Metabolomics and the risk assessment of GMO crops

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Outline

- Development to GM crops and current safety assessment of crop composition
- Overview of omics in agriculture
- Challenges of using omics in safety assessment

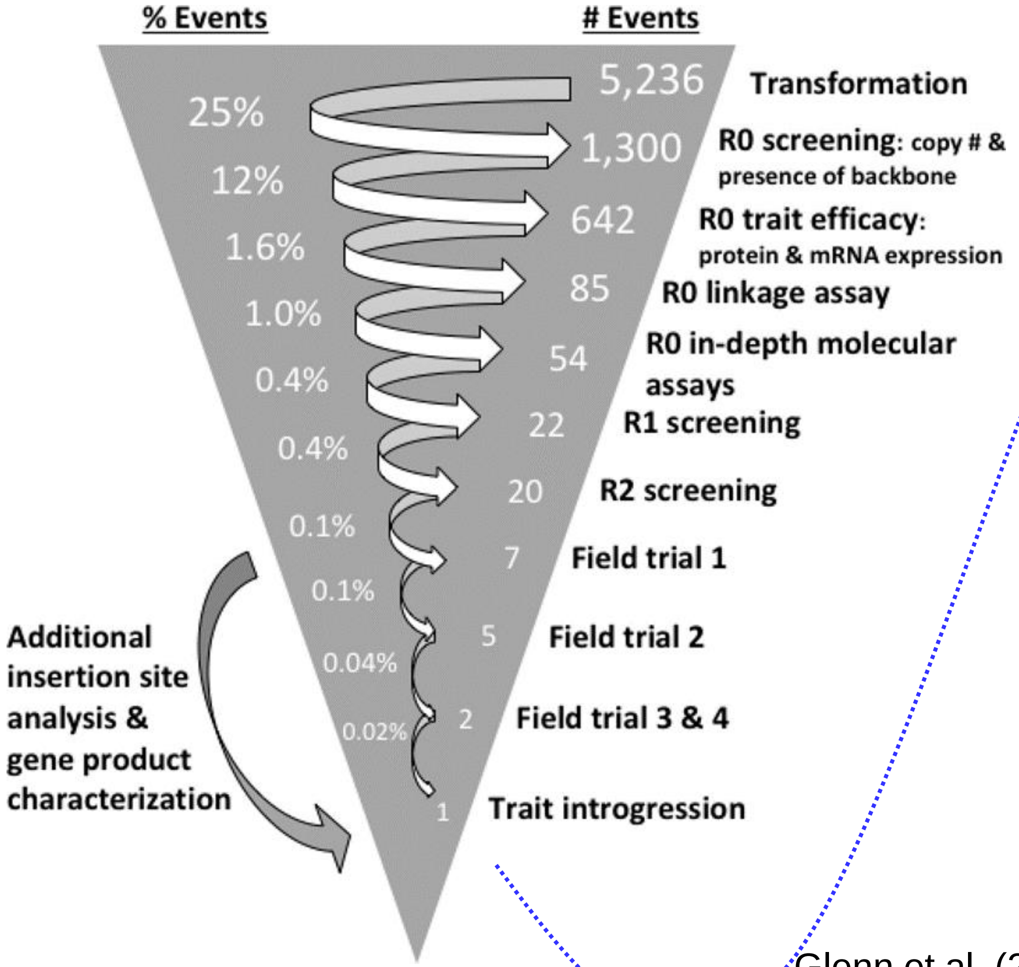
Development of a GM crop requires significant time and resources

Discovery	Phase 1	Phase 2	Phase 3	Phase 4
• Gene and trait identification • High throughput screening • Model plant testing	• Proof of concept • Crop transformation • Gene optimization	• Trait development • Large scale transformation • Pre-regulatory data	• Regulatory data generation • Trait integration • Product development	• Regulatory submission • Seed bulk-up • Premarketing • Product development

