Evaluating the occurrence of unintended effects through nutritional compositional analysis studies

Workshop on Molecular and Genetic Basis of Potential Unintended Effects in Modified Plants
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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.

Why Compositional Analysis?

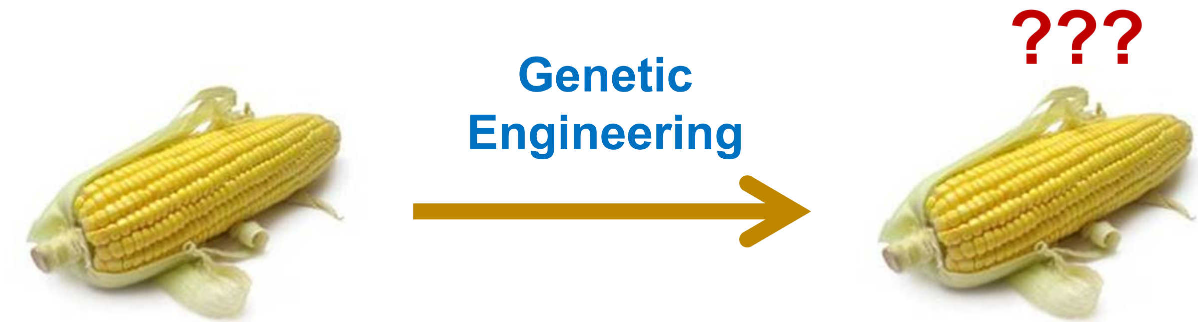
Genetic Engineering

???

Transformation process?

Gene or gene products?

Why Compositional Analysis?



Transformation process?

Gene or gene products?

- **Compositional analysis is used to:**
  - Assess the <u>nutritional status</u> of the resultant food/feed (nutrients, antinutrients, toxicants, appropriate secondary metabolites); show *substantial equivalence* to its conventional (non-GM) counterpart
  - Provide evidence for where an <u>unintended change</u> in nutrition may have occurred

Concept of “Substantial Equivalence”

Objective: to demonstrate *substantial equivalence* of the GM crop to the traditional crop having a history of safe use as feed and food.

The concept of substantial equivalence, “…embodies the idea that existing organisms used as food, or as a source of food, can be used as the basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new.”

(OECD 1993)

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

Replicated field trials at multiple locations:

- Randomized complete block design
- All plots treated the same
- Local agronomic practices
- Treatments:
    - o GM Crop
    - o Nontransgenic, near-isogenic comparator
    - o Often conventional (non-GM) “reference varieties” are included

Sample plant tissues relevant to food and feed

Analyze samples for key nutritional components

Choosing nutritional components to measure:

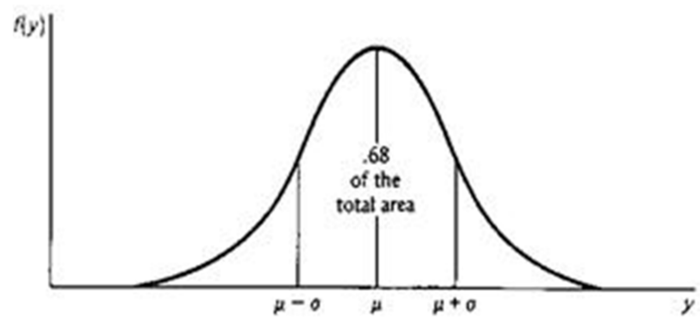
- **Starting point: Organisation for Economic and Cooperative Development (OECD) consensus documents**
  - "Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*)*:* Key Food and Feed Nutrients, Anti-Nutrients and Secondary Metabolites"

Components	Kernels	Silage / Forage
Proximates	Food, Feed	Feed
Minerals	Food	
Calcium	Feed	Feed
Phosphorus	Feed	Feed
Vitamins	Food	
Amino acids	Food, Feed	
Fatty acids	Food, Feed	
Phytic acid	Food, Feed	
Raffinose	Food	
Furfural	Food	
Ferulic acid	Food	
p-coumaric acid	Food	

