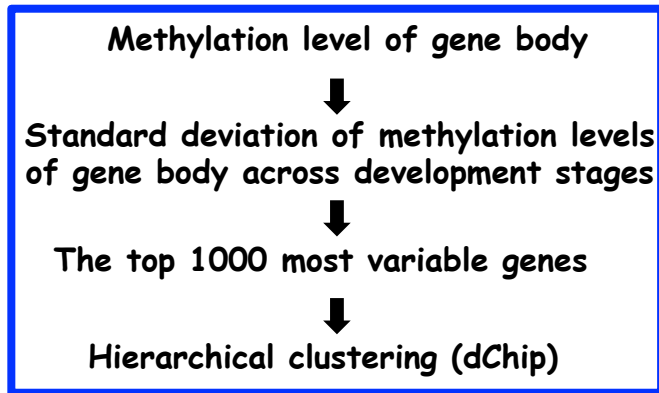
In CG context: genic region

In CHG and CHH context: intergenic region

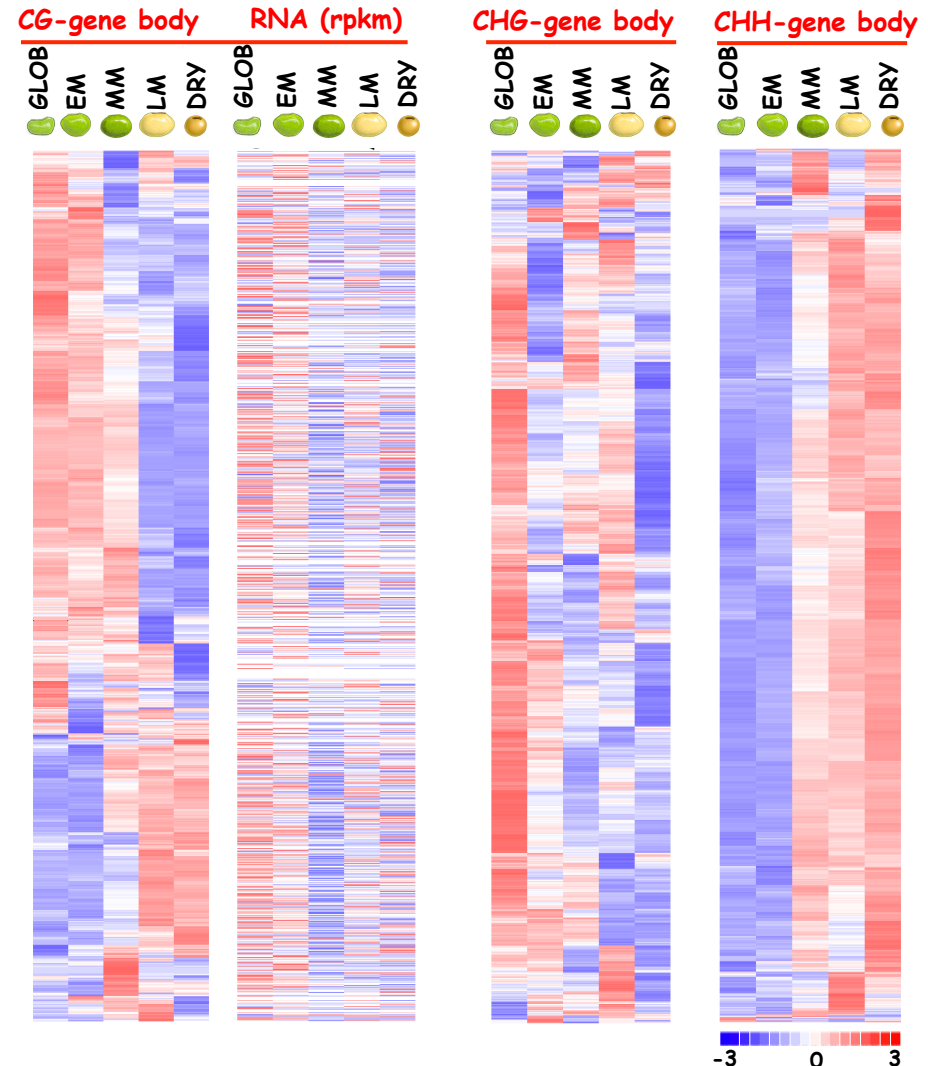
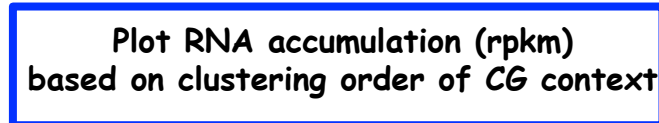
Do Methylation Changes Correlate With RNA Accumulations Patterns?



Work flow for DNA methylation clustering



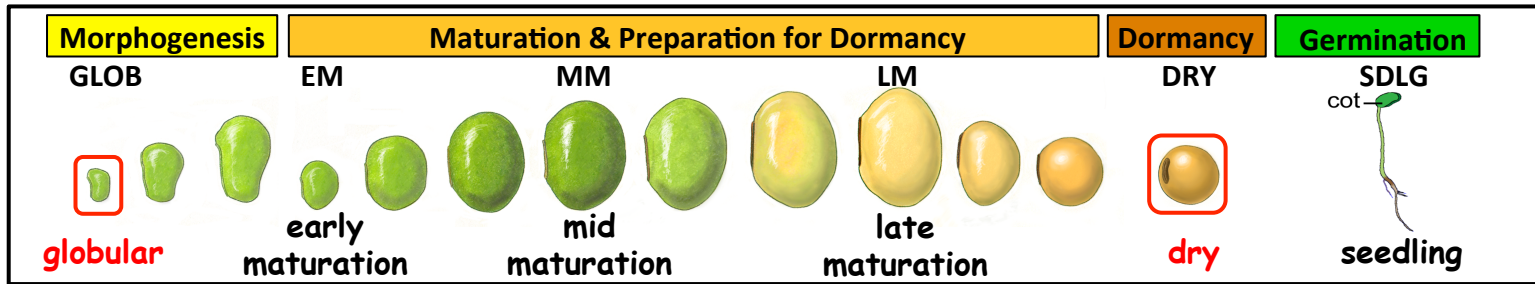
RNA accumulation



Methylation levels in genes are different in CG, CHG and CHH contexts.

There is no obvious similar clustering patterns between CG-gene body methylation and RNA accumulation.

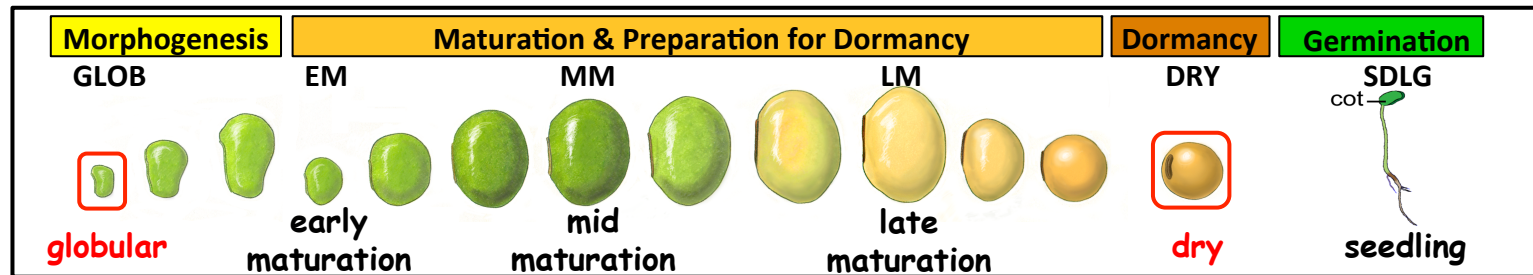
What Approach Did We Apply To Find DMRs In 1 Kb Upstream Of Genes Between Distinct Developmental Stages, GLOB Vs. Dry?



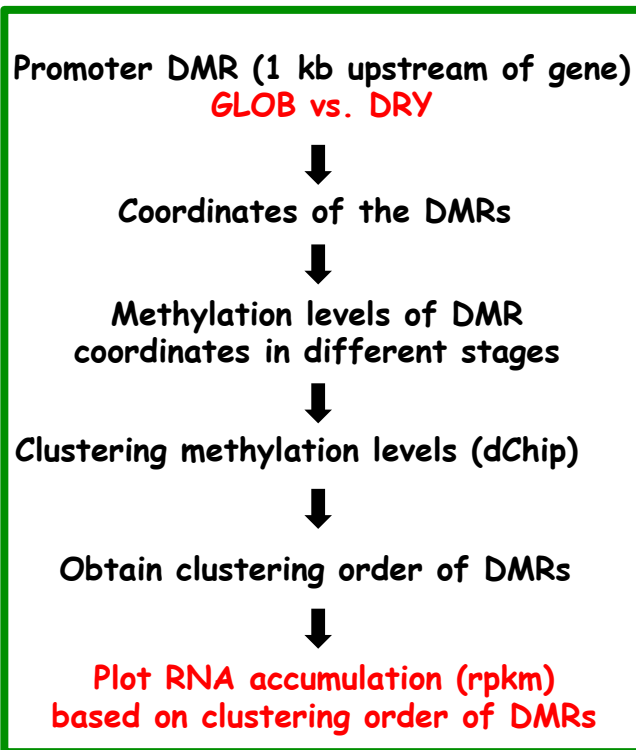
- Consider the length of regulatory element:
50 bp windows in 1kb upstream of genes, 25 bp step
- Criteria for DMRs
 - Common C # ≥ 5
 - Coverage per C $\geq 5x$
 - $|Z\text{-score}| \geq 10$ ($p < 7 \cdot 10^{-23}$)
 - $|\text{Methylation difference of one window}| \geq 50\%$
- Testing false positive
 - Divide dry seed data equally
 - Half DRY data vs. the other half DRY data
 - No DMRs

We applied stringent criteria with very rare false positive.

