OHIO UNIVERSITY

Seeds of Hope: Back to the Future

BOB GOLDERG

9/9/17

Agriculture is Facing the Perfect Storm

Population Growth & Increased Demand For Food

Reduced Land For Agriculture

Scarcity of Natural Resources (e.g., Water)

Climate Change

Expanding Pest Habitats

And…….Political Headwinds

YES 37 Right To Know GMO
LABEL GENETICALLY ENGINEERED FOODS

NO ON 37
STOP THE DECEPTIVE FOOD LABELING SCHEME

GMOs?

GMO

Vermont
VTRightToKnow.org
Right To Know GMOs
LABEL GENETICALLY ENGINEERED FOODS

US rethinks crop regulation

Committee begins study to guide oversight of gene-edited organisms.

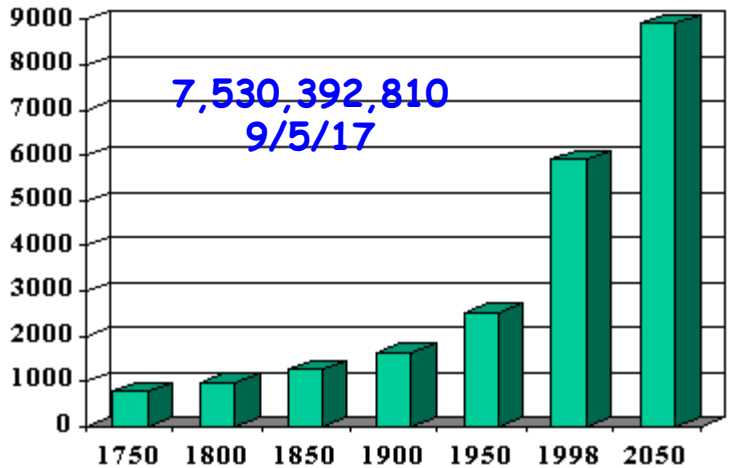
Congress Passes GMO Food Labeling Bill

Colorado, Oregon Reject GMO Labeling

L.A. backpedals on proposal to ban growing genetically modified crops

Justices Back Monsanto on Biotech Seed Planting

Regardless…….We Face a Major Challenge in Agriculture….

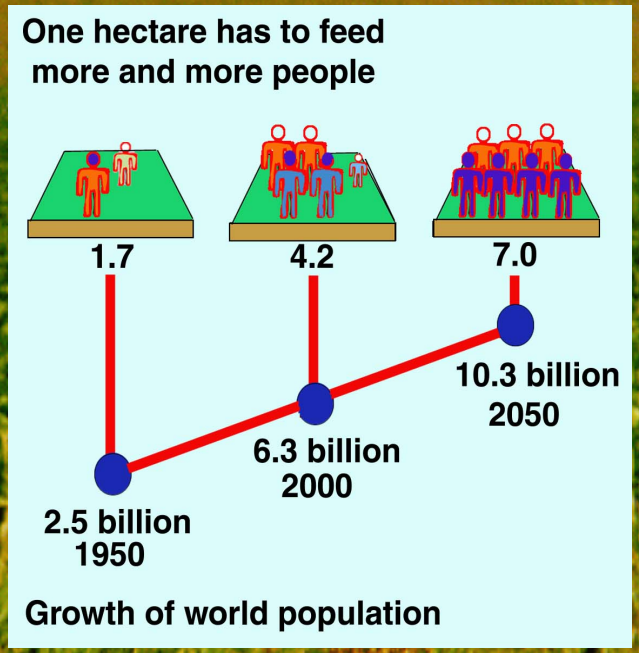


World Population (millions)

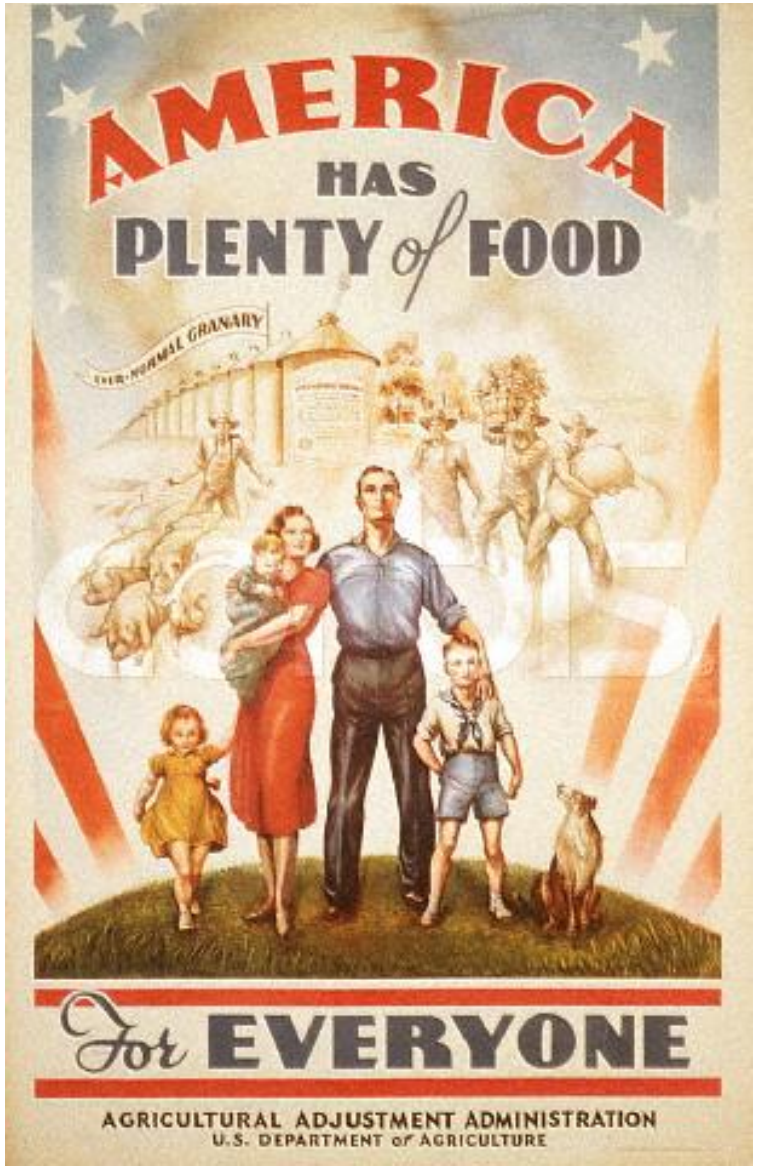
OVER THE NEXT 50 YEARS WE NEED TO PRODUCE MORE FOOD THAN IN THE WHOLE OF HUMAN HISTORY

AND DO IT WITH FEWER INPUTS, LESS ARABLE LAND, & CLIMATE CHANGE!!!!

Thus…..Crop YIELDS MUST Be Increased Using Every Discovery & Technique Available!



We Have Faced Similar Challenges Over The Past 100+ Years



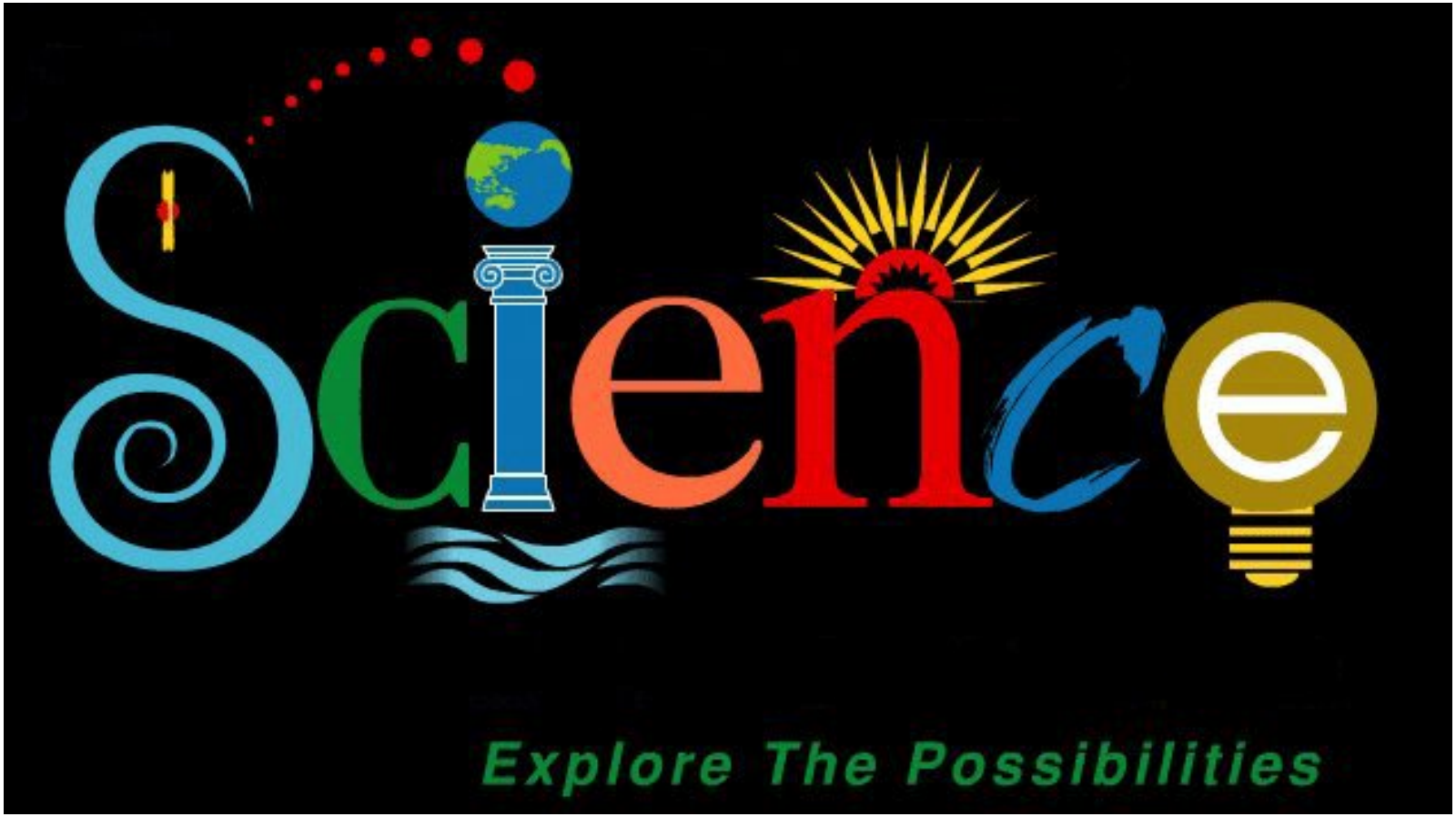
CROP YIELD INCREASES HAVE "ROCKETED UPWARDS" OVER THE LAST 100 YEARS AND CONTRIBUTED TO A LONGER AND "BETTER" LIFE

% Farm Workers	% Income on Food		Bushels/Acre	Life Span
55%	50% →	• 1900	30	← 48 Years
		• 1920	30	
		• 1940	40	
		• 1960	60	
	22% →	• 1980	100	
1.5%	7% →	• 2017	171	← 80 Years

1930: 30 bushels/acre
1930: 1 farmer fed 10 people

2017: 171 bushels/acre
2017: 1 farmer feeds 200 people

Conclusion: Crop yields increased significantly over the past 100 years and lead to a similar reduction in food costs!!!!!



And Not Succumbing To Anti-Science Forces of "Darkness"

Why Seeds? Our Food Is Derived from Fourteen Crops & Over Half Produce Seeds for Human and Animal Consumption