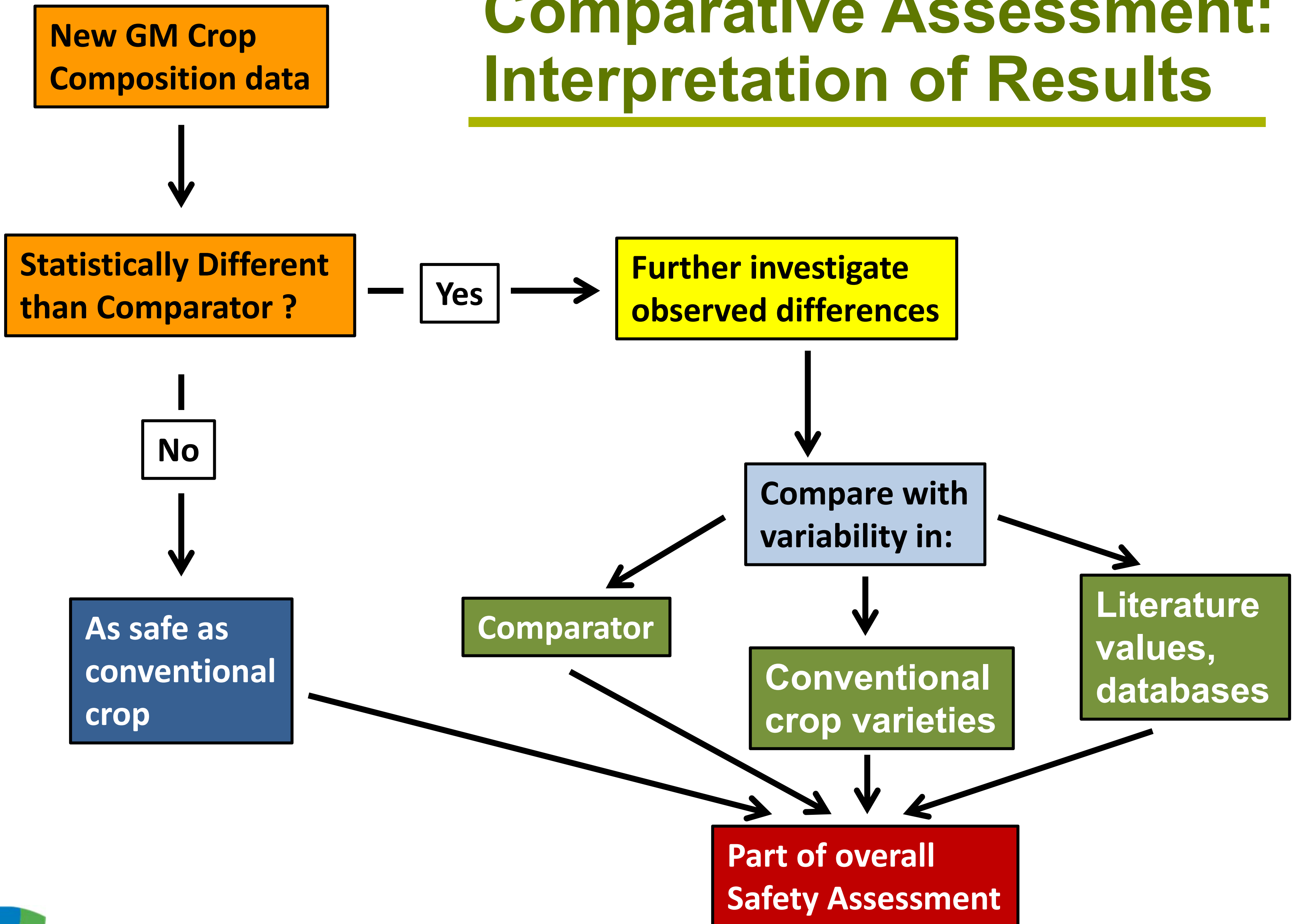
Compositional Analysis Studies

- Statistical comparison between the GM crop and the comparator (GM crop with trait-specific herbicides and comparator)

- Compare levels in the GM crop with levels recorded for the crop within the study (ranges for reference lines, if included in trial design), recorded in the literature, or in available databases.



Comparative Assessment: Interpretation of Results



The GM crop is place in context of the crop commodity

Compare to variability in the global crop commodity

Concept of “Substantial Equivalence”

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Substantially Equivalent ≠ Identical

Two conventionally bred cultivars (varieties) of a crop are considered to be substantially equivalent to one another, despite any differences in nutritional status.

Two commercial, non-GM maize hybrids are substantially equivalent to one another

- Two *commercial, non-GM maize hybrids* grown in a single field trial (one location, RCBD with 4 replicates)
- **55 statistical comparisons** made (Tukey's at $\alpha = 0.05$)
- **21 statistically significant differences** in component levels were detected
- Substantially equivalent regardless of these differences

(At $\alpha = 0.05$, one would expect about 3 significant differences due to chance alone.)

