Waiter, There's a Gene in My Soup! Finding Biotechnology and Eating It Anyway

What's In This Stuff Anyway? Composition of Food and Feed Crops

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January 25, 2011

Sponsored by ILSI
International Food Biotechnology Committee (IFBiC)

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Outline

- Introduction
- Role of Composition in Safety Assessment
  - Importance of natural variability
  - ILSI crop composition database, Version 4.0
- Improved Nutrition Crops
  - Golden Rice, an update
- Future Prospects
  - Eliminating gossypol in cottonseed
  - Improving potato processing
- Conclusions

Assessment of Food/Feed Safety

- Standard - "Reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption"
- Food is not inherently safe
- Considered to be safe based on experience
- Not absolute but relative, comparable safety:

. . . "as safe as" . . .

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International Guidelines for Evaluating Biotech Product Safety

- *International Food Biotechnology Council (1990)*
- *OECD Group of National Experts on Safety in Biotechnology, 1993, 1994, 1996*
- *OECD Task Force on the Safety of Novel Foods and Feed, 1998-present*
- *FAO/WHO Expert Consultations, 1991, 1996, 2000, 2001, 2003*
- *CODEX Task Force on Foods Derived from Biotechnology, 1999-2004*
- *US FDA, 1992, 2000, 2002*
- *Council for Agricultural Science and Technology (CAST), 2001*
- *ENTRANSFOOD, the EU Thematic Network on the Safety Assessment of Genetically Modified Food Crops, 2000-2003*
- *European Food Safety Authority (EFSA), 2002, Guidance Documents GMO Panel, 2003-2007*
- *International Life Science Institute (ILSI), Task Forces,1996 - present*

A rigorous, integrated process to assess safety of all elements of the biotech crop/food

- **Parent Crop**
  - Identity, Phenotypic & Agronomic Performance
  - Geographical Distribution
  - History of Safe Use
  - Compositional Analysis
- **Donor, Transgene(s) and Delivery Process**
  - Description of Donor
  - Description of Vector DNA
  - Transgene Delivery Process
  - Characterisation of Introduced DNA
  - Characterisation of Insertion Site
- **Characterisation of Gene Product(s)**
  - Structure, Identity and Characterisation
  - Mode of Action/ Specificity
  - Toxicity
  - Allergenicity
- **Safety Assessment of New GM Crop/Food**
  - Identity, Phenotypic & Agronomic Performance
  - Compositional Analysis
  - Nutritional Analysis
  - Safety Analysis (Animal Studies)

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The Importance of Food Crop Analysis

Why should food crops be analyzed?

- Crop plants and their commodities such as oil, protein and starch are the raw materials for the manufacture of food products
- Nutrients derived from food crops are essential for normal growth and development
- Compositional analysis ensures a safe and plentiful food supply

Materials For Analysis

- Test – A variety or hybrid that contains a gene(s) that confer desired agronomic or nutritionally enhanced properties.
- Control – A conventional variety or hybrid with a history of safe use that is isogenic or near isogenic with the test but without the trait.
- References – Conventional varieties or hybrids that are grown commercially in the geographies of the field trials.

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What Nutrients Should Be Analyzed?

- Major constituents: oil, protein, ash, carbohydrate and moisture (proximates) and fiber
- Other common nutrients: amino acids, fatty acids, vitamins, minerals
- Antinutrients: Trypsin inhibitor, gossypol, lectins, phytic acid, and other
- The list can vary depending on the properties of the crop and its contribution to the food supply*.

* *OECD consensus documents are an important resource.*

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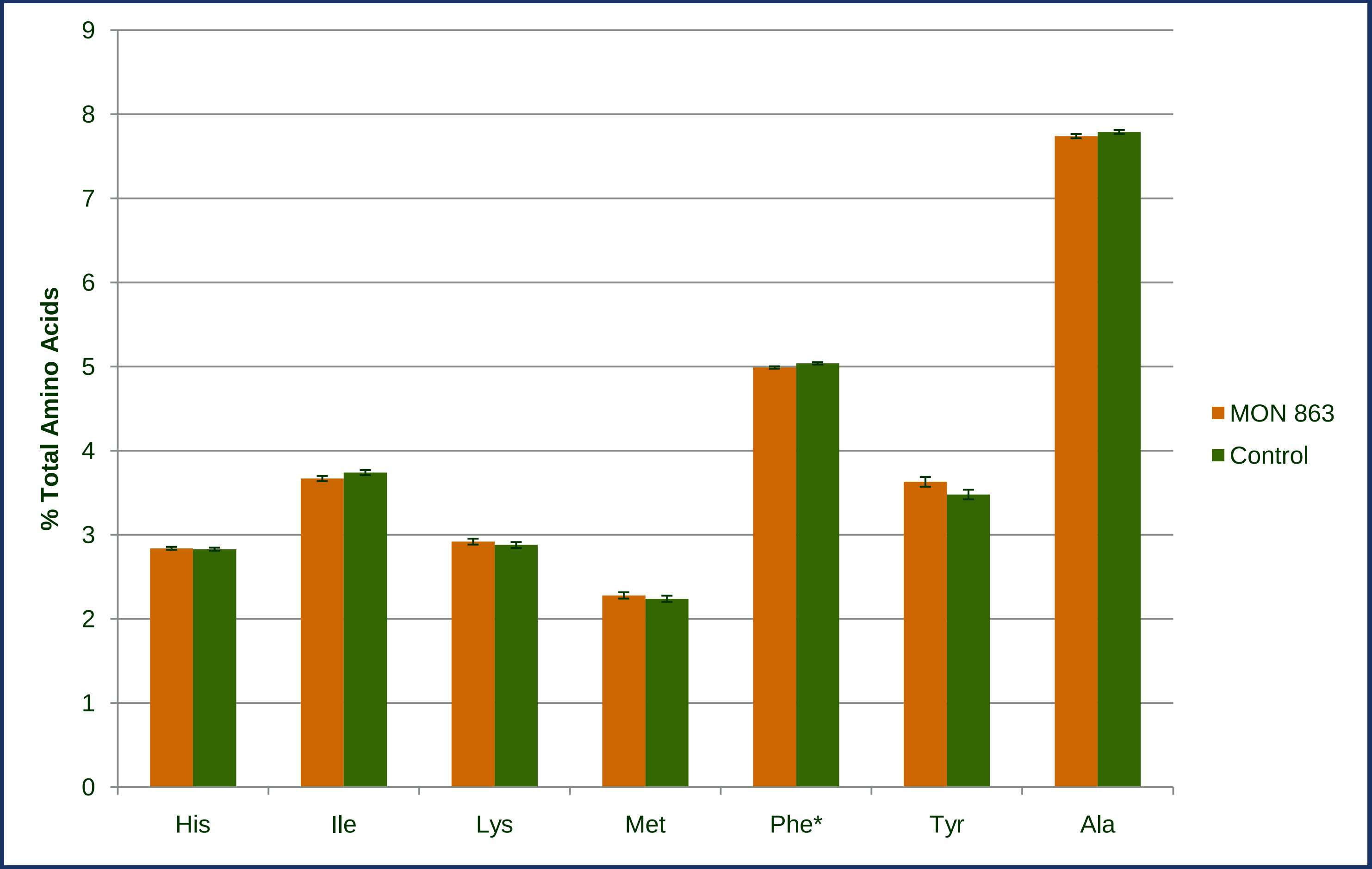
Amino Acid Composition for MON 863 Insect Protected Maize Grain*



* Statistically significant difference ($p < 0.05$); George, C. et al., *J. Agric. Food Chem.*, 2004, 52, 4149-4158.

Statistical Results for Grain from MON 863*

	Phenylalanine (% Total AA)	Iron (mg/kg dw)
MON 863 (Mean+/-SE)	4.99 +/- 0.014	21.73 +/- 0.046
Control (Mean+/-SE)	5.04 +/- 0.014	21.29 +/- 0.0049
Mean Diff (Mean+/-SE)	-0.093	-0.021 +/- 0.0068
Significance ($p<0.05$)	0.038	0.013
Mean Diff (% of control)	-0.95	-5.26
MON 863 (range)	4.80- 5.35	23.99-25.42
Reference varieties (99% Tolerance Interval)	4.53, 5.66	12.52, 35,06

George, C. et al.,** ***J. Agric. Food Chem.**, 2004, 52, 4149-4158.**

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Assessing Biological Relevance

"The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance."

Codex Alimentarius. 2003. Guideline For The Conduct Of Food Safety Assessment Of Foods Derived From Recombinant-DNA Plants. CAC/GL 45-2003.

