TAM66274: Ultra-low Gossypol Cottonseed

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Cotton Breeders Tour, Texas A&M Univ.
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Cotton Production and Exports 1,000 Metric Tons (2014-15)

	Production	Exports
World	**25,970**	
China	6,532	16
India	6,432	849
USA	3,553	2,330
Pakistan	2,308	120
Burkina Faso	290	256 (Gold: #1 and Cotton: #2)
Mali	231	152 (Gold: #1 and Cotton: #2)
Cote d'Ivoire	183	174
Cameroon	109	98
Benin	131	125 (Cotton: #1)
Chad	48	41
Togo	38	33
Tanzania	82	44
Egypt	114	33
Zimbabwe	54	38
Nigeria	44	11

Sources: FAS/USDA - May, 2015; PRB – 2016; http://atlas.media.mit.edu/en/

Cotton Production 1,000 Metric Tons (2014-15) & Global Hunger Index (2018)

	Production	Global Hunger Index
World	**25,970**	
China	6,532	25th
India	6,432	103rd
Pakistan	2,308	106th
Burkina Faso	290	89th
Mali	231	90th
Cote d'Ivoire	183	85th
Cameroon	109	71st
Benin	131	80th
Chad	48	118th
Togo	38	80th
Tanzania	82	95th
Egypt	114	61st
Zimbabwe	54	107th
Nigeria	44	103rd

Sources: FAS/USDA - May, 2015; PRB – 2016; http://atlas.media.mit.edu/en/

Rampant protein-calorie malnutrition in many poor countries

- cereal-based diets do not provide enough protein

• Brown rice	7.3% Protein (75 - 95% calories in some countries)
• Maize	9.8%
• Millet	11.5%
• Sorghum	8.3%
• *Wheat*	*10.6%*
• Cassava	1.0%
• Yam	2.0%

Ultra-low Gossypol Cottonseed (ULGCS)

From Conception to Deregulation of TAM66274

(https://www.aphis.usda.gov/brs/aphisdocs/17_29201p.pdf)

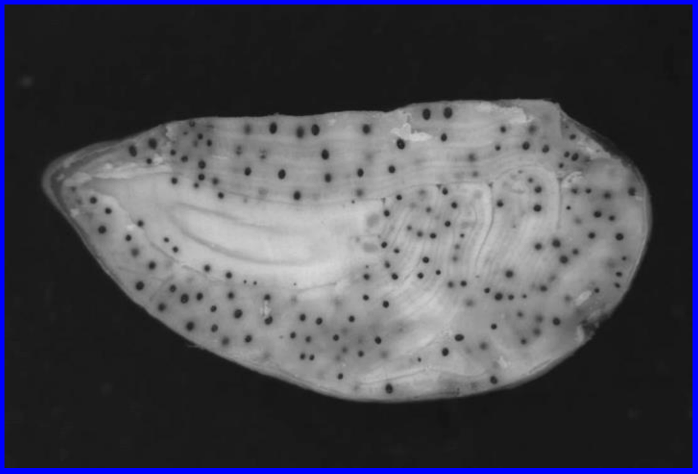
Global Cotton Lint Production in 2014 = 26 MMT

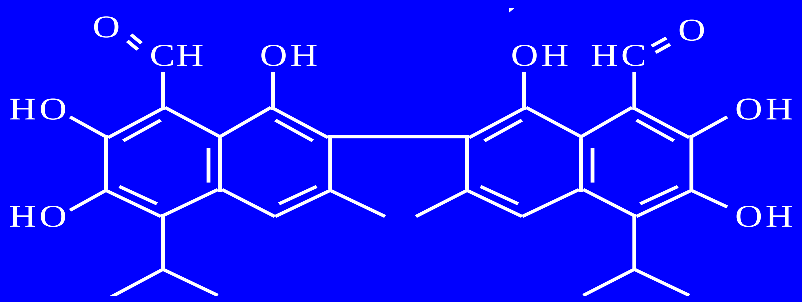
Global Cottonseed Production in 2014 = 47 MMT (~10.5 MMT Protein)

Can meet the protein requirements of 575 million people/year (50 g/day)

Presence of toxic gossypol prevents the use of cottonseed as food & also limits its use as feed

- Protein content of the seed is about 22.5%; good quality protein.
- Its use for human consumption or animal feed is limited by the presence of gossypol in the seed glands.
- Gossypol is toxic to non-ruminant animals, including pigs, chicken and humans. It damages heart and liver.
- Meal used as feed for the cattle – gossypol binds to lysine thus reducing the nutritional value of the meal.
- Gossypol typically removed during the processing of cottonseed to obtain edible oil.





Gossypol

Current Use of Cottonseed in Human Nutrition

- Cottonseed Oil (gossypol removed during the processing)

- Feed for cattle either directly as seed or as meal following oil extraction (in limited quantities)

Gossypol is Toxic to Monogastric Animals

Cows are Inefficient in Converting Crude Protein into Animal Protein

- 3.66 kg of crude protein is converted to 0.83 kg of milk protein per day (PCR = **4.4**)

- Feed conversion ratio (FCR): **5.8**
- Protein Conversion Ratio (PCR): **20**

FCR: Feed consumed / weight gain

PCR: Feed Protein consumed/ Edible Animal Protein Produced

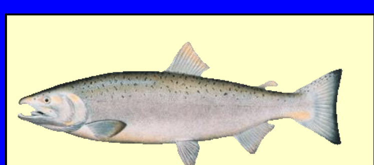
Protein Conversion Ratio

20

5.7

4.7

2.6



4.6

5.7

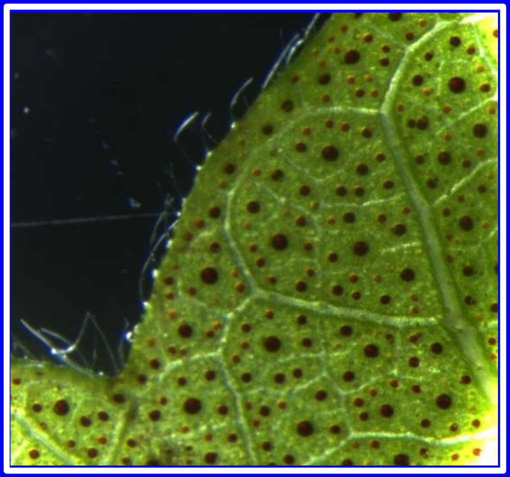
8.8

7.7

Tilman and Clark (2014) Nature 515:518
Claude Boyd (2005) Global Aquaculture Advocate 73-74

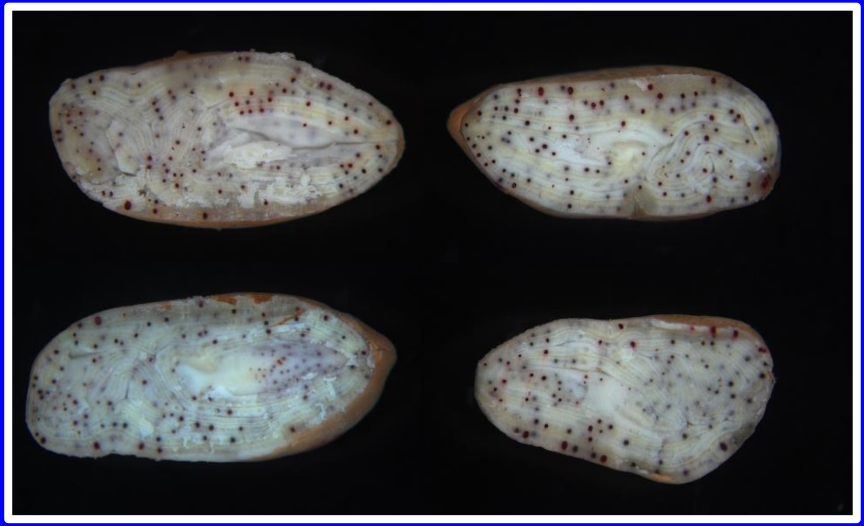
Attempts to Eliminate Gossypol Using Plant Breeding

- McMichael discovered a GLANDLESS mutant of cotton in 1954
- Seed from glandless cotton was gossypol-free
- National and International breeding programs launched to transfer the trait to commercial varieties
- Animal nutrition studies showed glandless cottonseed to be a relatively good source of feed for pigs, chicken, and shrimp
- Glandless cottonseed was even considered fit for human consumption