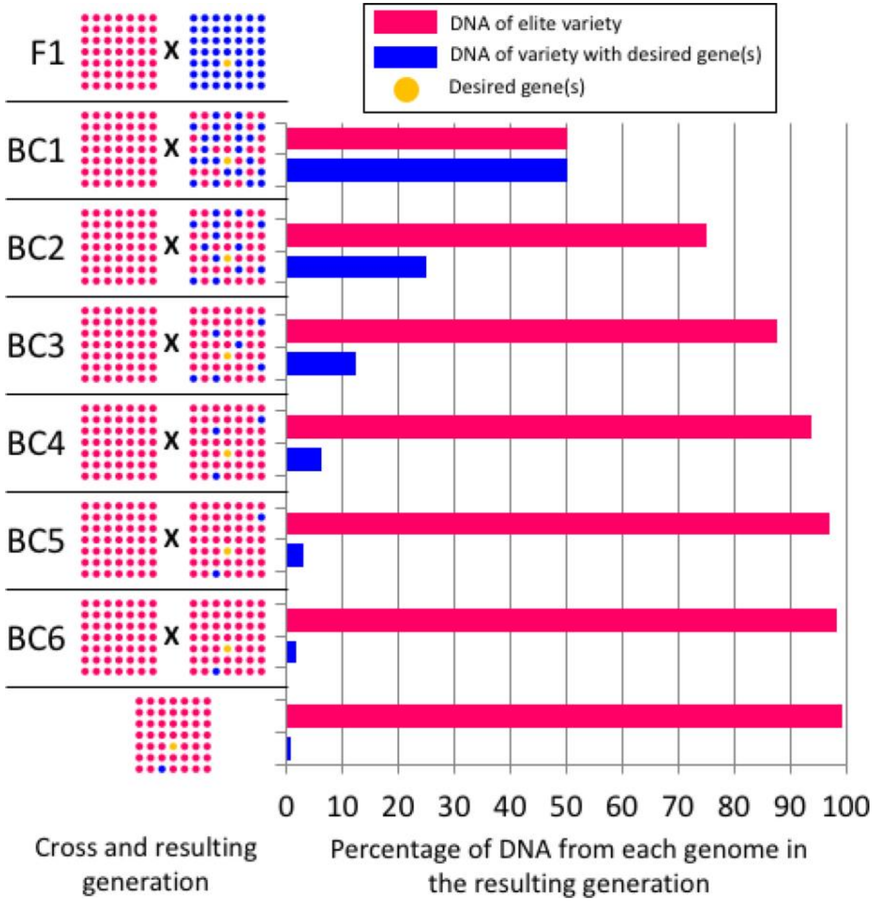
Average 13 Years and USD 136 Million

Event selection and commercial breeding into elite germplasms ensure efficacy, safety and minimize any unintended effects

Event Selection



Commercial breeding into elite germplasms



Glenn et al. (2017) Crop Science 57: 2906

Guidelines for compositional studies are harmonized internationally

Important principles in compositional studies

Growth conditions

Compare Test (GM crop) to a conventional counterpart grown and harvested under the same conditions

Components to assess

Assess components important for safety and nutrition for changes that can affect health consequences

Results interpretation

Natural variation in component levels is critical to understanding any statistically significant differences

Organisation for Economic Co-operation and Development

OECD consensus documents suggest the tissues and components to analyze for food and feed safety

Proximate Grain + (Soy and corn forage)	*Proteins, Total Fat, Ash, Carbohydrates by calculations, Fibers (NDF, ADF, TDF)*			
Nutrients	*Amino acids, Fatty acids, Minerals, Vitamins*			
Antinutrients	*Phytic acid, Raffinose, Lectin, Trypsin Inhibitor*	*Phytic acid, Raffinose*	*Phytic acid, Sinapine, Glucosinolates, Tannins*	*Gossypol, Cyclopropene fatty acids*
Other Compounds	*Isoflavones, Endogenous Allergens*	*Ferulic acid, p-Coumaric acid, Furfural*		