Seed Crops

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

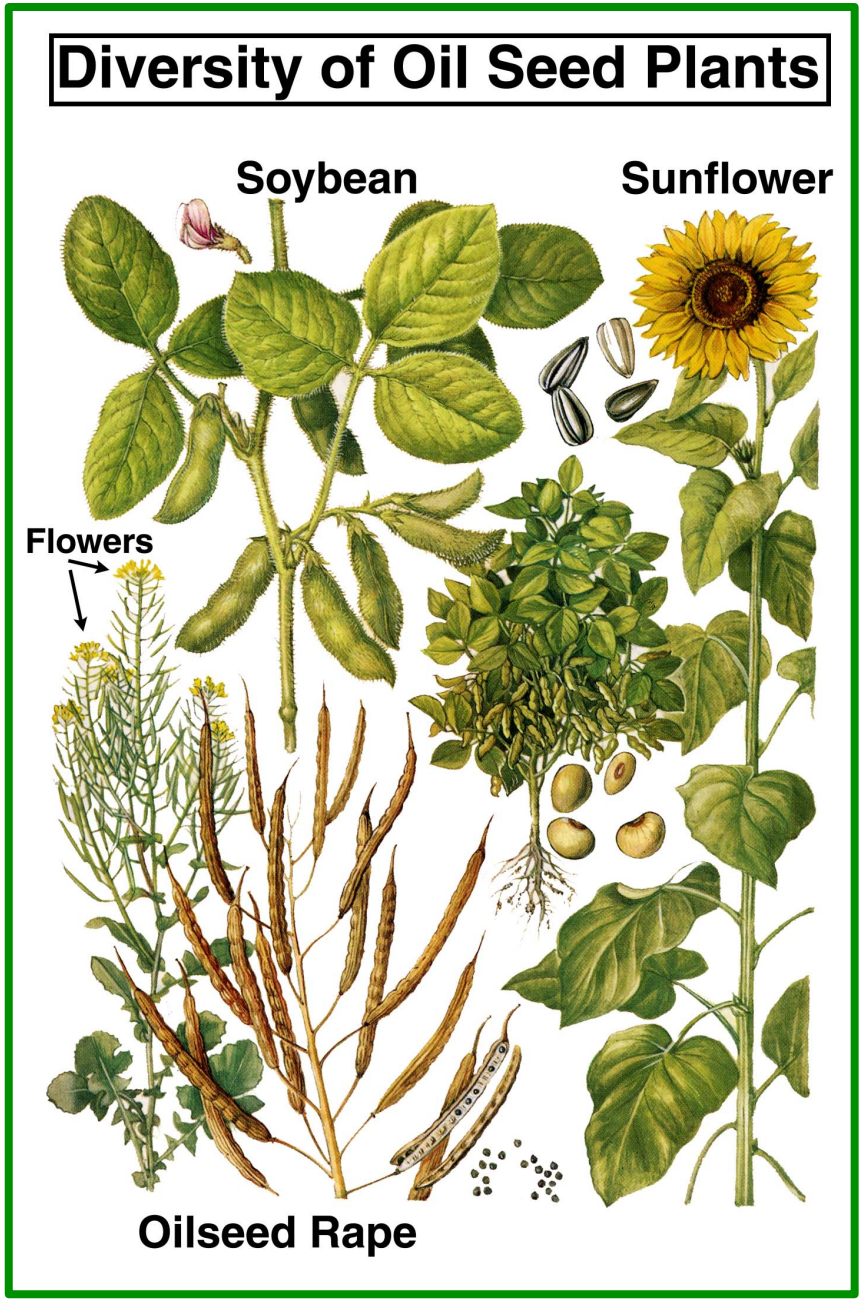
Non-Seed Crops

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

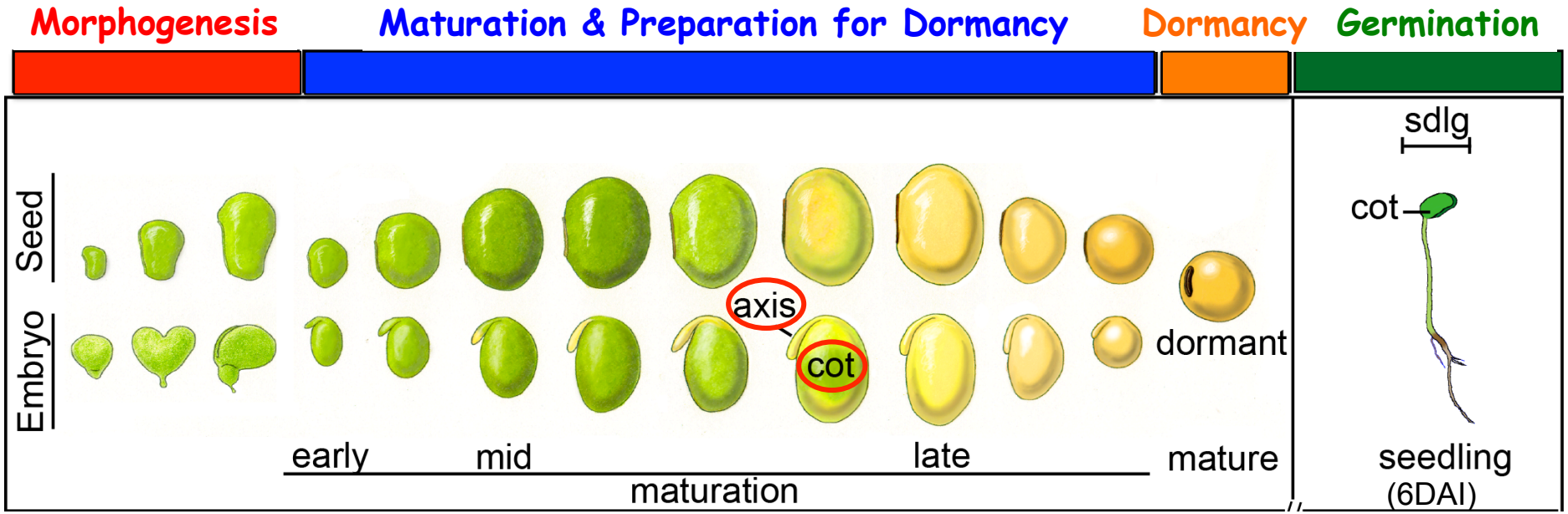
In Some World Populations 75% of Calories Are Derived from Seeds!

Why Soybean?

- Second Largest US Crop (77M Acres)
- Total Crop Value $42 Billion (50% Value Exported)
- The Second Highest Edible Oil (44 million tons consumed worldwide)
- Important Biofuel Source (~30% of US Soybean Oil Production for Biodiesel)
- Excellent Model Plant (Transformation, Knockdowns, Genetics)
- Genome Sequenced
- Seed Gene Expression Data



Question…What Are the Genes Required to Make a Seed?
And…… How Are They Wired in a Plant Genome?

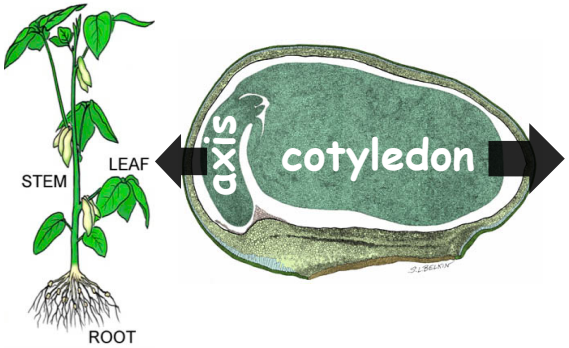


Differentiation of Tissues/organs

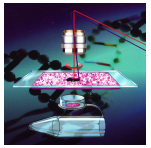
Storage Protein and Oil Deposition
Preparation for Dormancy

Dormancy and Germination

Food Reserve Accumulation



Finding the Players - Mapping the Seed Using Laser Capture Microdissection (LCM) and RNA-Seq From Fertilization Through Dormancy

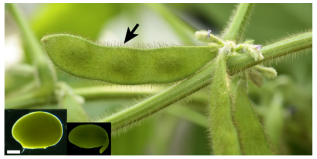


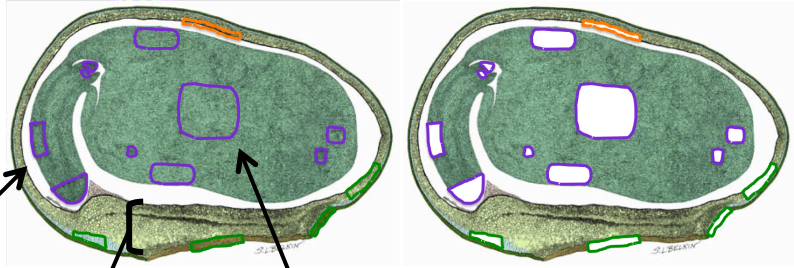
835 Seeds
42 hrs

Collect → **Fix & Section** → **Laser Capture Microdissection (LCM)** → **Library Construction** → **RNA-Seq**

71,225 Sections
(n=835 Seeds)
189 hrs

26,000 Captures
875 hrs



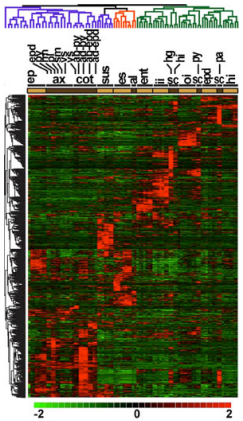


Endosperm (Nourishment)

Seed Coat (Protection)

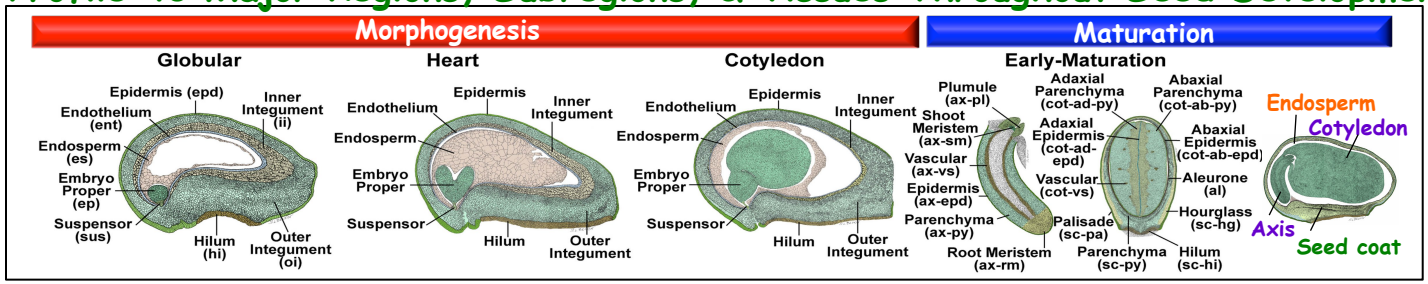
Embryo (Next Generation)

Time from Collecting to LCM = ~1,100 person hours

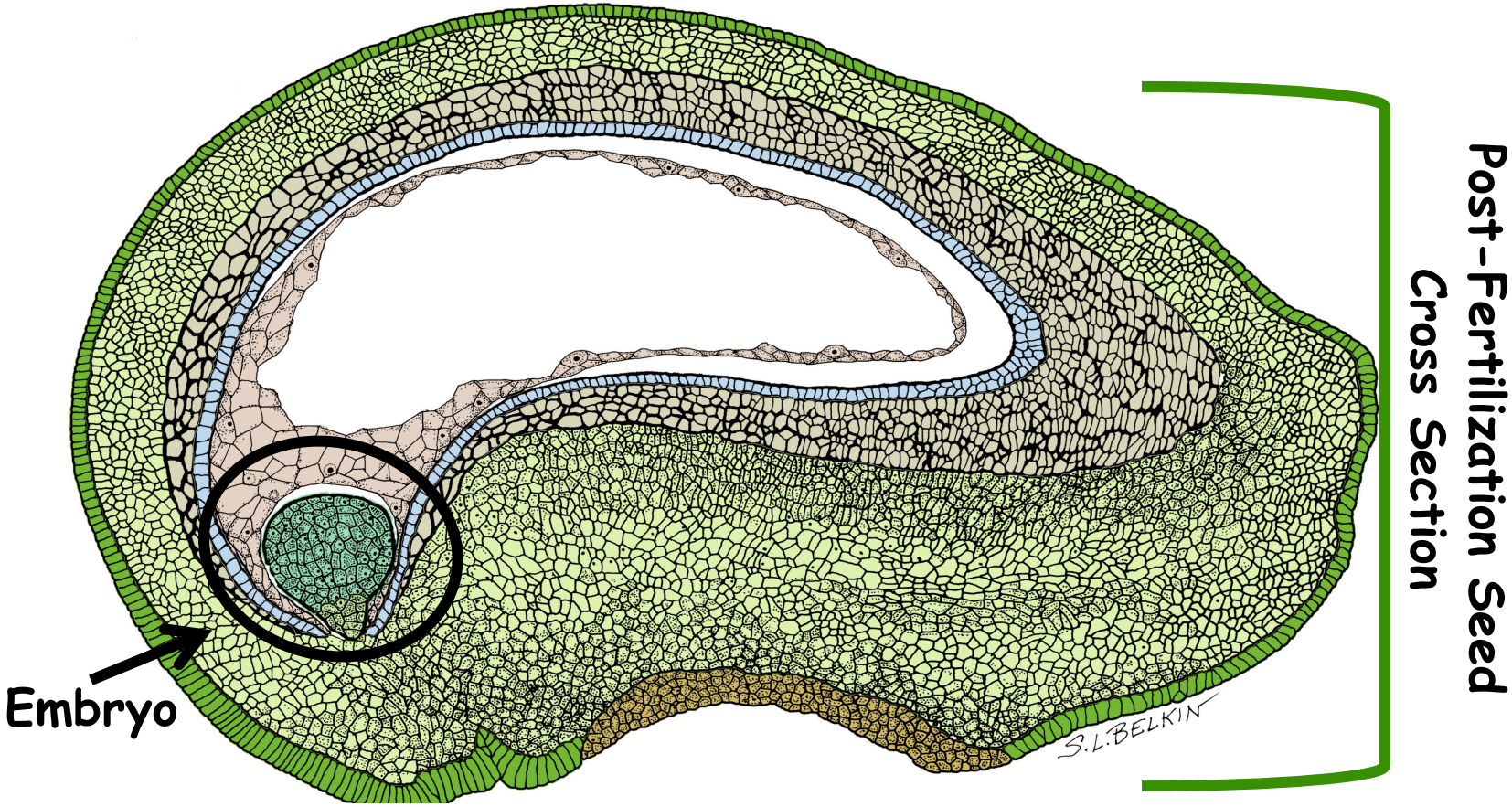


~230 Datasets

Profile 40 Major Regions, Subregions, & Tissues Throughout Seed Development



Using LCM to Capture Two Embryo Regions Shortly After Fertilization



Comprehensive Atlas of Biological Processes and Regulators During Soybean Seed Development

Globular Stage

EMBRYO

EMBRYO PROPER
Biological Processes:
Regulation of transcription
Regulation of timing of organ formation
Leaf & Root development
Pattern specification process
Transcription Factors:
GRF1a, GRF1b, **CUC2**, **STM**, AIL6

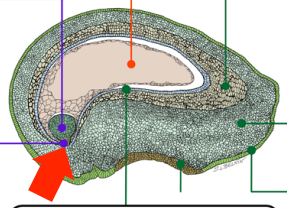
SUSPENSOR
Biological Processes:
Protein phosphorylation
Regulation of asymmetric cell division
Root cap development
Response to stress
Thiazole bp
Transcription Factors:
WOX9a, AP2-EREBP, ARF7, **Lec1-1**,RDR1

SEED COAT

ENDOTHELIUM
Transcription Factors:
JKDa, ZF-TAZ, SOM, AP2-EREBP, C2C2-Dof

ENDOSPERM

ENDOSPERM
Biological Processes:
Nucleoside transport
Oxidation-reduction process
Transcription Factors:
AGL62, AGL6, NAC2, NAC83, BLH1



HILUM
Biological Processes:
Formation of organ boundary
Regulation of cell proliferation
Hormone-mediated signaling
Regulation of transcription
Spermine bp
Transcription Factors:
DAG1, NPY2, KAN2, BEL1, C2C2-Dof

SEED COAT

INNER INTEGUMENT
Biological Processes:
Cell wall biogenesis
Transcription Factors:
NAC87, C3H, NAC100, IDD4, PHD

OUTER INTEGUMENT
Biological Processes:
Jasmonic acid bp
Meristem development
Response to nitrate
Defense response
Cytokinin cp
Transcription Factors:
IDD4, WOX1, SPL12a, ASHR2, SPL12b