Wild-type 'Glanded' Cotton

Mutant 'Glandless' Cotton

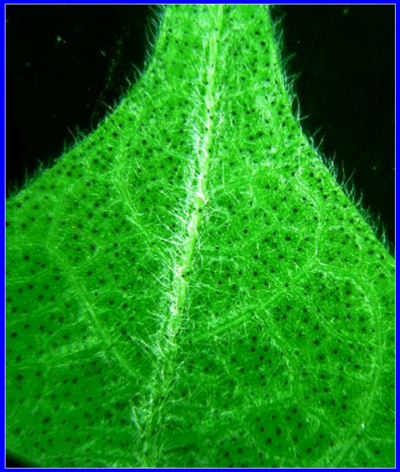
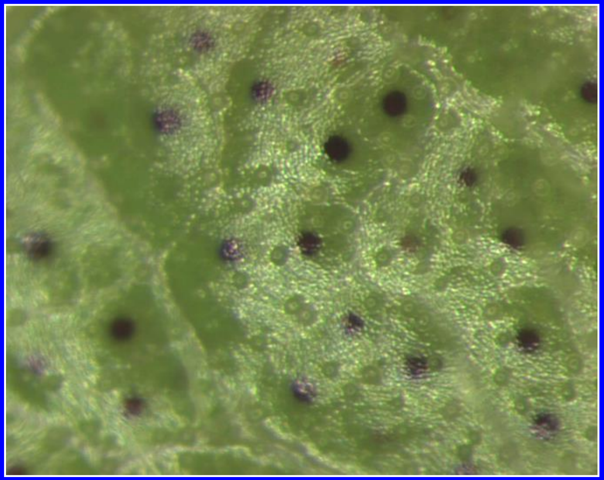


Gossypol: 10,000 ppm

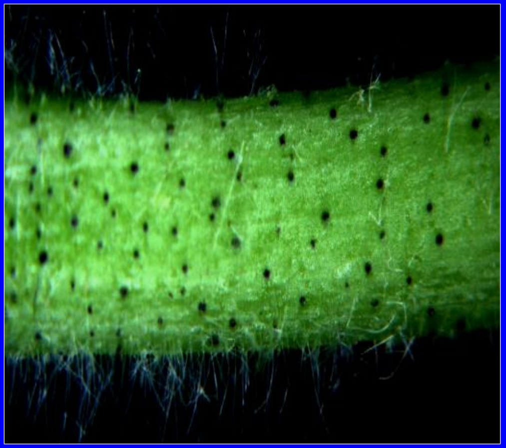
Gossypol: 0-10 ppm

Gossypol and other related terpenoid aldehydes present in the glands in leaves, stem, and floral parts confer resistance to insect pests

These terpenoids are also induced in response to pathogens and serve as phytoalexins





Traditional Insect Pests that Cause Greater Damage to Glandless Cotton

Cotton Bollworm (*Helicoverpa zea*)
Tobacco Budworm (*Heliothis virescens*)
Beet Armyworm (*Spodoptera exigua*)

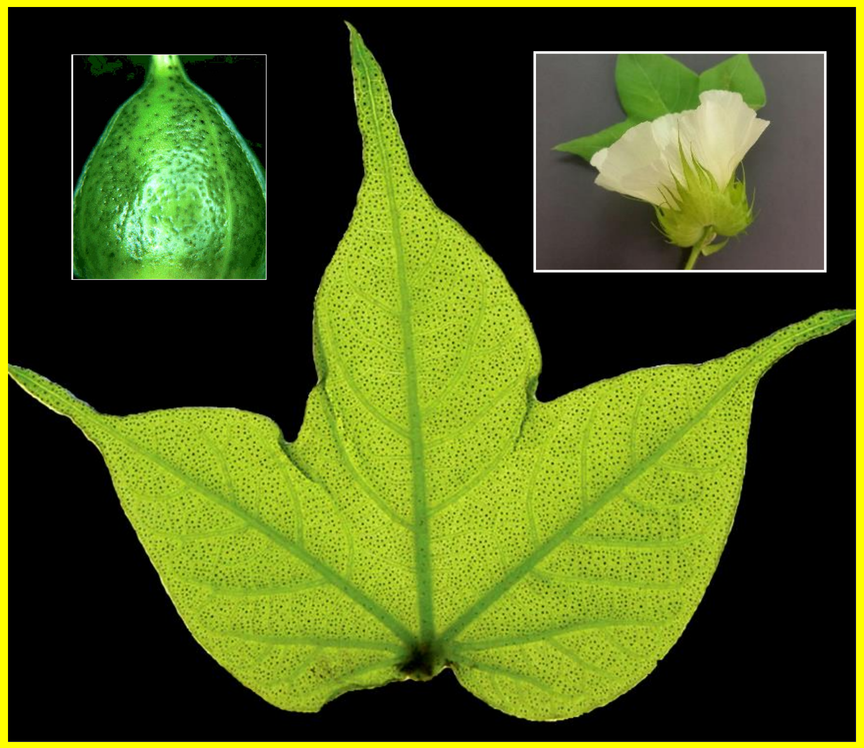
Non-traditional Insects that Damage Glandless Cotton

Black Fleahopper (*Spanogonicus albofasciatus*)
Pillbug (*Porcellia sp*.)
Spotted Cucumber Beetle (*Diabrotica undecimpunctata*)
Grape Colaspis (*Maecolaspis flavida*)
Green Dock Beetle (*Gastrophysa cyanea*)
Blister Beetle (*Epicauta vittata*)

- Currently, there is no large-scale cultivation of glandless cotton.

Desired Trait in Cotton

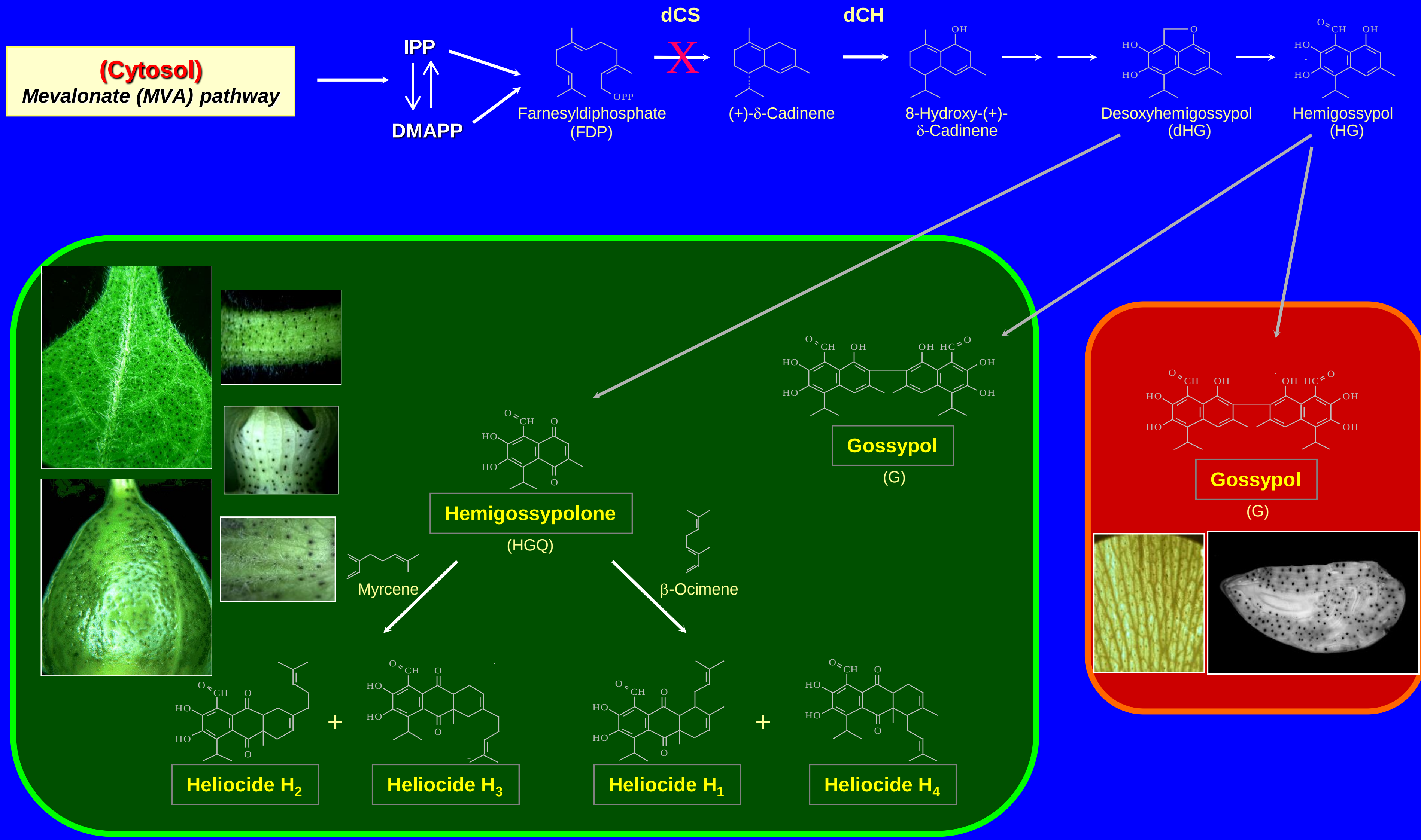
Seeds:
Gossypol <450 ppm (0.45 µg/mg)
(FDA Guidelines)