Component	Significance
Protein	Significant
Total Fat	NS
Ash	NS
Carbohydrates	NS
Starch	NS
Fibers (ADF, NDF, Total)	0 out of 3
Minerals	3 out of 8
Vitamins	2 out of 7
Trypsin Inhibitor	NS
Phytic Acid	NS
Raffinose	Significant
Ferulic Acid	Significant
p-Coumaric Acid	Significant
Total Inositol	NS
Amino Acids	9 out of 18
Fatty Acids	3 out of 8

Unintended Effects

Unintended Effect – a change that results from insertion of the trait or its action but that was not a part of the original, intended outcome.

- *Expected* – can be predicted due to expected phenotypic characteristics conveyed by the new trait(s)
  - Example: the intended increase in percent content of a particular fatty acid leads to reduction in one or more other fatty acids

- *Unexpected* – cannot be predicted by current knowledge of crop biology and composition
  - Example: the intended increase in percent content of a particular fatty acid is associated with a change in Riboflavin

Unintended Effects

An *unintended effect* is observed in constant association with the GM trait(s) of interest and are repeatable – across locations, years, etc.

A statistically significant difference does not automatically indicate an unintended effect:

- GM crop and its near-isogenic comparator may not be as similar as assumed
- For a typical compositional analysis study, levels of 55 or more components are compared between the GM crop and its non-GM comparator.
  - At the 0.05 probability level, one expects about 3 statistically significant differences due to random chance alone (Type II error).
  - These differences are not repeatable / not consistent
- Other differences may arise due to unforeseen or unpredicted experimental error:
  - Plot-to-plot differences that were uncontrollable with experimental blocking or unpredicted effects between plots (inter-plot interference)
  - These differences are not repeatable / not consistent

How does measurement of these components address the purpose of compositional analysis studies?

- Nutritional Assessment –
  - Measurement of components for which the crop (food) is an important dietary source
  - Measure what is impactful in the diet of humans and animals

- Unintended Effects –
  - The components are metabolites or end products of various major pathways within the plant.
  - Unintended changes in metabolic pathways are likely to be detected as changes in component levels.

Note: composition studies do not verify that an unintended effect has occurred, but only identify where further assessment may be needed.

The Shikimate Pathway and Aromatic Amino Acid Biosynthesis in Plants

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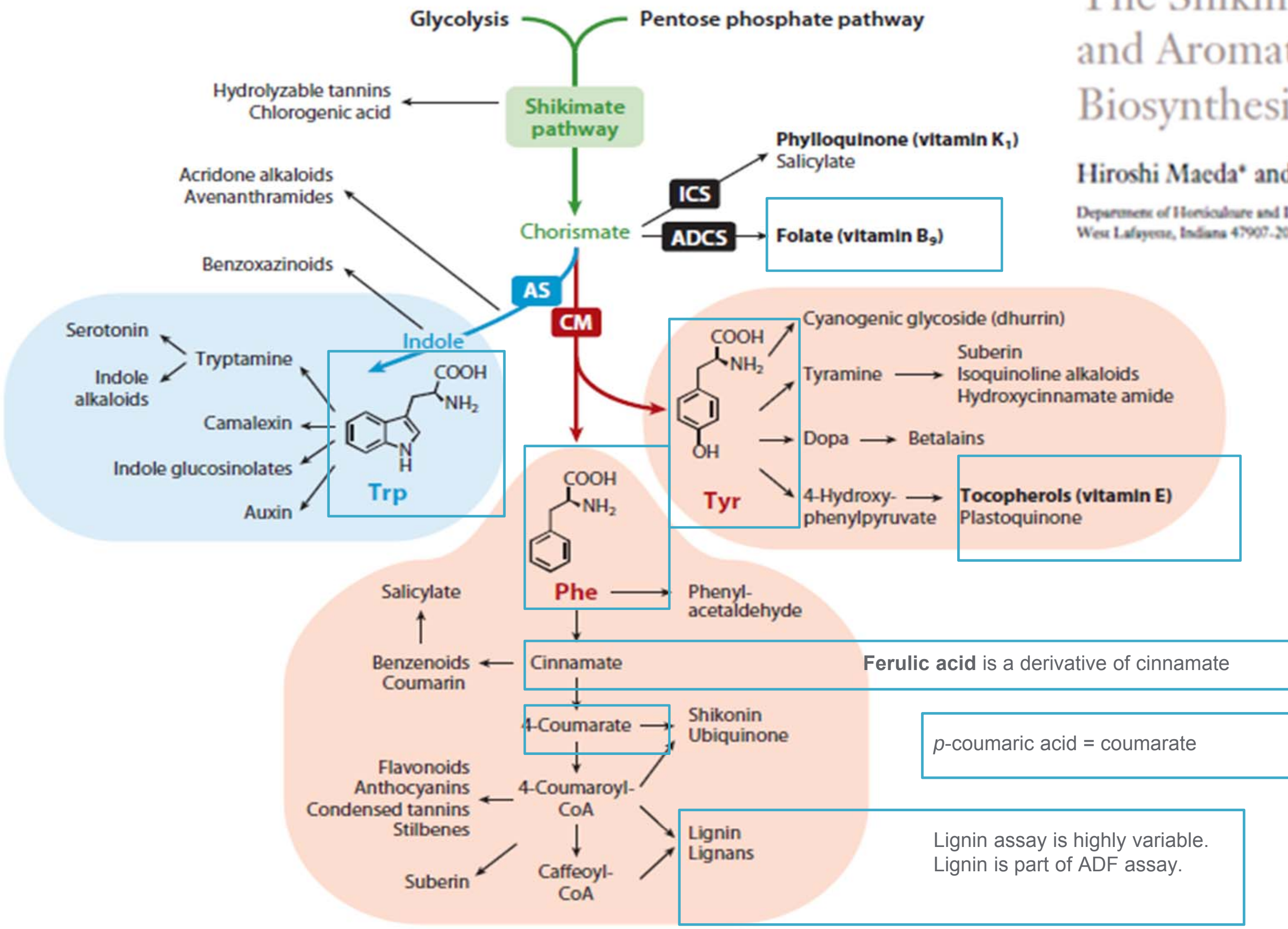