EPIDERMIS
Biological Processes:
Multiorganism cp
Response to stress
Seed coat development
Proanthocyanidin bp
Alcohol mp
Transcription Factors:
TTG2, TCP3a, TINY2, TCP3b, ANT

Heart Stage

EMBRYO

EMBRYO PROPER
Biological Processes:
Regulation of transcription
Leaf development
Regulation of timing of organ formation
Photosynthesis
Pattern specification process
Transcription Factors:
ARIDa, ARIDb, GRF1b, JUMONJI, ANT

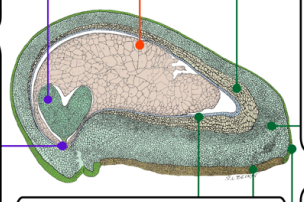
SUSPENSOR
Biological Processes:
Defense response
Response to stress
Root cap development
Protein targeting to membrane
Ethylene bp
Transcription Factors:
WOX9a, **WOX9b**, COR47, AP2-EREBP, RDR1

SEED COAT

ENDOTHELIUM
Transcription Factors:
JKDb, JKDa, SOM, HB17, AGL6

ENDOSPERM

ENDOSPERM
Biological Processes:
Endosperm development
Regulation of cell differentiation
Response to stress
Transcription Factors:
MYB98, **AGL62**, MIF1a, ARF2, ML1



HILUM
Biological Processes:
Formation of organ boundary
Regulation of cell proliferation & cell size
Hormone-mediated signaling
Regulation of transcription
Transcription Factors:
SRS7, SHI, DAG1, HEC3, **SUP**

SEED COAT

INNER INTEGUMENT
Transcription Factors:
NAC87, GT2, C3H, PHD, XERICO

OUTER INTEGUMENT
Biological Processes:
Negative regulation of catalytic activity
Proteolysis
Regulation of transcription
Jasmonic acid bp
Carbohydrate mp
Transcription Factors:
MYB-related, SDG39, SPL12a, IDD4, WOX1

EPIDERMIS
Biological Processes:
Regulation of transcription
Response to salicylic acid stimulus
Response to UV-B
Seed coat development
Transcription Factors:
TCP3a, TINY2, TCP2, TCP3b, SBP

Cotyledon Stage

EMBRYO

EMBRYO PROPER
Biological Processes:
Regulation of transcription
Leaf morphogenesis
Gibberellic acid mediated signaling pathway
Pattern specification process
Seed dormancy process
Transcription Factors:
YAB1a, WRI1, **YAB1b**, AP2-EREBP, HB31

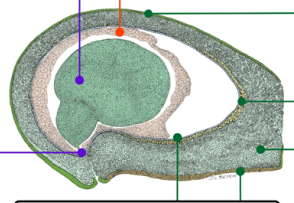
SUSPENSOR
Biological Processes:
Response to stress
Defense response
Root cap development
Protein targeting to membrane
Ethylene bp
Transcription Factors:
WOX9b, **WOX9a**, COR47, AP2-EREBP, GRP-ARR-B

SEED COAT

ENDOTHELIUM
Transcription Factors:
JKDb, JKDa, HV17, AGL6, TT16

ENDOSPERM

ENDOSPERM
Biological Processes:
Regulation of defense response
Transcription Factors:
RL6, MIF2a, HDG11, MIF2b, MYB98



HILUM
Biological Processes:
Formation of organ boundary
Regulation of cell size
Regulation of transcription
Hormone-mediated signaling
Post-embryonic development
Transcription Factors:
HEC3, DAG1, SRS7, AGL5, C2C2-Dof

SEED COAT

EPIDERMIS
Biological Processes:
Response to UV-B
Response to salicylic acid
Flavonoid bp
Oligopeptide transport
Transcription Factors:
TINY2, MYB5, SBP, MYB17, WLIM1

INNER INTEGUMENT
Biological Processes:
Carbohydrate mp
Xylan bp
Hydrogen peroxide cp
Transcription Factors:
GT2, NAC87, C3H, Trihelix, NAC74

OUTER INTEGUMENT
Biological Processes:
Negative regulation of catalytic activity
Carbohydrate mp
Proteolysis
Sucrose transport
Jasmonic acid bp
Transcription Factors:
GBF6, MYB-related, bZIP44, bZIP9, bHLH

Early Maturation Stage

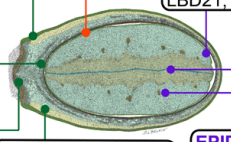
SEED COAT

PALISADE
Biological Processes:
Cell wall modification
Transcription Factors:
GL2, SEP, MYB12, GT2, E2F-DP

PARENCHYMA
Biological Processes:
Sucrose transport
Negative regulation of catalytic activity
Lipid oxidation
Cellular response to salicylic acid stimulus
Proteolysis
Transcription Factors:
bZIP44, HAT22, MYB-related, WOX1, AP2-EREBP

ENDOSPERM

ALEURONE
Biological Processes:
Gibberellin bp
Seed dormancy process
Regulation of cell differentiation
Regulation of defense response
Multicellular organismal development
Transcription Factors:
ID2, HB22, PHD, HDG11, NAC75



HILUM
Biological Processes:
Glucuronoxylan mp
Secondary cell wall biogenesis
Formation of organ boundary
Transcription Factors:
HEC3, **SUP**, MYB61, ZFP11, GARP-ARR-B

HOURGLASS
Biological Processes:
Developmental cell growth
Carbohydrate mp
Sporopollenin bp
Transcription Factors:
AXR3, AP2-EREBP, MYB86a, ERF4, MYB86b

EMBRYO COTYLEDON

ABAXIAL EPIDERMIS
Transcription Factors:
KNAT3, WOX3, TCP5a, TCP5b, MYB113

ADAXIAL EPIDERMIS
Transcription Factors:
ASL1

VASCULAR
Biological Processes:
Regulation of cell size
Transcription Factors:
bHLHa, MYB59, LBD21, bHLHb, bHLHc

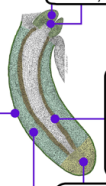
EPIDERMIS
Transcription Factors:
WOX9b, WOX8b,BBM, JKC, MYB5

PARENCHYMA
Transcription Factors:
RL6, ZFP10, bHLH

EMBRYO AXIS

PLUMULE
Transcription Factors:
TCP1a, LFY2, PTL, TCP1b, G2-like

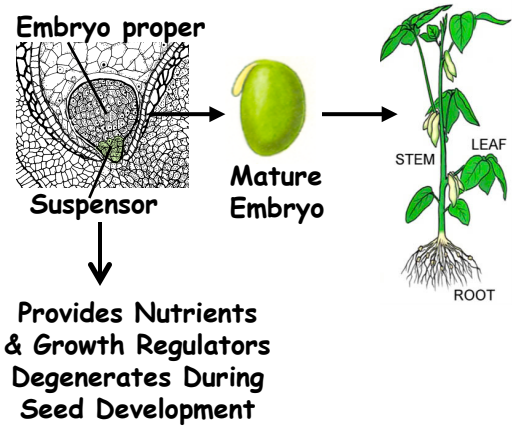
SHOOT MERISTEM
Biological Processes:
Pattern specification process
Cotyledon development
Maintenance of floral meristem identity
Regulation of transcription
Transcription Factors:
SBLH8a, BLH8b, **STM,** DAG1, AP2-EREBP



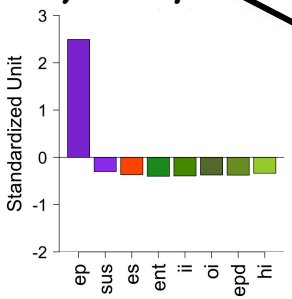
Vascular
Transcription Factors:
GL3, EGL1, C2C2-Dof, LBD39, MYB4

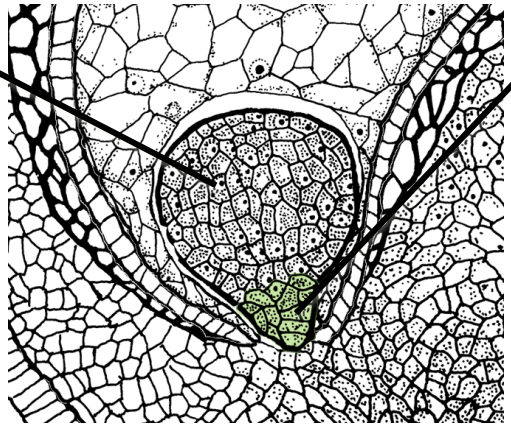
ROOT MERISTEM
Transcription Factors:
PLT1, PLT2, TLP6, SMB, PLATZ

For Example - Two Embryo Regions Shortly After Fertilization Express Different Genes and are Specified to Follow Different Developmental Pathways

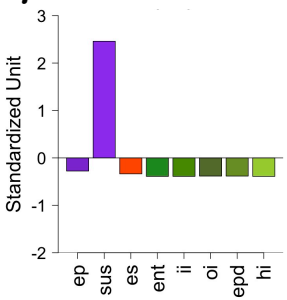


127 mRNAs more than 5-fold up-regulated in ***Embryo Proper***





741 mRNAs more than 5-fold up-regulated in ***Suspensor***



Embryo Proper

GO Terms: Regulation of organ formation timing, Pattern specification process, Leaf & Root development, Reproductive process, Regulation of transcription

Metabolic Processes: Fatty acid bs, Glycolipid desaturation, Auxin bs, Calvin-Benson-Bassham cycle

Transcription Factors: GRF1a, GRF1b, CUC2, STM, AIL6

Suspensor

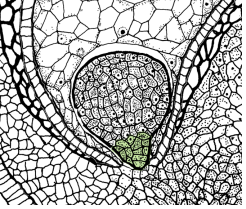
GO Terms: Transport, Cellular component movement, Asymmetric cell division, Gibberellin bp, Root cap development, Response to stress, Defense response, Thiazole bp

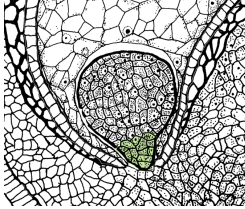
Metabolic Processes: Phenylpropanoid bs, Ethylene bs, Jasmonic acid bs

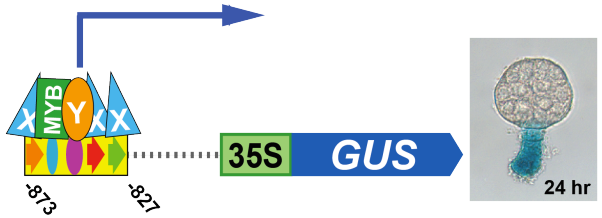
Transcription Factors: WOX9-like, AP2-EREBP, ARF7, Lec1-like

The Beginning of a Seed Gene Network

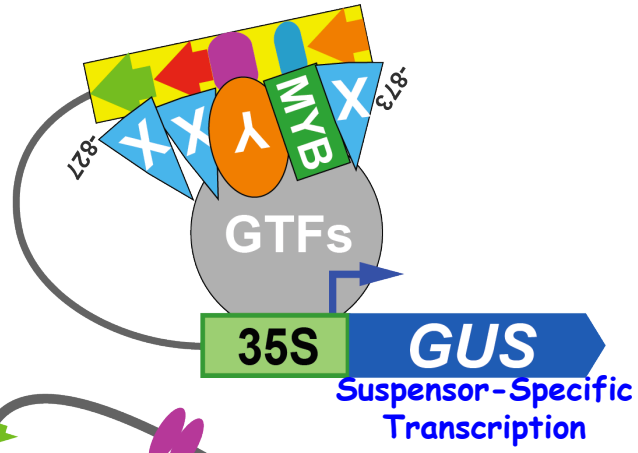
A Unique Cis-Control Module Activates Genes Specifically Within the Suspensor After Fertilization!!