Is there any correlation between promoter CG-DMRs And RNA accumulation In GLOB Vs. DRY



Work flow

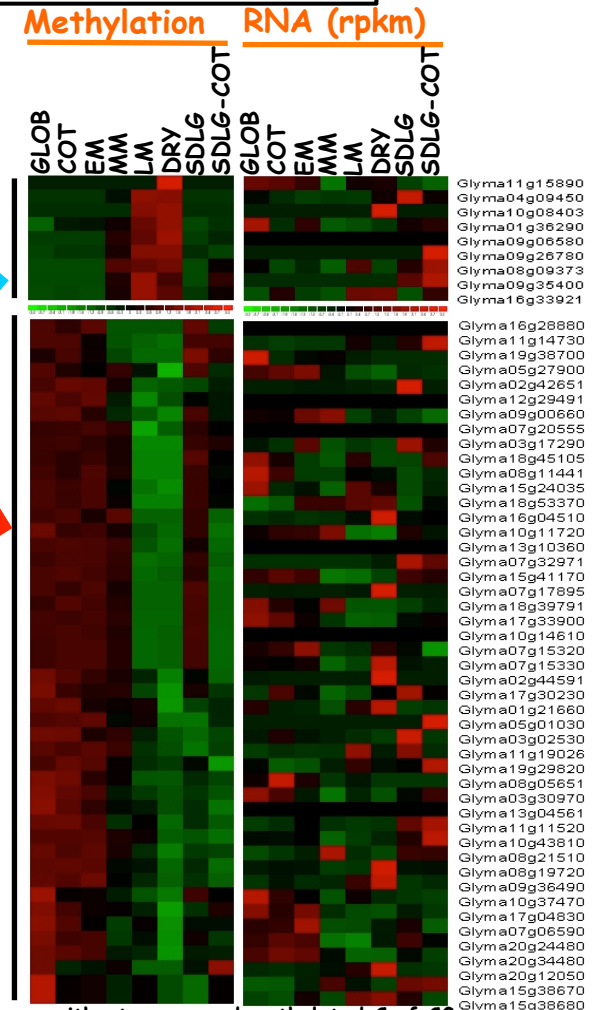


Hypo methylation
in GLOB

13 genes

Hyper methylation
in GLOB

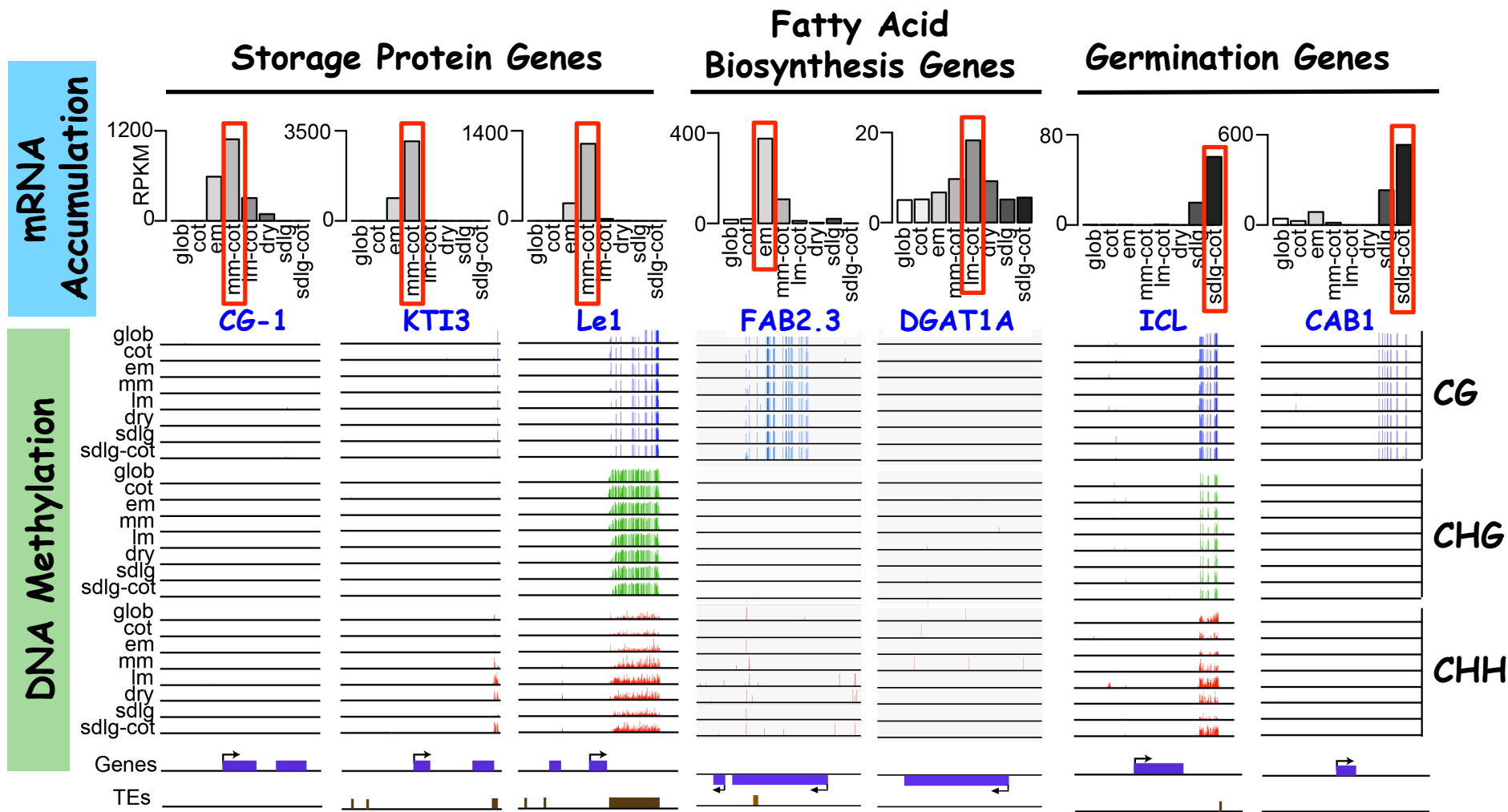
74 genes



There is no obvious similar clustering patterns between DMRs and RNA accumulation.

* Genes without sequenced methylated C of CG context in BS-seq data are not shown here

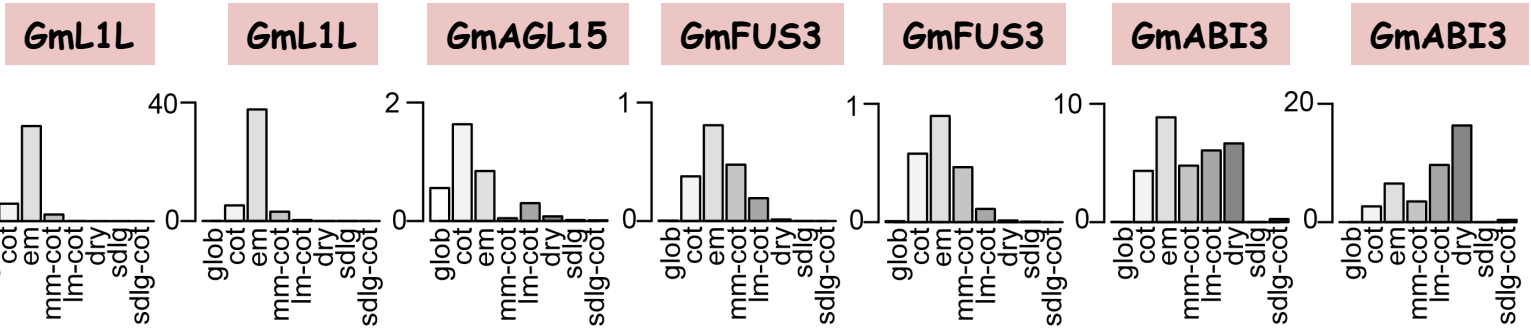
Do Major Seed Developmental and Germination Gene Activities Correlate With DNA Methylation Changes?



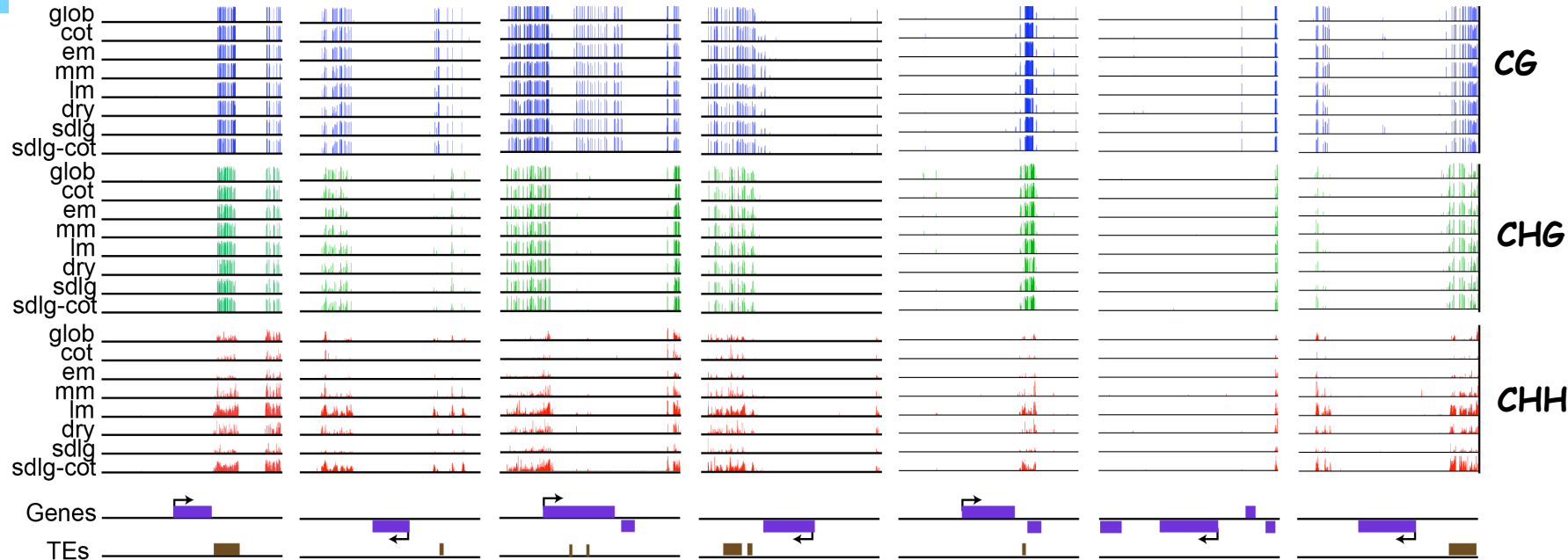
Activation And Repression of These Genes are NOT Correlated With Methylation Changes

Is the Expression of Major Seed Regulatory Genes Correlated With Methylation Changes?