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Natural Variability – A Key Concept

Nutrient, anti-nutrient and secondary metabolite levels vary with genetic background and environmental conditions (*G x E*)

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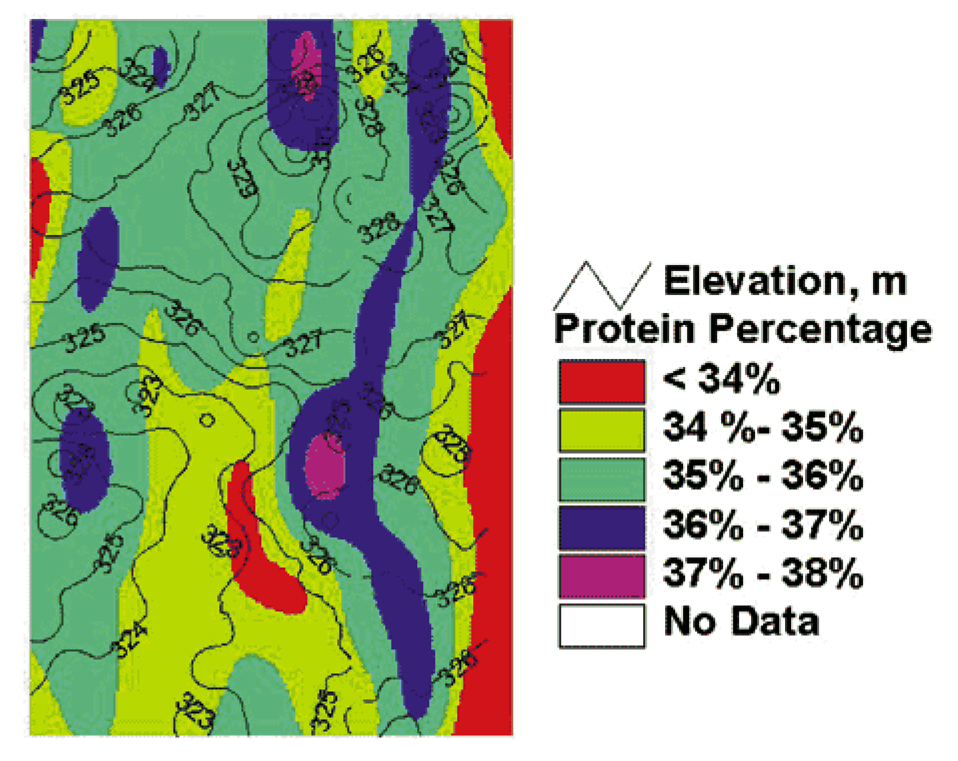
Effect of Environment on Total Protein Content of a Single Soybean Genotype in a Single Field

Distribution of protein content in a 50-acre soybean field near Perry, IA (1998).

Total Protein Range: 33-38%



http://www.ipm.iastate.edu/ipm/icm/1999/5-5-1999/soycompvar.html

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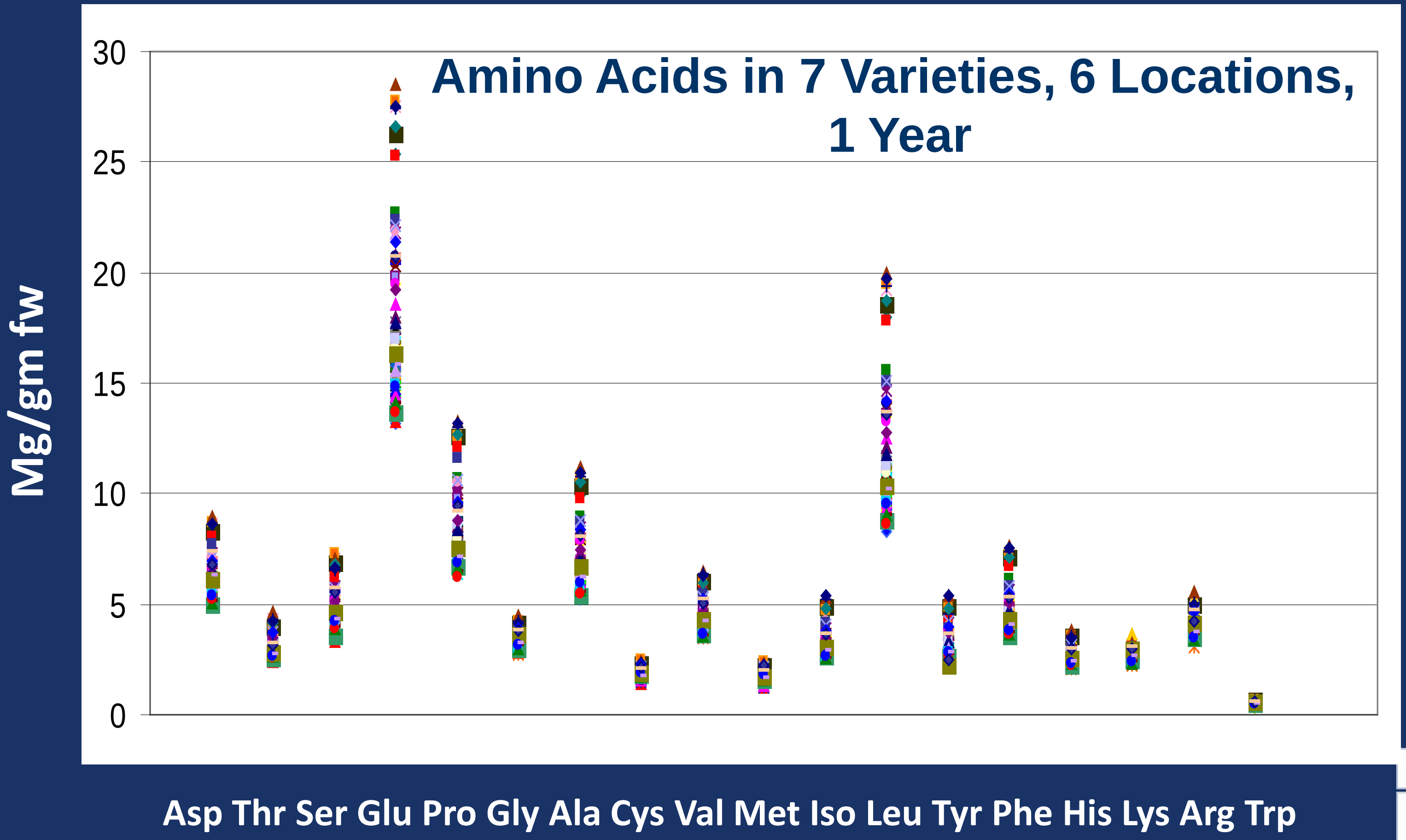
Natural Variability – Conventional Maize Hybrids



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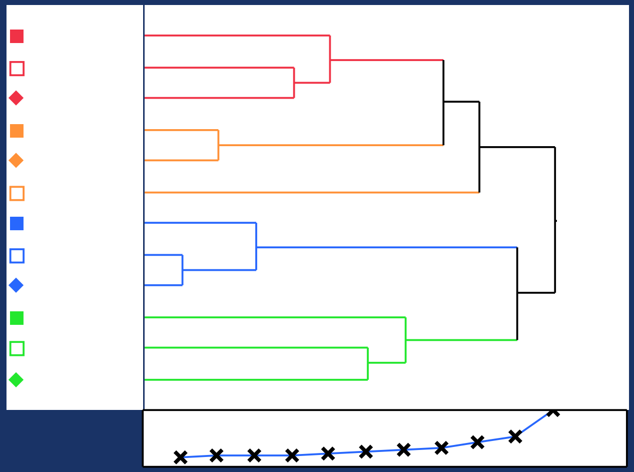
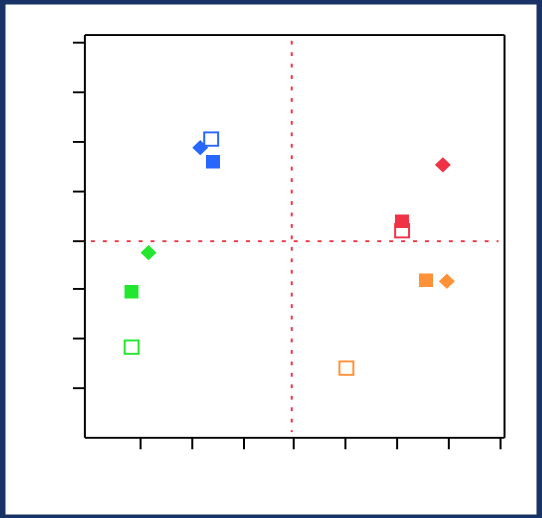
Reynolds et al. 2005, *J. Agric. Food Chem.*, 53, 10061-10067.

