Comparative Assessment Studies: Design and Interpretation of Results

GM food and feed safety assessment: Training workshop for regulators
7-8 August, 2014

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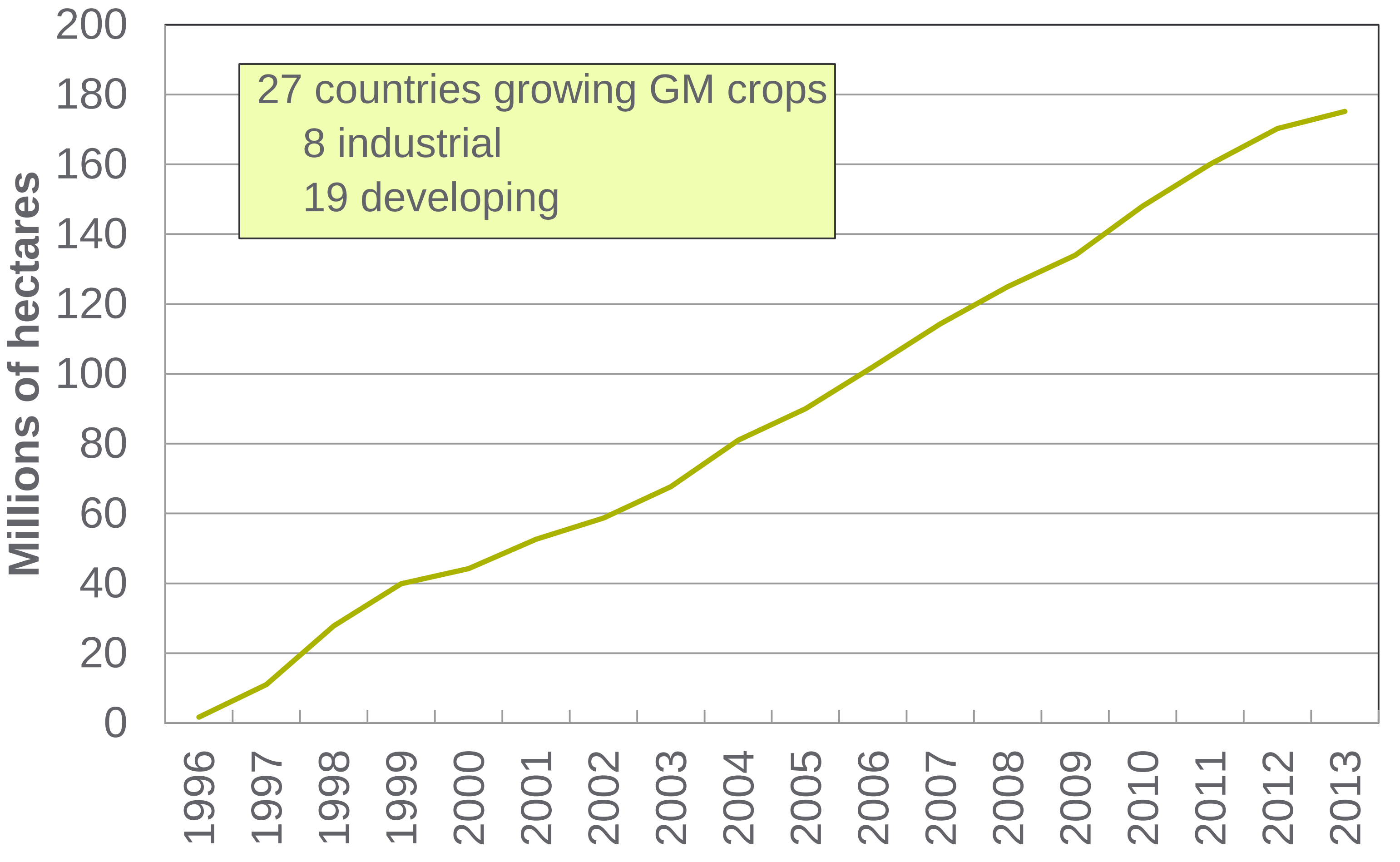
Compositional Data Analysis in the Safety Assessment of GM Crops

- Introduction to the comparative assessment: compositional analysis
- Choosing components to be assessed as they pertain to safety
- ILSI Crop Composition Database
- Design of field studies to control variability
- Interpreting compositional analysis data

Global area of GM crops: 1996 to 2012



James, Clive. 2013. Global status of commercialized biotech/GM crops:2013. *ISAAA Brief* No. 46-2013. ISAAA. Ithaca, NY.

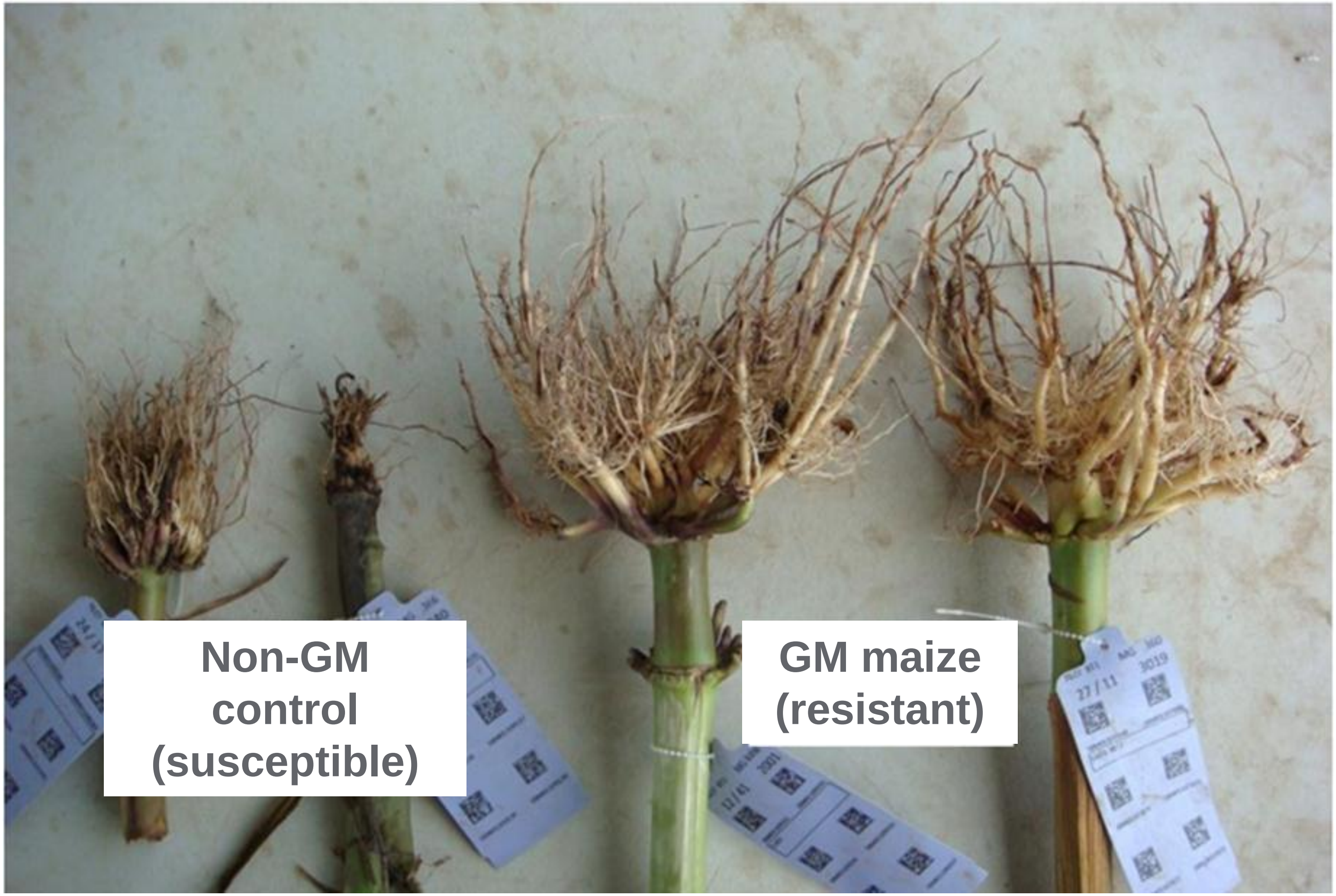
World-wide adaptation of biotech crops

- 100-fold increase in area planted to GM crops since 1996 (1.7 million ha in 1996 vs. 175 million ha in 2013)

- Biotech crops fastest adopted crop technology in recent history – it delivers benefits (James, 2012)

Crop inputs	↓
Convenience and Flexibility	↑
Yield and Quality	↑

Damage caused by *Diabrotica* spp. (corn rootworm)



Brazil - Beans Resistant to Golden Mosaic Virus

Yield lost because of the virus would be able to feed 5-10 million people.