mRNA Accumulation



DNA Methylation

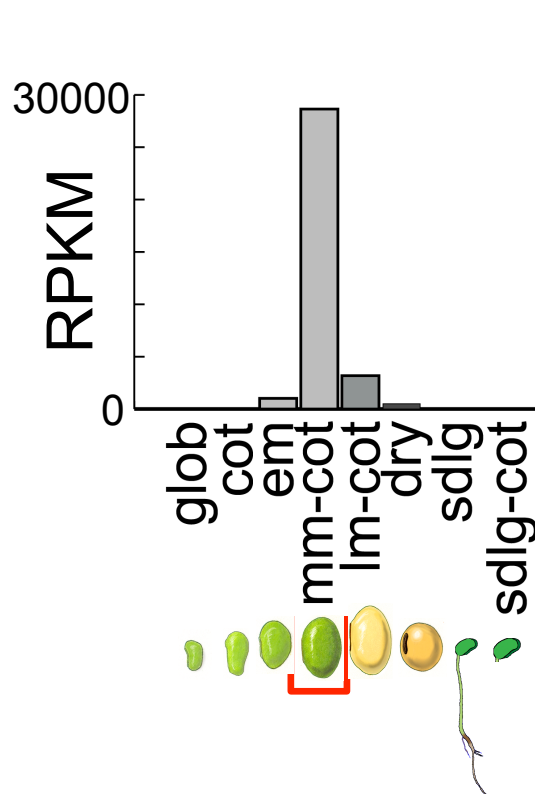


Major Regulators Of Seed Development Are Not Correlated With DNA Methylation Changes

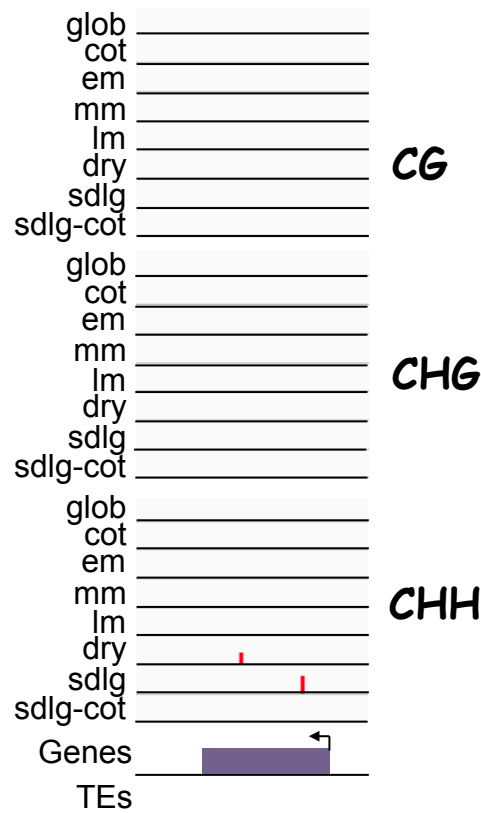
Do Other Epigenetic Changes Correlate with Gene Activity During Seed Development?

Seed Storage Protein - Glycinin 4 Glyma10g04280

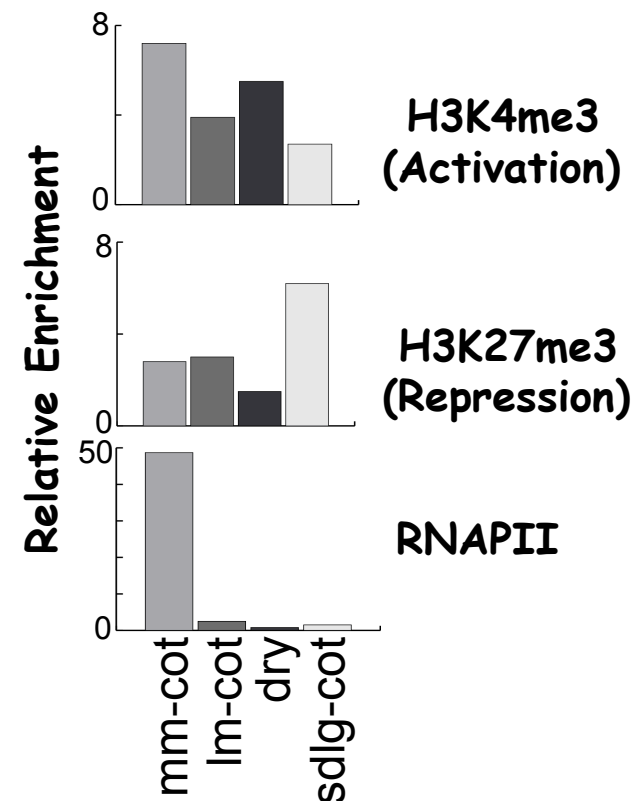
mRNA Accumulation



DNA Methylation



Histone Modification

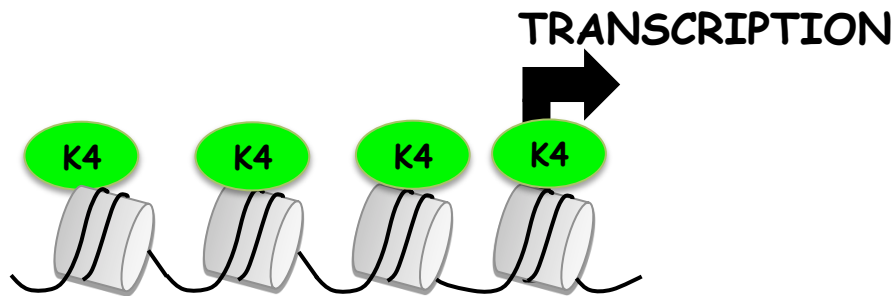


How Do Histone Modifications Correlate with Methylation Patterns Across the Entire Genome?

- H3K4m3 and H3K27m3

- **Active Chromatin Marks**

H3K4me3 (mediated by Trithorax, TrxG)

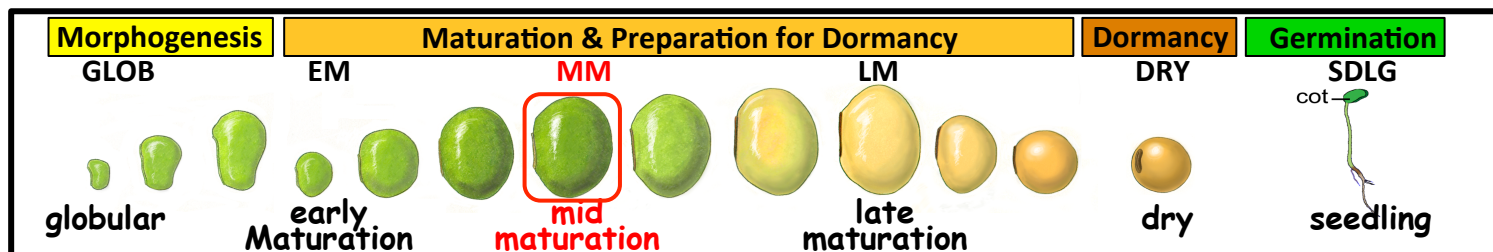


- **Repressive Chromatin Marks**

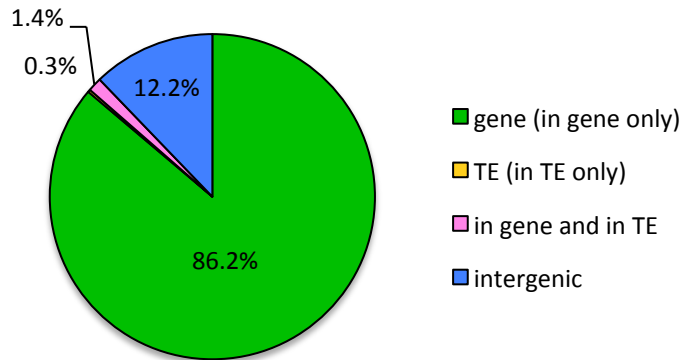
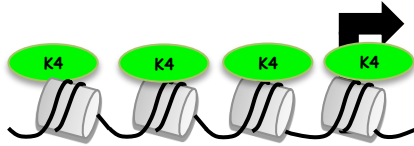
H3K27me3 (mediated by Polycomb, PcG)



- **MM stage in this study**

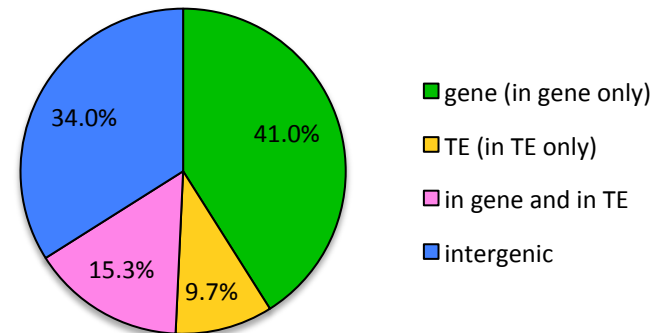


Where do H3K4m3 and H3K27m3 markers locate?



47,697 intervals

- DNA with H3K4m3 marker
 - Most in genes
 - Small portion in intergenic regions
 - Tiny amount in TEs



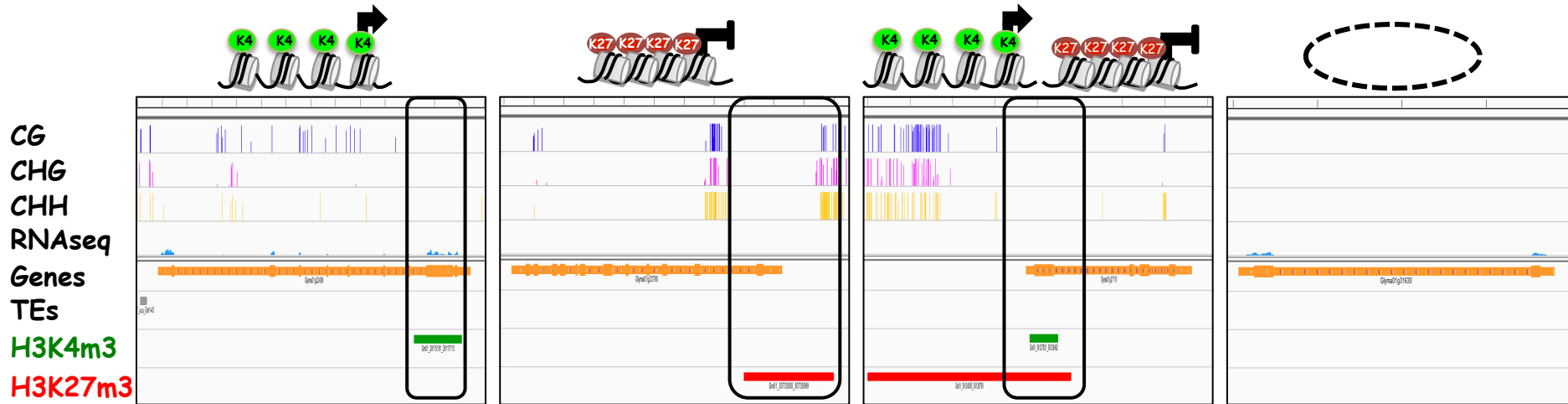
15,672 intervals

- DNA with H3K27m3 marker
 - Half in genes
 - Small portion in TEs.
 - ~1/3 in intergenic regions.