Leaf & other Organs:
Full Compliment of Gossypol and Related Terpenoids for Defense Against Insects and Diseases

Seed-specific, RNAi-mediated Silencing of δ-cadinene synthase Gene

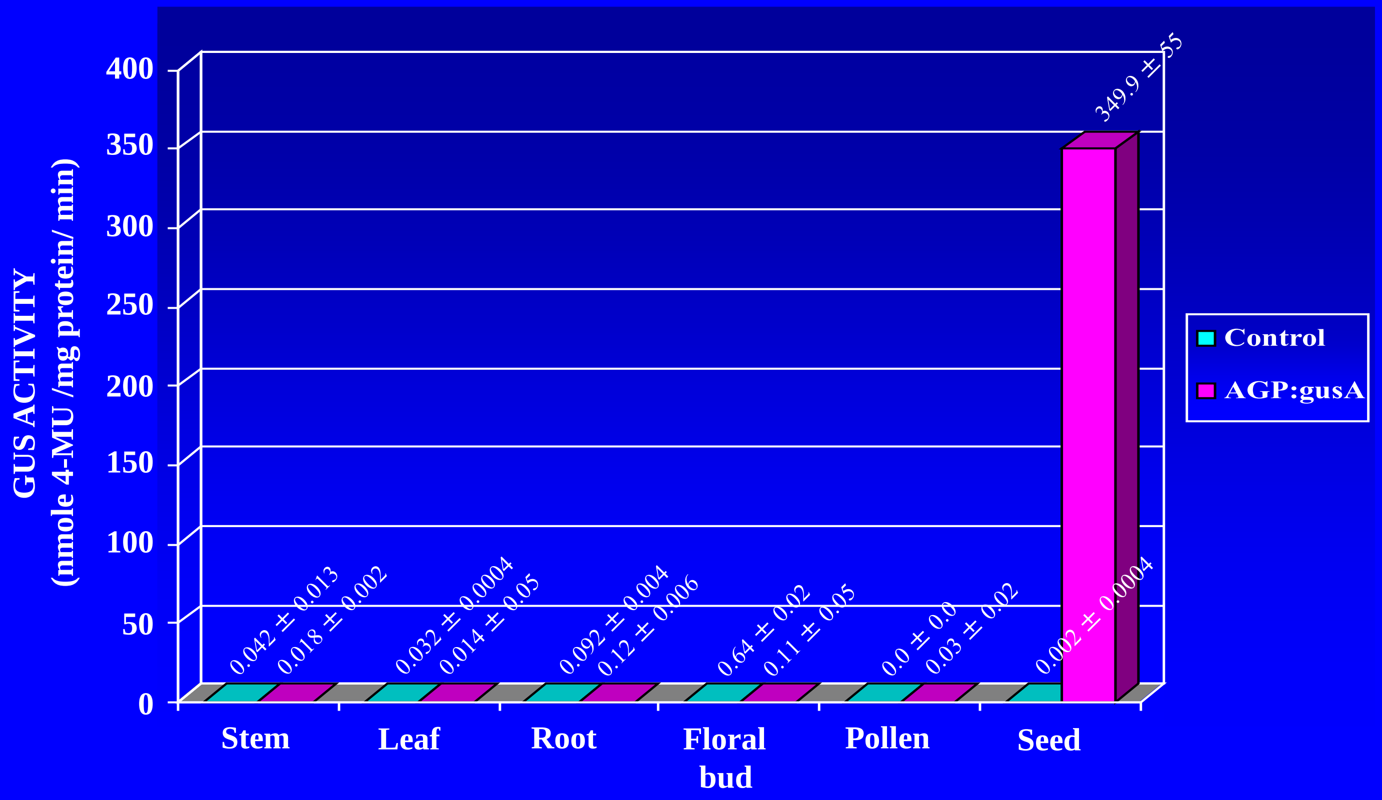
(α-Globulin Promoter::RNAi against δ-Cadinene Synthase)



dCS = δ-cadinene synthase

dCH = δ-cadinene hydroxylase

Seed-specific expression of AGP::*gus*A in cotton

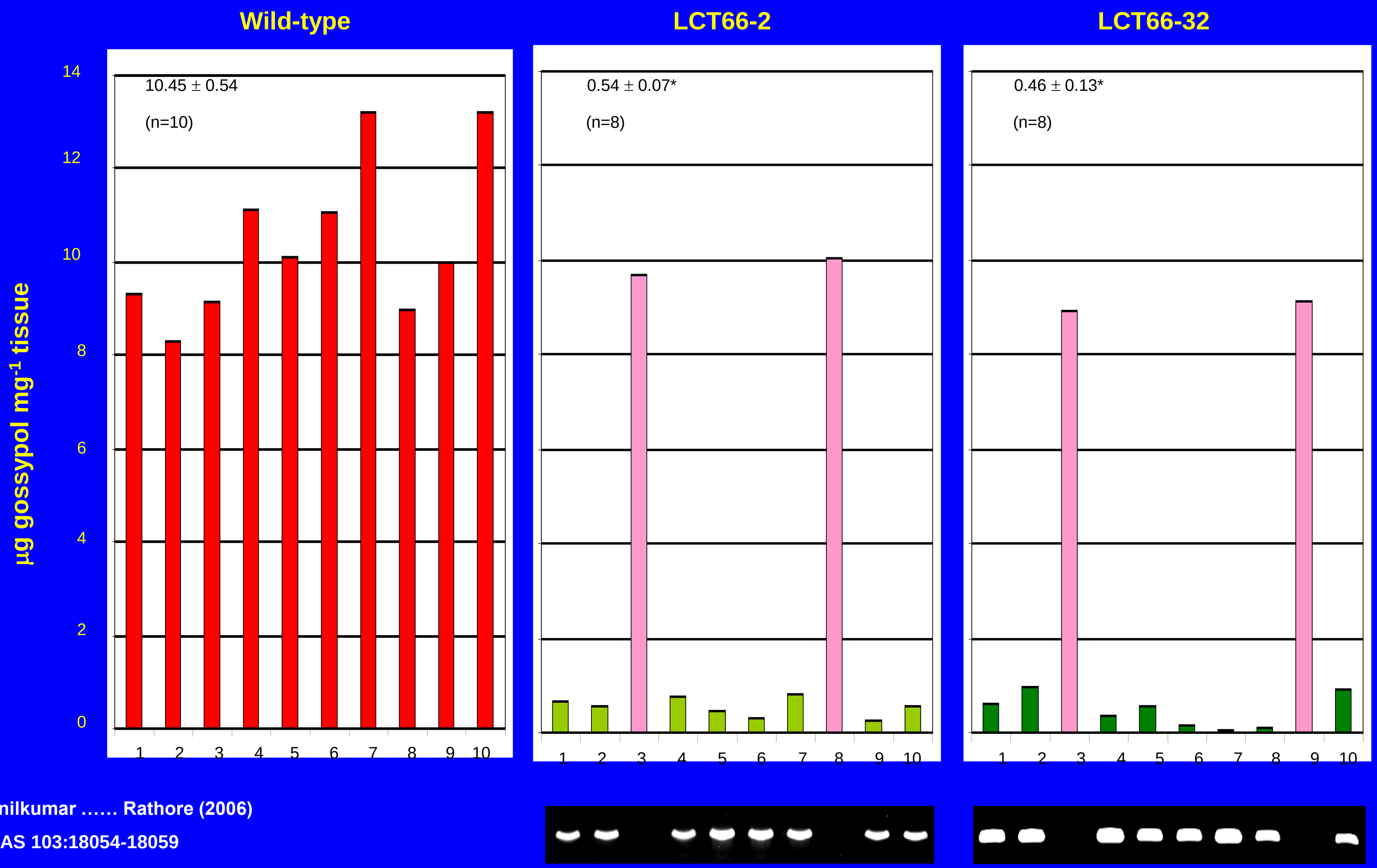


Sunilkumar …… Rathore (2002)