Photos: Franciso Aragão, EMBRAPA

Golden Rice: biofortified with beta-carotene

www.goldenrice.org

Many countries require comprehensive safety assessment of the GM crop

- General guidelines for the safety assessment have been laid out by the Codex Alimentarius Commission in 2003 (established by FAO/WHO)
- Regulations vary by country or region, although the *general* approach to the safety assessment remains fairly consistent with Codex
- Requirements for the safety assessment continue to evolve; crop composition continues to be an important part of the assessment

Comparative Assessment

Safety assessment

- Mode of action
- Agronomic evaluation
- Compositional analysis
- Protein expression studies
- Allergenicity assessments
- Molecular characterization
- Specific toxicity studies
- Environmental risk assessment

Comparative assessment

Comparative assessments contribute to the overall safety assessment.

Goal of Comparative Assessment: Composition

- Document nutritional status of the novel crop: as nutritious as the conventional crop having a history of safe use
  - Assesses the nutrition of the new GM crop and puts it in the context of the natural variability within the conventional crop

- Check for evidence of and document changes / effects:
  - **Intended effects -** product or activity of the transgene
  - **Unintended effects -** insertion of DNA or product(s) of transgenes could cause a change
  - Many of the nutritional components that are measured are end products of metabolic processes or are involved in these processes
  - Also investigated through molecular and agronomic studies

How to decide what Components to measure?

How to decide what Components to measure?

- Depends upon the crop
  - Valuable starting point - Individual crop consensus documents prepared by the Organisation for Economic and Co-operative Development (OECD)
  - Levels of key nutrients, anti-nutrients, toxicants, appropriate secondary metabolites (based on contributions the crop makes to the diet)

- Analysis should be on raw agricultural commodity

- Depends upon the nature of the trait(s) of interest
  - Some types of traits may directly influence metabolism (example: traits for stress tolerance)
  - Decisions on what to include should be hypothesis-driven

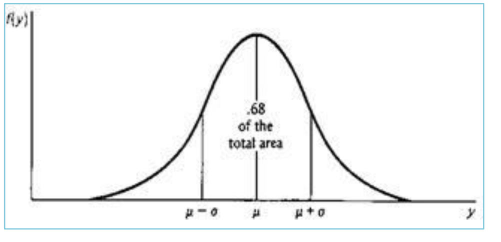
Experimental phase: Nutritional equivalence of GM crop to non-transgenic crop with history of safe use

Replicated field trials at multiple locations

Sample plant tissues relevant to food and feed

Analyze samples for key nutritional & anti-nutritional components

Compare GM crop to non-GM comparator (control)



Comparative Assessment: Interpretation of Results

New GM Crop Composition data

Statistically Different than Comparator ?

Yes

Further investigate observed differences

Compare with variability in:

Comparator

Conventional crop varieties

Literature values, databases

No

As safe as conventional crop

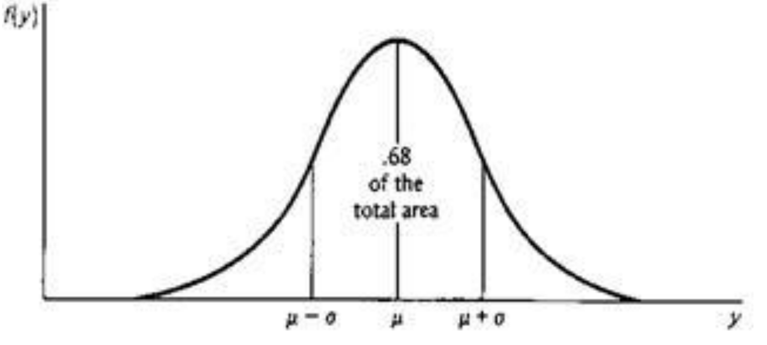
Part of overall Safety Assessment

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.

Statistical significance ≠ Biological relevance



Sources of Variation in Crop Composition

- Experimental Design

- Experimental Conduct

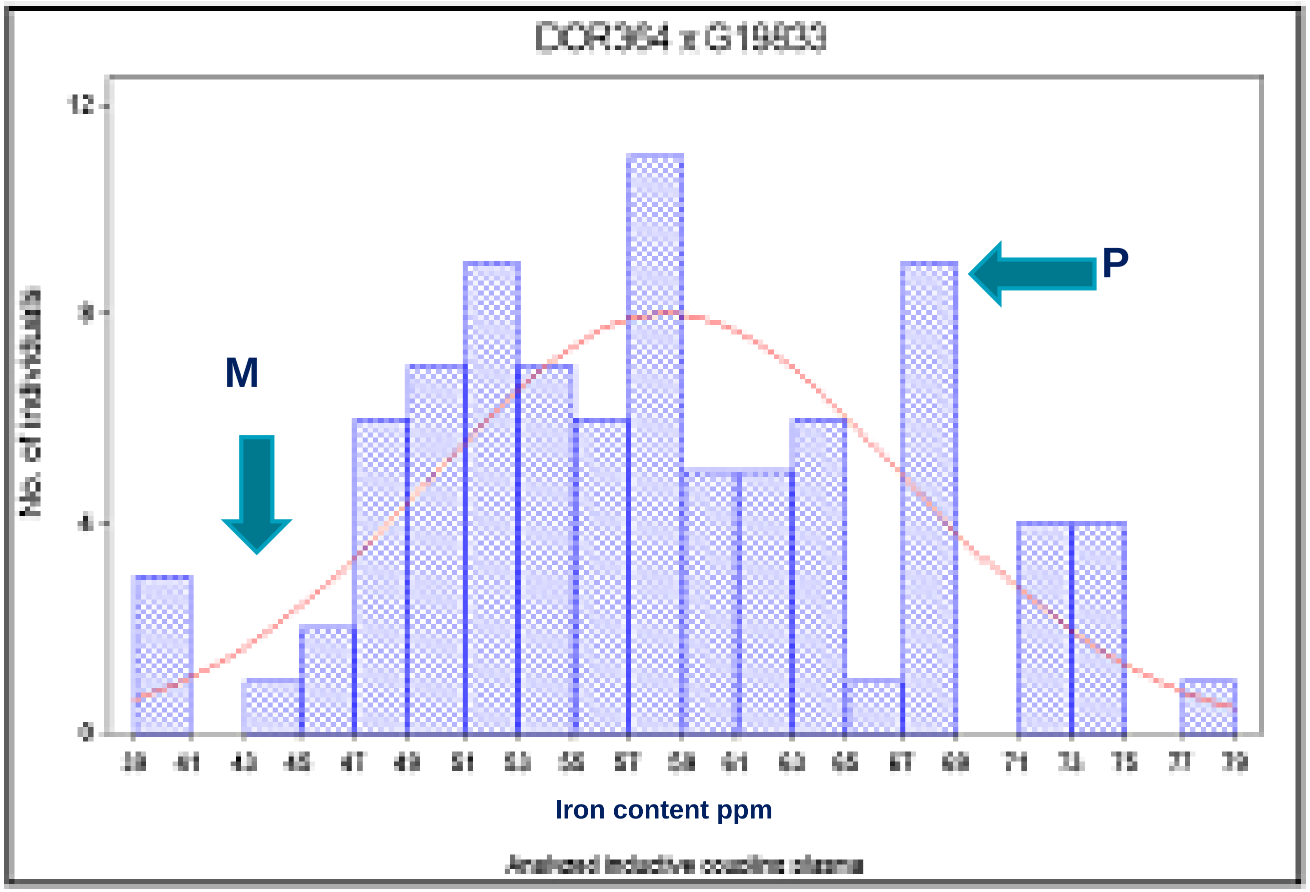
Sources of Variation in Crop Composition

- Experimental Design
  - Lack of proper treatment randomization
  - Improper placement of blocks (replicates) in the field
  - Plot size / number of plants
  - Not enough locations to capture environmental effects

- Experimental Conduct
  - Plots not all treated the same (except for the intended treatments)
  - Failure to control known (control-able) environmental factors
    - Examples: non-uniform weed or insect pressure, movement causing soil compaction
  - Bias in sampling / data collection methods