Validated methods are used for compositional analysis under GLP

Official Analytical Methods

AOAC, ISO, AOCS, AACC

Validated for accuracy & precision

Reproducible

Good Laboratory Practice

A quality system that ensure quality and integrity of data

Required by Regulatory agencies

Statistical Analysis

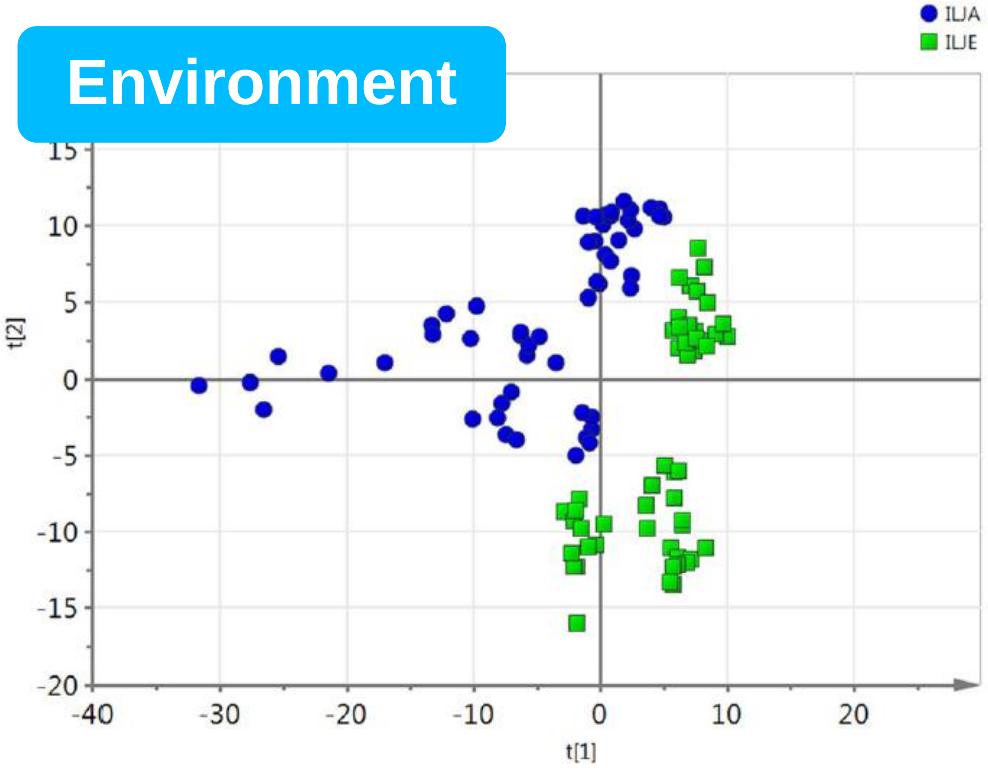
Combined Site Analysis

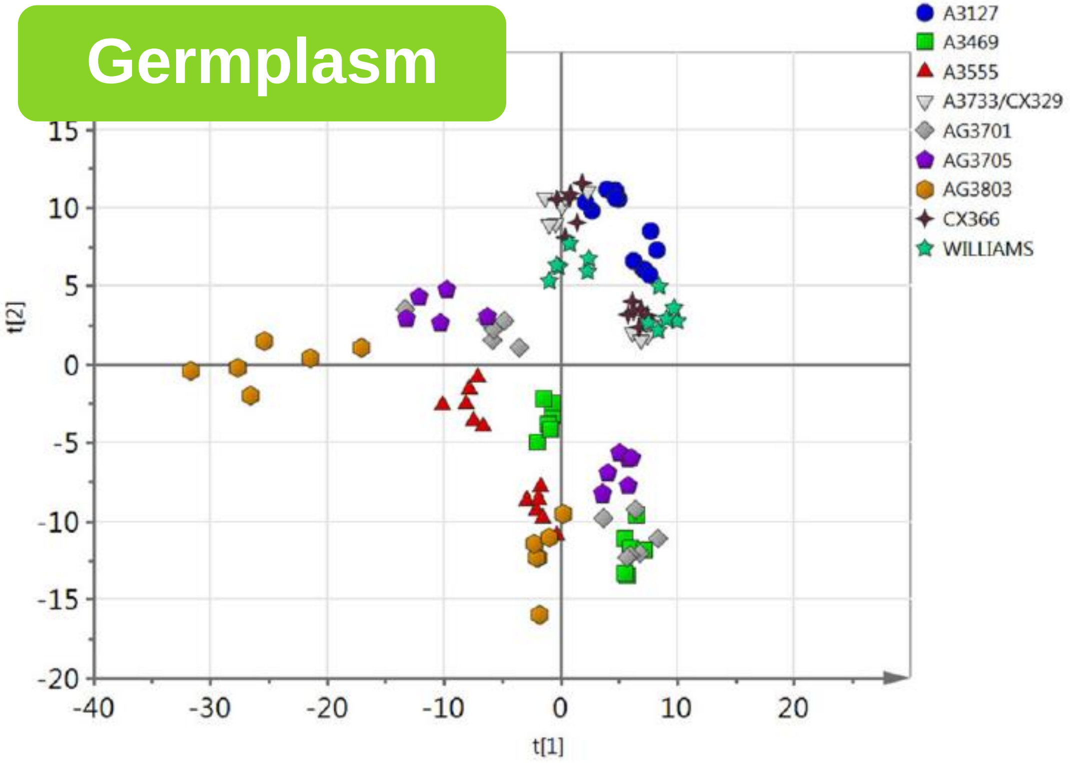
Linear Mixed Model

Mean comparison: Test vs Control ($\alpha = 0.05$)