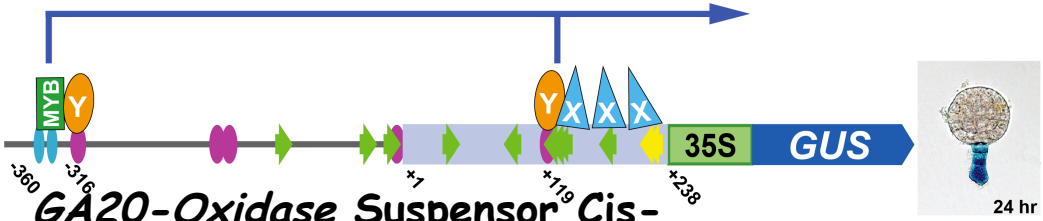




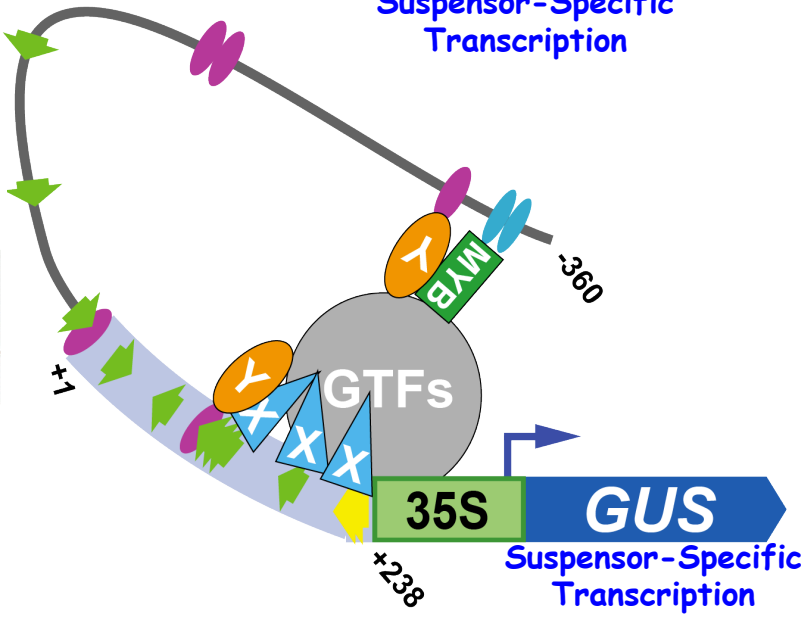


G564 Suspensor Cis-Control Module





GA20-Oxidase Suspensor Cis-Control Module

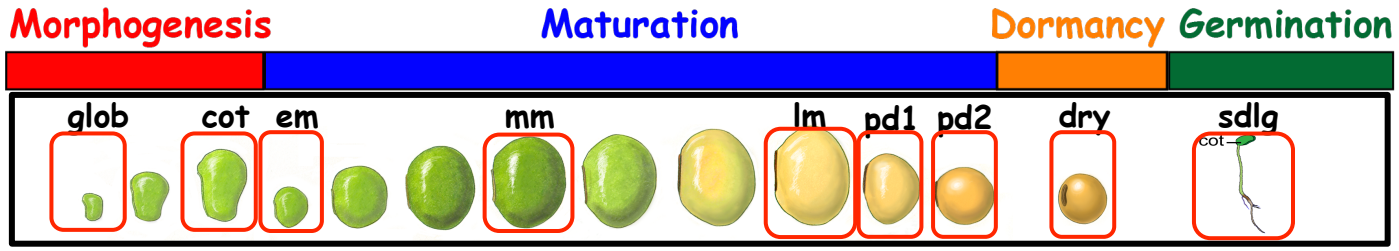


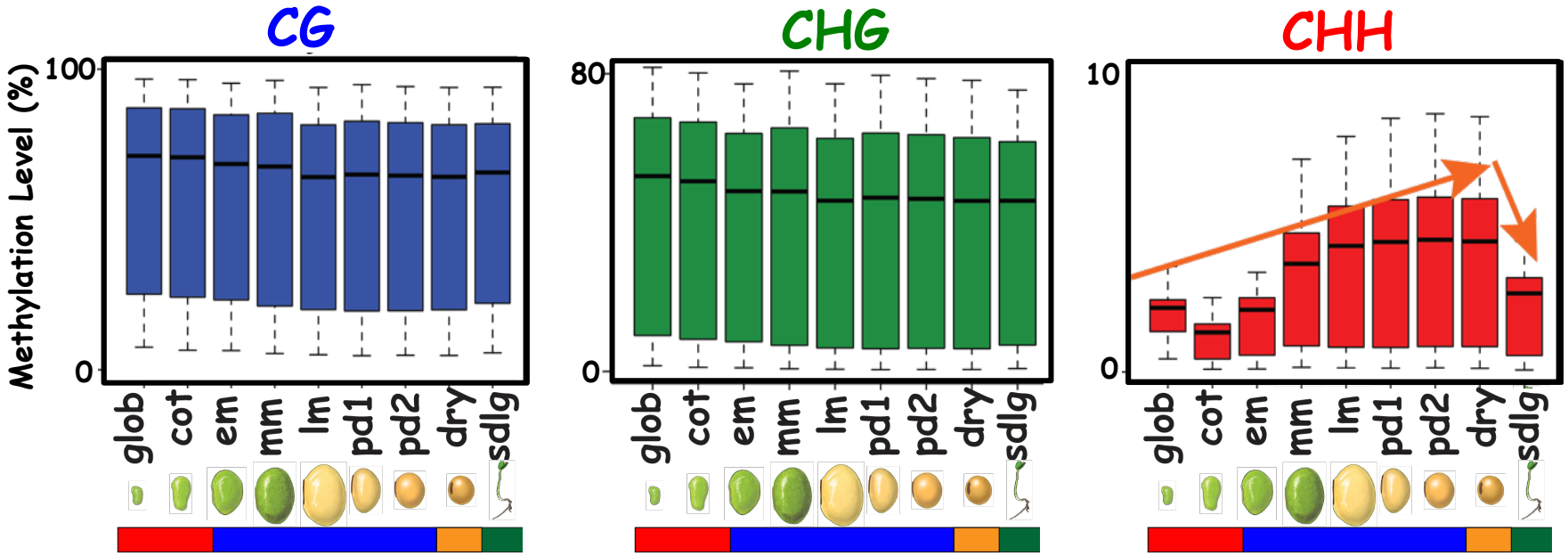
Constructs made	164
Transgenic plants generated	1106
Embryos dissected from seeds	7,742
Hours spent dissecting embryos	903 hr
Days spent dissecting embryos	90 days

- X : factor X binding to 10-bp motif
- Y : factor Y binding to Region 2
- Z : MYB TF binding to Fifth motif
- : general transcription factor complex
- : 47-bp region
- : 5' UTR
- : 10-bp motif (GAAAAGCGAA)
- : 10-bp motif with 1 mismatch
- : 10-bp motif with 2 mismatches
- : 10-bp motif with 3 non-adjacent mismatches
- : Fifth motif (RAGTTA)
- : Region 2 (TTGRDAAT) with 1 mismatch (3rd position cannot be A)

Henry et al., 2015
Kawashima et al., 2010

Are There Global DNA Methylation Changes During Seed Development and Germination?

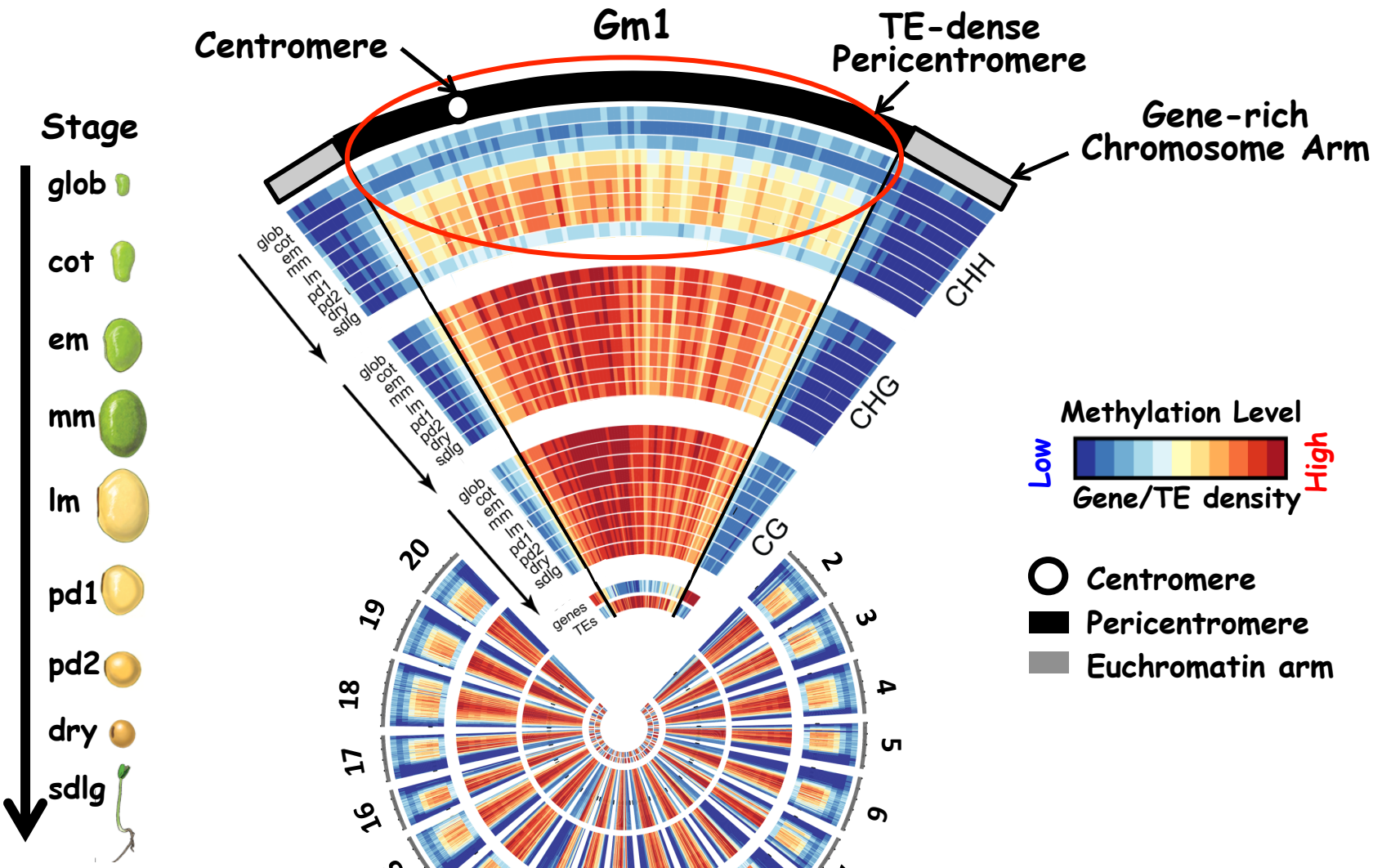




- There Are No Major Changes in CG and CHG Methylation
- CHH Methylation Increases During Seed Maturation Towards Dormancy…Then…Decreases After Germination

H = A, C, T

Developmental CHH Methylation Changes Are Located Mostly in Transposon-Dense Regions



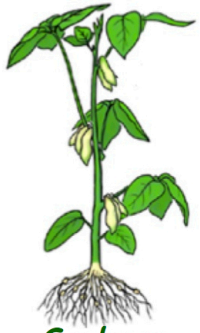
H = A, C, T

Are the CHH Methylation Changes During Soybean Seed Development Conserved in Other Plants?