Sources of Variation in Crop Composition

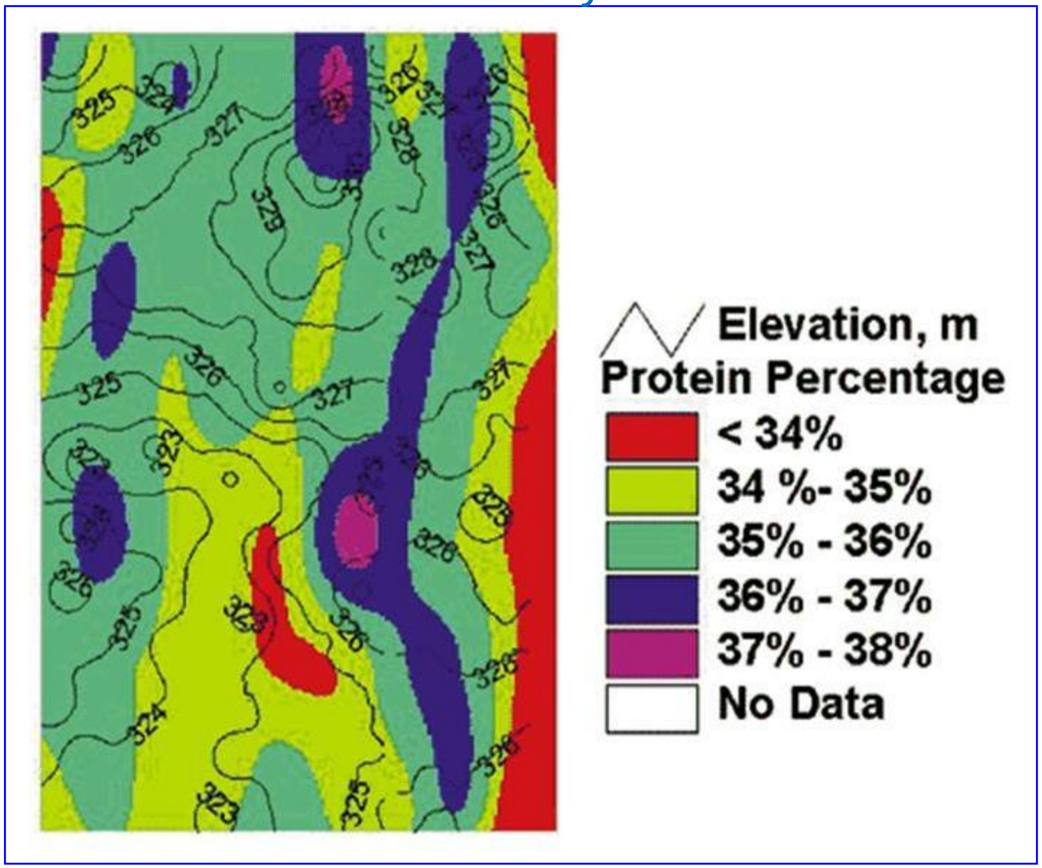
Fe content in common bean



Genotype

Sources of Variation in Crop Composition

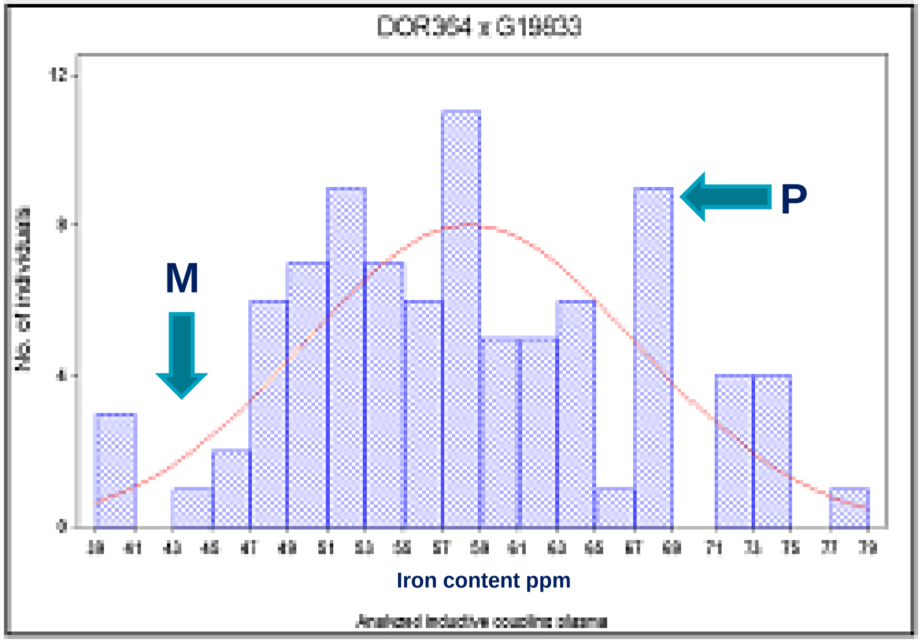
Protein content in soy across a field



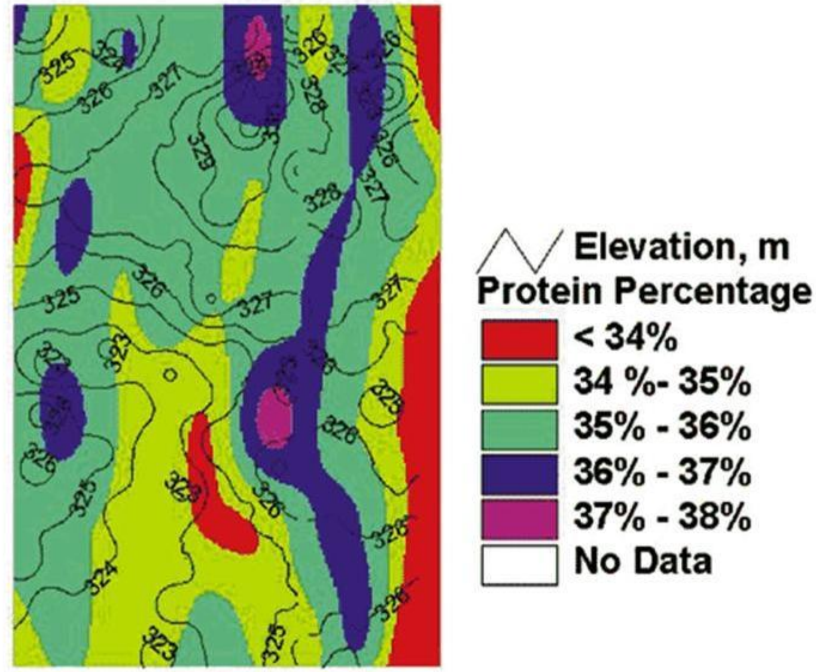
Environment

Sources of Variation in Crop Composition

Fe content in common bean



Protein content in soy across a field



Genotype x Environment

Genotypes differ in their response to various environmental factors.

Variability in a crop is expected and desirable

- Genetic background of a crop is diverse, and the food we eat on a daily basis is highly variable
- Crop breeders actively search out diversity
- Traditional breeding methods, used safely for thousands of years, can cause intended and unintended changes in genetics and composition
- Amount of variability depends on the specific crop component

Maize Landraces
From: Flint-Garcia, ILSI CCW, 2012

Variability in a crop is expected and desirable

- Some components do not act independently
  - Example: protein and starch tend to be negatively correlated

- **Crop variability captured in:**
  - the control genotype within the study
  - reference varieties within the study
  - literature
  - private and public data bases

Maize Landraces
From: Flint-Garcia, ILSI CCW, 2012

Role of Crop Composition Databases

- These data are important tools in helping to describe the boundaries of natural variability for each crop (considered to be safe levels)
- Easily-accessed and *updatable* compilations of crop composition data
- Help to capture the variability of component levels across broad geographies, multiple environments, and multiple years

From: Blair, ILSI CCW, 2012

Extracting Data From the Database

How to access the ILSI Crop Composition Database (CCDB) and how to navigate through the different screens

www.cropcomposition.org

Welcome Page (Screen 1)

International Life Sciences Institute

ILSI IS A GLOBAL NETWORK OF SCIENTISTS DEVOTED TO ENHANCING THE SCIENTIFIC BASIS FOR PUBLIC HEALTH DECISION-MAKING.

Crop Composition Database

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Welcome to the ILSI Crop Composition Database

Version 4

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 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 has been released.

Results from your search of Version 4 (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

Primary Search Criteria (Screen 2)

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

Search Crop Composition Database v4.2

Primary Search Criteria

The first step in searching the Crop Composition Database is to select your primary search criteria to filter the data sets.

You must select one Crop Type and one Tissue Type. You can further filter your results by optionally choosing one or more Crop Years, and Locations.

If you make no selections other than Crop Type and Tissue Type, all data sets for the chosen Crop-Tissue selection will be included.