The locations of H3K4m3 marker and H3k27m3 marker are quite different.

How Many Genes Have H3k4m3/H3k27m3 Histone Markers In The Soybean Genome?

- Types of Histone markers H3K4m3 and H3K27m3 around genes

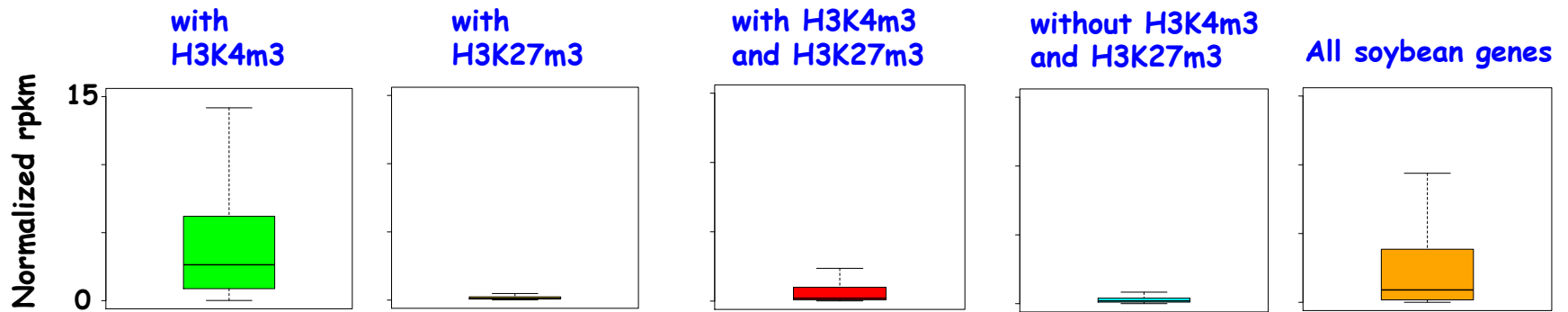


Type	With H3K4m3	With H3K27m3	With both markers (Bivalent chromatin*)	Without both markers
Gene number	31879	4206	6092	11998
% in the genome	59%	8%	11%	22%

- The majority of soybean genes have active Histone marker H3K4m3.
- Small fraction of genes have repressive marker H3K27m3
- Small fraction of genes have bivalent chromatin markers.

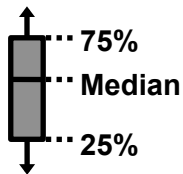
The variation of number of genes with different histone markers are large.

How Do Seed Gene Expression Patterns Correlate With Histone Markers?

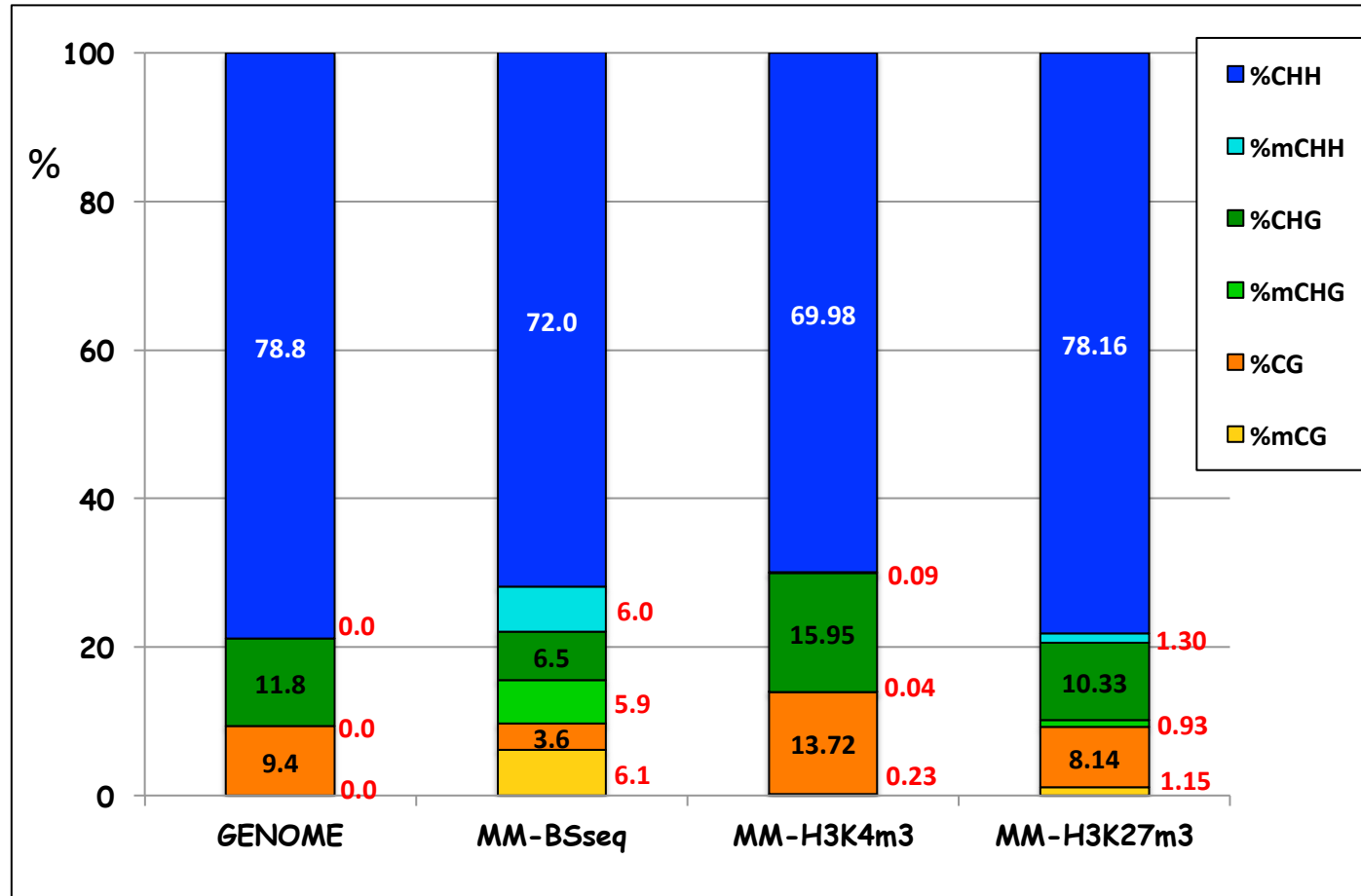


- Genes with H3K4m3 have relatively higher expression level.
- Genes without H3k27m3 markers have relatively lower expression level.

Genes with H3K27m3 have low CHH methylation in gene body, and the variation is large.



How Many Cytosines Are Methylated In The Pull-down DNA?



* Percentage of methylated Cytosine is shown in red.

Fewer mC in the pull-down DNA, especially in H3K4m3

What Is The Correlation Between Methylated Cytosine Sites In The Histone Marked DNA Regions vs. The Soybean Genome?

- Evaluate the fold change of mC sites:

Ratio of

(% of mC sites from the pull-down DNA) : (% of mC sites from the MM genome)

- mC sites from the pull-down DNA vs the MM genome

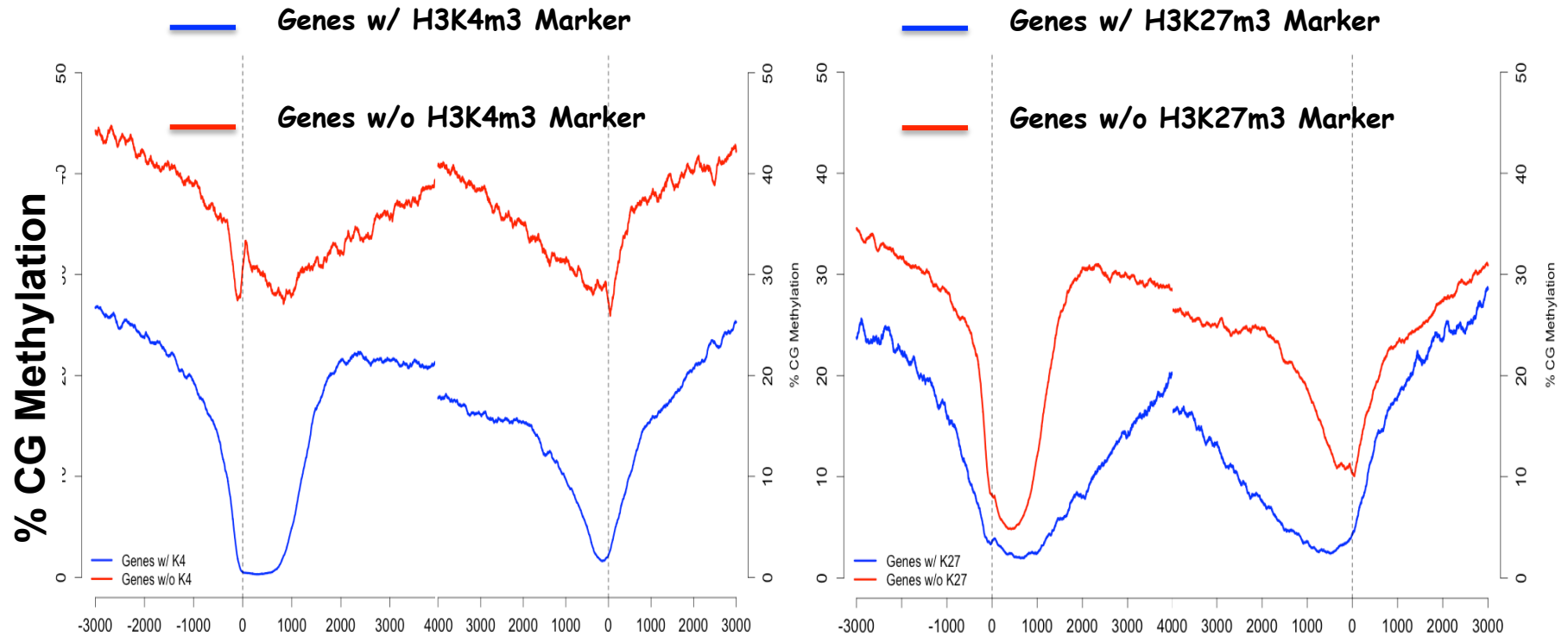
	H3K4m3
CG	1 : 66
CHG	1 : 148
CHH	1 : 27