Transgenic Research 11: 347-359

U.S. Patent #7,626,081

Gossypol Levels in Individual Wild-type and T1 Cottonseeds

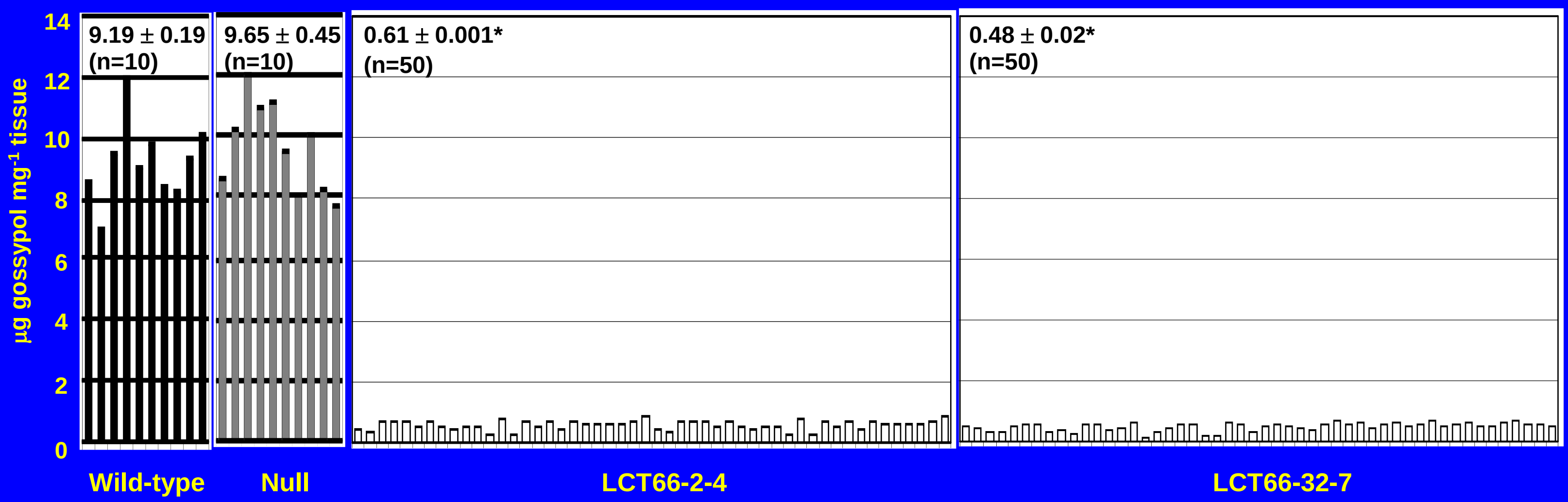


Sunilkumar …… Rathore (2006)

PNAS 103:18054-18059

U.S. Patent #7999148

Gossypol Levels in Individual Wild-type, Null Segregant, and T2 Cottonseeds

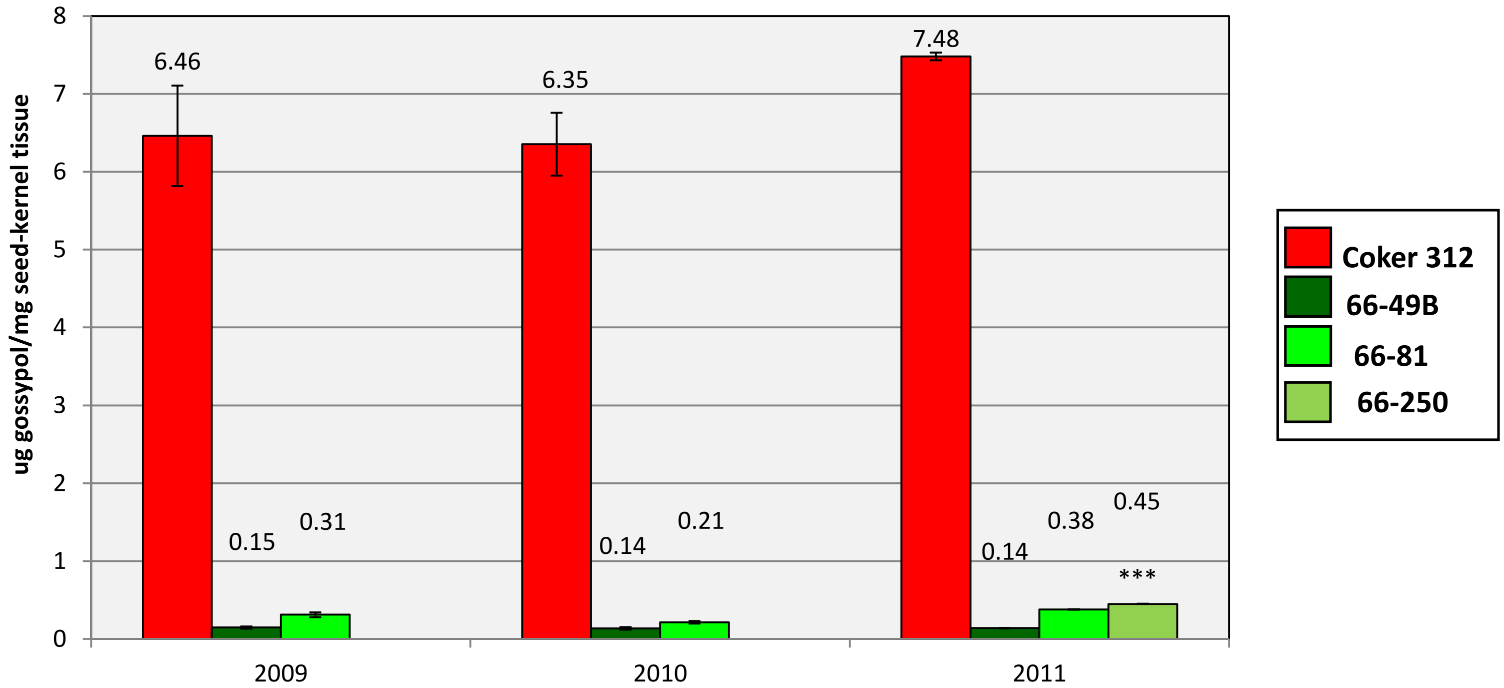


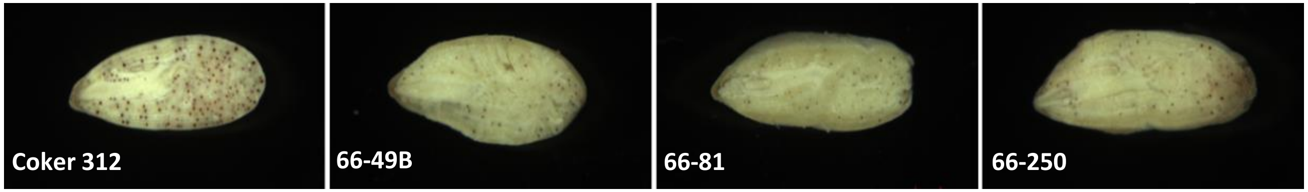
Sunilkumar …… Rathore (2006)