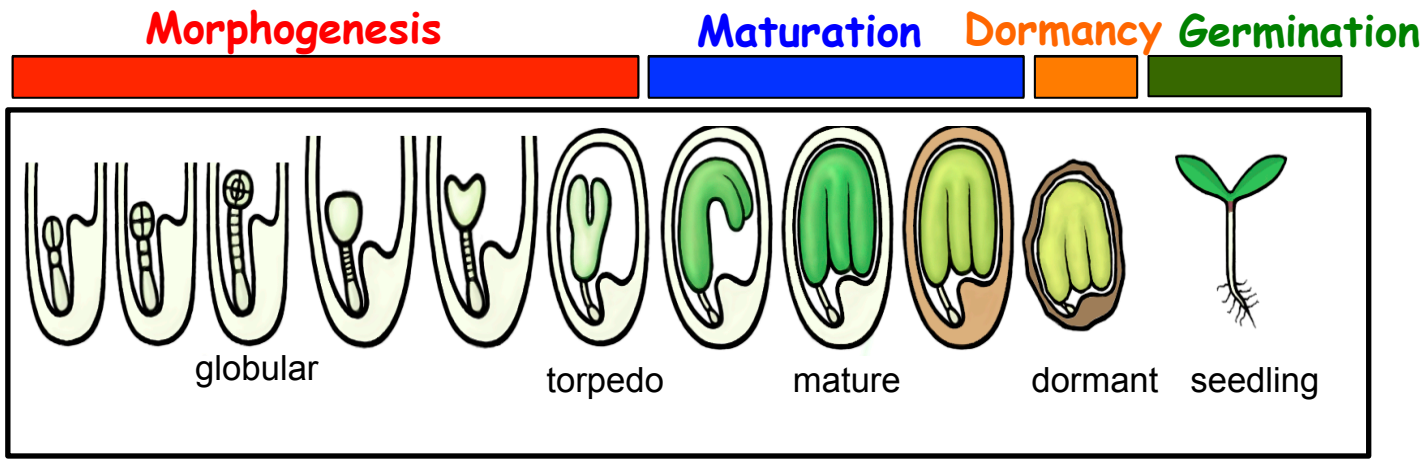
Arabidopsis thaliana

Arabidopsis

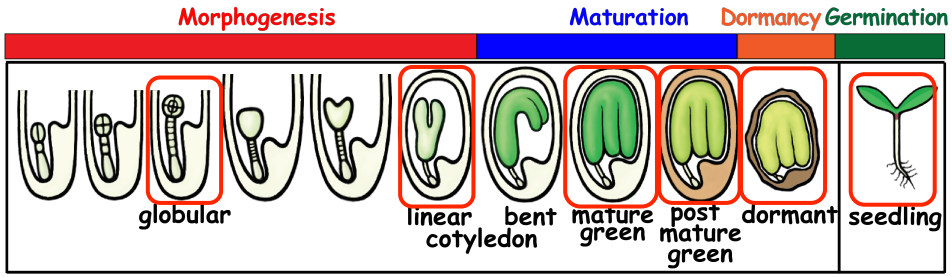
Soybean

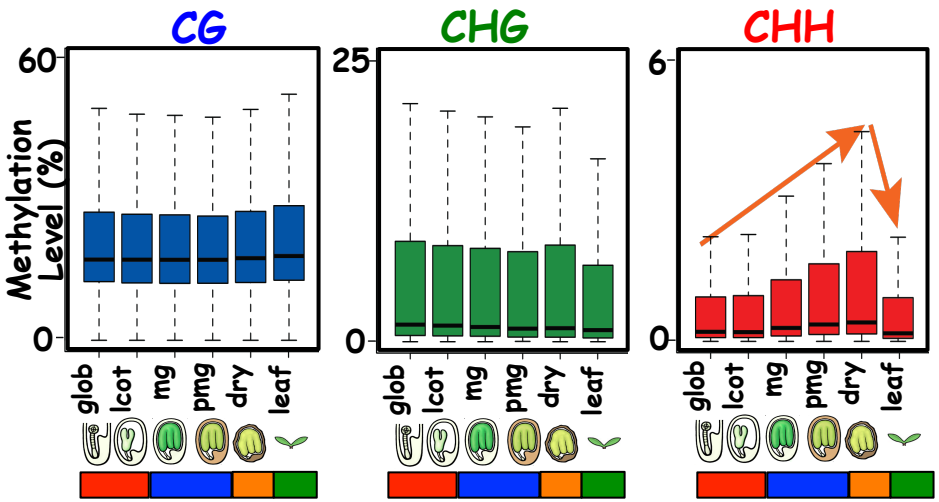


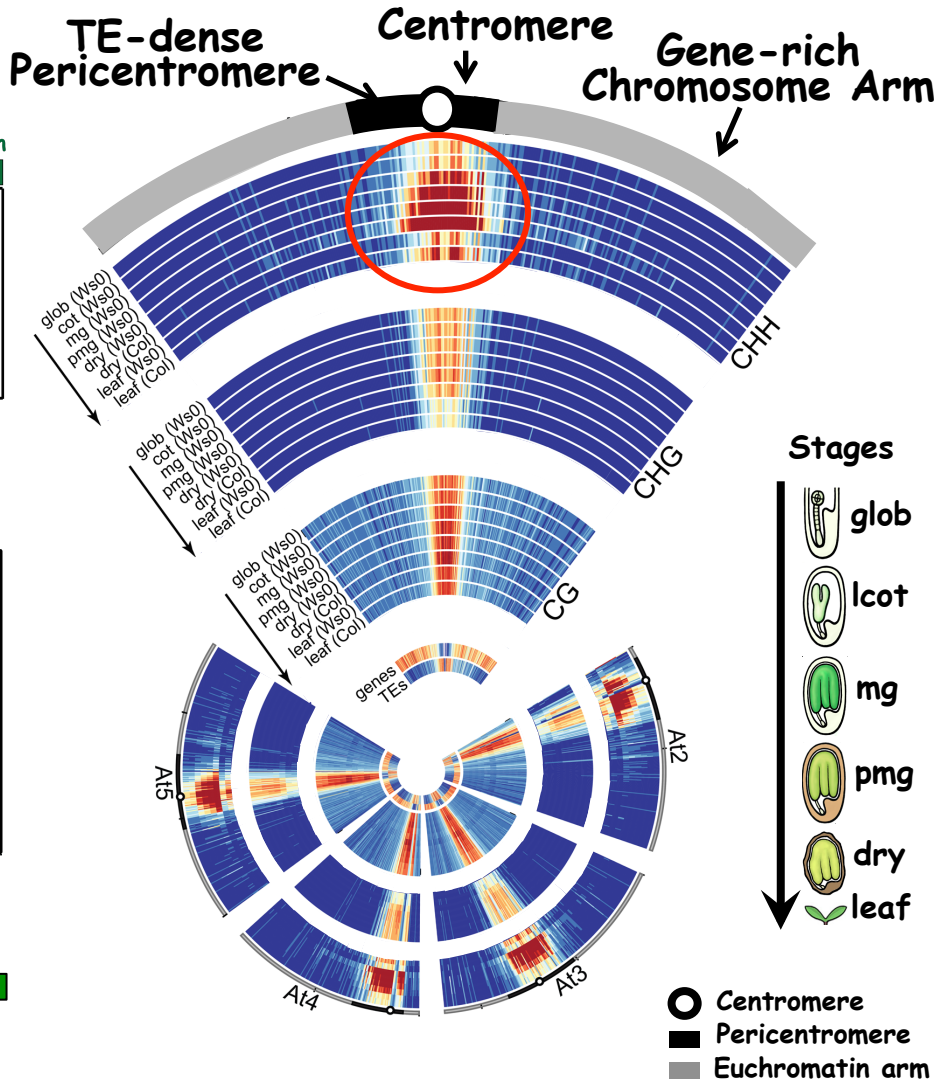
Soybean (Glycine max)



Do the DNA Methylation Changes Occur During Arabidopsis Seed Development?







CHH Changes Are Conserved in Seed Development!

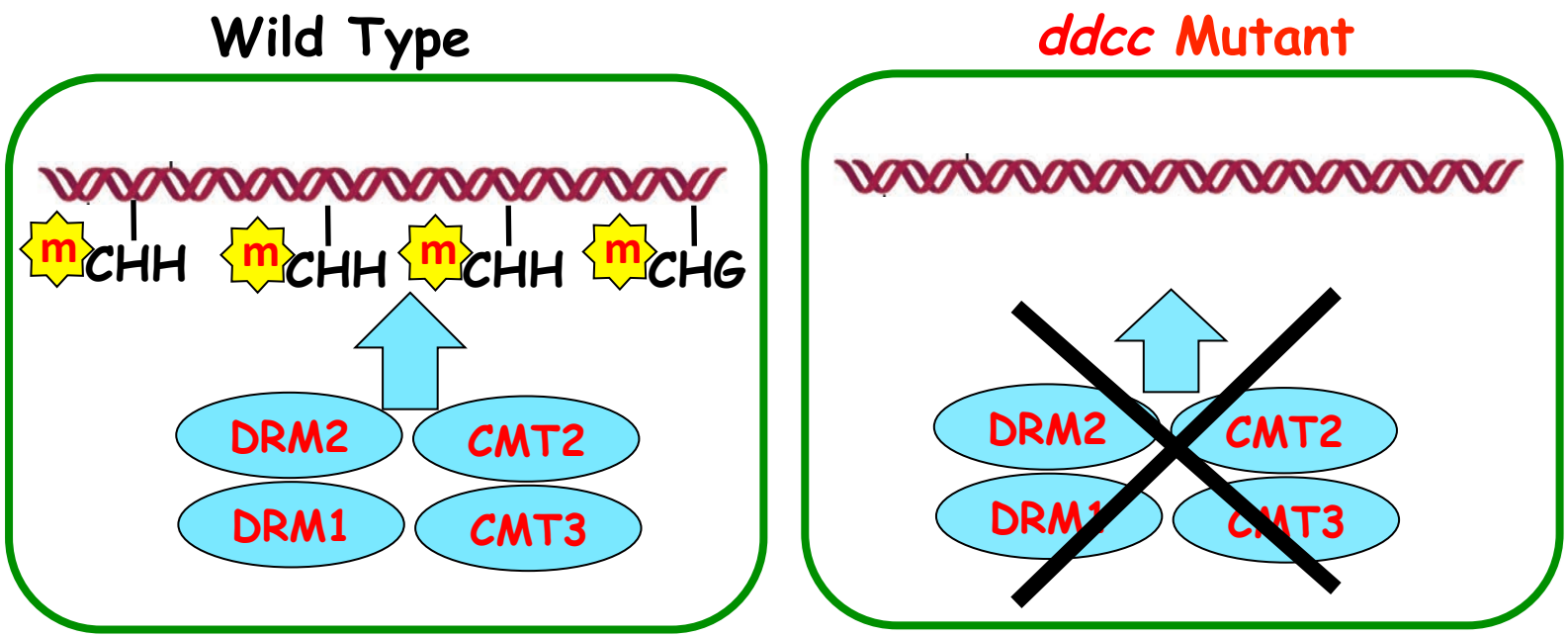
What Is the Biological Relevance of Increasing CHH Methylation During Seed Development?

1. Play a Role in Desiccation?
2. Play a Role in Dormancy?
3. Play a Role in Seed Morphogenesis?
4. Play a Role in Germination?
5. Affect Gene Activities?
6. Reinforce Transposon Silencing?

How to Functionally Test These Hypotheses?

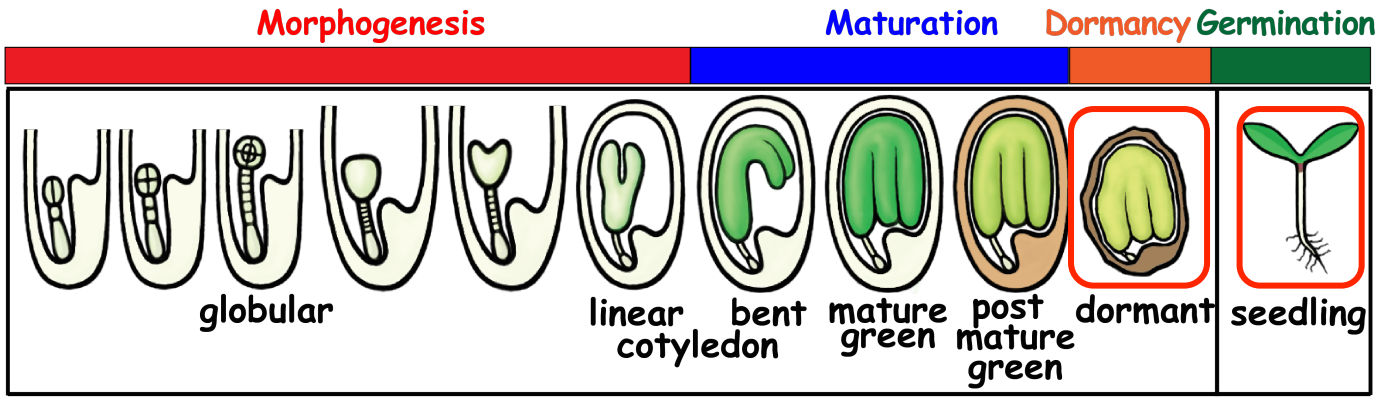
Use a Mutant Arabidopsis Plant Without CHH Methylation

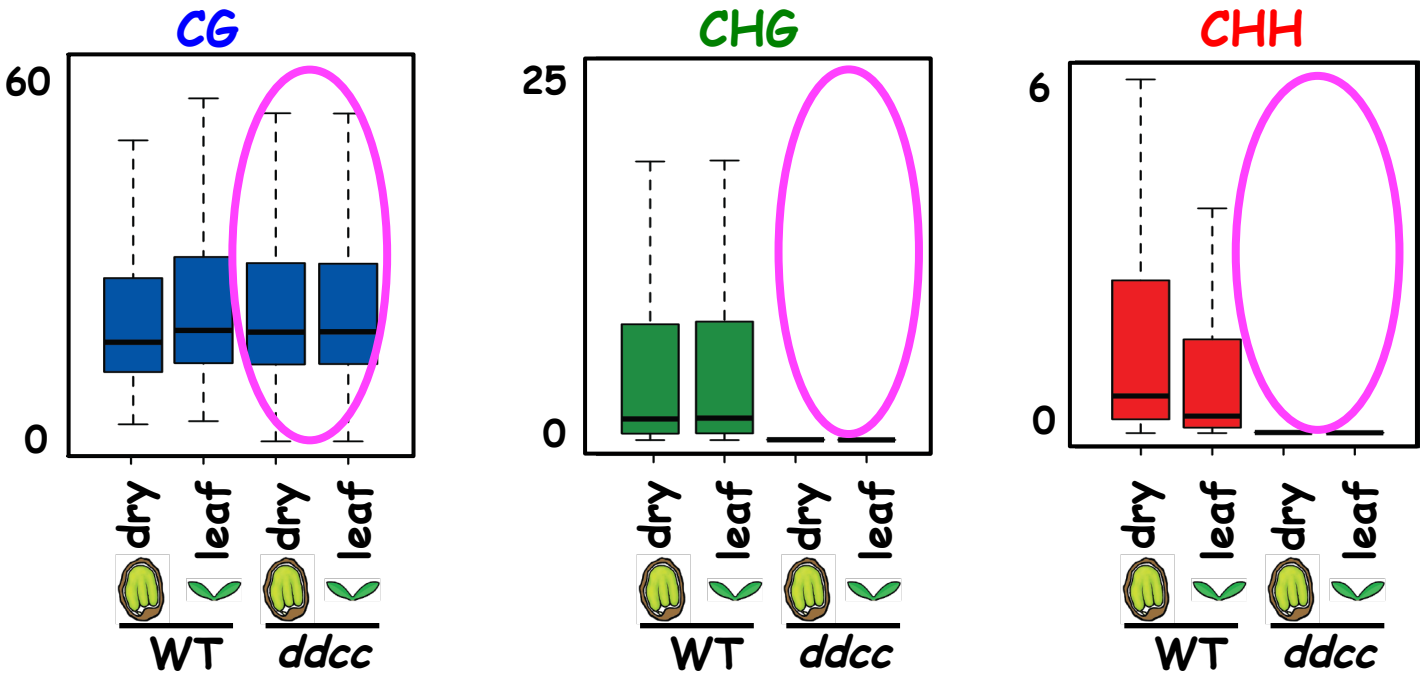
- Arabidopsis CHG/CHH Methyltransferases: DRM1 DRM2 CMT2 CMT3
- Arabidopsis ddcc mutant (drm1 drm2 cmt2 cmt3)



Hume *et al.*, 2014

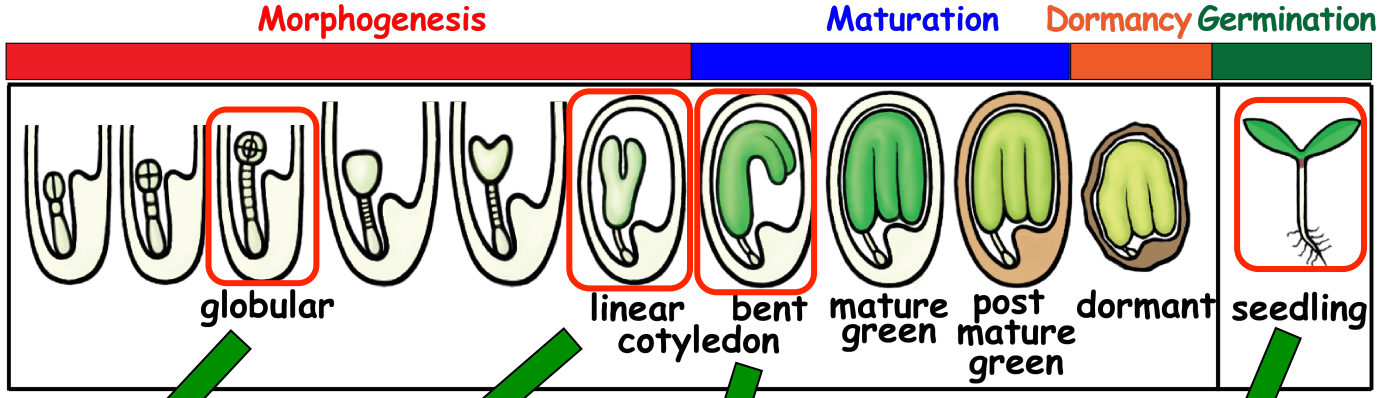
What is the DNA Methylation Level in ddcc Seeds?