	H3K27m3
CG	1 : 5
CHG	1 : 6
CHH	1 : 5

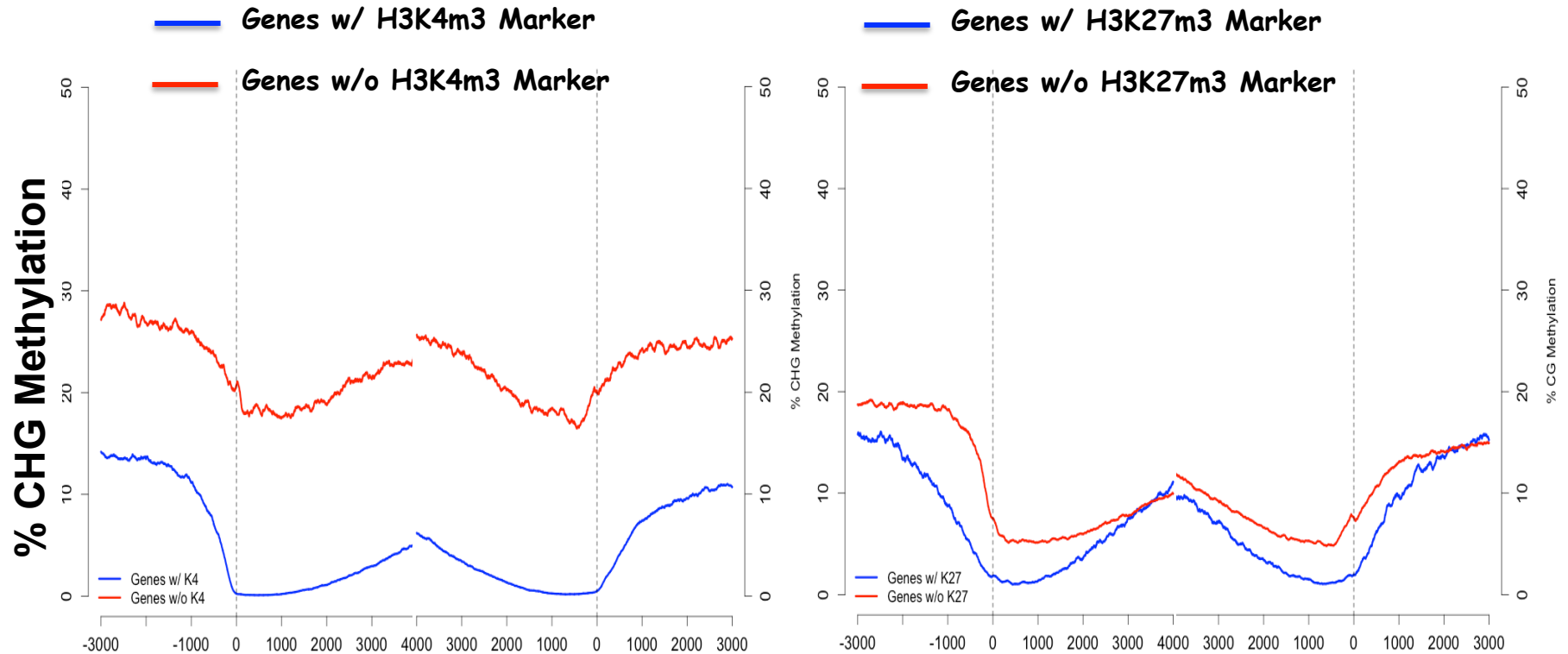
- The degrees of decreased mC site in three contexts of DNA pull-down by H3K4m3 are different.
 - CHH has the smallest decreased ratio.
 - CHG has the largest decreased ratio.
- The degrees of decreased mC site in three contexts of DNA pull-down by H3K27m3 Ab are similar, ~5x fewer as compared to the soybean genome.

Fewer mC sites in the pull-down DNA, especially in H3K4m3

Is there any different CG methylation status around genes with/without H3K4m3 vs the ones with/without H3K27m3?

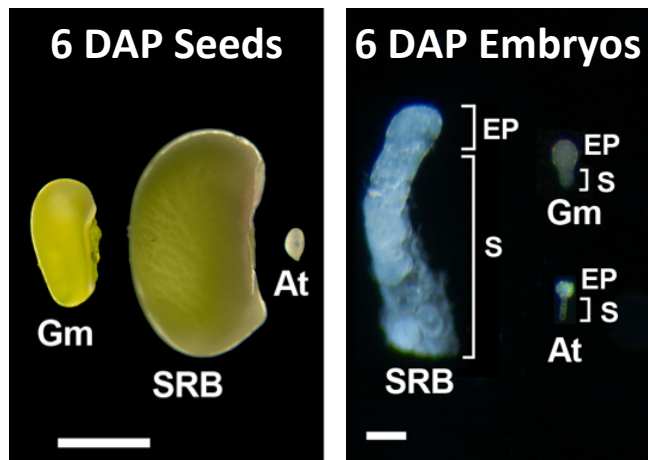
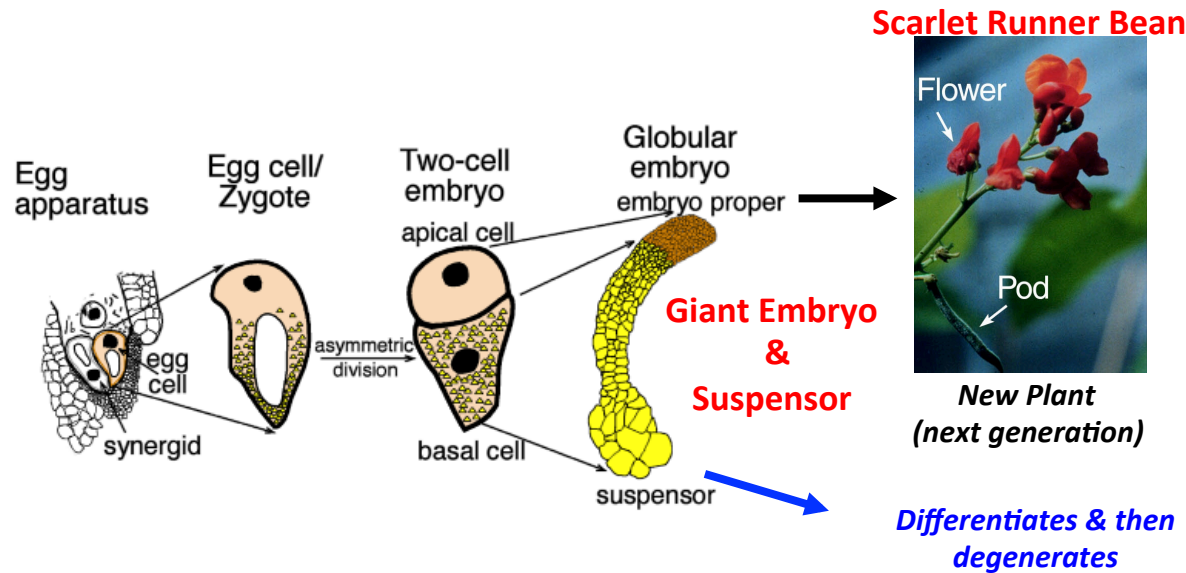


Is There Any Different CHG Methylation Status Around Genes With/Without H3k4m3 Vs The Ones With/Without H3k27m3?

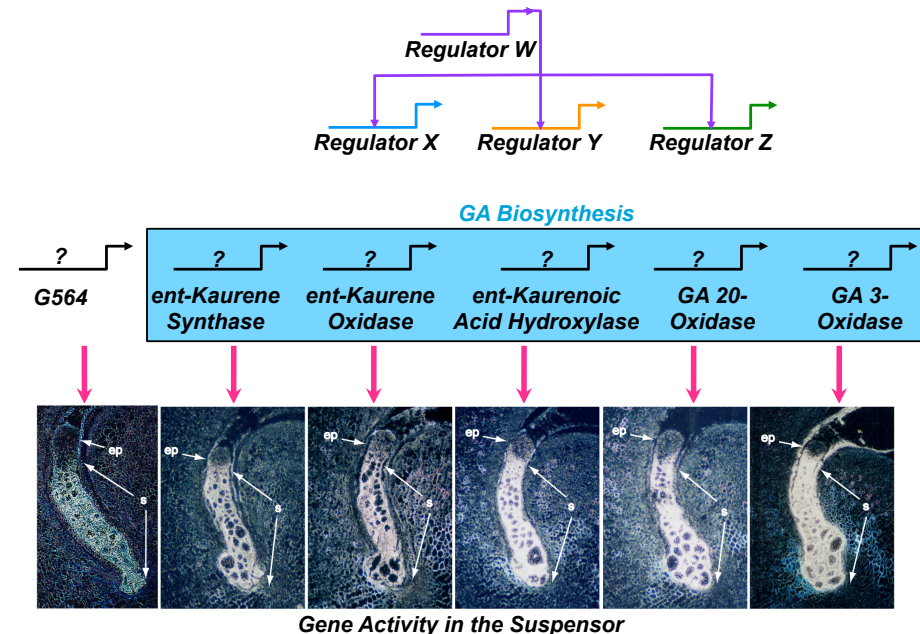


Genes Marked With H3K4m3 Have Lower Methylation Levels Than Those Marked With H3K27m3 & Methylation Levels Are Lower at Beginning and End of Genes