PNAS 103:18054-18059

U.S. Patent #7999148

Seed gossypol content of field-grown plants

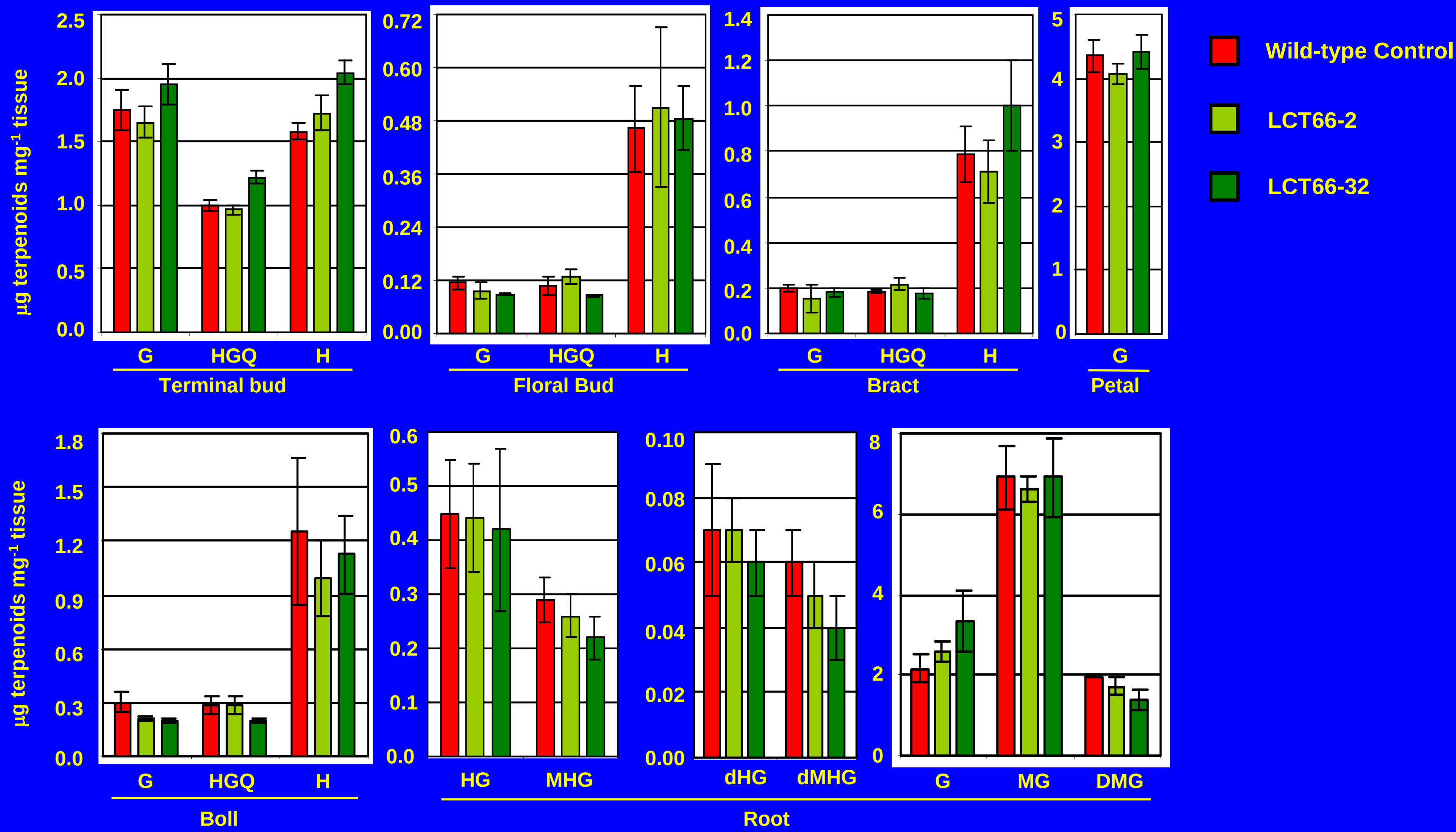




ULGCS Trait is stable under field conditions

Palle ----- Rathore (2013) Plant Biotech. J.

Levels of Gossypol and Related Terpenoids in Terminal Bud, Floral Bud, Bract, Petal, Boll, and Root Tissues



ULGCS trait is completely tissue specific

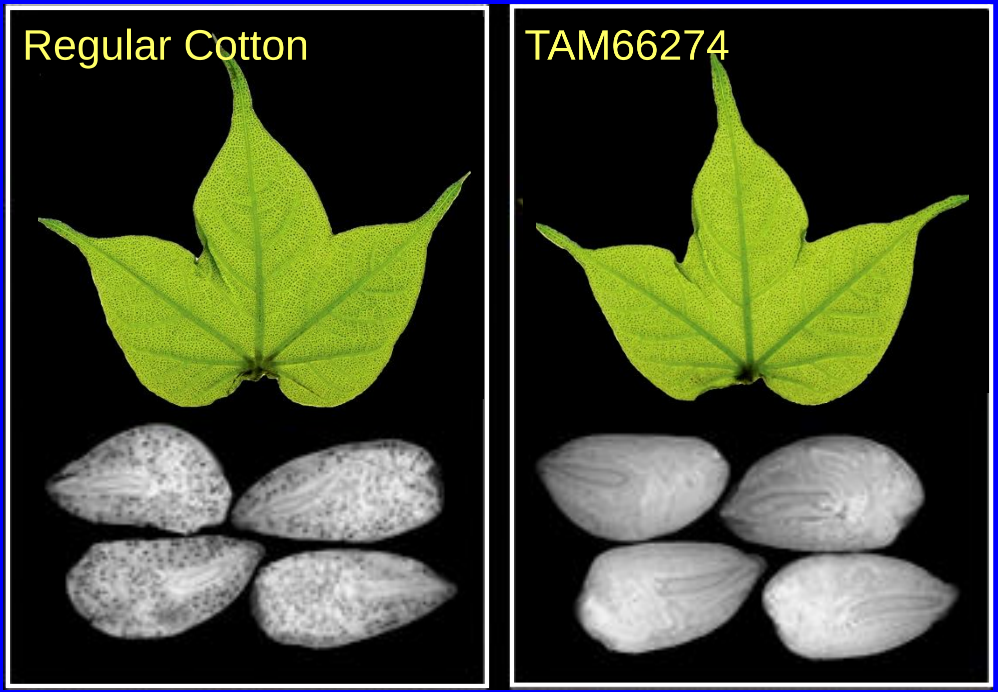
Sunilkumar …… Rathore (2006)
PNAS 103:18054-18059
U.S. Patent #7999148

Deregulation of TAM66274
(Steps since 2006)

- ✓ Generate many more additional events, test for desired phenotype
- ✓ Molecular analyses – Southerns, insert localization, silencing of the target gene
- ✓ Biochemical analysis
- ✓ Inheritance analysis
- ✓ Agronomic performance trials (8 trials in 3 states)
- ✓ Seed composition analysis
- ✓ Fiber quality analysis
- ✓ USDA-APHIS petition & FDA dossier submitted (2017)

TAM66274: Ultra-low Gossypol Cottonseed (ULGCS)

(Seed-specific, RNAi silencing of δ-cadinene synthase gene)



- Deregulated by USDA-APHIS (Oct. 17, 2018)
- Awaiting FDA approval

- **Gossypol levels reduced from ~10,000 ppm to ~300 ppm, well below levels considered safe for human consumption by FDA (450 ppm) and FAO/WHO (600 ppm)**

- **Foliage and Floral parts contain normal levels of gossypol for protection against pests**

https://www.aphis.usda.gov/brs/aphisdocs/17_29201p.pdf

Commercial Cotton	Glandless Cotton	TAM66274
Seed Gossypol: 10,000 ppm Insect Protected Foliage	Seed Gossypol: 10 ppm No insect protection	Seed Gossypol: 300 ppm Insect Protected Foliage & Floral Tissue

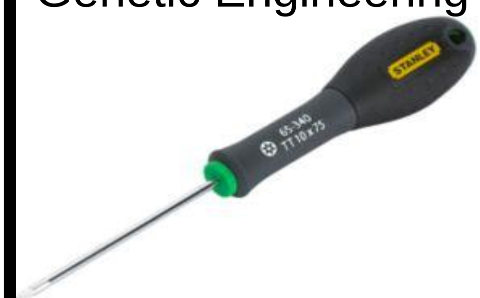
Seed kernel

Leaf

Problem: Seed Gossypol

Plant Breeding

Genetic Engineering



Can gene editing technology (CRISPR/Cas9) be used to achieve the same objective?

- Targeting the δ-cadinene synthase gene will not work (large gene family, RNA-seq analysis shows expression of at least 10 genes in the embryo)

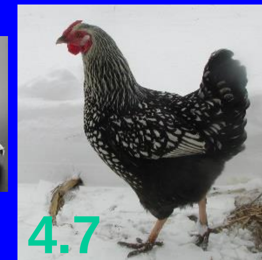
- Even if another, single copy target gene is identified, simple knockout of this gene will not achieve tissue specificity

- CRISPR interference is a possibility, but the plant will still be considered transgenic because of the need for tissue-specific expression of guide-RNA and CRISPR through a transgenic cassette

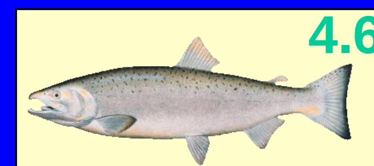
STV474 x TAM66274 – BC3F4 Planted for Seed Increase in North Carolina in 2019