Crop Source / Crop Type / Tissue Type

Help

Crop Type

Corn - Field - Maize - Zea mays

Tissue Type

Grain

Crop Year

Help

Crop Year(s)

All Years
2005
2004
2003
2002

Location

Help

Country(s)

All Countries
ARGENTINA
AUSTRALIA
BRAZIL
BULGARIA

Region(s)

All Regions

Analyte Filters (Optional)

View Summary of Search Results >

BY SUBMITTING SEARCH, YOU AGREE TO THE TERMS OF USE

Summary of Search Results (Screen 3)

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 total number of samples and the number of samples with data below LOQ (denoted as X < LOQ) reported for each analyte. Expanding an Analyte Type also reports the minimium, maximum, and mean values for the samples in the primary unit of measure; these minimum, maximum, and mean values derive from data that is above LOQ for that analyte.

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. If secondary units of measure (or multiple units of measure for a single analyte) are

Results matching your query criteria

Help

Analyte Type	Analyte	Samples	Min	Max	Mean	Units
Amino Acids	-	-	-	-	-	-
Bio Actives	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-
Fatty Acids	-	-	-	-	-	-
Fiber	-	-	-	-	-	-
Minerals	-	-	-	-	-	-
Other Metabolites	-	-	-	-	-	-
Proximates	-	-	-	-	-	-
Vitamins	-	-	-	-	-	-

More Below

Summary of Search Results (Screen 3)

Query Summary

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

Query Criteria

Help

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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. 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.

LOQ = limit of quantitation, ND = not determined. In those cases where all measured values for a specific

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	184(98<LOQ)	0.05	0.69	0.19	ppm FW
	Sodium	1152(935<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	-	-	-	-	-	-

More Below

Summary of Search Results (Screen 3)

r measure for a single analyte) are referred, Version 4.0 of the ILSI-CCDB requires that data with secondary units of measure be generated and viewed using an output report.

ou can use this information below when defining the specific Analytes ou would like to display in your final eport.

OQ = limit of quantitation, ND = not determined. In those cases where all measured values for a specific analyte are below LOQ, the data is eported as not determined (ND).

	Sodium	1152(935<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	-	-	-	-	-	-

reate Report from Search Results

hoose the Analytes you would like o display in your report output.

ou may choose individual Analytes r may select an Analyte Type and hoose to display all Analytes for the nalyte type chosen.

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

o remove a selected analyte click n the red 'X' next to the anaylte ame.

Analytes to show in output Help

Analyte Type: Choose One | Analyte | Units

Add Analyte(s)

Analyte List Help

elect Fields for Report Output.

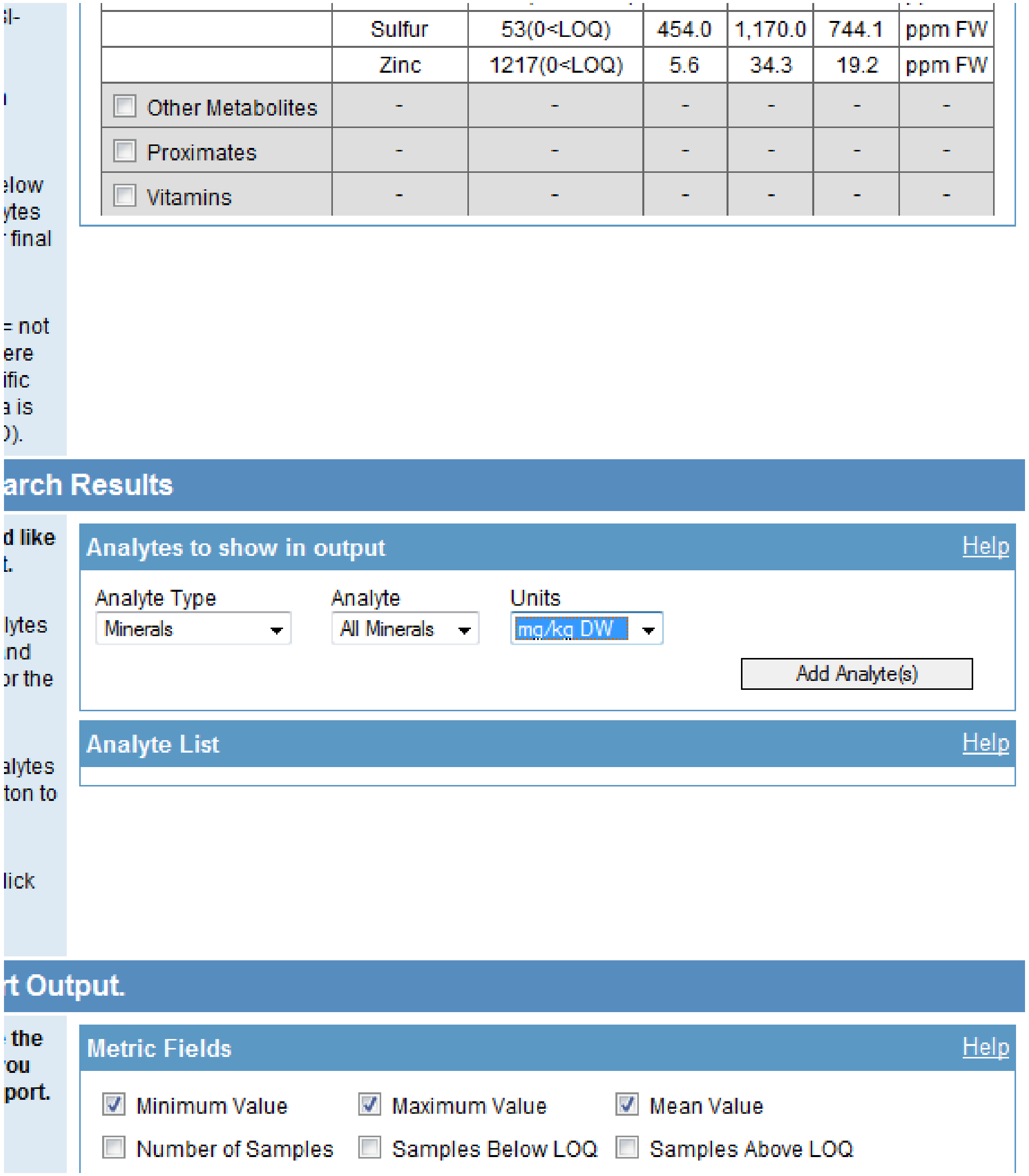
n this section you can choose the netric fields and other fields you vould like to display in your report.