Figure 1

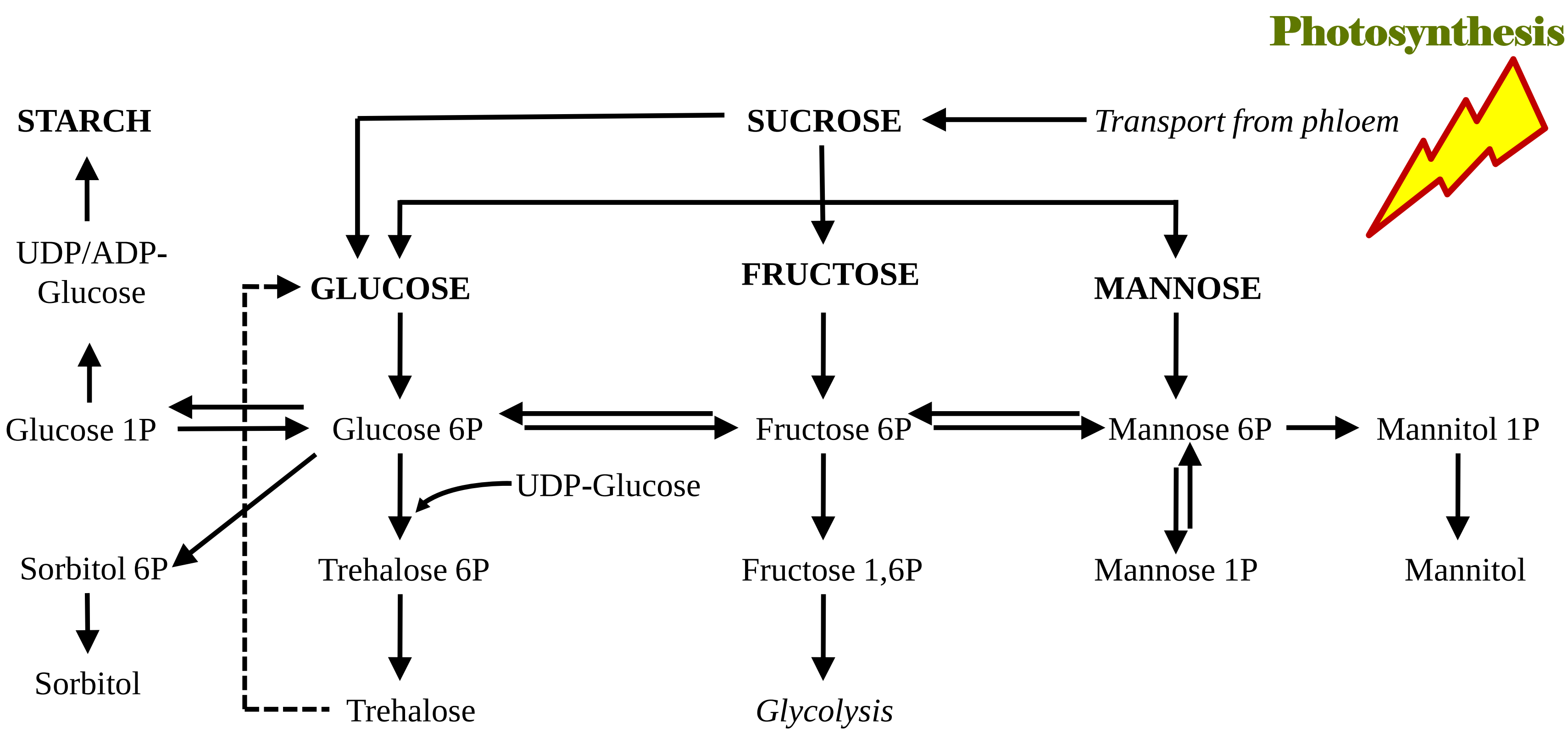
The aromatic amino acid pathways support the formation of numerous natural products in plants. The shikimate pathway (shown in *green*) produces chorismate, a common precursor for the tryptophan (Trp) pathway (*blue*), the phenylalanine/tyrosine (Phe/Tyr) pathways (*red*), and the pathways leading to folate, phylloquinone, and salicylate. Trp, Phe, and Tyr are further converted to a diverse array of plant natural products that play crucial roles in plant physiology, some of which are essential nutrients in human diets (**bold**). Other abbreviations: ADCS, aminodeoxychorismate synthase; AS, anthranilate synthase; CM, chorismate mutase; CoA, coenzyme A; ICS, isochorismate synthase.