Possible uses of meal from ULGCS to combat protein malnutrition

- Feed for poultry and swine



- Feed for aquaculture species

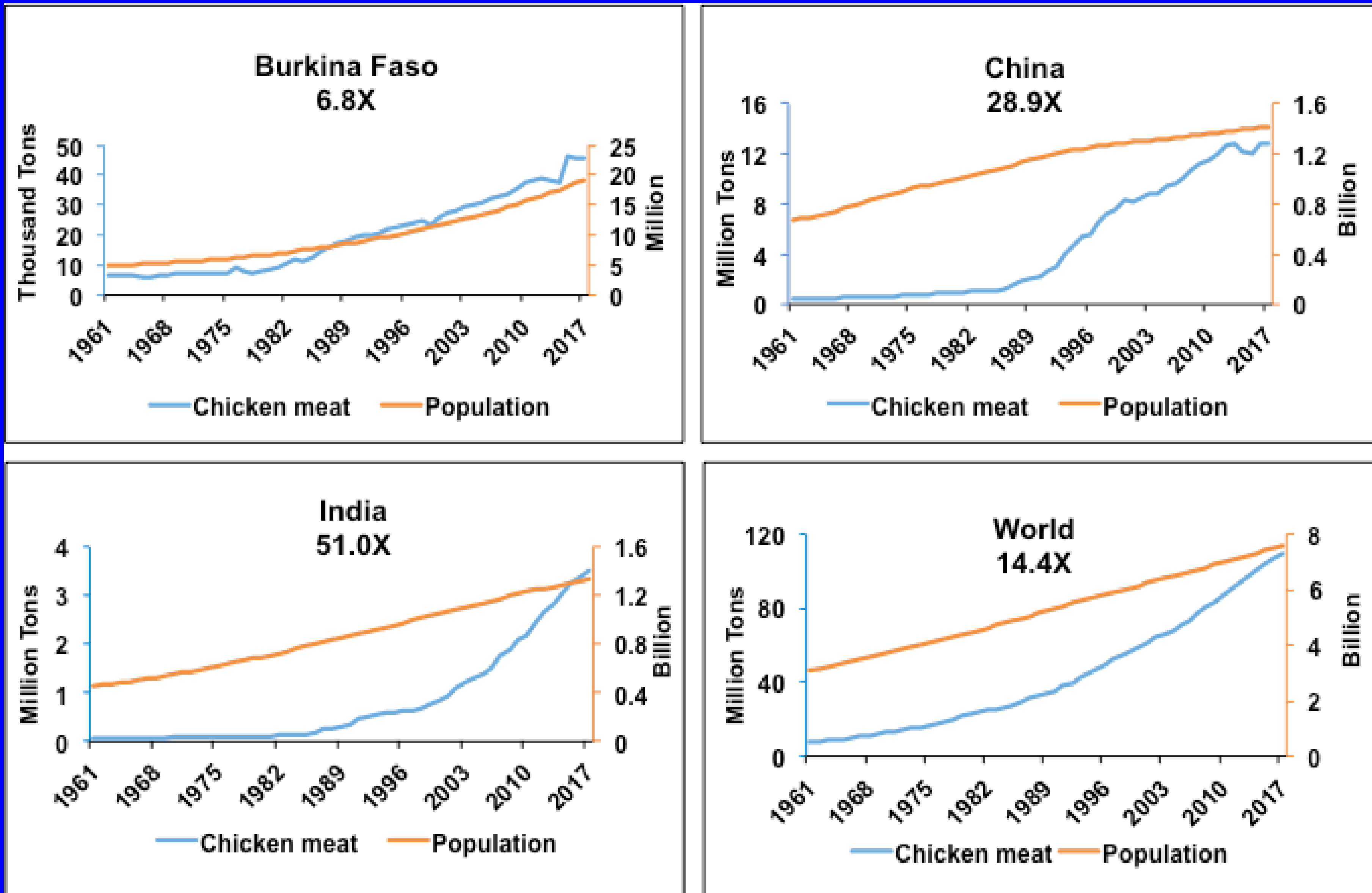


- Direct use as food

  (fortify flour for protein enrichment,

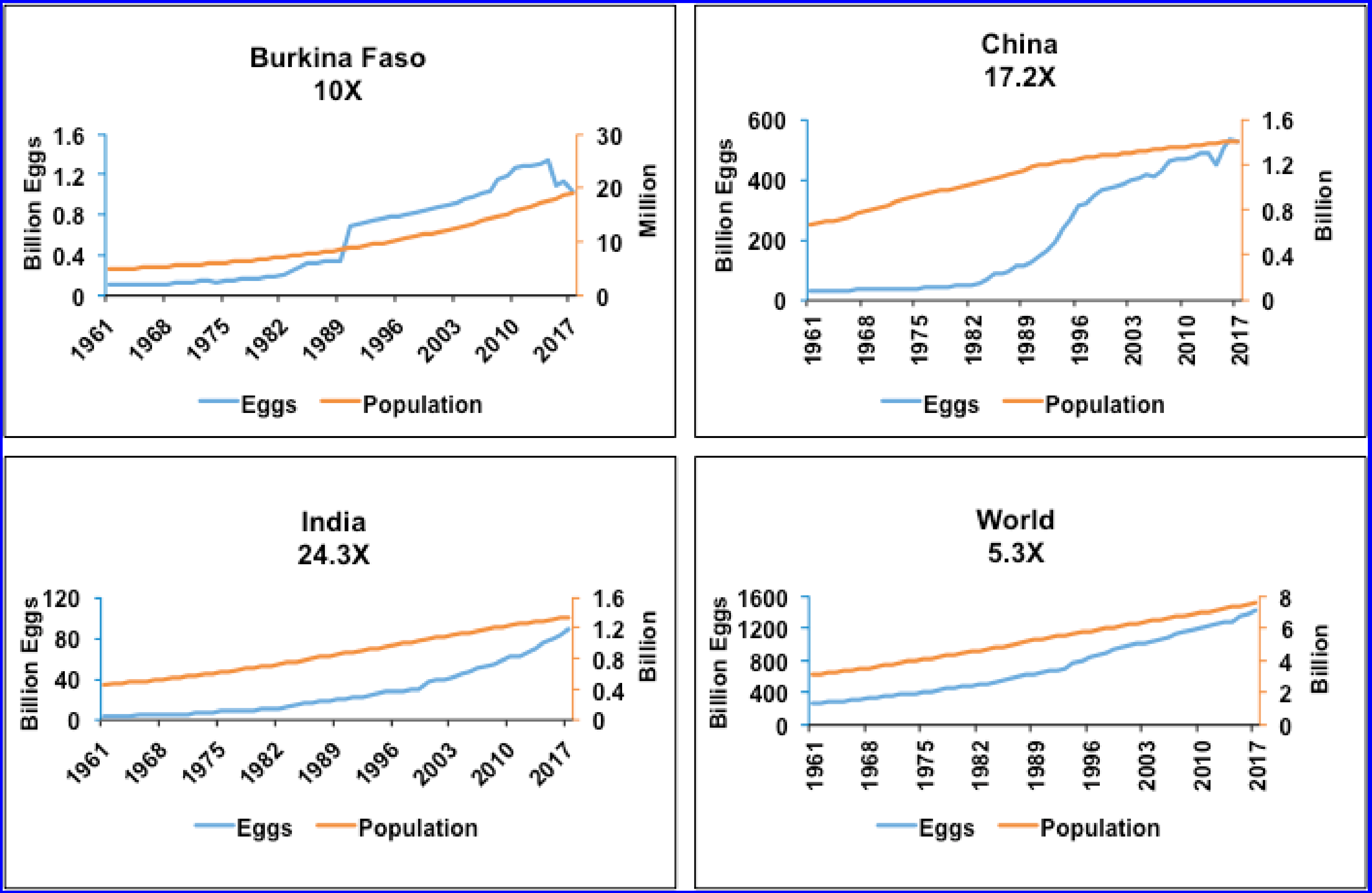
  Paruthi Paal)

Rising Demand for Feed by Poultry Industry (Broiler Production)



FAOStat (2019)