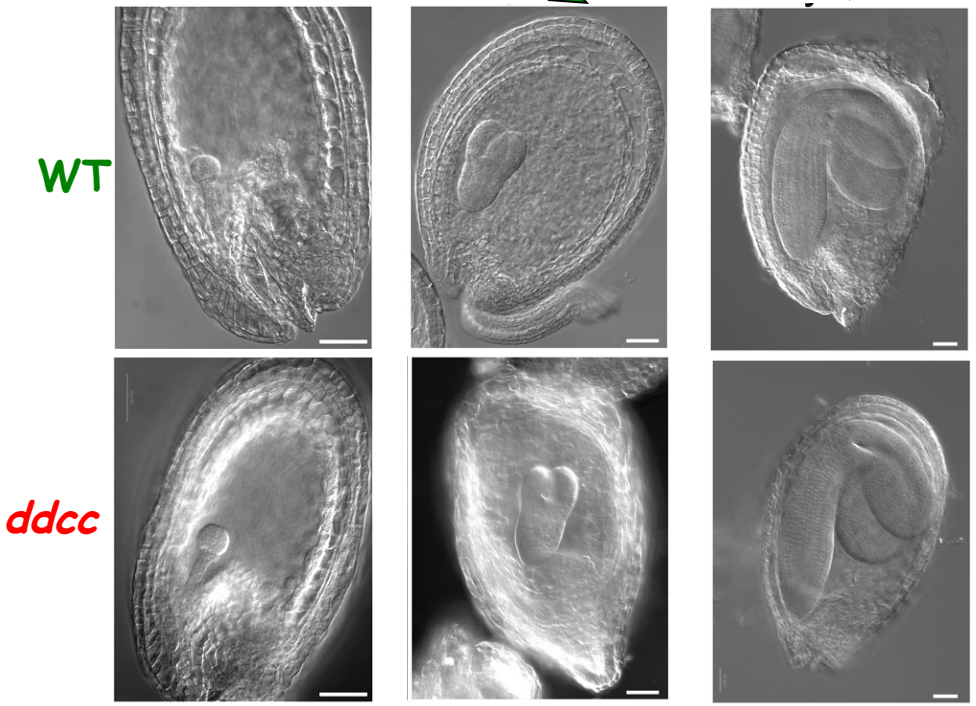


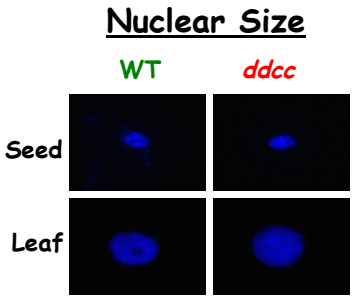


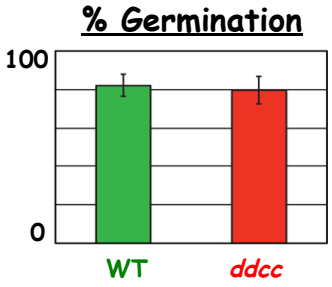
There Is No CHG and CHH Methylation!

The ddcc Mutant Seeds Develop and Germinate Normally!



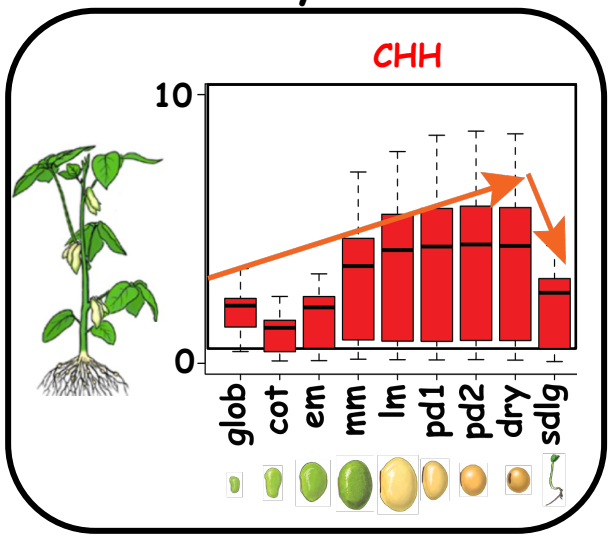




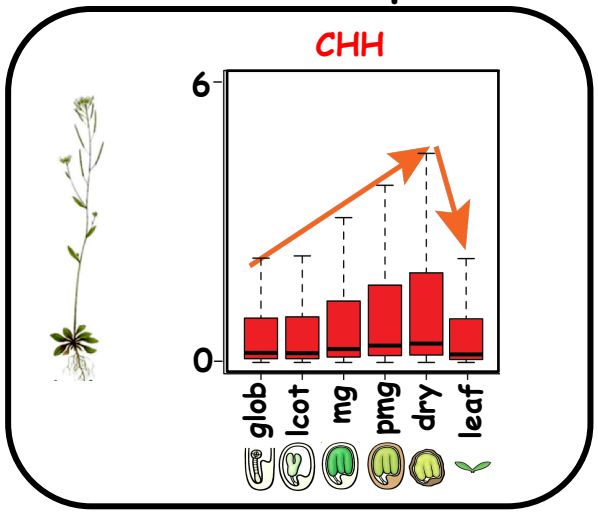


What Is the Biological Relevance of Increasing CHH Methylation During Seed Development?

Soybean



Arabidopsis



X Play a Role in Desiccation

X Play a Role in Dormancy

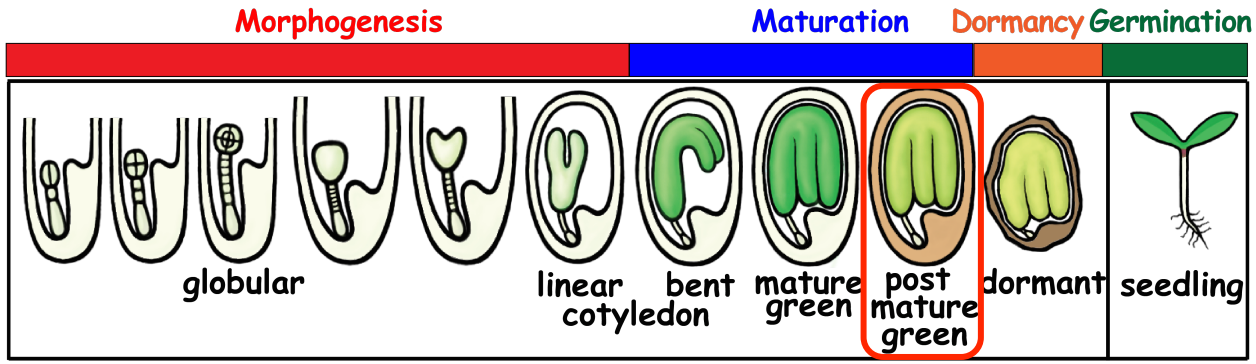
X Play a Role in Seed Morphogenesis

X Play a Role in Germination

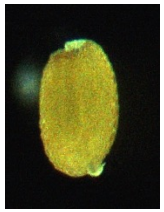
? Affect Gene Activities

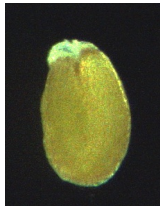
? Reinforce Transposon Silencing

Are Gene Activities Different in ddcc Mutant Seeds?



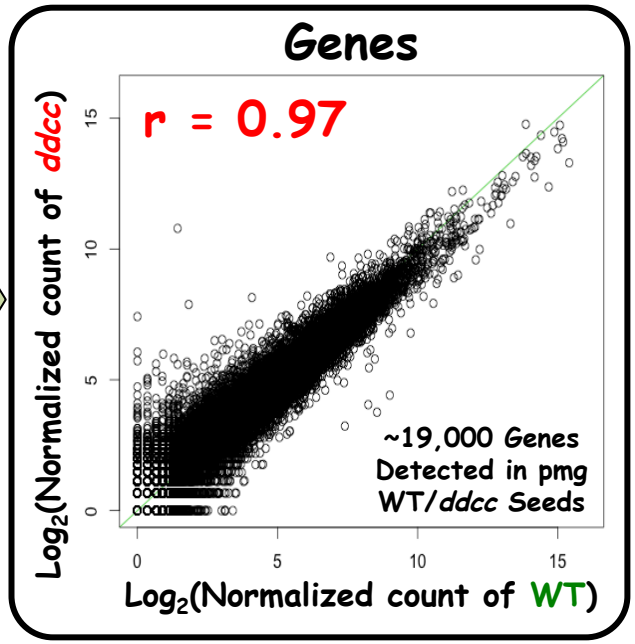
WT pmg Seed

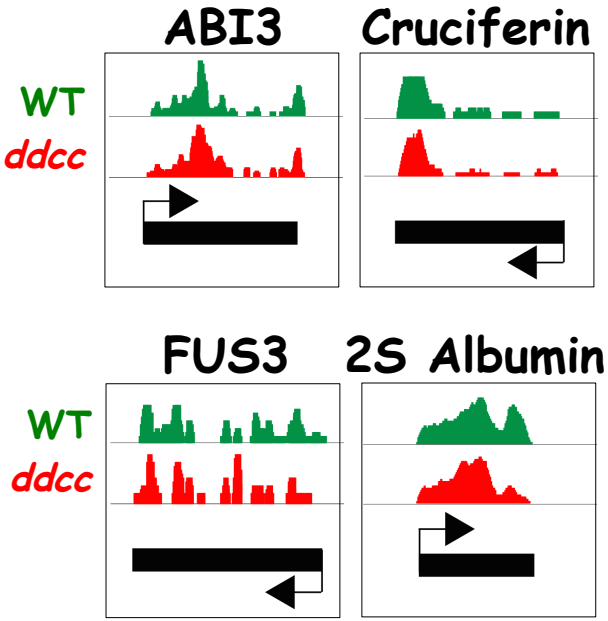




ddcc pmg Seed

RNA-Seq

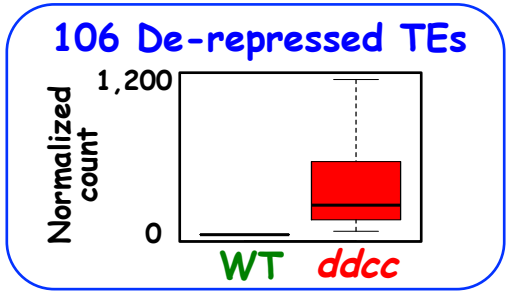


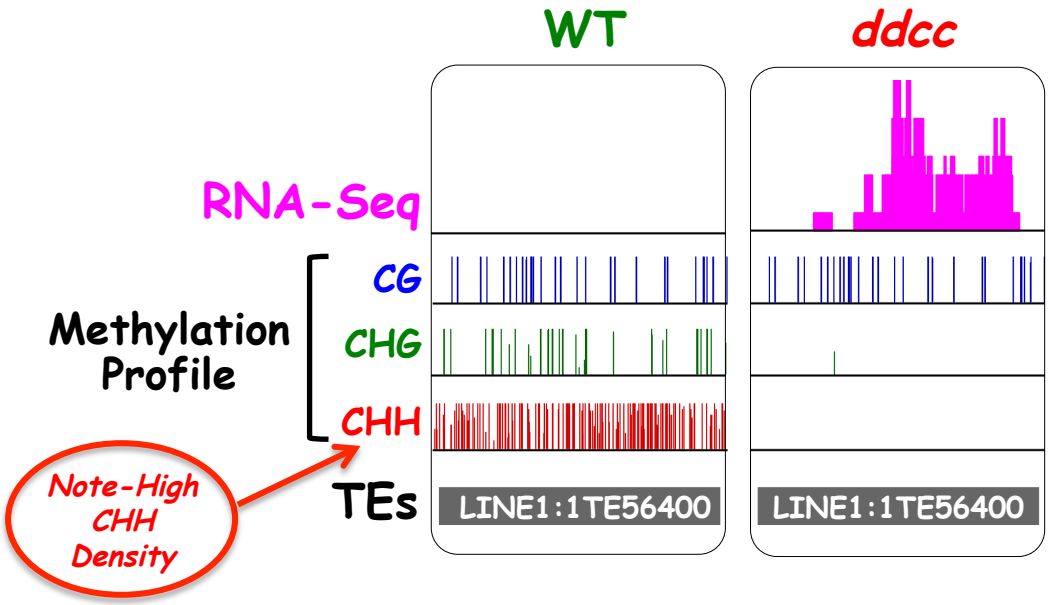


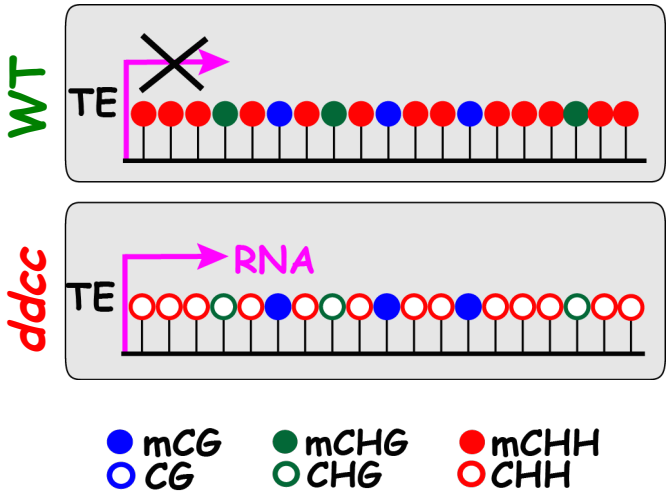
Gene Expression Is Not Affected in ddcc Mutant!

Are TE Activities Different in ddcc Mutant?