Multivariate Analysis of Seed Composition for Insect-Protected (IP) and Herbicide-Tolerant (HT) Soy*

Hierarchical Cluster Analysis (HCA)



Principal Component Analysis (PCA)



77T = IP Soy
R2T= HT Soy
77C= Control

Brazil Northern - BrN
Brazil Southern - BrS

Closed symbols = IP, HT Soy
Open symbols = Control

Conclusion: Composition of GM varieties is contained within the same natural variability as non-GM varieties

*Harrigan, G. G. et al., 2010. *Nature Biotechnology*, 28, 402-404

Characterization of Natural Variation in Biochemical Composition

- Variation in the level of biochemical components for a crop occurs as a result of the interaction of genetic background and environmental conditions
- Any comparison of biotech to conventional crops must include the consideration of natural variation
- An understanding of the natural variation in compositional analytes can enhance the scientific basis for the concept of substantial equivalence

Proposal: Development of a comprehensive, public database

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ILSI Crop Composition Database

- Task Force convened by the ILSI International Food Biotechnology Committee (IFBiC) in 2001
- Members of the Task Force 7:
  - Bayer CropScience
  - BASF (2004)
  - Dow AgroSciences
  - Monsanto
  - Pioneer/DuPont
  - Renessen
  - Syngenta

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Status of Database

- **Database overview (v3.0)**
  - Conventional crops (maize, soy, cotton)
  - Tissues (forage, seed/grain)
  - Analytes (94 unique)
  - Data (3K samples, 115K data points)
  - Meta-data (method, country, location, year, variety, source)
  - 2009 usage (30K visits, 122 countries)

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Database Improvements

	Version 3.0	Version 4.0*
• Language	Perl	Java
• Hardware	Early 2004	Late 2008
• GUI	Outdated	Simple, Intuitive
• Speed	5 – 10 min	2 – 5 sec
• Units of measure	Limited	Flexible
• Data preview	No	Yes
• LOQ	No	Yes
• Data output	Monitor	Monitor, PDF, CSV

*Alba, R. et al., 2010, J. Food Comp. Analysis; launched Sept. 2010.

Welcome page (Screen 1)

Address http://www.cropcomposition.org/query/index.html

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Crop Composition Database

Home | Database Search | Privacy Policy | Terms of Use | Contact Us

Welcome to the ILSI Crop Composition Database

Version 4.0

The ILSI Crop Composition Database is a project of the International Life Sciences Institute.

The first three versions of the database have represented a compilation of crop analyses from a number of companies engaged in agricultural life sciences. Through ILSI, the participants have standardized and pooled their crop data in order to make the data available to scientists from academia, government agencies, and industry, and to the general public.

It is envisioned that future versions of the database will include other publicly available data that meet the acceptability criteria of ILSI and are submitted from scientists and other researchers representing a variety of public and private organizations.

NOTE: With the Version 4.0 upgrade, the system has been completely redesigned for increased speed and efficiency. No additional data were incorporated into the database from Version 3.0; however, minor differences in search results may be apparent due to the requirements associated with restructuring of the database. You will not be able to go back to Version 3.0 to replicate earlier search results now that Version 4.0 has been released.

Results from your search of Version 4.0 (the current version of the database) represent analyses of only conventionally bred crops.

- Database Search
- Register for Updates
- Other Web Sites of Interest
- About the ILSI-CCDB
- How to Cite this Database
- Frequently Asked Questions
- Help

www.cropcomposition.org

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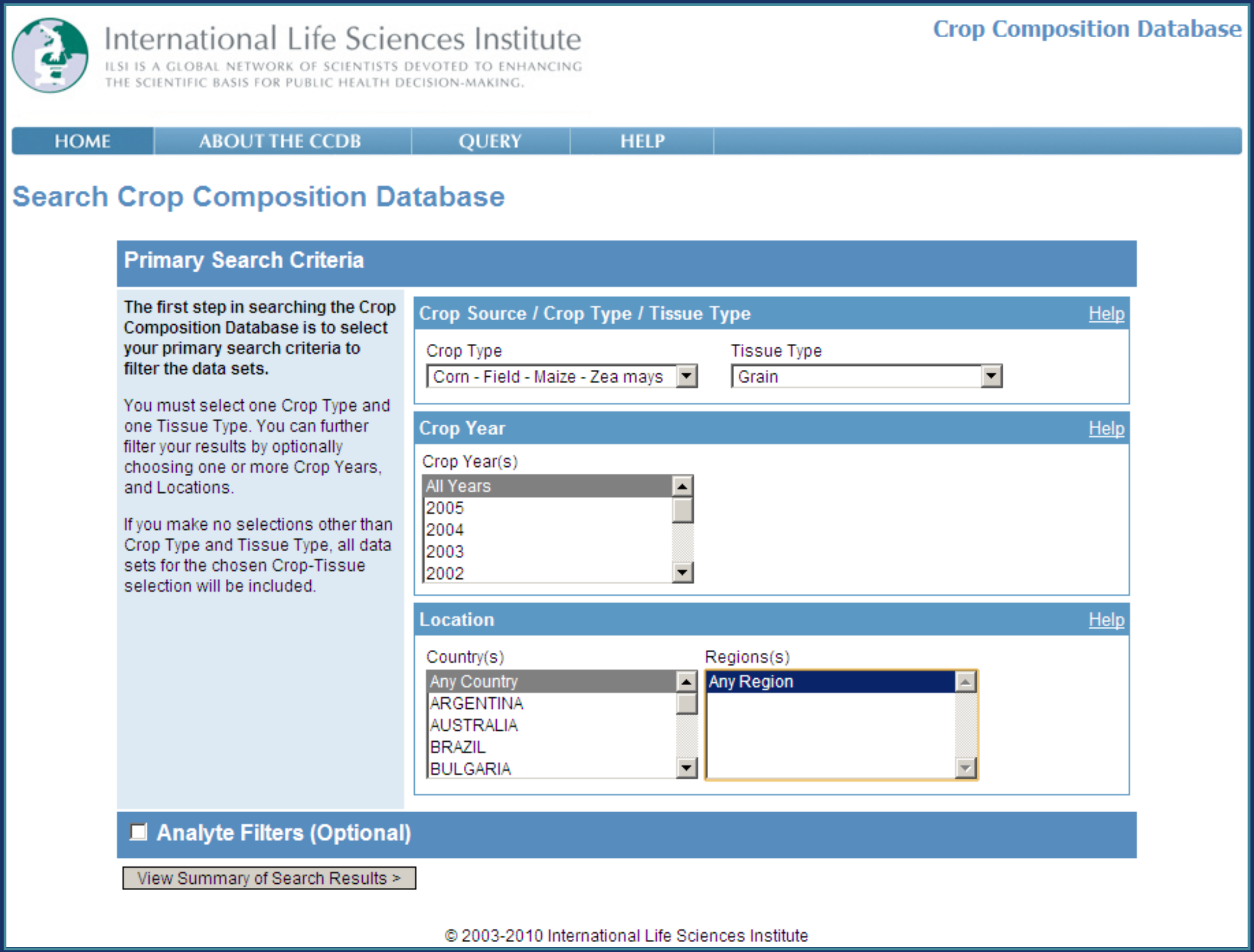
Primary Search Criteria (Screen 2)



www.cropcomposition.org

Data Summary & Output (Screen 3)

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Crop Composition Database

HOME | ABOUT THE CCDB | QUERY | HELP

Search Crop Composition Database

Query Summary

The Query Summary shows the criteria that was used to filter the result set.

Query Criteria Help

Crop Type:	Corn - Field - Maize - Zea mays
Tissue Type:	Grain

Summary of Search Results

The Summary of Search Results shows the results of your initial search grouped by the Analyte Types for the Data Sets that were found.

You can expand each Analyte Type to see the number of samples, and the min, max, and mean values for the samples in the primary unit of measure for each.

All analytes in the database have been assigned a primary unit of measure, which is shown in the right column of the new Summary of Search Results tool. To maximize performance and simplify usage of this tool, composition data is only allowed to be viewed in the primary unit of measure. If secondary units of measure (or multiple units of measure for a single analyte) are preferred, Version 4.0 of the ILSI-CCDB requires that data with secondary units of measure be generated and viewed using an output report.

You can use this information below when defining the specific Analytes you would like to display in your final report.

Results matching your query criteria Help

Analyte Type	Analyte	Samples	Min	Max	Mean	Units
☐ Amino Acids	-	-	-	-	-	-
☐ Bio Actives	-	-	-	-	-	-
☐ Carbohydrates	-	-	-	-	-	-
☐ Fatty Acids	-	-	-	-	-	-
☐ Fiber	-	-	-	-	-	-
☑ Minerals	-	-	-	-	-	-
	Calcium	1309(5<LOQ)	10.9	169.0	41.3	ppm FW
	Chloride	53(0<LOQ)	320.0	800.0	526.6	ppm FW
	Copper	1211(2<LOQ)	0.64	14.78	1.55	ppm FW
	Iron	1215(0<LOQ)	9.21	44.70	19.40	ppm FW
	Magnesium	1217(0<LOQ)	534.6	1,726.6	1,057.9	ppm FW
	Manganese	1216(0<LOQ)	1.43	13.00	5.51	ppm FW
	Phosphorus	1309(0<LOQ)	1,323.0	4,743.7	2,902.8	ppm FW
	Potassium	1217(0<LOQ)	1,629.0	5,366.7	3,422.0	ppm FW
	Selenium	123(34<LOQ)	0.05	0.69	0.18	ppm FW
	Sodium	1145(928<LOQ)	0.15	654.00	29.00	ppm FW
	Sulfur	53(0<LOQ)	454.0	1,170.0	744.1	ppm FW
	Zinc	1217(0<LOQ)	5.6	34.3	19.2	ppm FW
☐ Other Metabolites	-	-	-	-	-	-
☐ Proximates	-	-	-	-	-	-
☐ Vitamins	-	-	-	-	-	-

Create Report from Search Results

Choose the Analytes you would like to display in your report output.