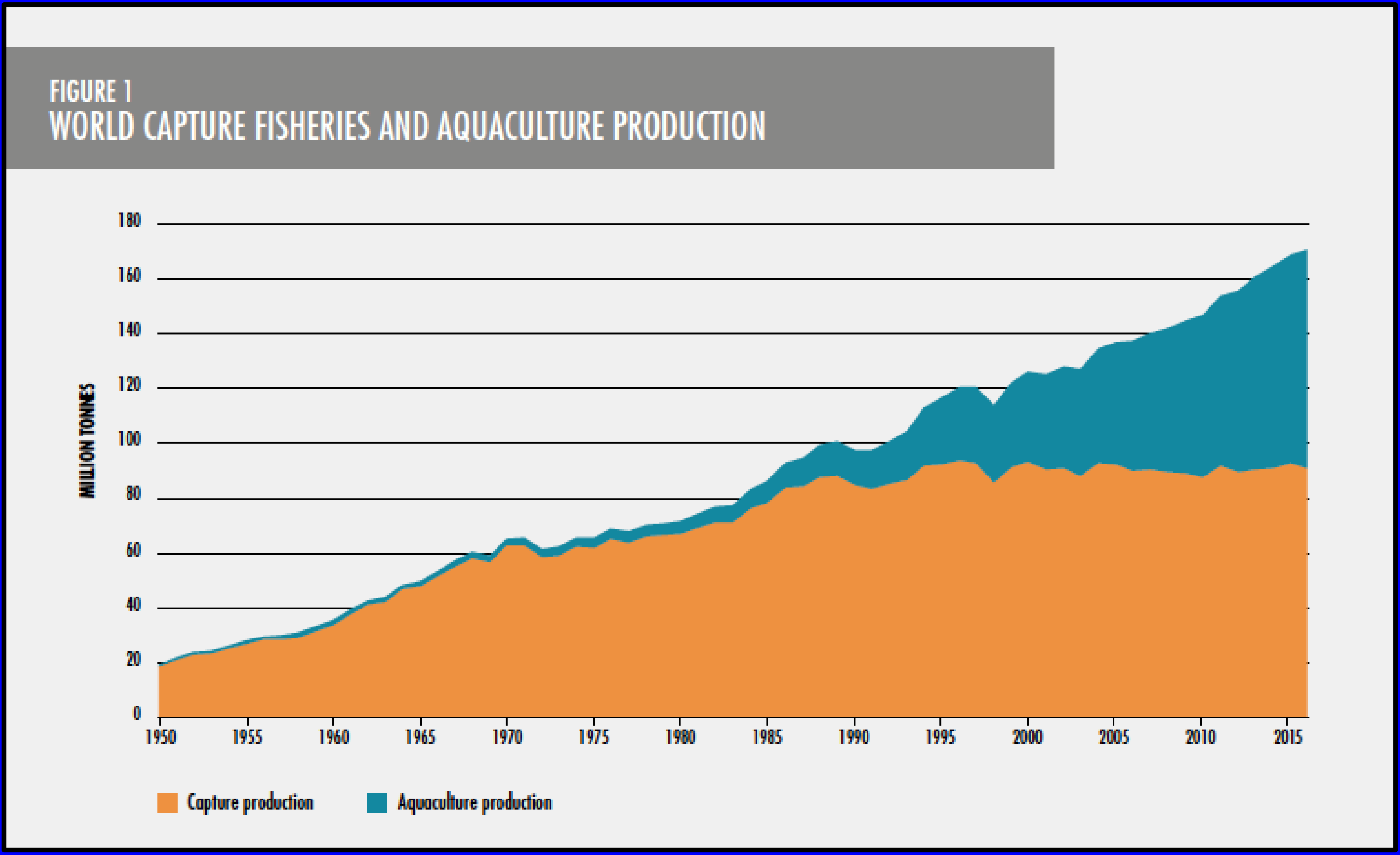
Rising Demand for Feed by Poultry Industry (Egg Production)



Protein in Global Cottonseed Output = Protein in 1.7 Trillion Eggs
Global Annual Egg Production = 1.4 Trillion

FAOStat (2019)

Aquaculture Meets Fish Demand as Ocean Catches Decline



Source: The State of Fisheries and Aquaculture; FAO 2018

Rising Demand for Feed by Aquaculture Industry

- **Aquaculture is the fastest growing food production system globally (8% growth)**

- **Aquaculture projected to supply >60% of fish for human consumption by 2030**

- **Demand for fishmeal and fish oil for aquaculture expected to increase, however, the supplies likely to decrease**
  - **Fishmeal price will rise by 90%**
  - **Fish oil price will rise by 70%**

- **Great need for substitutes for fishmeal and fish oil**

  - **ULGCS meal (41% protein) and oil could serve as a good substitute**
  - **Some cottonseed is already used in aquafeeds**

Shrimp Feeding Study: Richardson et al. (2016)
Southern Flounder Feeding Study: Alam et al. (2018)

World Bank Report (2013) #83177-GLB
Agriculture and Rural Development Department, The World Bank Group (2011)

Identification and Cloning of Genes Involved in the Development of Glands in Cotton

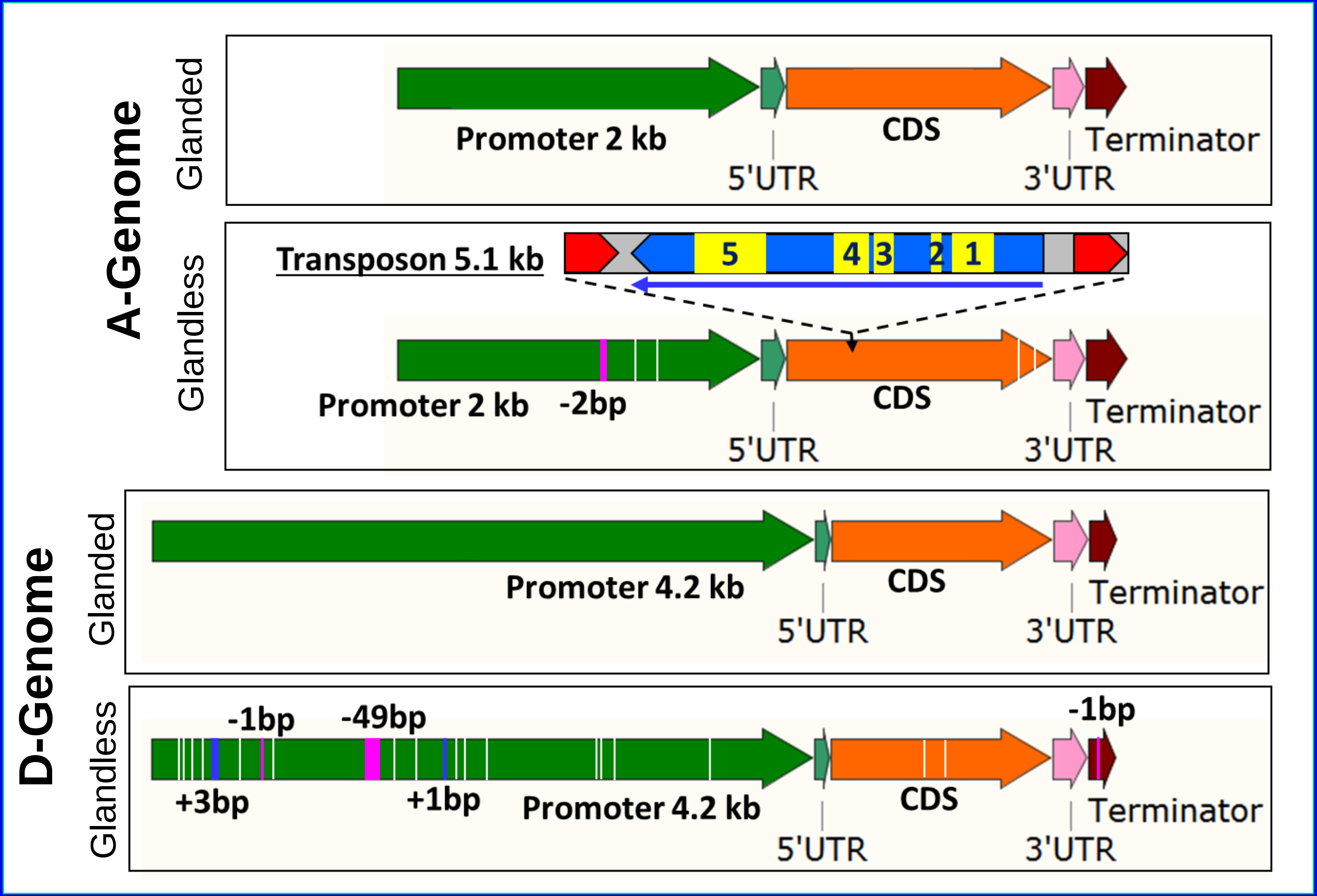
- Used comparative transcriptomics between normal, glanded cotton and glandless (Hopi) cotton 14 dpa embryos to identify three gene pairs involved in the development of glands in cotton

- Cotton Gland Formation (*CGF1, CGF2 & CGF3*) genes encode transcription factors

- Only the *CGF3* genes show sequence differences between glanded and glandless cotton