Metric Fields Help

☑ Minimum Value ☑ Maximum Value ☑ Mean Value

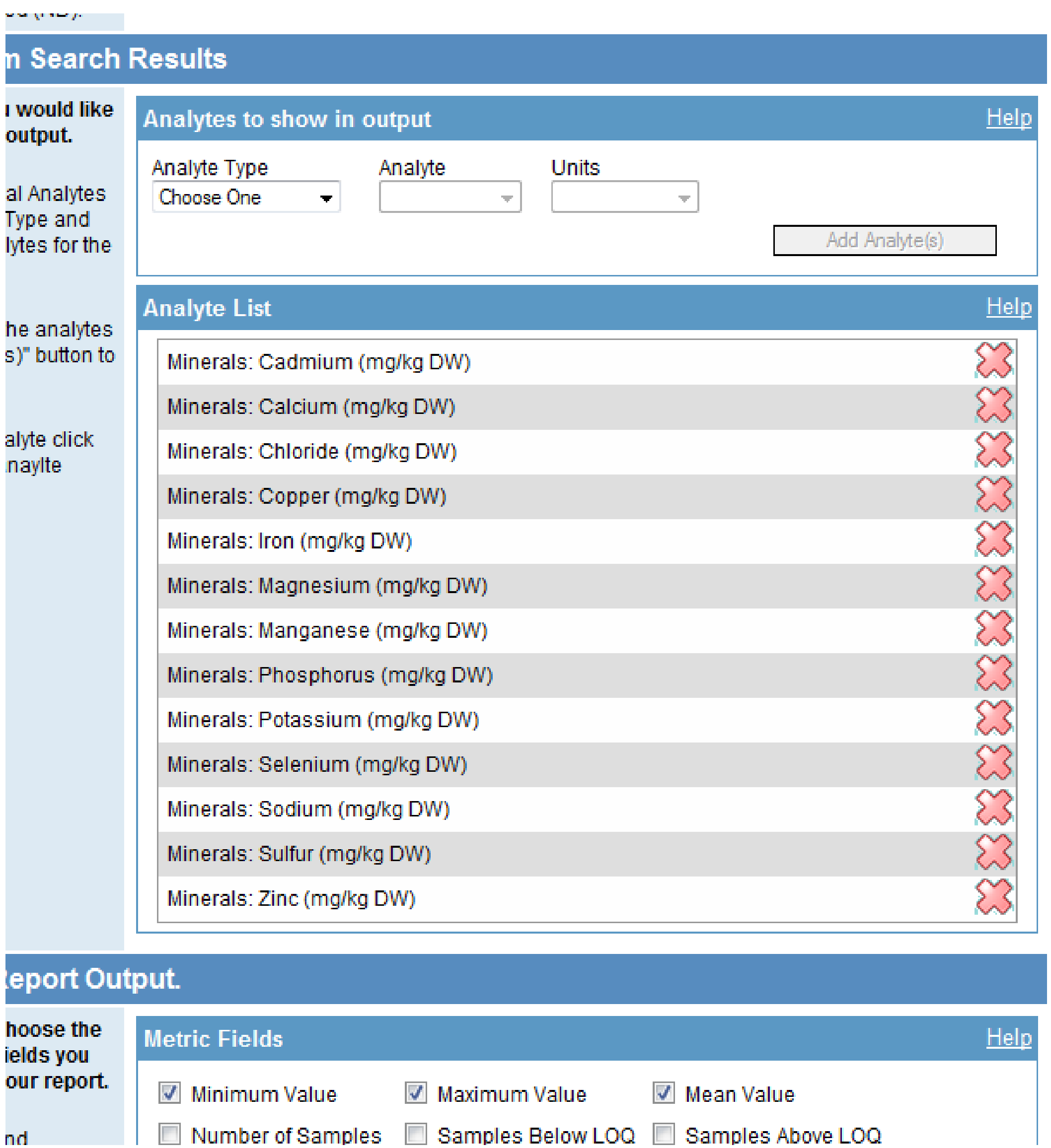
More Below

Summary of Search Results (Screen 3)



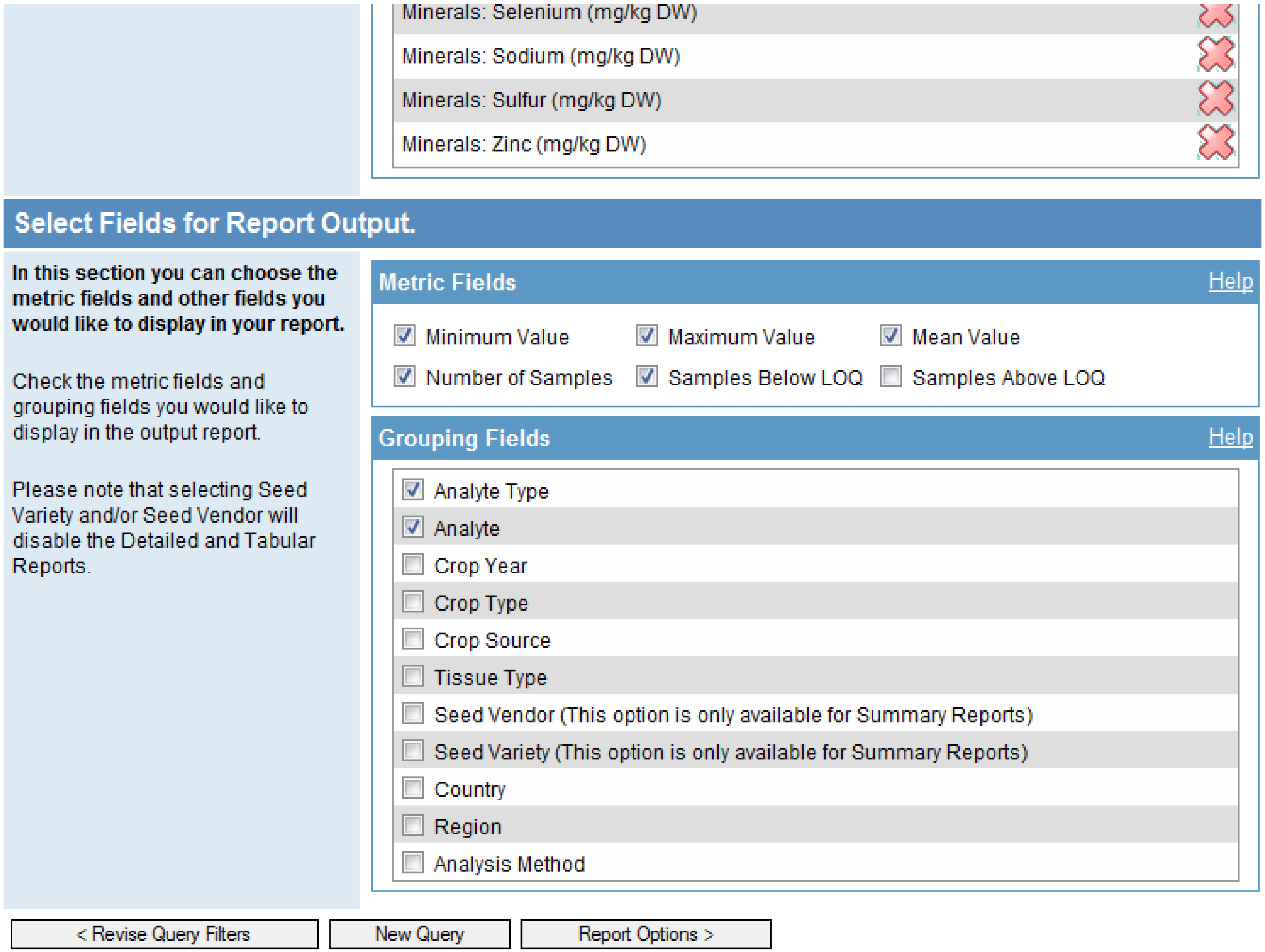
More Below

Summary of Search Results (Screen 3)

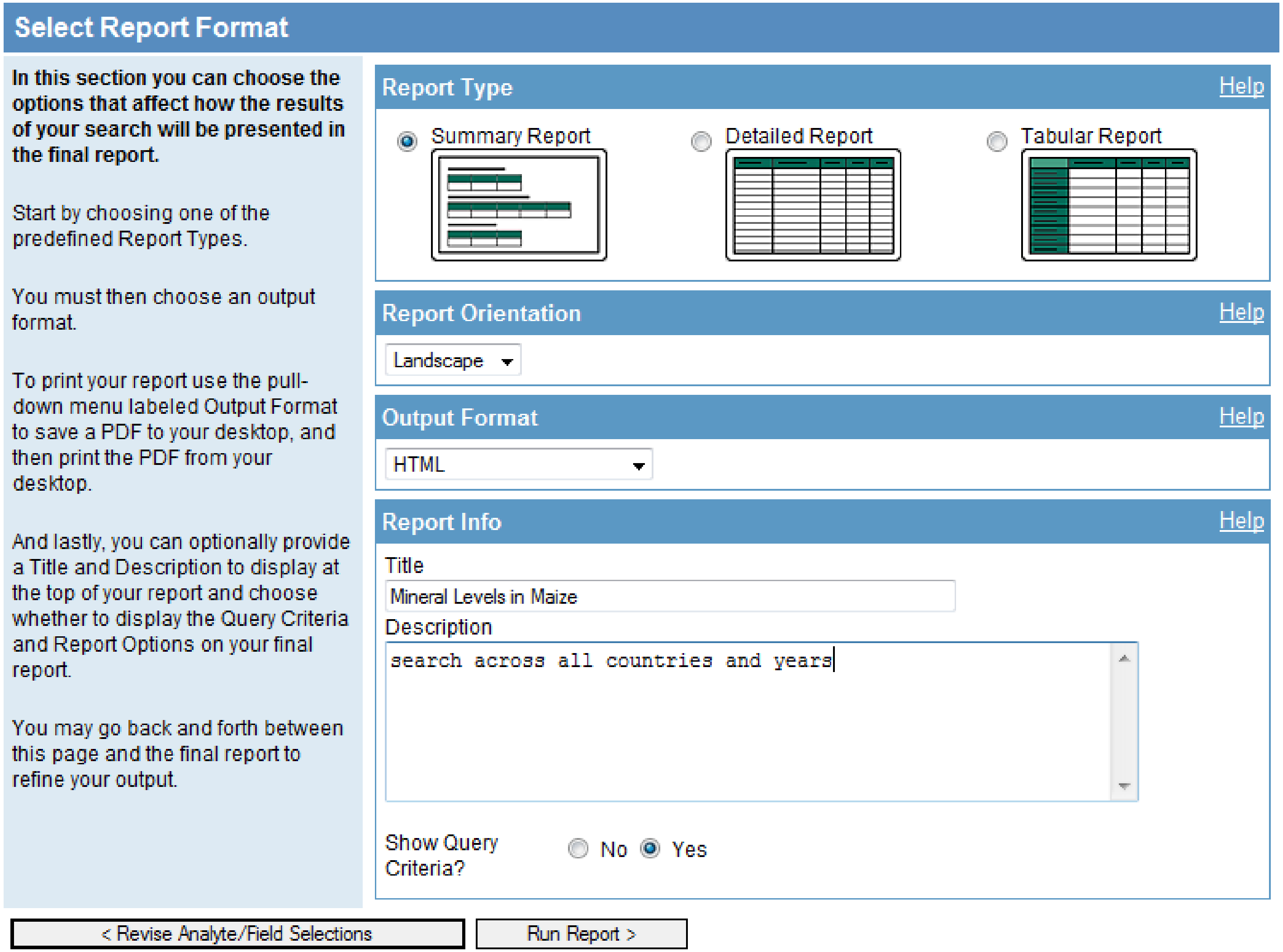


More Below

Summary of Search Results (Screen 3)