You may choose individual Analytes or may select an Analyte Type and choose to display all Analytes for the Analyte type chosen.

Once you have selected the analytes click on the "add analyte(s)" button to confirm your selection.

To remove a selected analyte click on the red 'X' next to the anaylte name.

Analytes to show in output Help

Analyte Type: Choose One | Analyte | Units

[Add Analyte(s)]

Analyte List Help

- Bio Actives: Phytic Acid (% FW) ✖
- Bio Actives: Phytic Acid (% DW) ✖

Select Fields for Report Output

In this section you can choose the metric fields and other fields you would like to display in your report.

Check the fields you would like to display and for the Grouping Fields you can click the up and down arrows to arrange the order in which to display the fields in your report.

Please note that selecting Seed Variety and/or Seed Vendor will disable the Detailed and Tabular Reports.

Metric Fields Help

- ☑ Minimum Value
- ☑ Maximum Value
- ☑ Mean Value
- ☑ Number of Samples
- ☑ Samples Below LOQ
- ☑ Samples Above LOQ

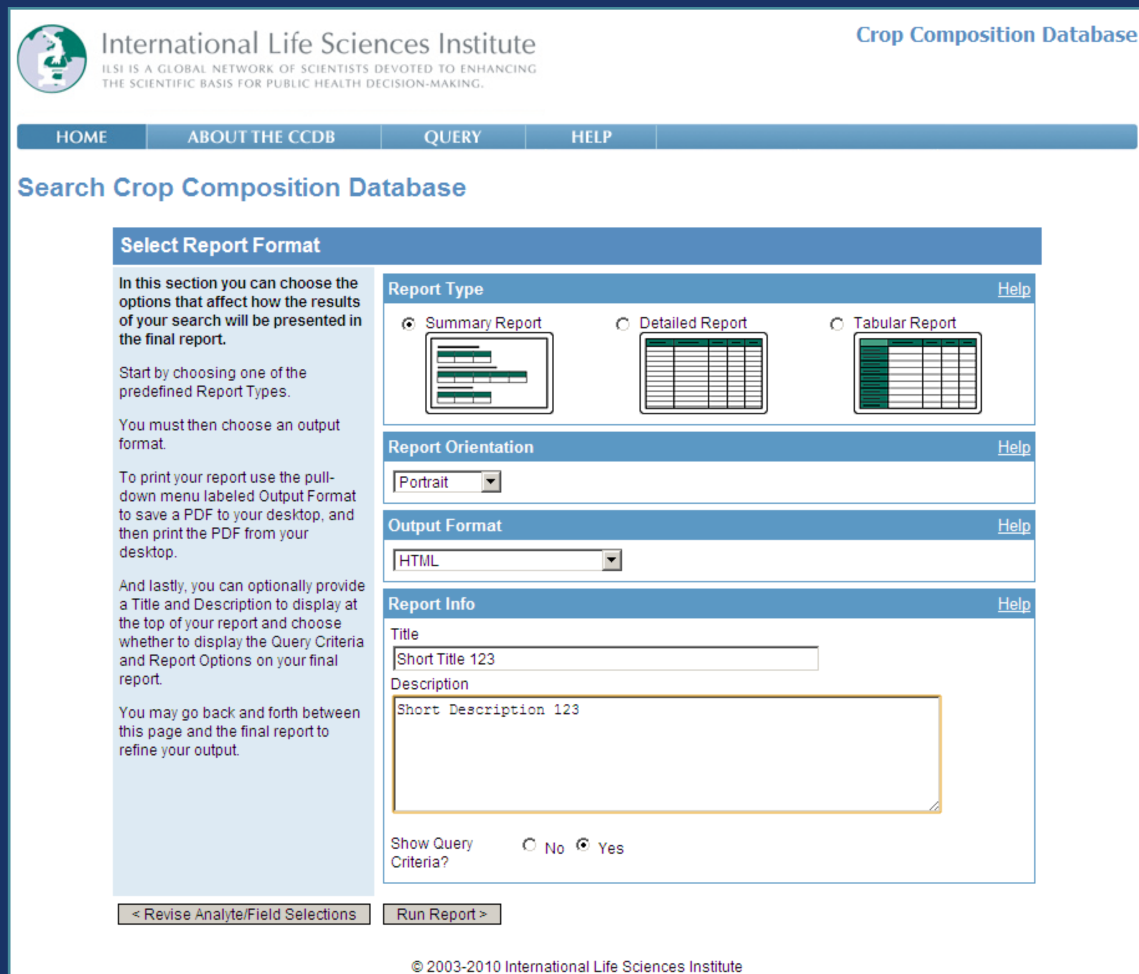
Grouping Fields Help

- ☑ Analyte Type
- ☑ Analyte
- ☐ Crop Year
- ☐ Crop Type
- ☐ Crop Source
- ☐ Tissue Type
- ☐ Seed Vendor (This option is only available for Summary Reports)
- ☐ Seed Variety (This option is only available for Summary Reports)
- ☐ Country
- ☐ Region
- ☑ Analysis Method

[< Revise Query Filters] [New Query] [Report Options >]

www.cropcomposition.org

Output Format (Screen 4)



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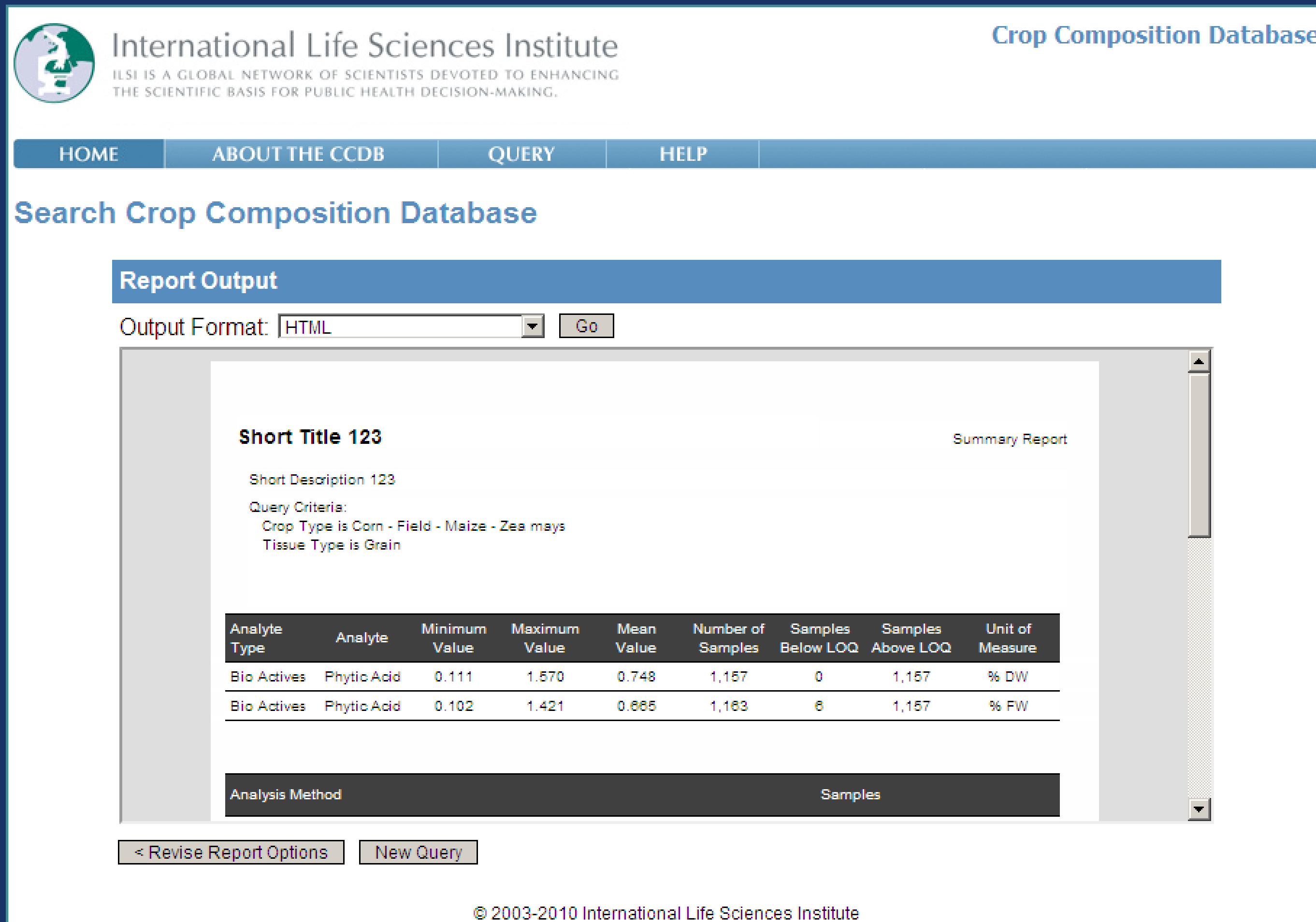
Output Example (Screen 5)