Plant metabolism is resilient to changes

- Kim et al. studied 136 single gene perturbations (mutations) in *Arabidopsis* using metabolomics
  - Measured 1,348 chemically identified metabolites; studied the changes in “metabolic networks” (pathways are not independent of one another)
  - Over 80% of the mutants (113 out of 136) showed changes in less than 10 metabolites; 23% showed no changed metabolites; only 2 mutants showed more than 50 changed metabolites
  - The single gene mutations were having little effect on metabolism of the plant
  - Concluded that this was evidence supporting the idea that, “…the overall metabolic network is highly robust against single gene mutations.”

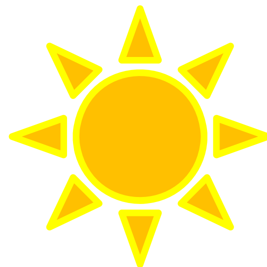
Kim et al. 2015. *Plant Physiol.* Vol. 167:1685-1698.

Plant metabolism is resilient to changes



Simplified interrelationships among key sugars and sugar phosphates in maize grain. Adapted from Stoop et al. 1996 and Paul et al. 2008. P = phosphate, diP = diphosphate, UDP = uridine diphosphate, ADP = adenosine-5-diphosphate.

Plants often have more than one method of adapting to environmental changes



Too much light

↓

Decline in efficiency of photosynthesis

↓

Chloroplasts generate reactive oxygen species

↓

Oxidative damage:

- Thylakoid membranes
- Proteins in electron transport of photosynthesis
- DNA

- Beta carotene absorbs extra light energy, scavenges reactive oxygens
- So do anthocyanins, ascorbate, α-tocopherol
- Chloroplast movement within the cell