RNA-Seq → Differentially Expressed TEs →

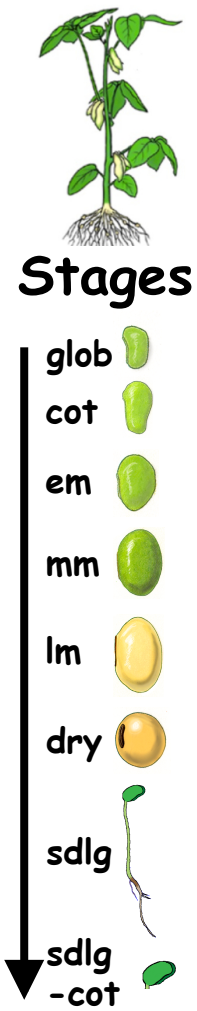


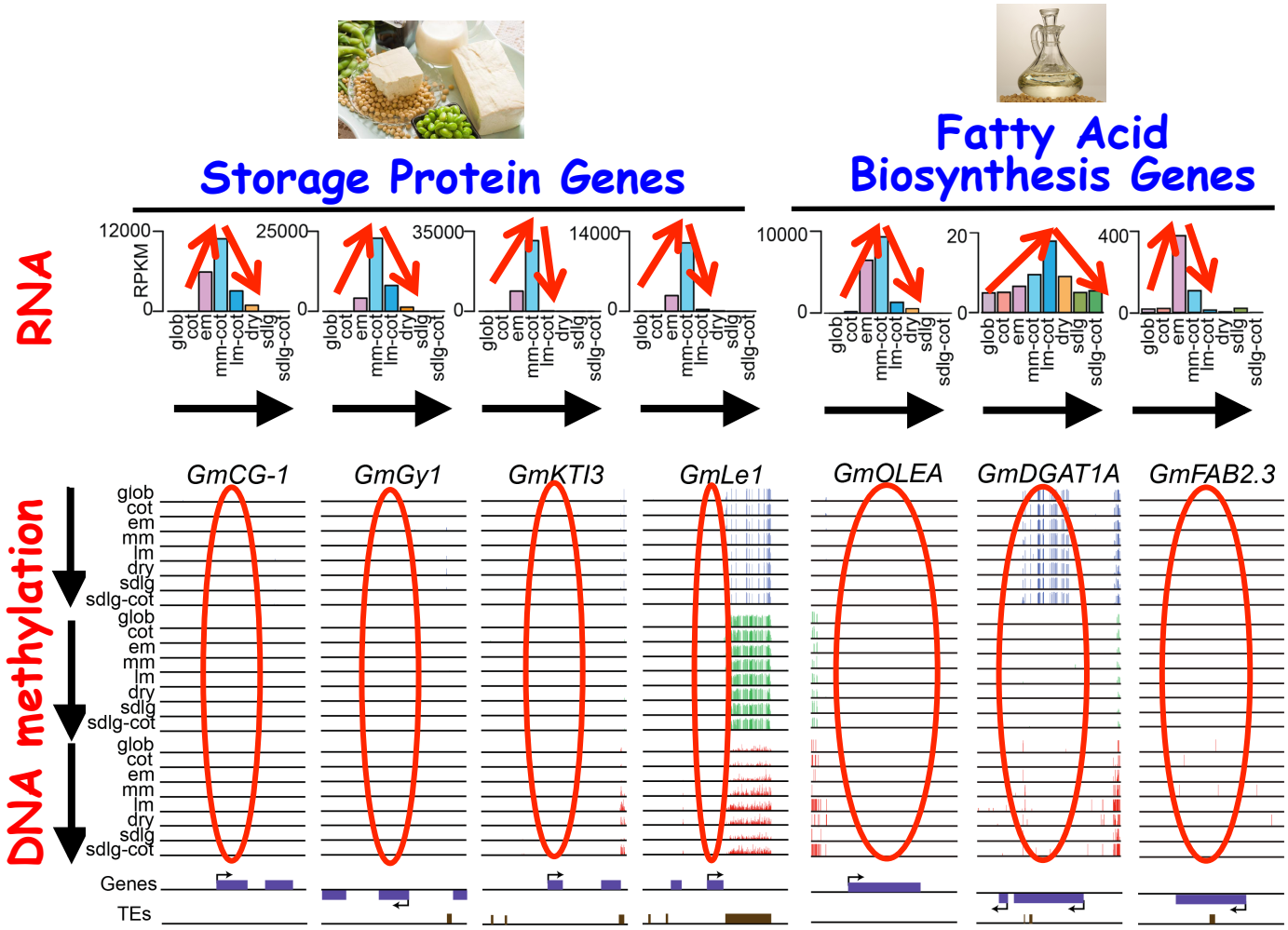




CHH Methylation May be a Failsafe Mechanism to Reinforce TE Silencing During Seed Development

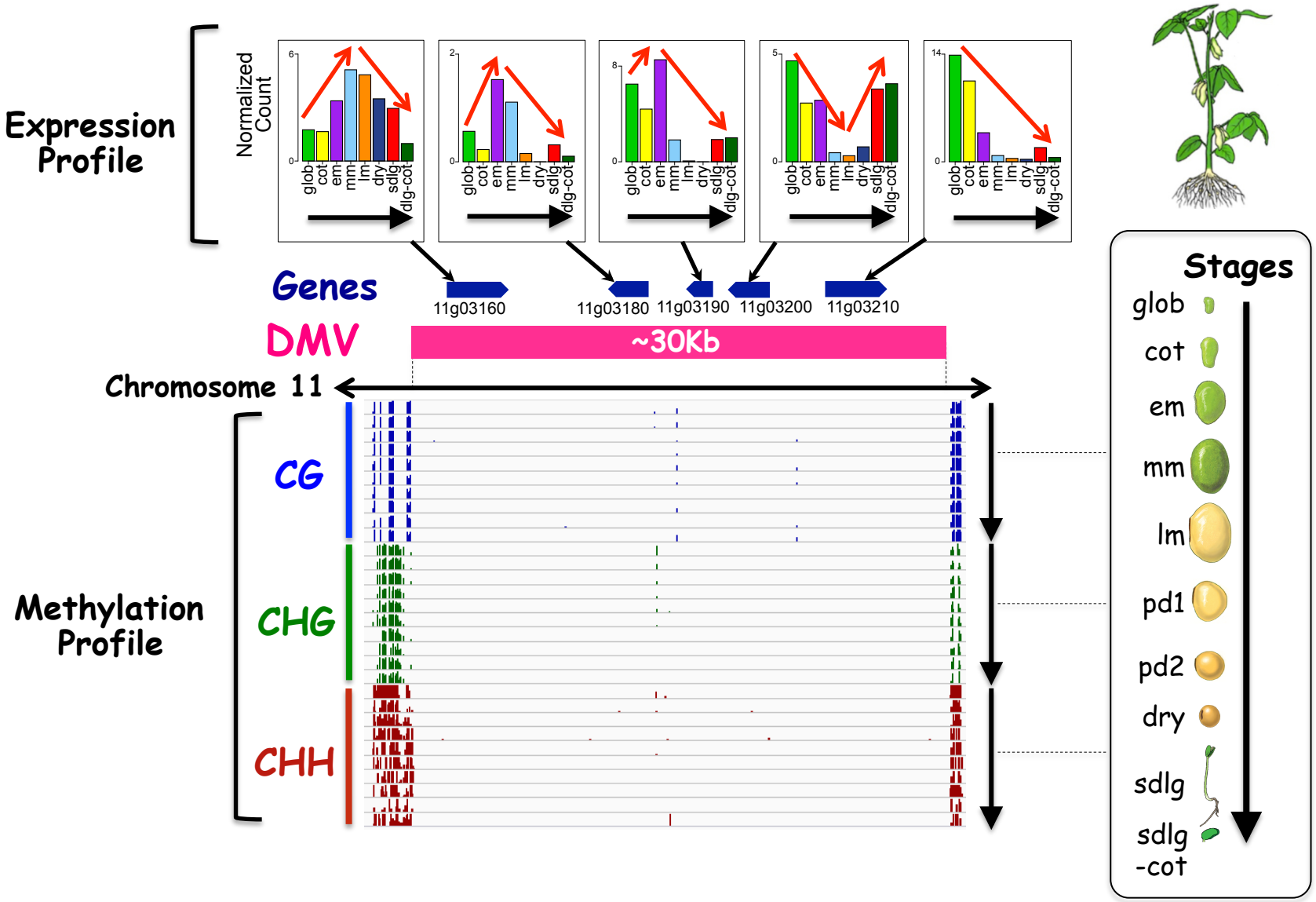
Are Expression Patterns of Major Soybean Food Reserve Genes Correlated With DNA Methylation Changes?





Activation and Repression of These Genes Are NOT Correlated With Methylation Changes!

Are There Other Seed DNA Methylation Valleys (DMVs) That Are Devoid of Methylation* in the Soybean Genome?

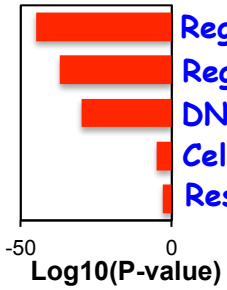


* DMV means an average methylation level <5% in all three contexts across ALL of seed development & germination

Yes…….Soybean Seed DMVs Contain Genes That Are Regulated During Development!

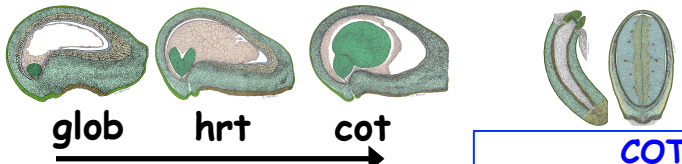
Total 21,669 DMVs identified in genome

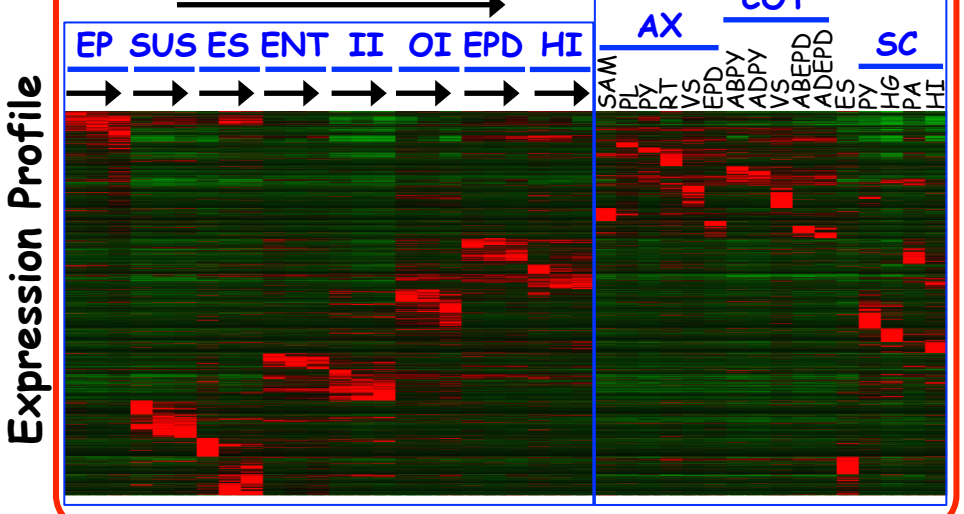
21% (~210Mb) of genome (~978Mb) are DMVs



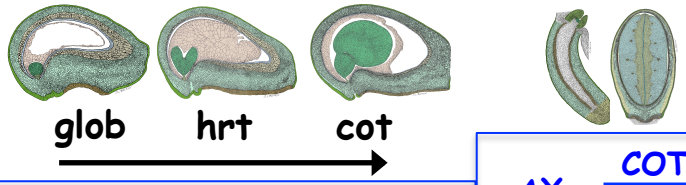
GO Analysis

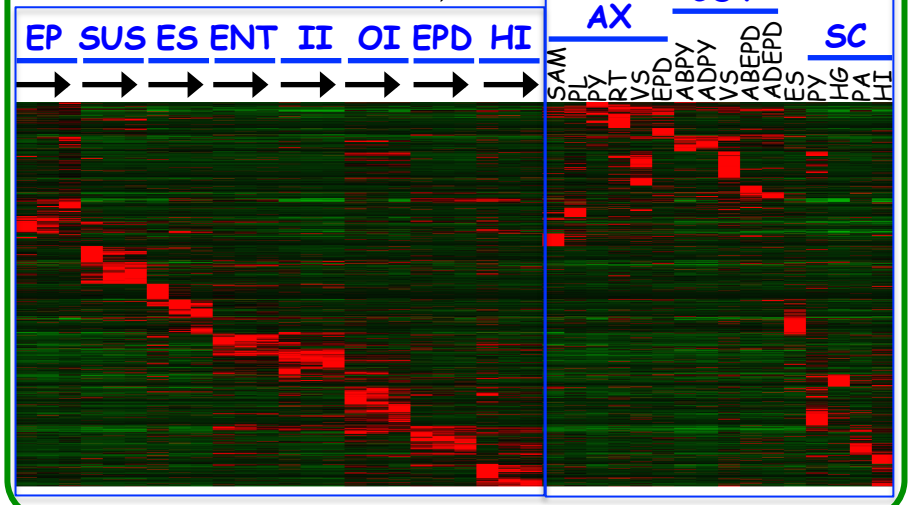
26% (14,328) of Genes in DMVs





38% (2,088) of Transcription Factor Genes in DMVs



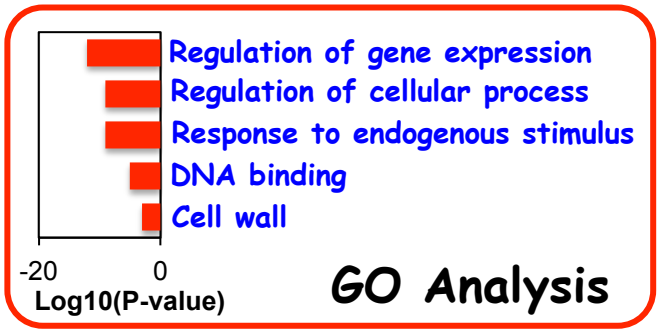


Are There Seed DMVs With Regulated Genes in Other Plant Genomes?