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Crop Composition Database

HOME | ABOUT THE CCDB | QUERY | HELP

Search Crop Composition Database

Report Output

Output Format: HTML [Go]

Short Title 123

Summary Report

Short Description 123

Query Criteria:
Crop Type is Corn - Field - Maize - Zea mays
Tissue Type is Grain

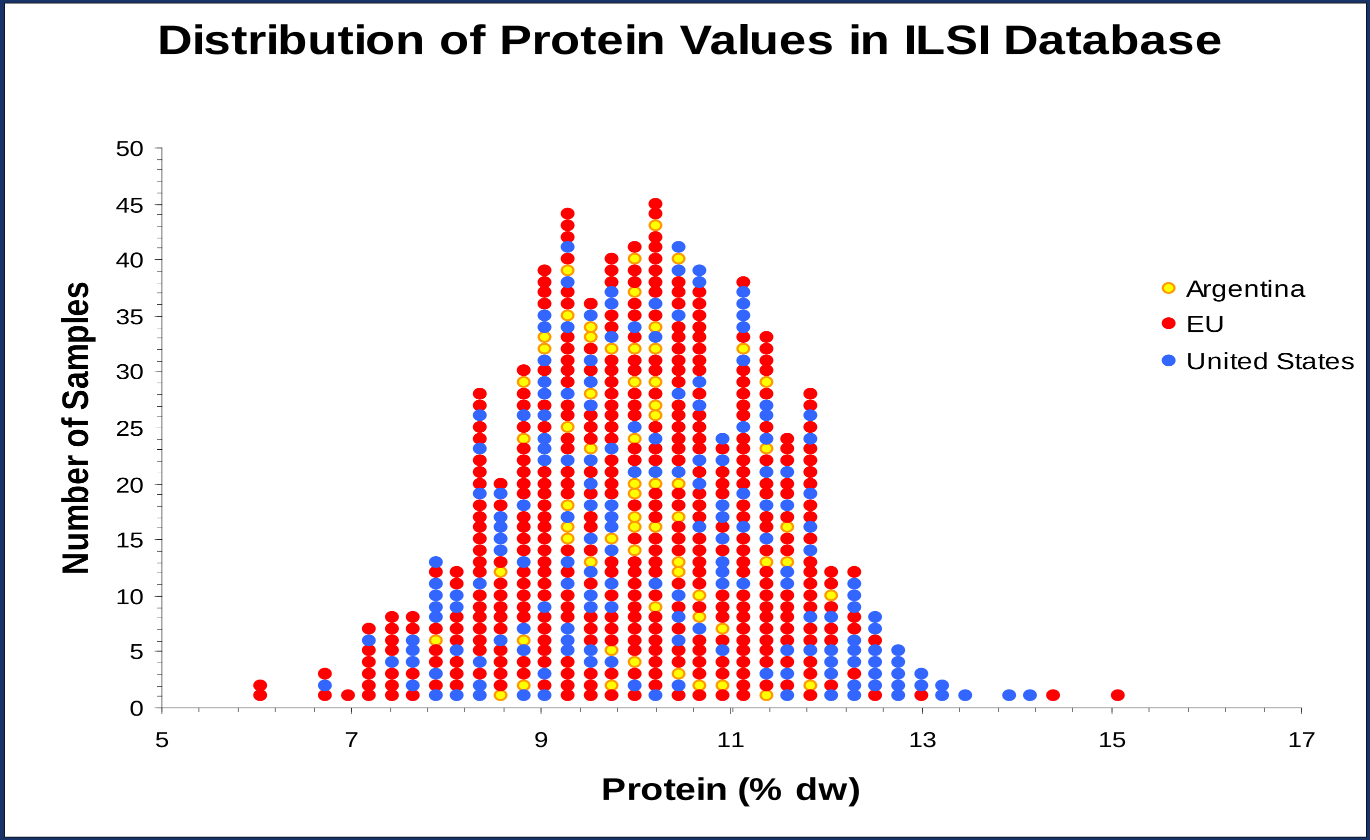
Analyte Type	Analyte	Minimum Value	Maximum Value	Mean Value	Number of Samples	Samples Below LOQ	Samples Above LOQ	Unit of Measure
Bio Actives	Phytic Acid	0.111	1.570	0.748	1,157	0	1,157	% DW
Bio Actives	Phytic Acid	0.102	1.421	0.665	1,163	6	1,157	% FW

Analysis Method	Samples

< Revise Report Options | New Query

www.cropcomposition.org

Natural Variability of Maize Protein*



* Ridley et al., *J. Food Comp. Analysis*, 2004, 17, 423-438.

Future Plans

- **May 2011**

  Data upload tools

  Curator interface tools

- **August 2011**

  Complete upload of “new” existing data

- **CY2011**

  Add new crops, tissues, analytes

  ex: rice, canola, potato

Improved Nutrition Crops

Golden Rice –
An Update

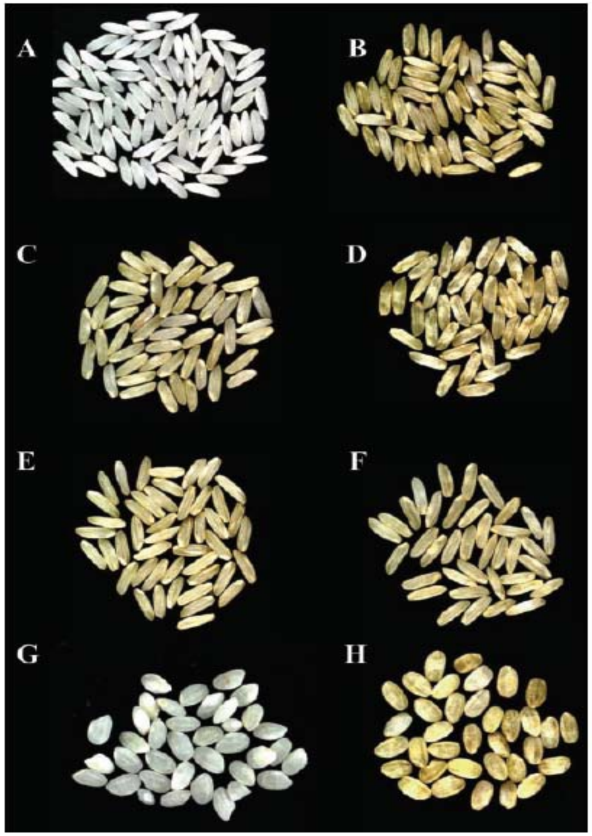


Figure 6. Examples of some T_2 seeds. A, IR64 wild type; B to F, transgenic IR64 lines; G, Taipei 309 wild type; H, transgenic Taipei 309 line.