Report Options (Screen 4)



Data: *Summary* Report (Screen 5)

Summary Report

Report Output

Output Format: HTML ▾ [Go]

Mineral Levels in Maize

search across all countries and years

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
Minerals	Cadmium	ND	ND	ND	47	47	0	mg/kg DW
Minerals	Calcium	12.7	208.4	46.5	1,309	5	1,304	mg/kg DW

[< Revise Report Options] [New Query]

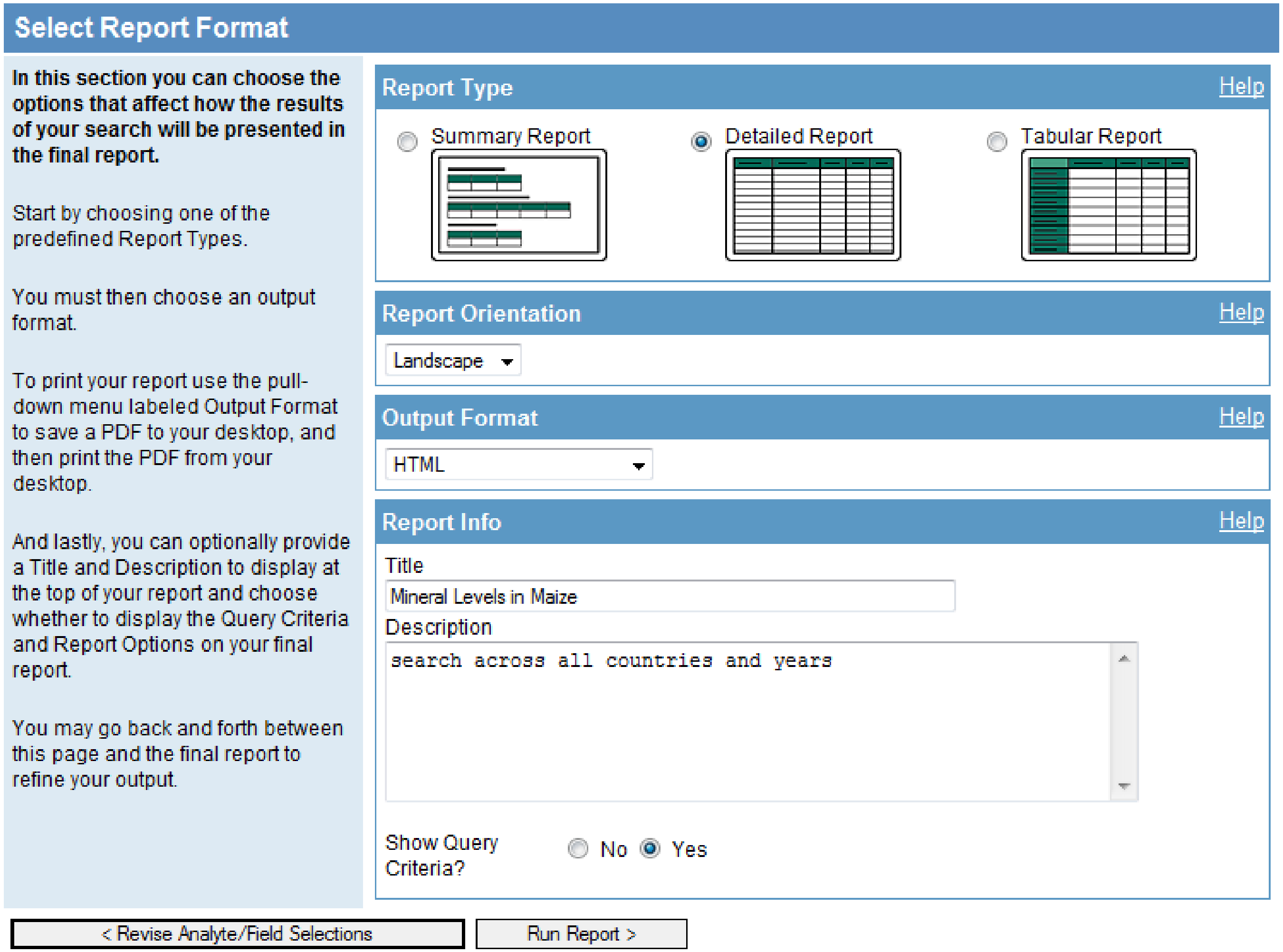
Data: *Summary* Report (Screen 5)

CSV Report (comma-delimited text)

	Summary Report						
	ILSI CCDB Version 4.2						
	Generated at 08/01/2014 10:41 AM Eastern Daylight Time						
Analyte Type	Analyte	Minimum Value	Maximum Value	Mean Value	Number of Samples	Samples Below LOQ	Unit of Measu
Minerals	Cadmium	ND	ND	ND	47	47	mg/kg DW
Minerals	Calcium	12.7	208.4	46.5	1,309	5	mg/kg DW
Minerals	Chloride	375.6	892.9	599.4	53	0	mg/kg DW
Minerals	Copper	0.73	18.50	1.74	1,211	2	mg/kg DW
Minerals	Iron	10.42	49.07	21.80	1,215	0	mg/kg DW
Minerals	Magnesium	594.0	1,940.0	1,190.1	1,217	0	mg/kg DW
Minerals	Manganese	1.69	14.30	6.19	1,216	0	mg/kg DW
Minerals	Phosphorus	1,470.0	5,330.0	3,271.3	1,309	0	mg/kg DW
Minerals	Potassium	1,810.0	6,030.0	3,848.1	1,217	0	mg/kg DW
Minerals	Selenium	0.05	0.75	0.21	184	98	mg/kg DW
Minerals	Sodium	0.17	731.54	32.36	1,152	935	mg/kg DW
Minerals	Sulfur	506.1	1,370.0	847.8	53	0	mg/kg DW
Minerals	Zinc	6.5	37.2	21.6	1,217	0	mg/kg DW

Copies easier into Excel

Report Options (Screen 4)



Data: *Detailed* Report (Screen 5)

Tabular Report

Report Output

Output Format: HTML [Go]