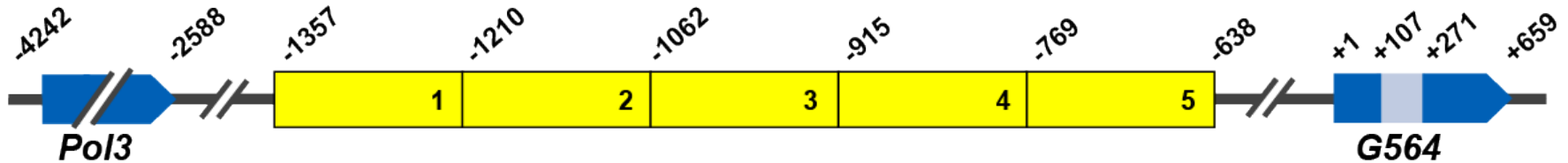
Scarlet Runner Bean as a System to Uncover Networks Controlling Suspensor Gene Activity



Size Comparison of 6 DAP Seeds and Embryos from Scarlet Runner Bean (SRB), Soybean (Gm) and *Arabidopsis* (At)



G564 Is Regulated Primarily at the Transcriptional Level



Scarlet runner bean



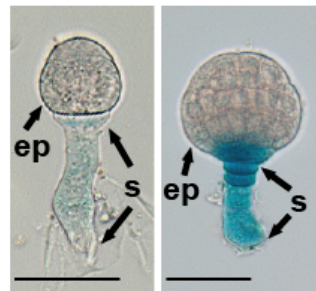
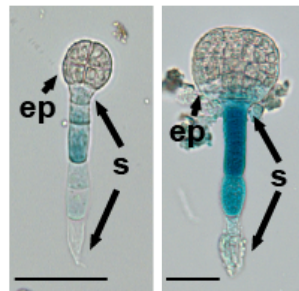
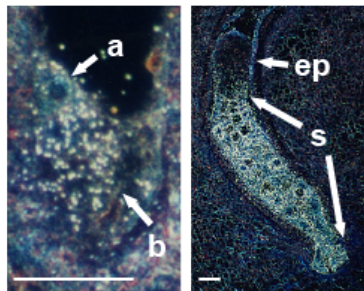
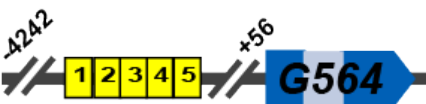
Arabidopsis



Tobacco



- Temporal and spatial suspensor-specific expression of the *G564* gene is controlled primarily at the transcriptional level.



~120 MYA

~150 MYA

- Suspensor transcriptional machinery is conserved in dicots.

How Do We Identify Functional Suspensor *cis*-Regulatory Elements?



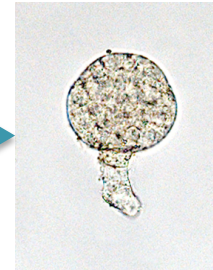
Transform
Tobacco with
promoter/*GUS*
construct

Promoter/*GUS* constructs

- Whole upstream region
- 5' deletions
- 3' deletions
- Gain of function
- Smallest sufficient fragment
- Mutagenesis ($G \leftrightarrow T$, $C \leftrightarrow A$)



Collect capsules 8 DAP



hand dissect
embryos from seeds

Incubate embryos in
GUS buffer at 37°C

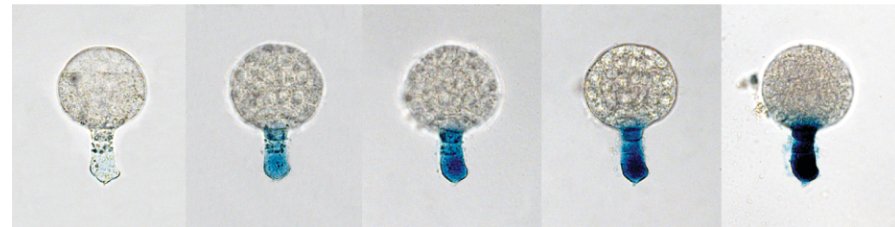
10 min

1 hr

2 hr

4 hr

24 hr

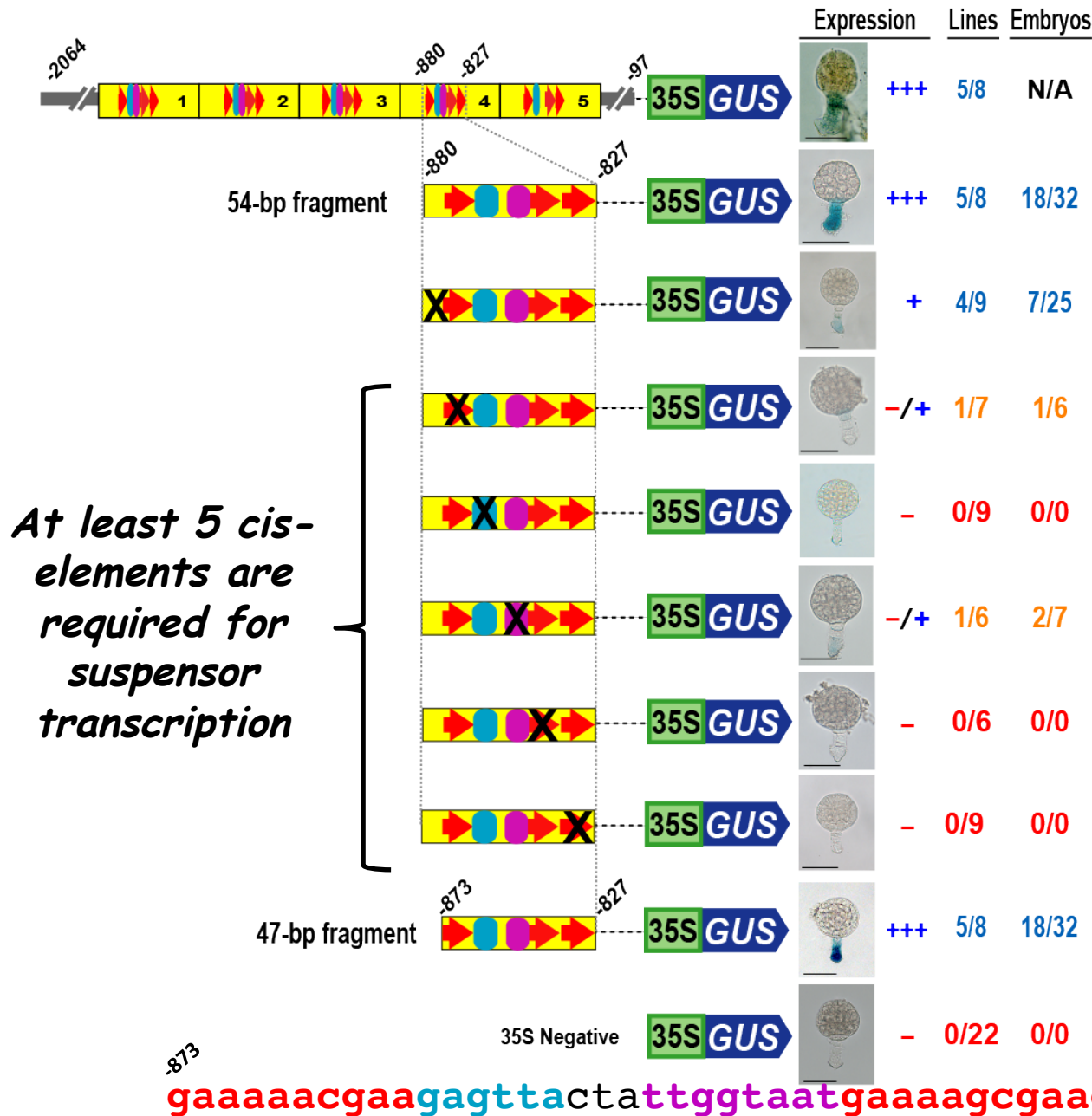


Take
pictures

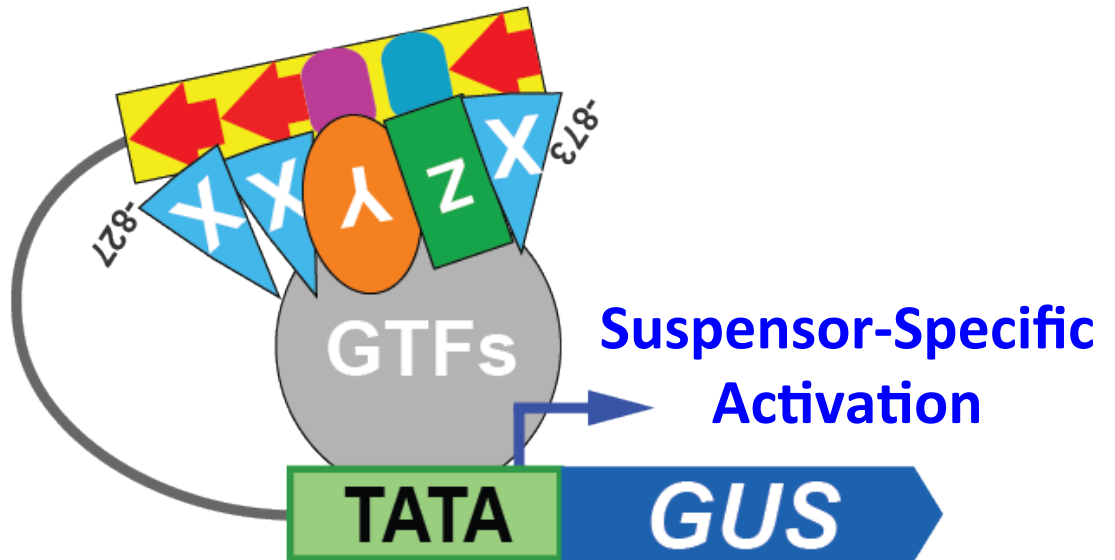
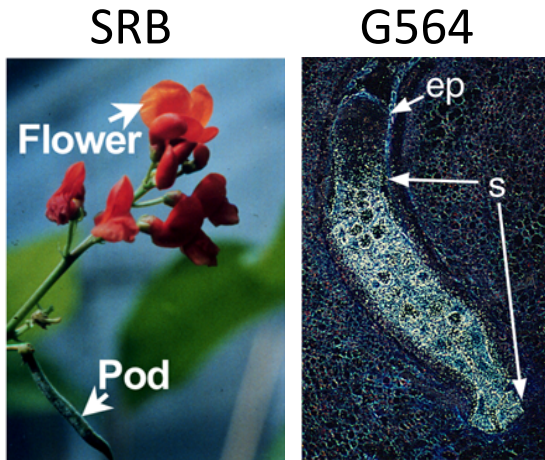
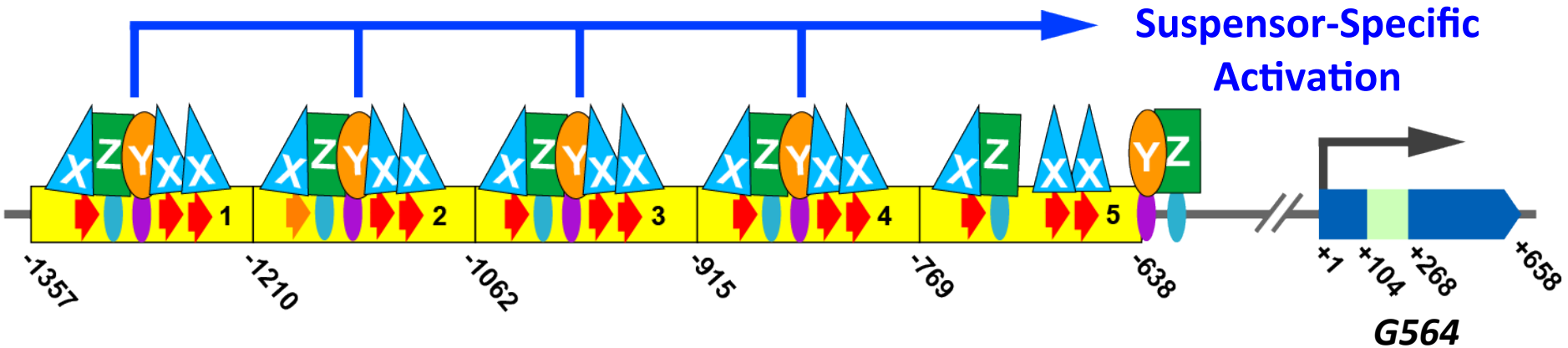
*How many times did we carry out
this procedure to study 2 upstream regions?*