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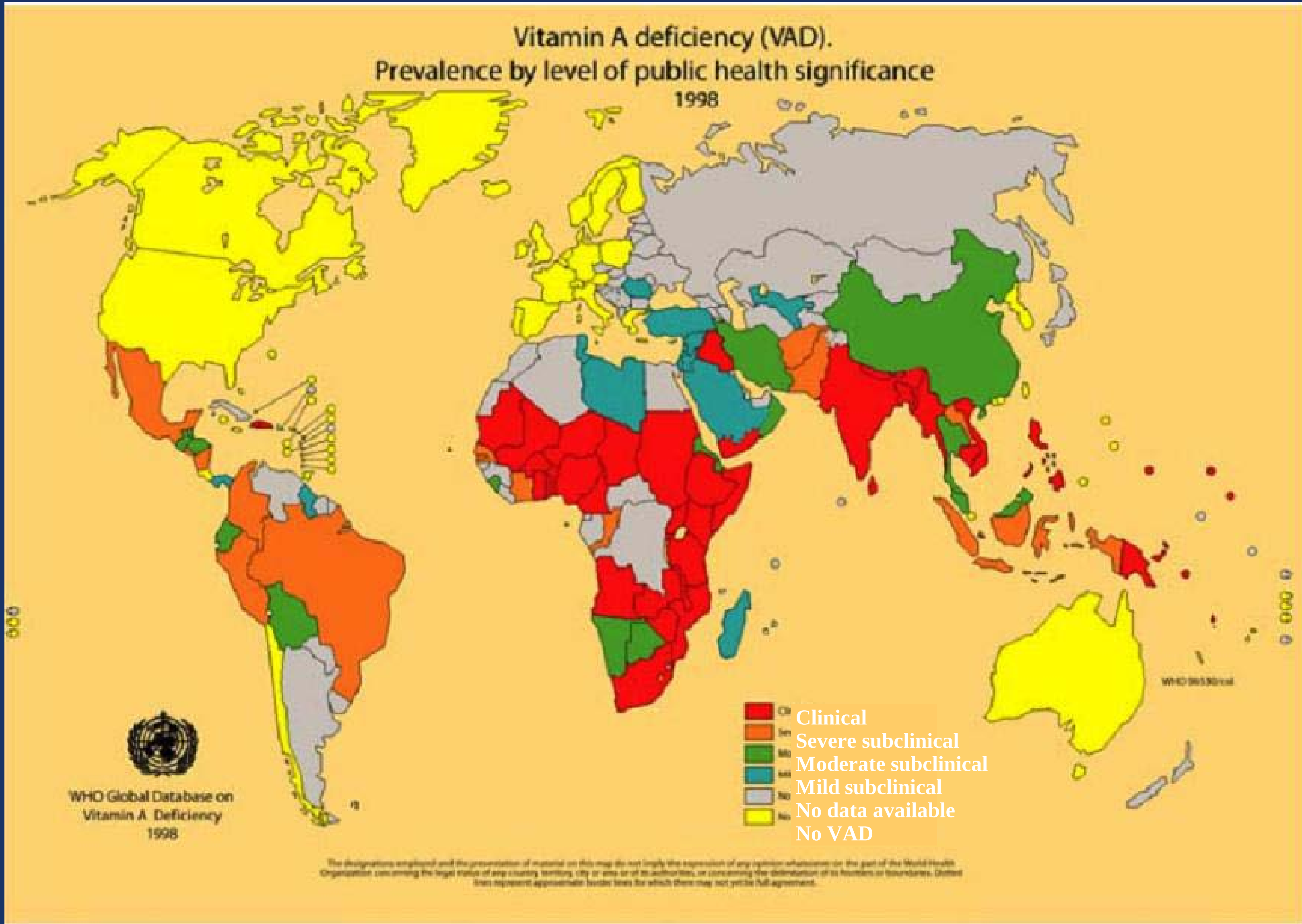
Vitamin A deficiency (VAD).
Prevalence by level of public health significance
1998
Clinical
Severe subclinical
Moderate subclinical
Mild subclinical
No data available
No VAD
WHO Global Database on
Vitamin A Deficiency
1998

Consequences of Vitamin A Deficiency

- 1.3-2.5 million deaths/year of children < 5 yrs old
- 2 million children/year have clinical vision problems
- 250,000 children/year become blind
- VAD common among poor, rice-consuming populations
  - Dietary diversity is poor
  - Rice is not a source of vitamin A
- Supplementation, fortification, and injections of vitamin A have not solved the problem

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ILSI IFBiC Task Force 4 Publication

JFS R: Concise Reviews/Hypotheses in Food Science

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Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology: Case Studies

EXECUTIVE SUMMARY OF A TASK FORCE REPORT BY THE INTERNATIONAL LIFE SCIENCES INSTITUTE, WASHINGTON, D.C.

During the last 2 decades, the public and private sectors have made substantial international research progress toward improving the nutritional value of a wide range of food and feed crops. Nevertheless, significant numbers of people still suffer from the effects of undernutrition. In addition, the nutritional quality of feed is often a limiting factor in livestock production systems, particularly those in developing countries. As newly de-

studies examine the principles and recommendations published by the Intl. Life Sciences Inst. (ILSI) in 2004 for the safety and nutritional assessment of foods and feeds derived from nutritionally improved crops (ILSI 2004). One overarching conclusion that spans all 5 case studies is that the comparative safety assessment process is a valid approach. Such a process has been endorsed by many publications and organizations, including the 2004 ILSI

- Existing comparative assessment process provides a framework for safety assessment
- Compositional analysis should involve targeted metabolic pathway

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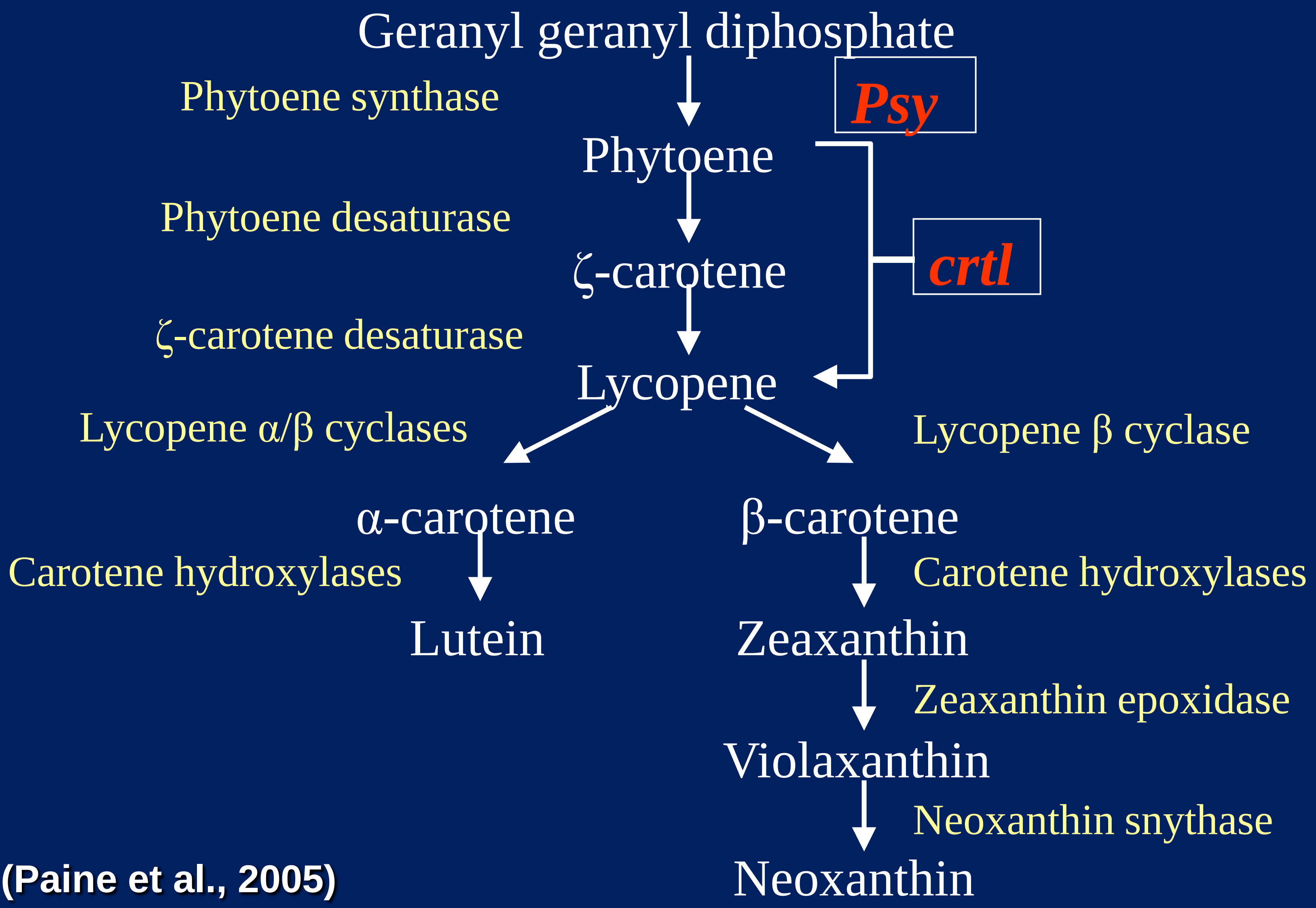
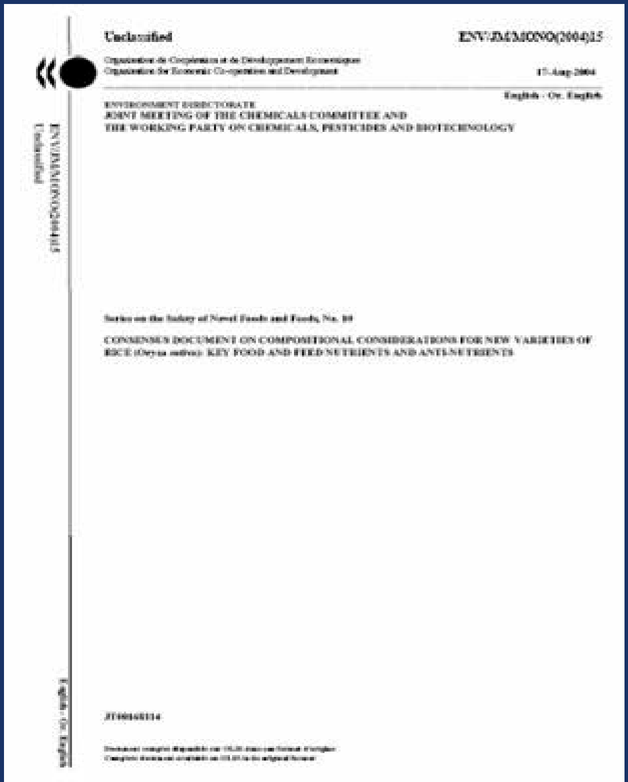
Carotenoid Biosynthesis in Plants