Mineral Levels in Maize Detailed

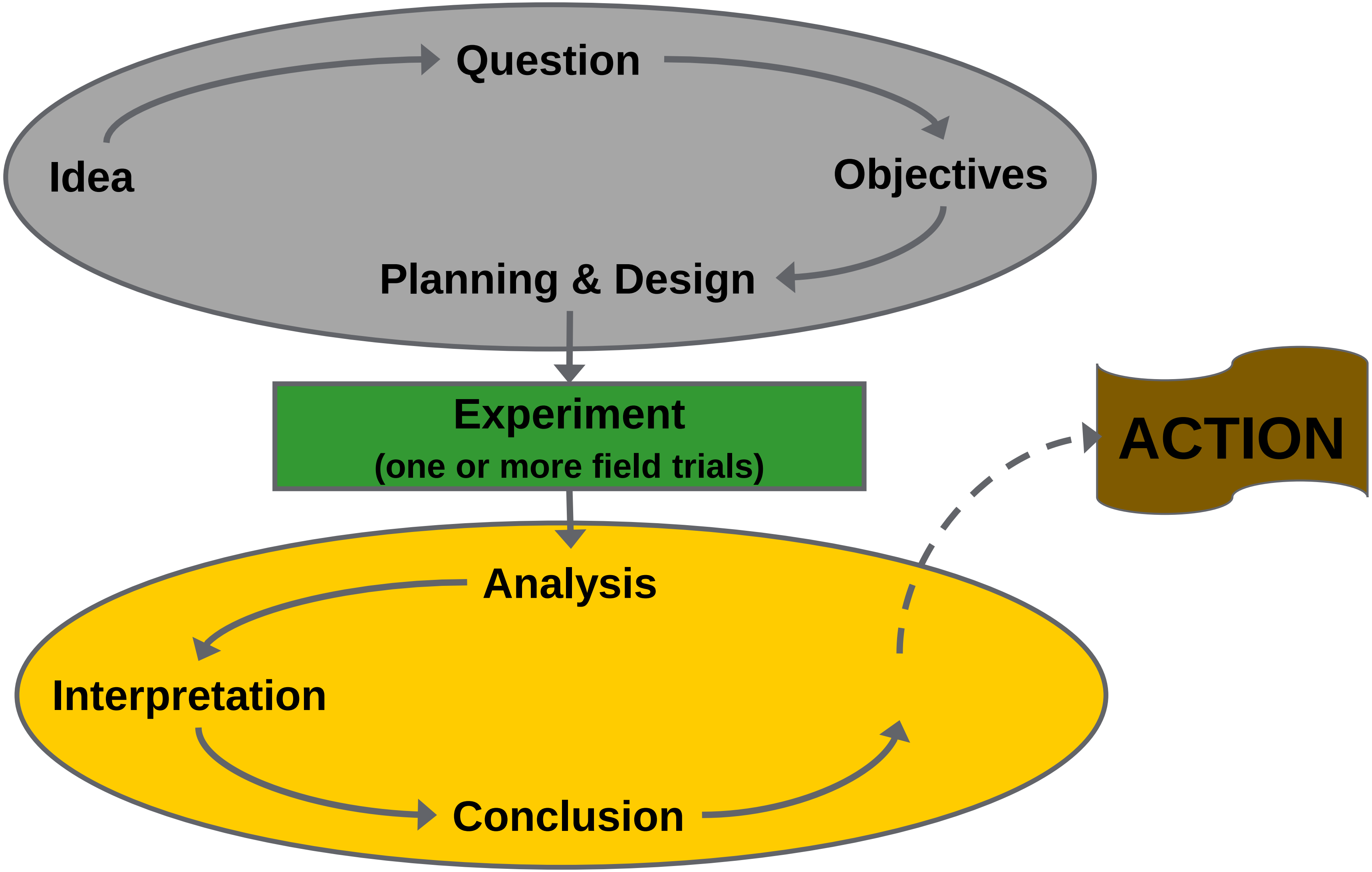
search across all countries and years

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

Analyte Type	Analyte	Value	Units
Minerals	Cadmium	<LOQ (7.52)	mg/kg DW
Minerals	Cadmium	<LOQ (7.72)	mg/kg DW
Minerals	Cadmium	<LOQ (7.74)	mg/kg DW
Minerals	Cadmium	<LOQ (7.77)	mg/kg DW

[< Revise Report Options] [New Query]

Overview of the General Trial Process



Why do we conduct experiments?

The objective is to determine if there are **<u>real</u>** differences among responses to treatments,

and . . .

to estimate the magnitude of those differences if they exist

and . . .

to understand if the outcome is repeatable.

FACT:

As agricultural researchers, we work in some of the most variable experimental conditions in science.

Is The Difference Real?

Statistical inference of differences among treatments involves assigning some measure of probability to the inference.

How likely is it that an observed difference was a random occurrence?

***P* = 0.05** → **If the probability of something occurring at random is below 5%, then we say it is significant**

Note: setting the probability at *P* = 0.05 means that we are willing to accept that 1 in 20 comparisons will result in concluding that a difference occurred, when it actually occurred by chance (a "false positive").

Experimental Error

- Experimental error is the variation that exists among observations on experimental units (plots) treated alike
- Account for and remove known variation when possible
- Take steps to allow quantification of unknown variation

If Background Noise > Treatment Difference

Then ➢ **fail to detect a true difference**

Field Trial Design for Comparative Assessment Studies

- **Entries / Treatments:**

  - **Control** - typically a nontransgenic, near-isogenic genotype, but depends upon nature of trait and availability of the genotype

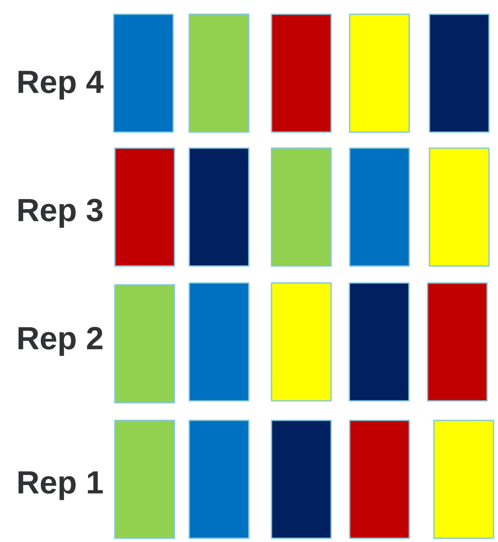
  - **GM event of interest**

  - **Reference varieties** normally included –
    - commercial varieties, other germplasm sources
    - currently these are usually non-GM, but this may change
    - normally at least three; helps to assess variability in the crop

Field Trial Design for Comparative Assessment Studies

- **Number of locations:**
  - Need to be able to represent environmental growing conditions normal for that crop
  - Three locations usually a minimum
  - One year sufficient if environmental conditions can be represented

- **Randomized Complete Block Design (RCBD)** is typical
  - Multiple replications at each field trial site (4 is usually a minimum) and reps arranged into “blocks”
  - Each entry appears once and only once in each block; assigned randomly to each block



Statistical Model:

$$Y_i = U + E_i$$

Statistical Model:

Treatment Mean

$$Y_i = U + E_i$$

Observed value

Error

Background Noise

Observations:

7 9 10 6 14

$$7 = 9.2 + E_i$$

Statistical Model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

Statistical Model:

Error

$$Y_{ijk} = U + T_i + \underbrace{L_j + B(L)_{jk} + LT_{ij}}_{\text{Identified sources of variability}} + e_{ijk}$$

Experimental Error

A good experiment is one that both facilitates valid identification of experimental error and reduces experimental error.

Account for Experimantal Error

- appropriate design & analysis
- adequate replication
- randomization
- proper blocking

Reduce Experimental Error

- uniform exp. units
- size/shape of exp. units
- unbiased sampling
- precise measurements

Orientation of blocks within the field design:

- **Blocked *against* a gradient**
  - The blocks (reps) cannot account for the differences within the field
  - Could lead to false differences among treatments

- **Sources of gradients:**
  - slope
  - soil factors (O.M., parent material, water movement)
  - fertilizer dispersal
  - movement of equipment
  - crop pests
  - unknowns….

Orientation of blocks within the field design:

- **Blocked *with* a gradient**
  - allows statistical model to account for differences in the gradient
  - Error term becomes smaller; more likely detection of true differences

- **Problems:**
  - More than one gradient possible (probable)
  - Gradient(s) often unknown

Interpretation of Data from Compositional Analysis Studies

Statistical differences should be investigated to determine relevance to nutrition and health

- Place differences in context of natural variability

- First data scenario – example of how to interpret results from a comparative analysis study

- Three additional data scenarios –
  - Work in groups of 2 or 3
  - Discuss results and what types of things to consider in data interpretation
  - Formulate a conclusion – has equivalence between the novel GM crop and the conventional crop been demonstrated?