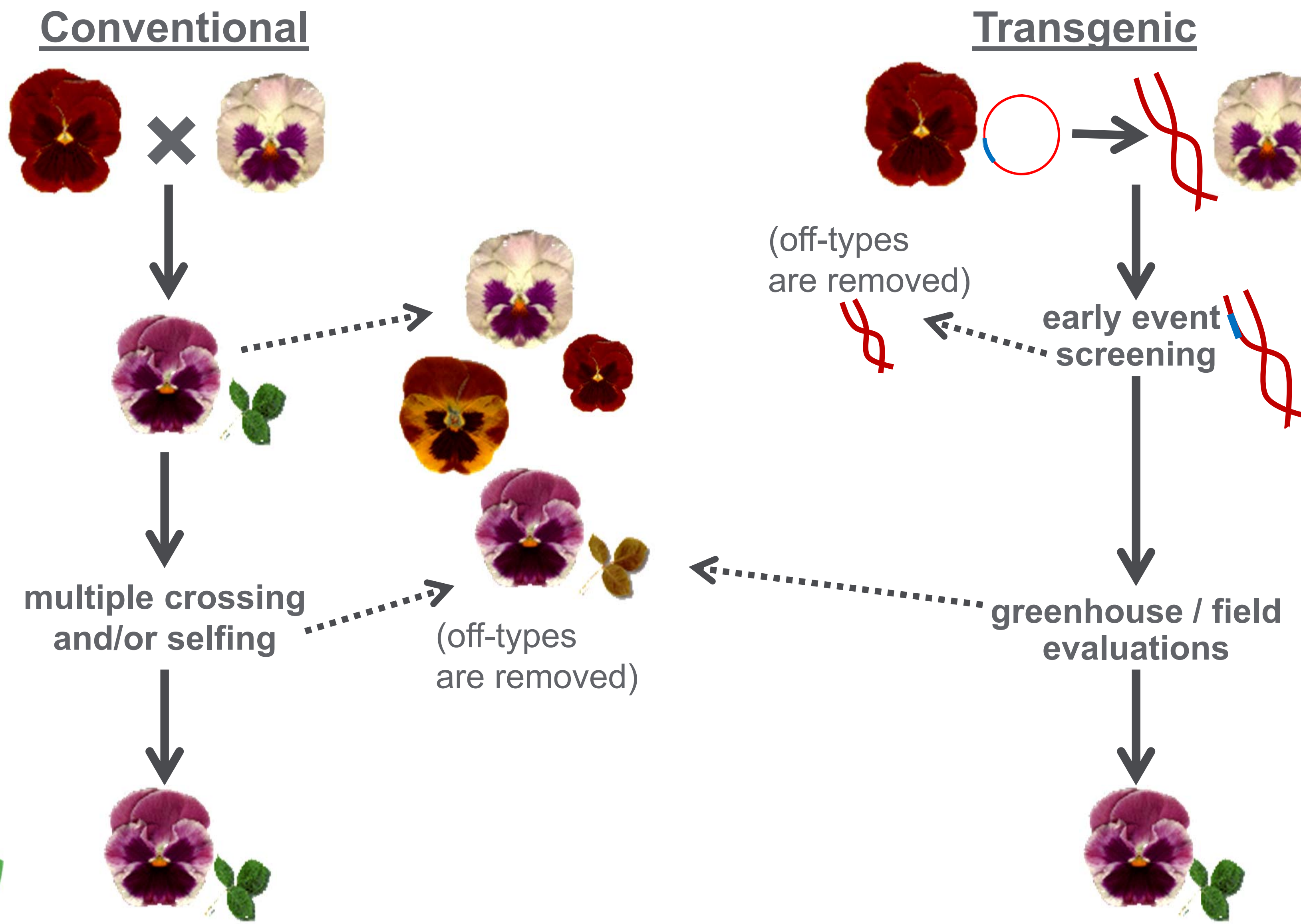
Sources of variation in component levels

- Component levels vary due to environment.
  - Temperature, solar radiation, moisture patterns, soil factors, etc.

- Component levels differ because of genetic background.
  - Most components differ; a handful are more conserved
  - Reynolds et al. 2005. *J. Agric. Food Chem.* 53:10061-10067

- Component levels differ because of genetics x environment interactions.
  - Harrigan et al. 2007. *J. Agric. Food Chem*. 55:6177-6185

Production of New Crop Cultivars



Conventional breeding produces off-type plants

Photos courtesy of Dr. Jason Wallace; Buckler Lab, Cornell Univ.

Interpreting the biological relevance of significant differences

- Need to consider magnitude of the difference in comparison to what is the normal variability for the component

- Component by component basis
  - A large change in one component may be unimportant, yet a small change in a particular component may have a more important effect

What do compositional analysis studies tell us about unintended effects?

- Levels of components in crops fluctuate widely due to environmental influences.
- Place suitable emphasis on comparisons of the GM crop with its comparator.
  - Statistically significant differences are not necessarily indicative of unintended effects.
- The statistically significant differences detected in a compositional analysis study are small compared to the variability within a single genotype reacting to environmental differences, and therefore are not biologically relevant to food and feed safety.

Thank You!