(Paine et al., 2005)

Compositional Analysis of Golden Rice 2

- OECD consensus document on rice
- Proximates
    - protein, crude fat, ash, carbohydrates, starch, NDF, ADF, crude fiber, lignin
  - Amino acids
  - Fatty acids
  - Minerals: Ca, P
- Golden Rice 2 specific analytes
  - α- and β- carotene
  - Lycopene
  - Xanthophylls
  - Vitamin A


Unclassified
ENV/JM/MONO(2004)15
Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development
17-Aug-2004
English - Or. English
ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY
Series on the Safety of Novel Foods and Feeds, No. 10
CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF RICE (Oryza sativa): KEY FOOD AND FEED NUTRIENTS AND ANTI-NUTRIENTS

Result: β-carotene – up to 31 μg/gm dry weight

Nutritional Analysis of Golden Rice 2

- Early estimates suggested that a single serving of Golden Rice 2 make a significant contribution to the RDA for vitamin A if:

  β-carotene is converted to vitamin A at 12:1

  Lower ratios could provide more vitamin A

- Remaining questions:

  In vivo conversion ratio

  Stability to storage and cooking

  Acceptability

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Golden Rice 2 as a Source of Vitamin A*

- β-carotene in Golden Rice 2 specifically labeled with deuterium (^{2}H)
- Rice containing ^{2}H labeled β-carotene fed to 5 healthy volunteers (3 women and 2 men)
- Reference dose of [$^{13}C_{10}$]-retinyl acetate administered one week before ^{2}H β-carotene
- Blood samples collected over 36 days and analyzed for [^{2}H]retinol (Vitamin A)
- Golden Rice 2 β-carotene was converted to retinol in 3.8 ± 1.7 to 1 ratio

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*Tang, G et al., Am. J. Clin. Nutr. 2009, 89, 1776-83.

Golden Rice 2: Conclusions

- VAD is a serious global health issue which adversely effects 10s if not 100s of millions of people
- Golden Rice 2 has the potential to dramatically help lower morbidity and mortality of VAD in rice-consuming populations
- Conversion of β-carotene in Golden Rice 2 to vitamin A in humans has been demonstrated
- The Rockefeller Foundation has provided funding to the International Rice Institute (IRRI) to shepherd Golden Rice 2 through the regulatory process
- IRRI anticipates that Golden Rice 2 may be approved in the Philippines and Bangladesh as early as 2013 and 2015, respectively.
- Additional information: www.goldenrice.org and www.irri.org

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Future Prospects

- ILSI IFBiC TF8 publication: Parrott et al., “Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops” Food Chem. Tox. (2010).
- Two approaches described in detail: Transcription factors and RNA interference (RNAi)
- RNAi – group of related natural phenomena mediated by formation of short (21-24 bp) ds RNA molecules which suppress the expression of a target gene
- Two applications of RNAi technology:
  - reduction of gossypol levels in cottonseed
  - improvement of processing properties in potato

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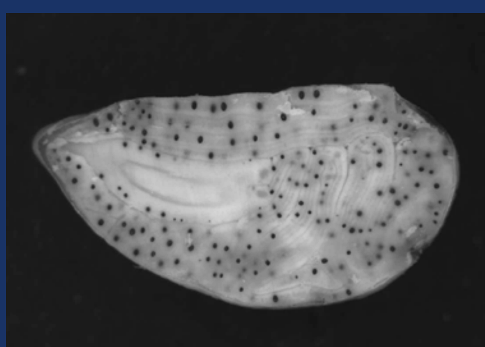
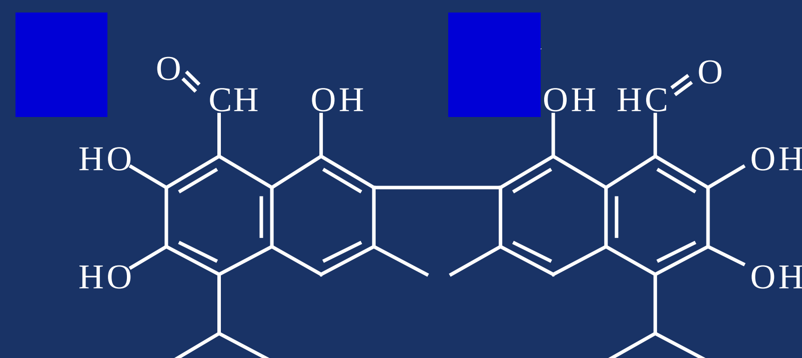
Cottonseed and Gossypol

- Protein content of cottonseed is about 26.9% dry weight*
- 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.
- Gossypol typically removed during the processing of cottonseed to obtain edible oil





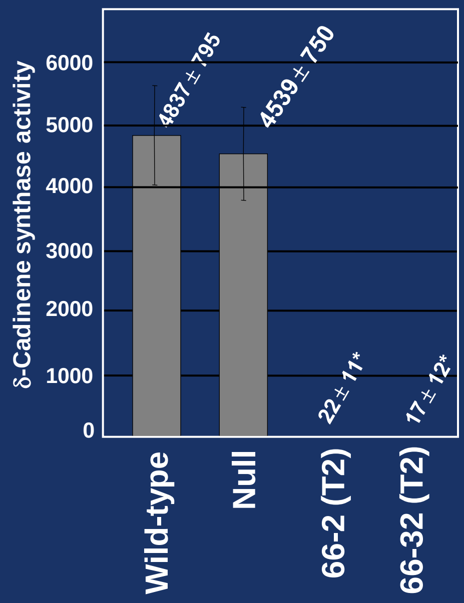
Gossypol

*ILSI crop composition database, V4.0, accessed Jan 17, 2011

Specific Suppression of Gossypol in Cottonseed*

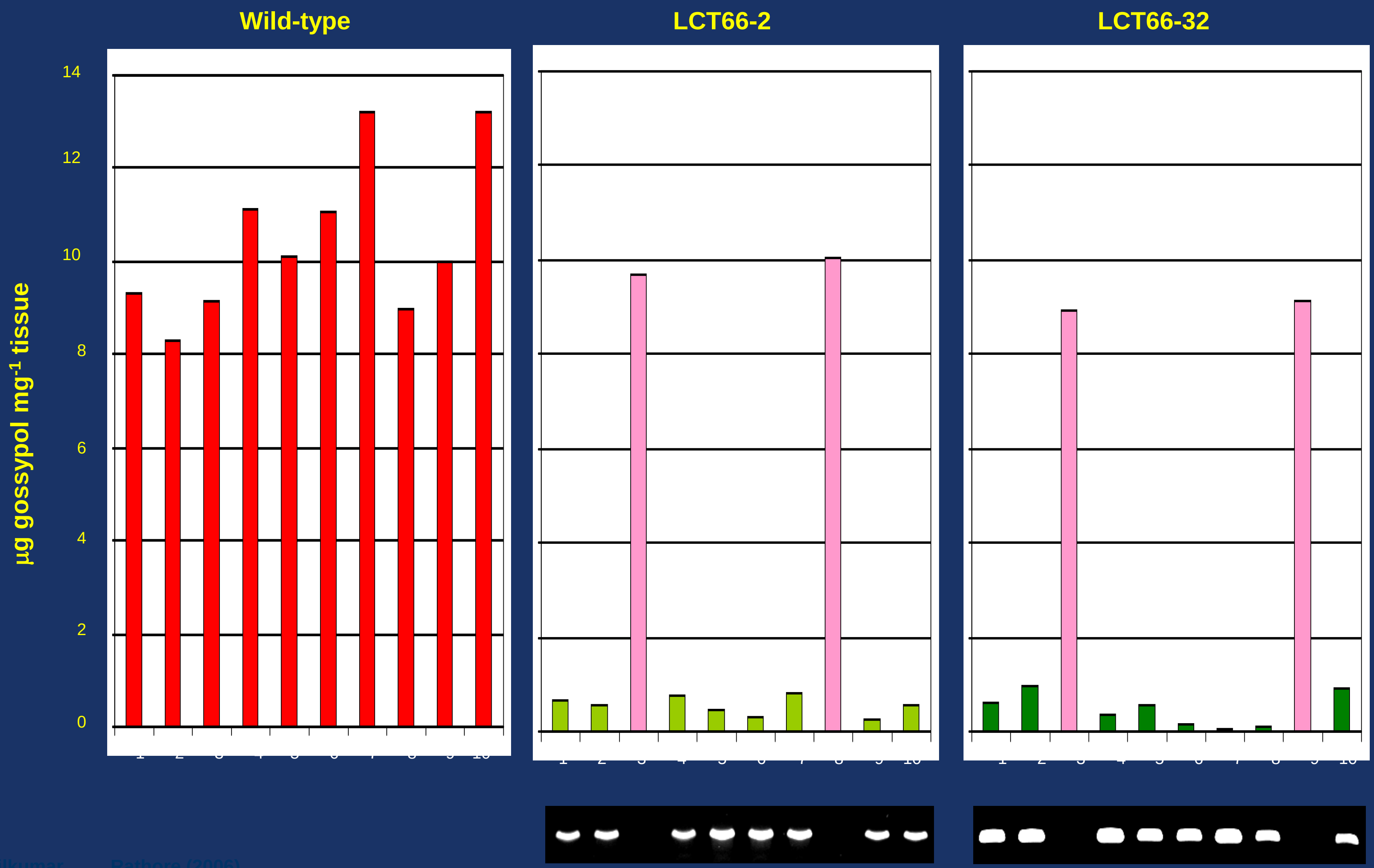
- δ-Cadinene Synthase is first committed step in gossypol biosynthesis