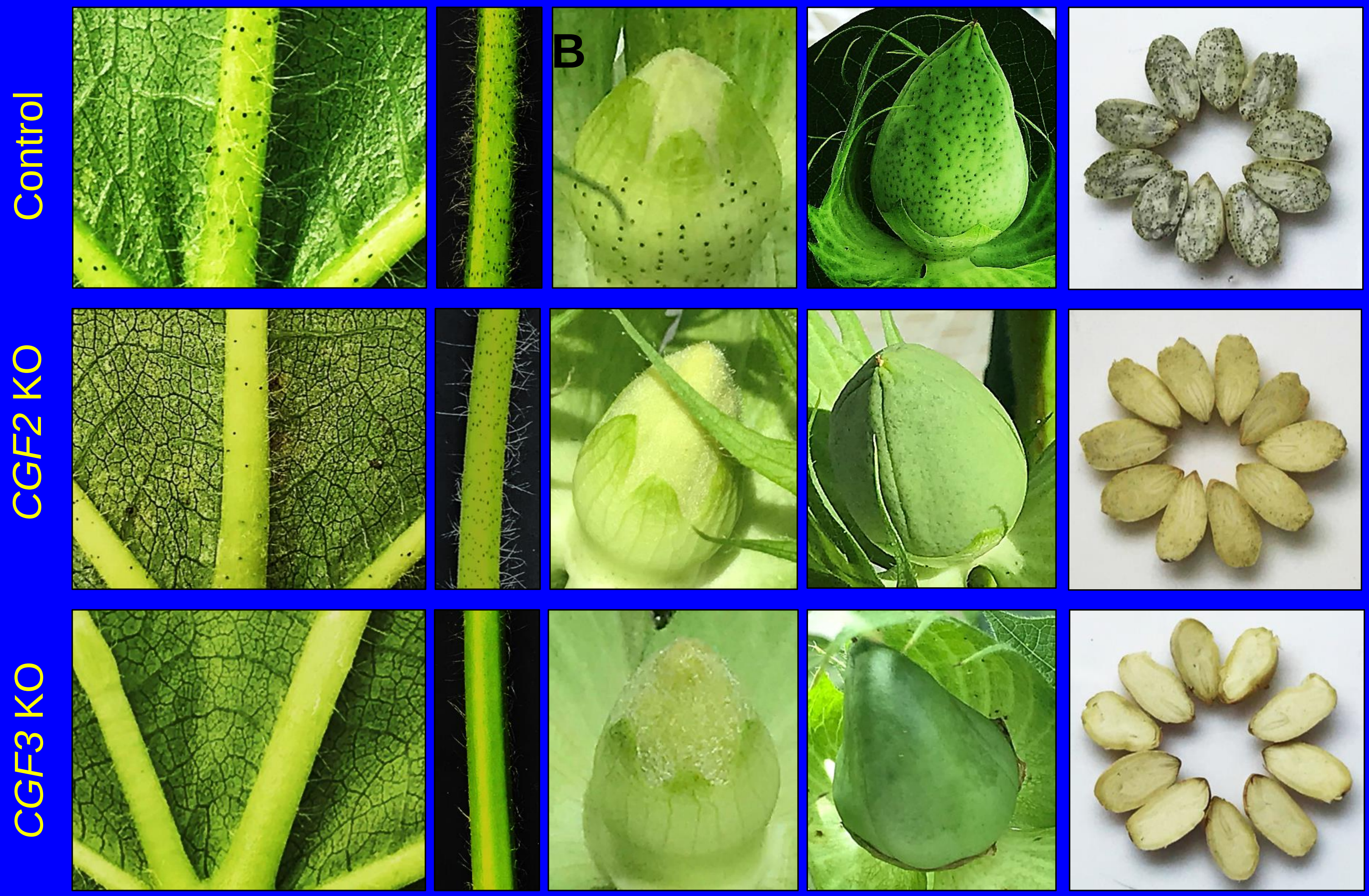
Janga et al. Plant Biotech. J. (2019)

CGF3, the Main Gene Involved in Gland Development in Cotton



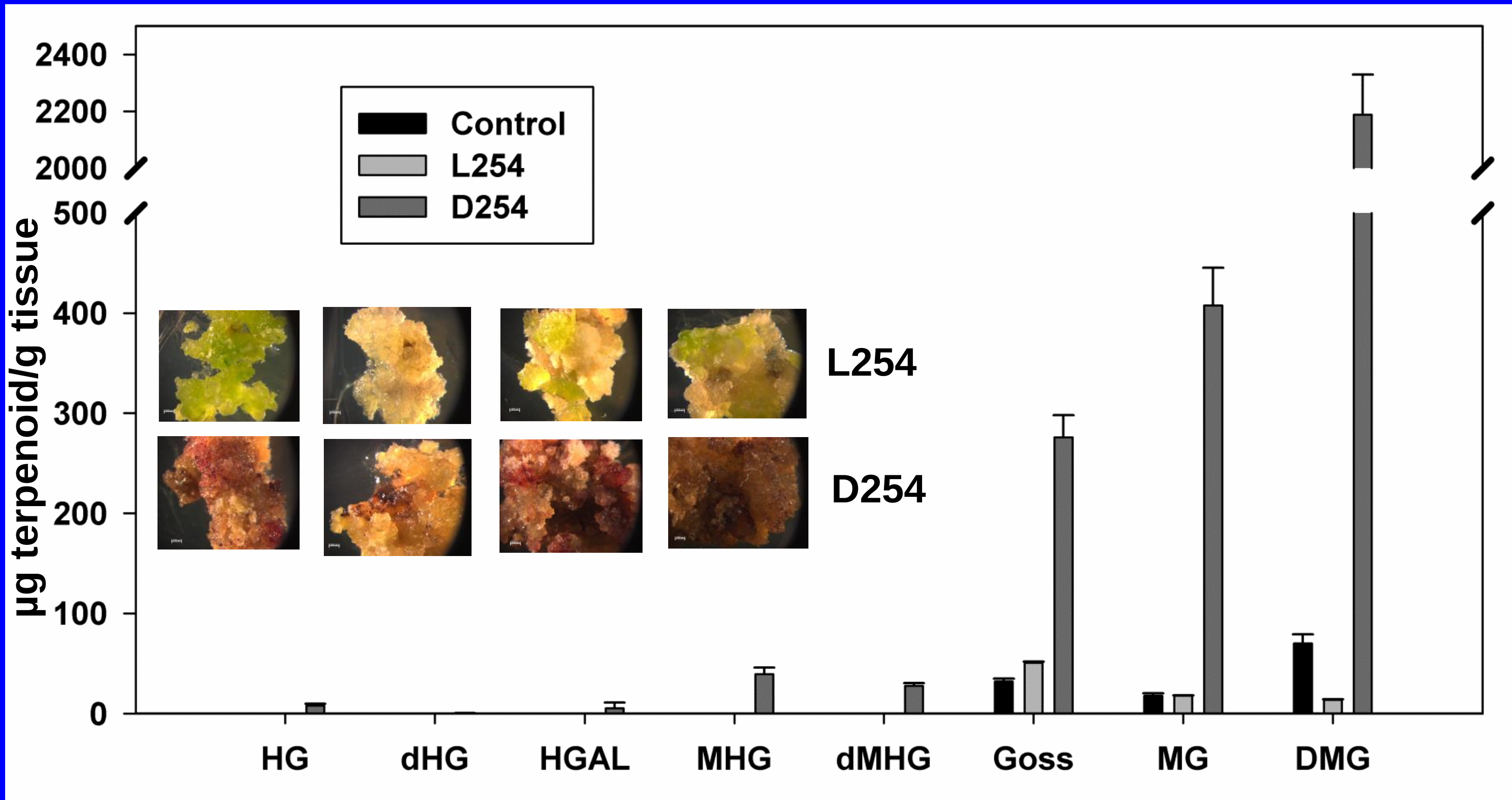
Janga et al. Plant Biotech. J. (2019)

CRISPR-Cas9-mediated, targeted knockout of *CGF2 & CGF3 genes*



Janga et al. Plant Biotech. J. (2019)

Overexpression of *CGF3* Gene in Cotton Cultures



HG: Hemigossypol
dHG: Desoxyhemigossypol
HGAL: Hemigossylic acid lactone
MHG: Methoxyhemigossypol
dMHG: Desoxymethoxy-hemigossypol
Goss: Gossypol
MG: Methoxygossypol
DMG: Dimethoxygossypol

New tool to increase glands (gossypol) in the foliage & flowers to boost the natural defenses of the cotton plant

Janga et al. Plant Biotech. J. (2019)

IPGB, TAMU

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Cotton Inc., TxCOT, TFFC, THECB & Texas AgriLife Research

Questions???