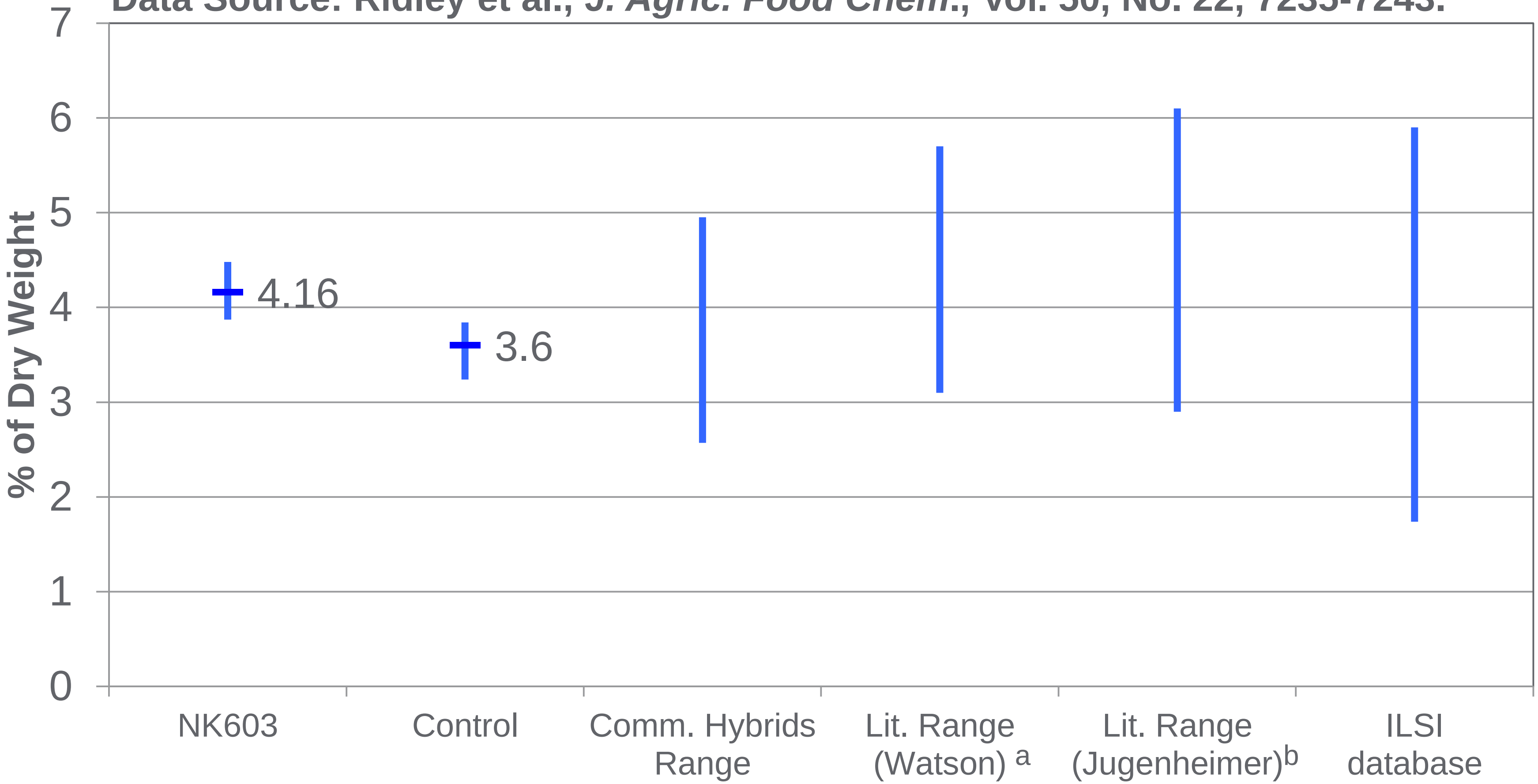
Composition of event NK603 maize (glyphosate-tolerant) at two replicated sites in the EU

Component (percent dry weight)	NK603 mean (range)	Control mean (range)	Comm. Hybrids (range)	Literature (range)
Protein	12.07	11.34		(6.0-12.0)
	(10.23-13.92)	(10.13-13.05)	(7.77-12.99)	(9.7-16.1)
Total Fat	4.16	3.60		(3.1-5.7)
	(3.87-4.48)	(3.24-3.84)	(2.57-4.95)	(2.9-6.1)
Ash	1.38	1.34		
	(1.23-1.65)	(1.25-1.50)	(1.02-1.94)	(1.1-3.9)
ADF	3.21	3.03		
	(2.63-3.87)	(2.30-3.68)	(2.46-6.33)	(3.3-4.3)
NDF	10.08	10.57		
	(8.50-12.00)	(9.35-11.63)	(8.45-14.75)	(8.3-11.9)
Carbohydrates	82.39	83.73		
	(80.49-84.57)	(81.93-84.92)	(82.18-88.14)	not reported

From: Ridley et al., J. Agric. Food Chem. 2002, 50, 7235-7249

Levels of total fat detected in NK603 maize compared to control

Data Source: Ridley et al., *J. Agric. Food Chem.*, Vol. 50, No. 22, 7235-7243.



[a] Watson, Stanley A. Structure and composition. In *Corn: Chemistry and Technology*; Watson, S. A., Ransted, P. E., Eds.;American Association of Cereal Chemists: Minneapolis, MN,1987; pp 53-82.
[b] Jugenheimer, R. W. In *Corn: Improvement, Seed Production, and Uses*; Wiley: New York, 1976; p 227.

Composition of event NK603 maize (glyphosate-tolerant) at two replicated sites in the EU

Component (mg/kg dry weight)	NK603 mean (range)	Control mean (range)	Comm. Hybrids (range)	Literature (range)
Calcium	0.0053	0.0053		
	(0.0050-0.0058)	(0.0050-0.0058)	(0.0039-0.0076)	(0.01-0.1)
Copper	1.89	1.83		
	(1.77-1.99)	(1.69-1.97)	(1.16-2.78)	(0.9-10)
Iron	22.73	21.81		
	(17.43-26.91)	(18.52-25.87)	(15.42-29.34)	(1-100)
Manganese	6.73	6.42		
	(5.18-7.90)	(5.63-7.32)	(3.86-10.47)	(0.7-54)
Potassium	0.36	0.38		
	(0.34-0.38)	(0.36-0.39)	(0.32-0.45)	(0.32-0.72)
Zinc	23.78	23.21		
	(15.95-31.45)	(17.87-29.88)	(13.51-27.98)	(12-30)

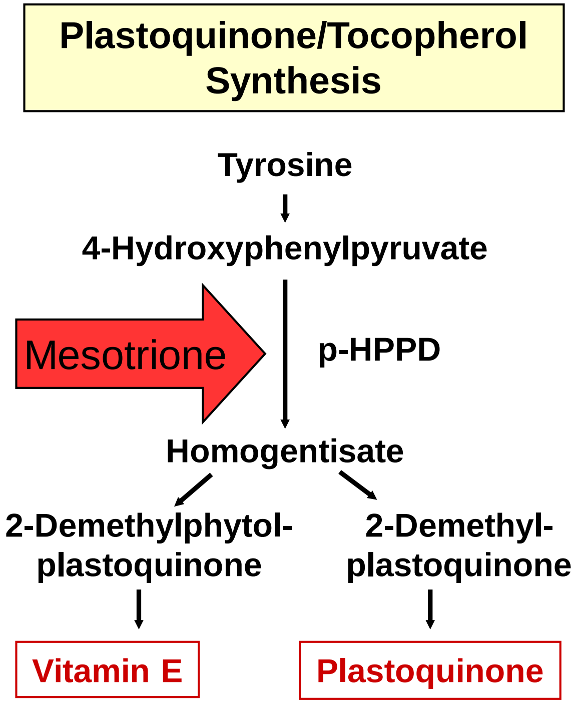
From: Ridley et al., J. Agric. Food Chem. 2002, 50, 7235-7249

Composition of event MON 88017 maize (glyphosate-tolerant) at two replicated sites in Argentina

Component (% of total fatty acids)	MON 88017 mean (range)	Control mean (range)	Comm. Hybrids (range)	Literature (range)
16:0 palmitic	11.52	11.71		
	(11.31-11.79)	(11.35-12.58)	(8.29-12.81)	(8.51-17.46)
18:0 stearic	2.05	2.07		
	(2.00-2.11)	(1.95-2.20)	(1.35-2.49)	(1.02-2.76)
18:1 oleic	26.61	32.12		
	(25.72-27.74)	(30.50-33.97)	(19.73-40.72)	(18.6-40.1)
18:2 linoleic	57.69	51.97		
	(56.22-58.80)	(49.67-53.98)	(45.41-65.50)	(43.1-65.6)
18:3 linolenic	1.12	1.08		
	(1.08-1.16)	(0.92-1.14)	(0.73-1.30)	(0.70-1.92)
20:0 arachidic	0.43	0.45		
	(0.41-0.44)	(0.42-0.49)	(0.30-0.53)	(0.279-0.720)

From: McCann et al., J. Agric. Food Chem. 2007, 55, 4034-4042

Mesotrione mode of action



Literature:
Overexpression of *hppd* may affect vitamin E levels

Event SYHT0H2 - includes *avhppd-03* gene from oat (less binding affinity for mesotrione)

Further investigation of these levels

Vitamin E isoforms	Relative Vitamin E activity
α-tocopherol	100%
β-tocopherol	50%
γ-tocopherol	10%
δ-tocopherol	3%

Composition of event SYHT0H2 soybean (mesotrione-tolerant) at eight replicated sites in the USA

(µg/g seed dry weight)	SYHT0H2 mean (range)	Control mean (range)	non-GM Reference Varieties mean (range)	Codex Alimentarius (standard ranges in soybean oil)
α-tocopherol	22.8	25.8	29.5	
	(9.96-62.8)	(9.34-60.5)	(11.5-77.1)	(9-352)
β-tocopherol	< LOQ	< LOQ	-	
	-	-	(< LOQ-7.79)	(undetectable-36)
γ-tocopherol	226	201	175	
	(183-268)	(154-244)	(127-236)	(89-2307)
δ-tocopherol	78.9	61.1	68.7	
	(51.8-107)	(31.2-84.5)	(32.0-112)	(154-932)

From: Kramer et al., J. Agric. Food Chem. 2014, 62, 3453-3457