Constructs made	164
Transgenic plants generated	1106
Embryos hand-dissected from seeds	7,742
Hours spent dissecting embryos	903 hr
Days spent dissecting embryos	90 days

What Sequences in the G564 Upstream Region Are Required for Suspensor-Specific Transcription?

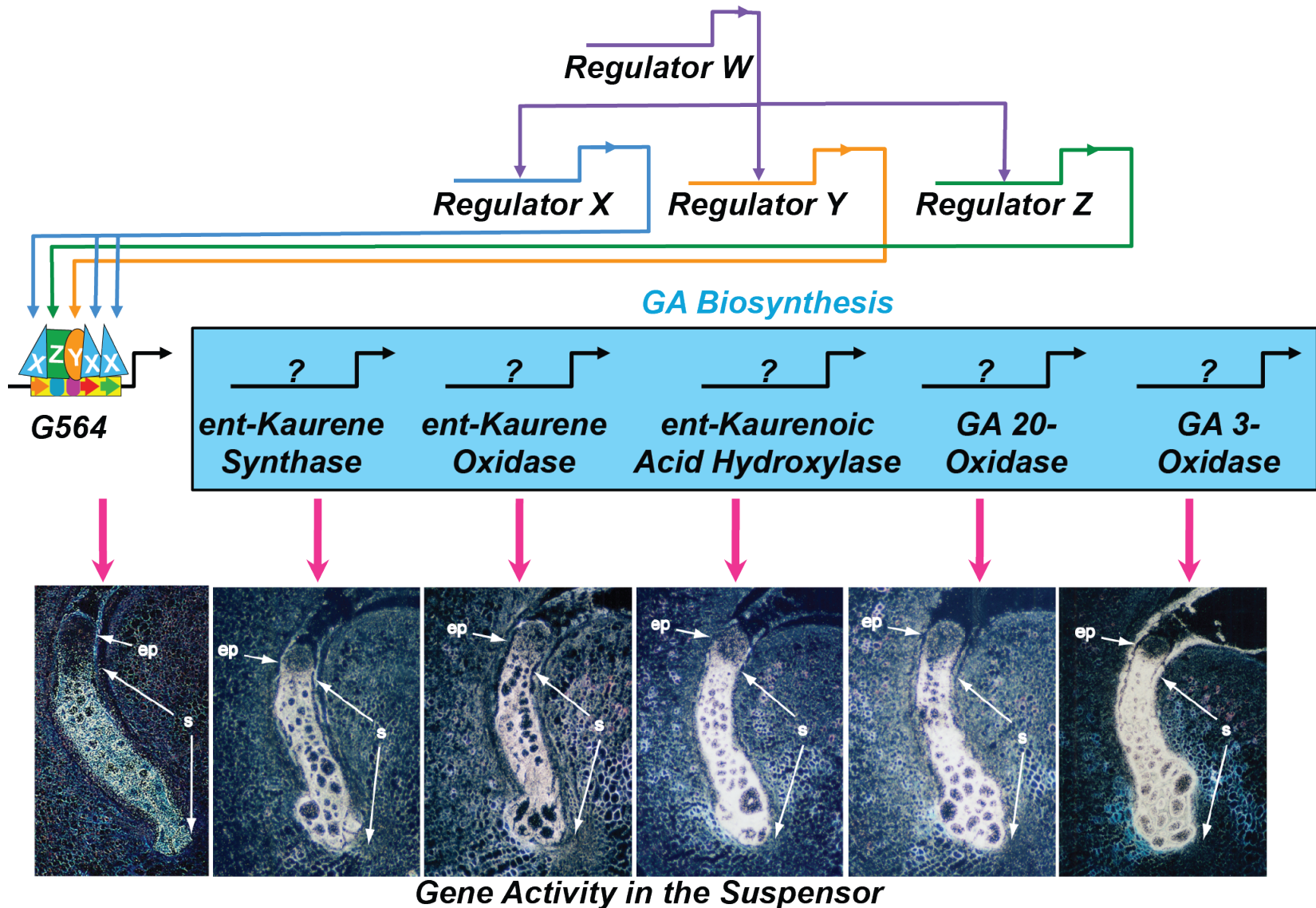


A model for activating suspensor transcription of G564



-  : factor X binding to the 10-bp motifs
-  : factor Y binding to Region 2
-  : factor Z binding to 4th motif
-  : general transcription factor complex

Identifying Regulatory Circuits that Control Suspensor-Specific Gene Activity



Are other suspensor-specific genes regulated by the same motifs as *G564*?

GA 20-Oxidase Gene Structure

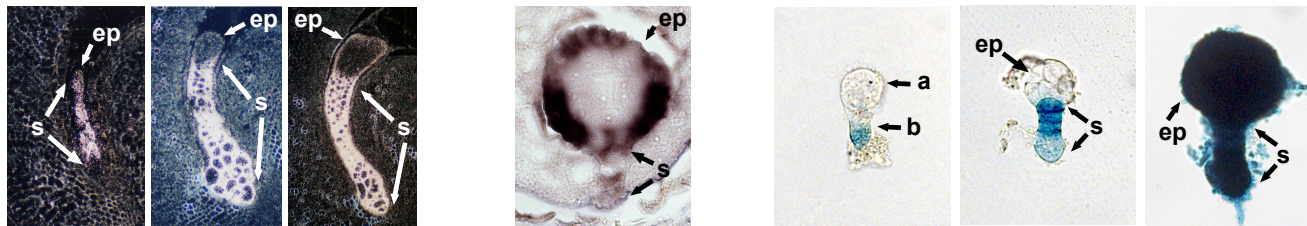


GA 20-Oxidase

Scarlet runner bean



Tobacco



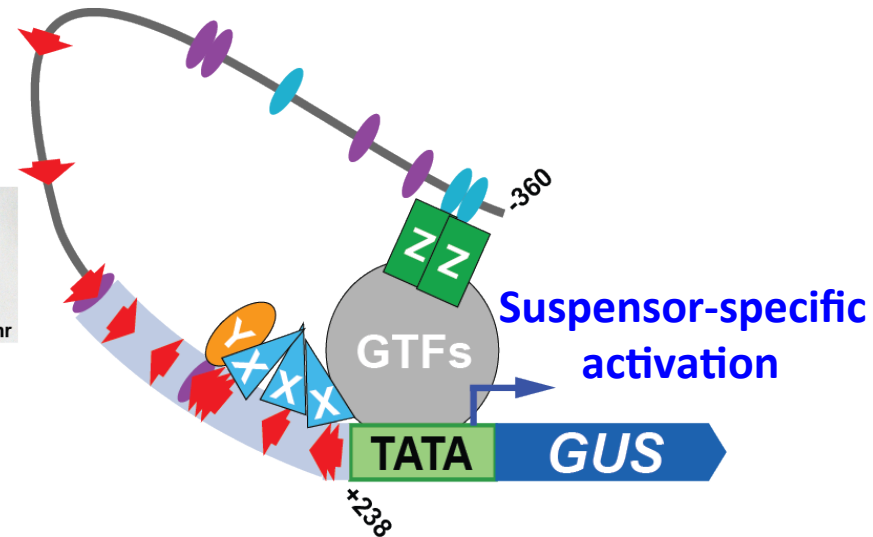
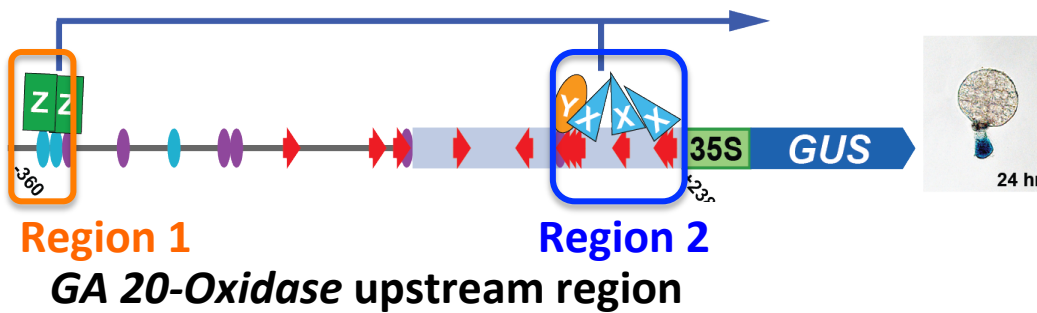
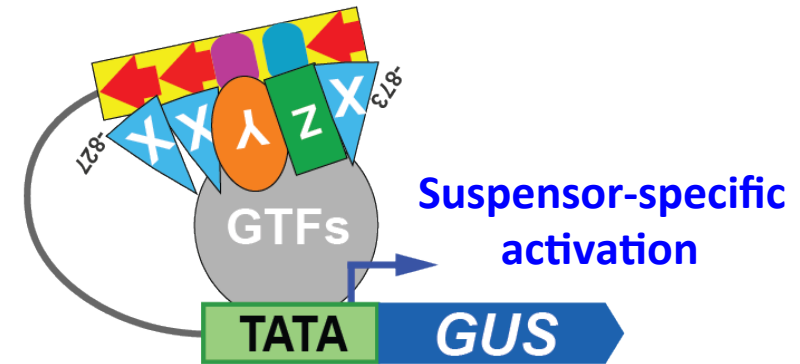
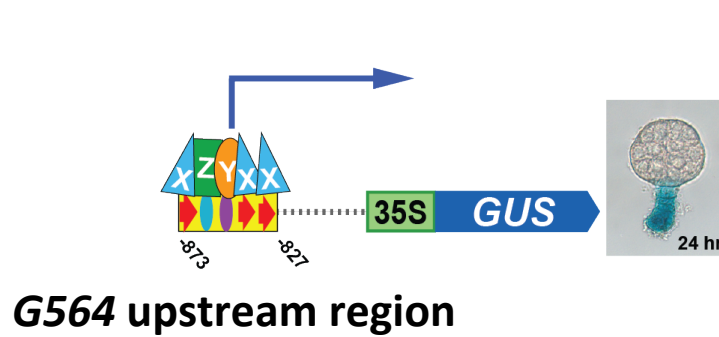
~150 MYA