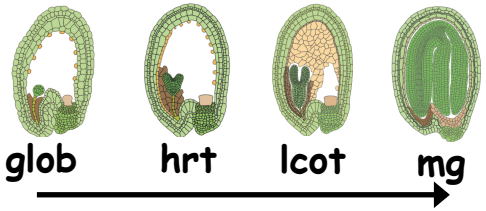
Arabidopsis

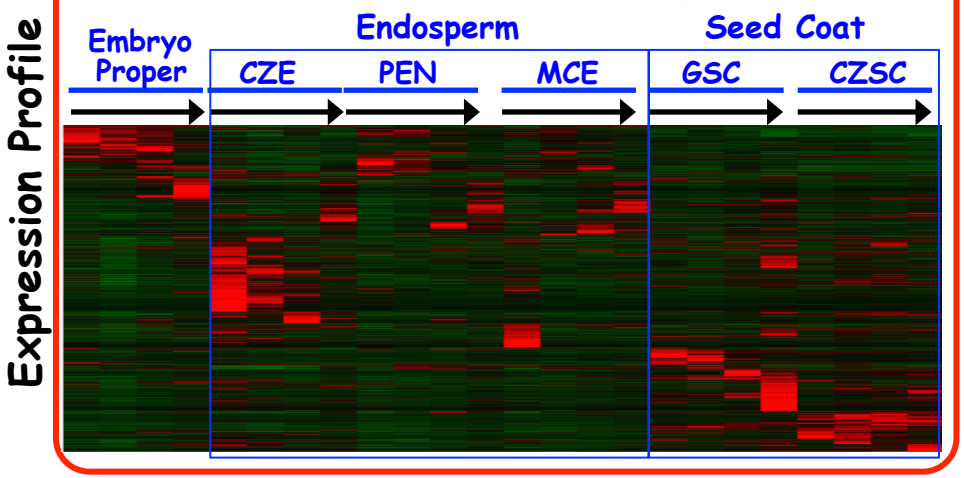
Total 4,829 DMVs identified in genome

41% (~49Mb) of genome (~120Mb) are DMVs

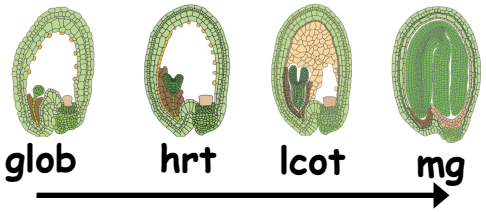


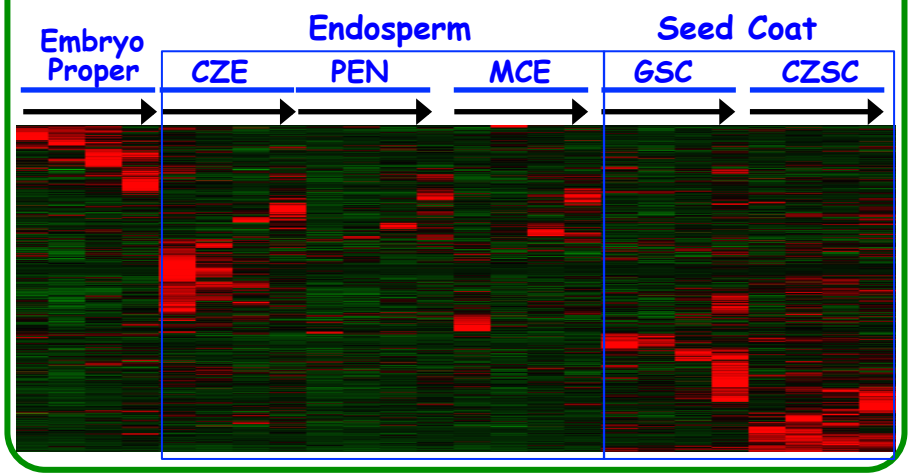
32% (8,710) of Genes in DMVs



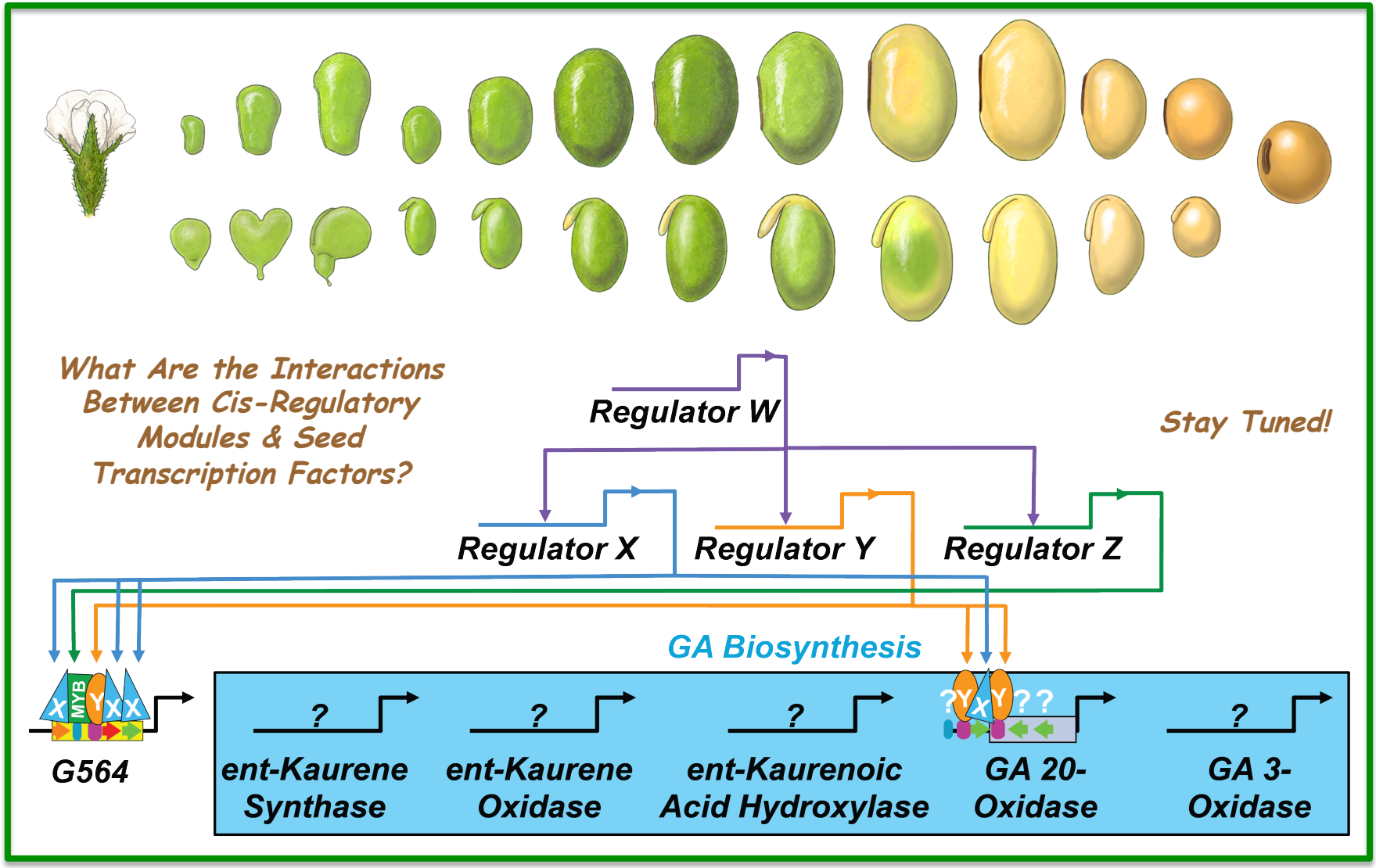


48% (835) of Transcription Factor Genes in DMVs





The Central Challenge is to Uncover the Regulatory Networks Required to Make a Seed!

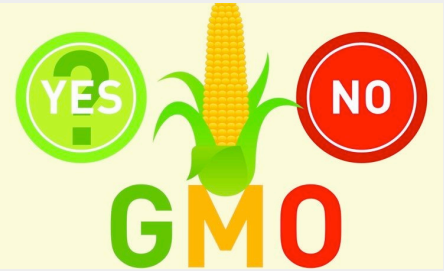


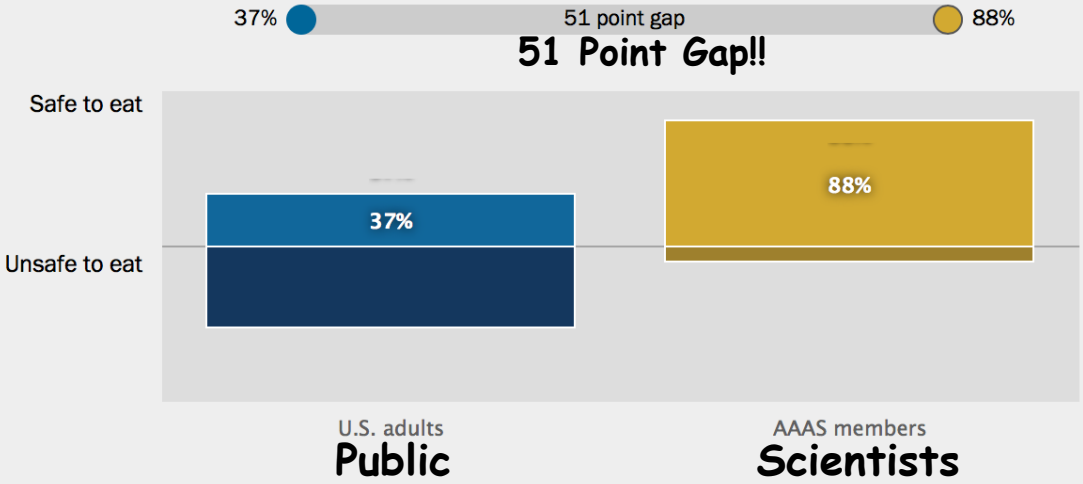
There Is Major Public Skepticism About GMOs!!!

PewResearchCenter

Public and Scientists' Views on Science and Society *2015*

▼ Safe to eat genetically modified foods

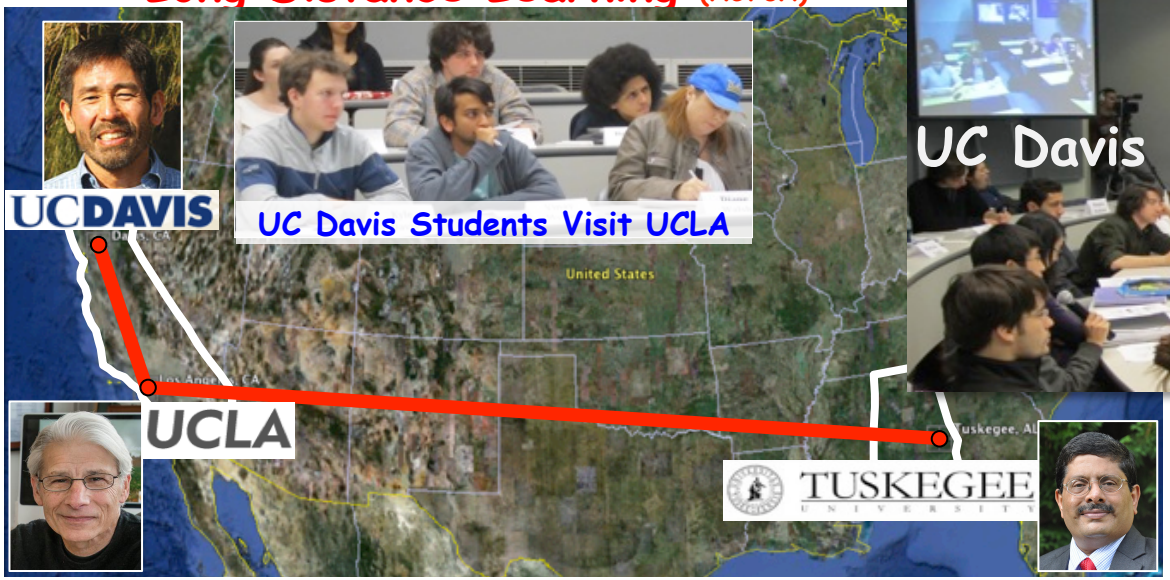




How Do We Change This?

Seeds of Hope: Using Long-Distance and Online Learning To Teach Genetic Engineering to Non-Science Students Across the US and the Globe!!

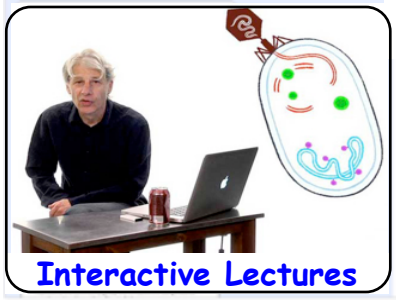
Long Distance Learning (HC70A)



UC Davis Students Visit UCLA

Tuskegee Students Visit UCLA

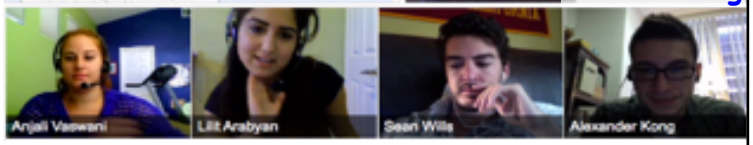
Online Teaching (MCDB70)



Interactive Lectures

Live Online Discussions

Dr. Goldberg



Participating Students

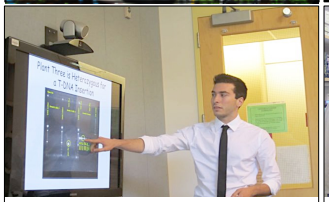
Gene Discovery Laboratory (HC70AL)

Weekly Meeting to Discuss Data

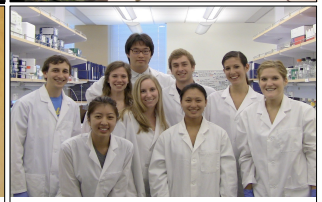
Identifying Knockout Arabidopsis Plants

Dissecting Embryos Under Microscope

Setting up PCR



Final Symposium



"Graduating" Class

Many Thanks To…..

UCLA

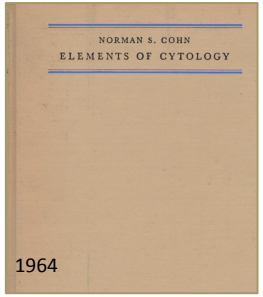
Matteo Pellegrini
Min Chen
Jer-Young Lin
Jungim Hur
Brandon Le
Kelli Henry
Allan Chung
Lauren Bowman

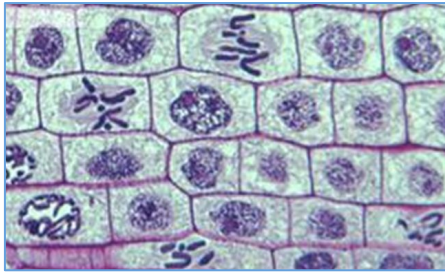
UC DAVIS

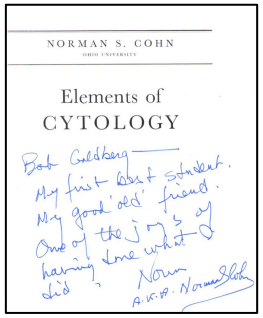
John Harada
Julie Pelletier
Ryan Kirkbride

NSF

AMERICAN SOCIETY OF PLANT BIOLOGISTS
INVENIAMVS VITALIA ARCANA PLANTARVM
1924







And Special Thanks To……

Professor Norman Cohn
Ohio University
1930-2016

Who Taught Me The Excitement of Discovery & The Art of Teaching