- RNAi and seed specific promoter used to suppress δ-cadinene synthase in cottonseed



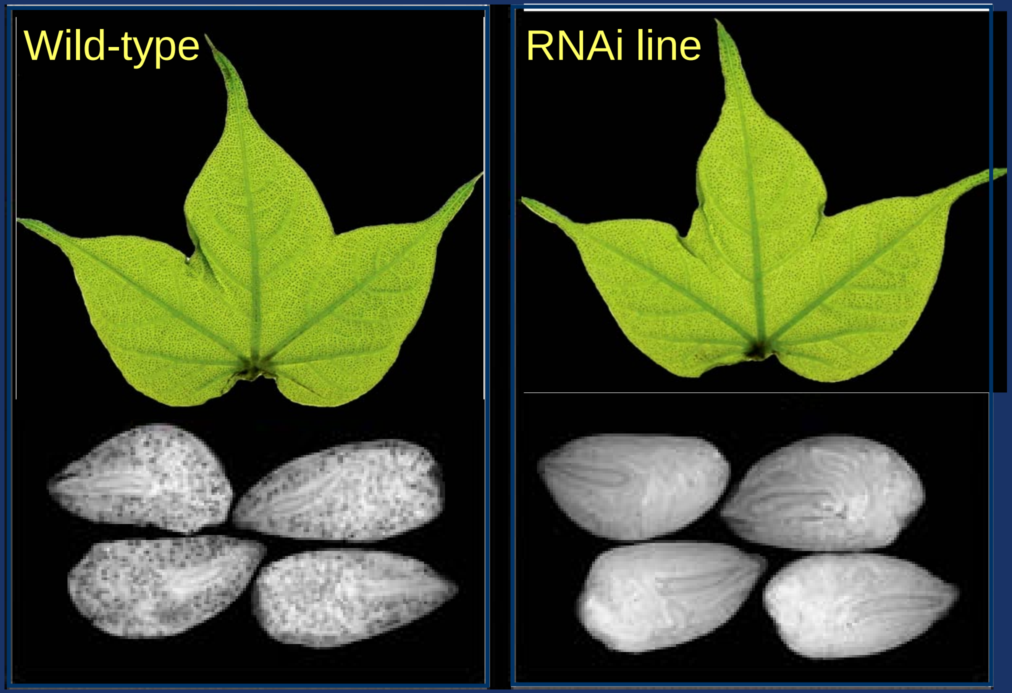
*Sunikumar et al., PNAS, 2006, 103, 18054-18059.

Gossypol Levels in Individual Wild-type and T1 Cottonseeds



Sunilkumar …… Rathore (2006)
PNAS 103:18054-18059

Reducing Gossypol in Cottonseed - Summary



- Some lines show 98% reduction in the seed-gossypol levels
- Non-seed tissues DO NOT show reduction in the levels of gossypol and other defensive terpenoids.
- Trait is stable and heritable (T1, T2, T3, T4 generations).

Improving Potato Storage and Processing*

- Potato (*Solanum tuberosum*) French Fry production in US based on Russet Burbank
- Higher yielding Ranger Russet market penetration limited by 1.) black spot bruise sensitivity, 2.) high levels of cold-induced sweetening
- Bruising due to tuber polyphenol oxidase (Ppo) and sweetening due to starch associated R1 and phosphorylase-L (Ph-L) genes
- Gene silencing of Ppo, R1 and PhL genes using inverted repeats for segments of these genes (RNAi)

*Rommens et al., J. Ag. Food Chem., 2006, 54, 9882-9887.

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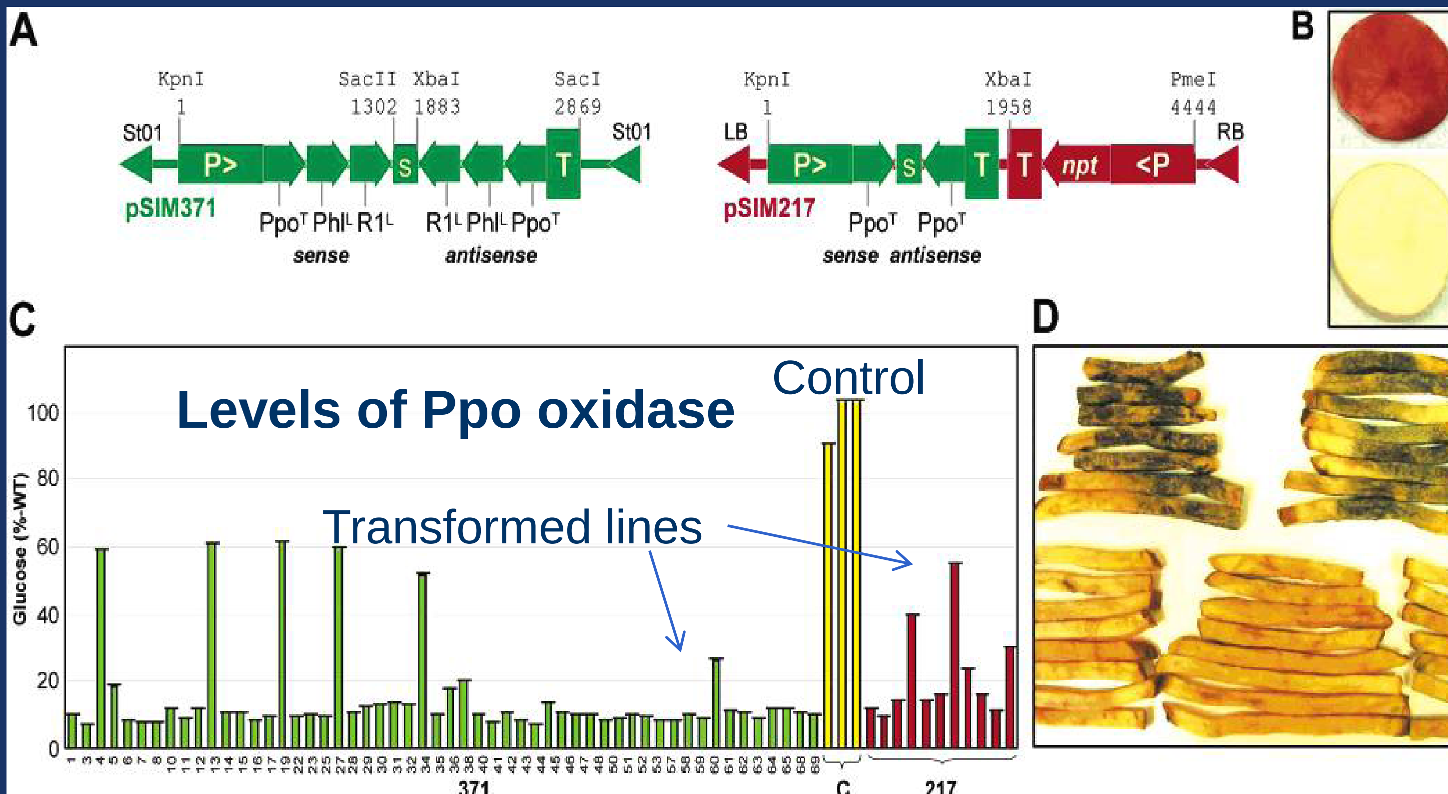
Results for Potato Gene Silencing



Black spot bruise tolerance and greatly reduced levels of cold-induced sweetening achieved plus improved aroma.

Conclusions

- Integrated, rigorous process has demonstrated the safety of biotech products
- ILSI crop composition database has led to an improved understanding of natural variability in crop composition
- Golden Rice 2 has great potential to improve global health associated with Vitamin A deficiency
- New technologies such as RNAi offer promising tools for future new products with grower/consumer benefits
- Work of ILSI IFBiC Task Forces continues to make important contributions to improved understanding of role of biotechnology in global nutritional health

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