▪ Temporal and spatial suspensor-specific expression of the GA 20-Oxidase gene is controlled primarily at the transcriptional level.

▪ **Suspensor** transcriptional machinery is conserved in dicots.

▪ GA 20-Oxidase is expressed first in the suspensor, then the embryo proper.

What Is a Model for Activation of Suspensor Transcription?



-  : factor X binding to the 10-bp motifs
-  : factor Y binding to Region 2
-  : factor Z binding to 4th motif
-  : general transcription factor complex

What are the factors the bind to these motifs?

What are The Common Functions of Suspensor Among Different Plant Species?



**~193M Reads from
Suspensor Obtained**

**17,389 Genes
Detected**

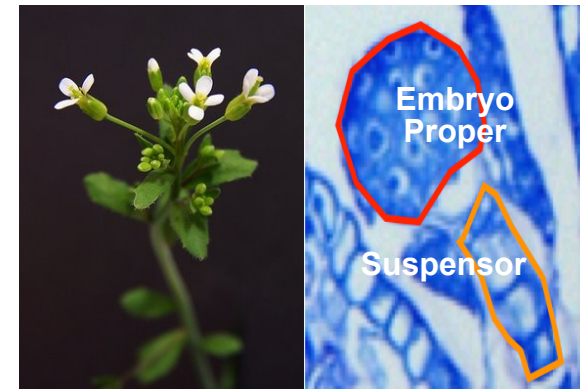
**1,000 TFs*
Detected**



**~100M Reads from
Suspensor Obtained**

**37,185 Genes
Detected**

**4,109 TFs
Detected**



**~30M Reads from
Suspensor Obtained**

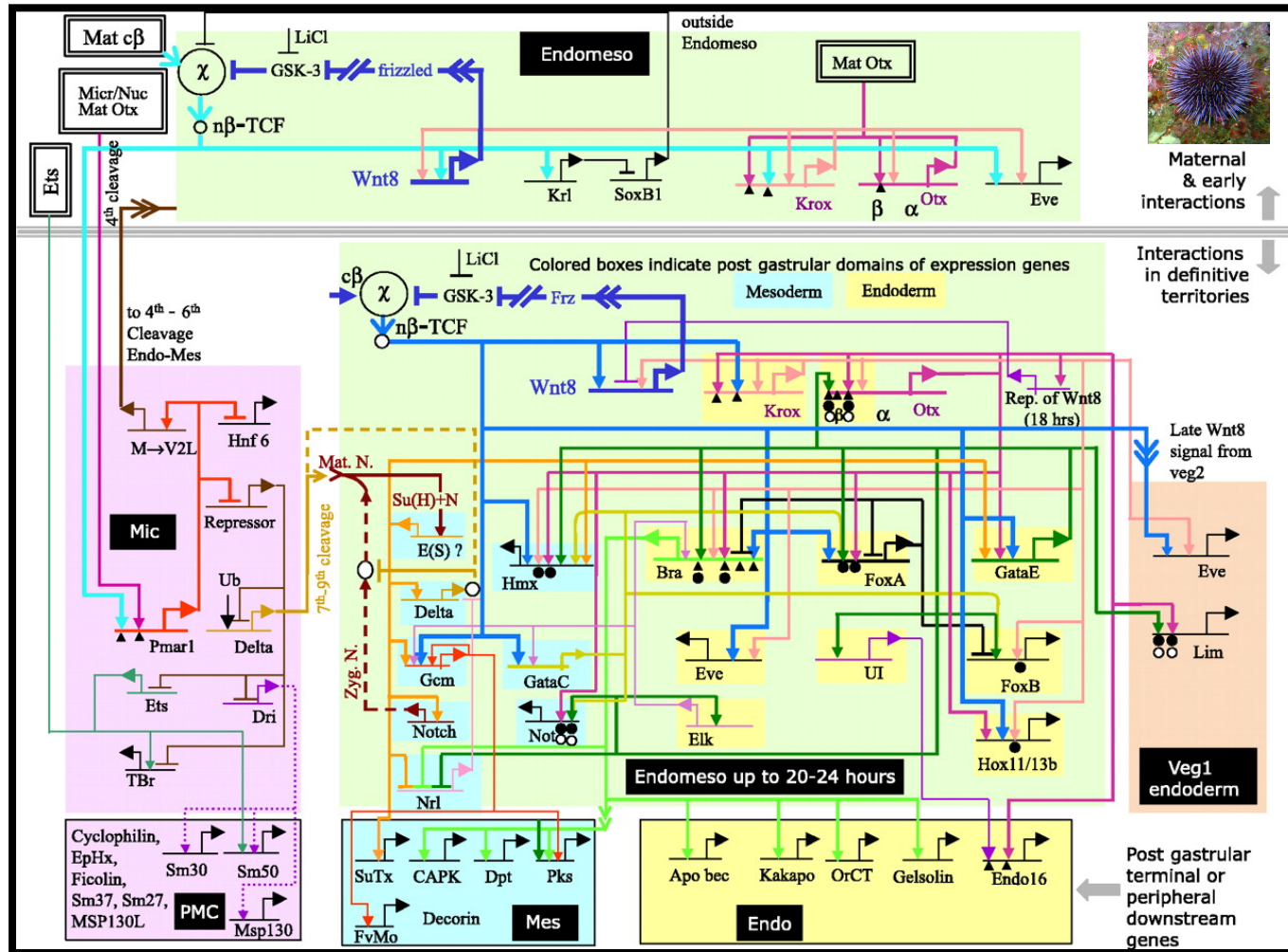
**17,069 Genes
Detected**

**1,123 TFs
Detected**

Common Genes and TFs?

*** Determined using AGI ID**

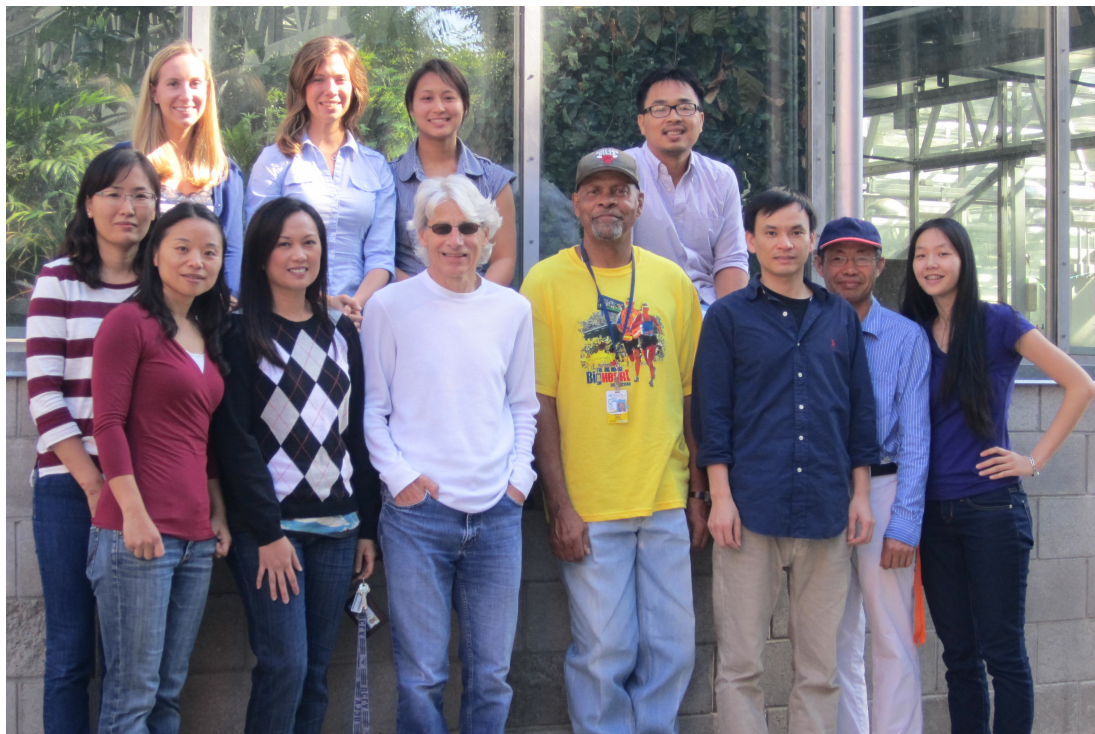
Ultimate Goal.....To Uncover Regulatory Genes and Circuits Driving Seed Differentiation and Development Using Genomics



Learn How To Make a Seed!!



- Big Seeds
- More Seeds
- More Yield
- Increased Food and Fuel



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