Chain of Custody of all samples maintained throughout entire process

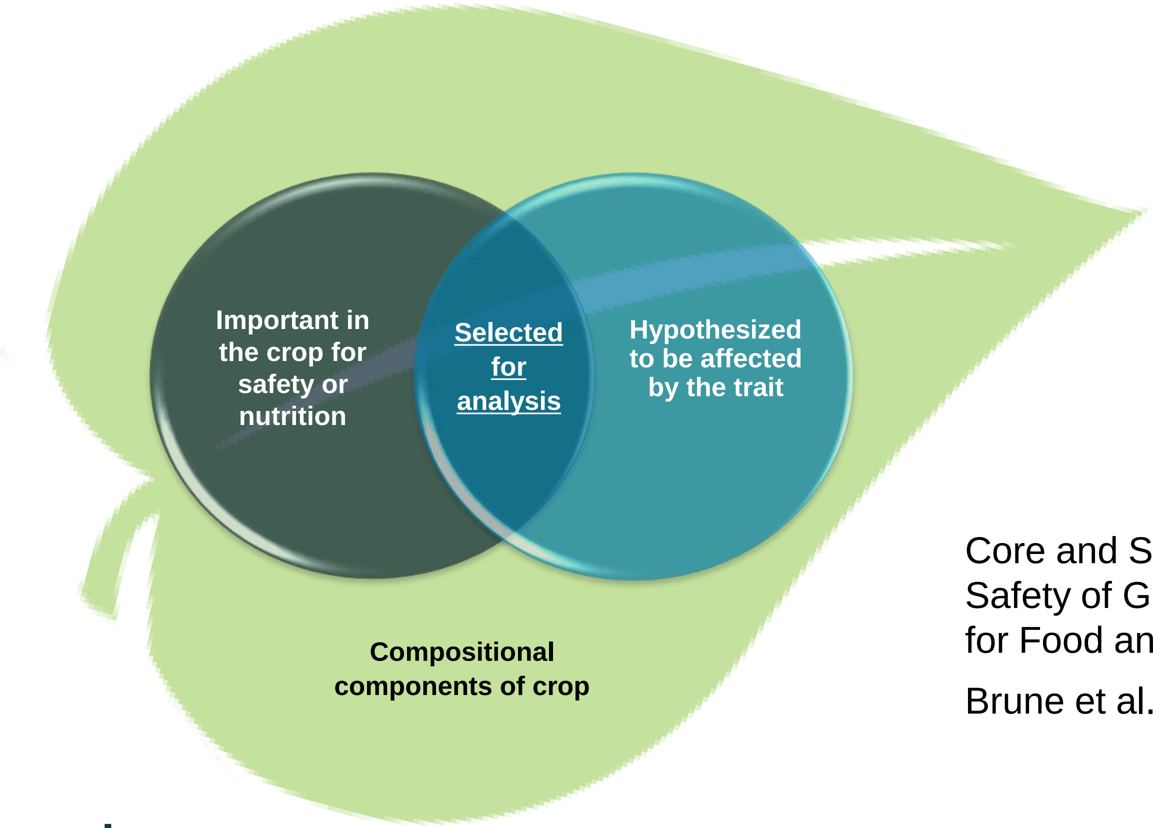
Environment and genetic background have much greater impact on the pant metabolites than the transgene





Kusano et al. (2015) Metabolomics 11: 261

Hypothesis-driven composition safety assessment is proposed based on 25 years of safe use



Core and Supplementary Studies to Assess the Safety of Genetically Modified (GM) Plants Used for Food and Feed

Brune et al., 2021 Journal of Reg Sci. 9: 45-60

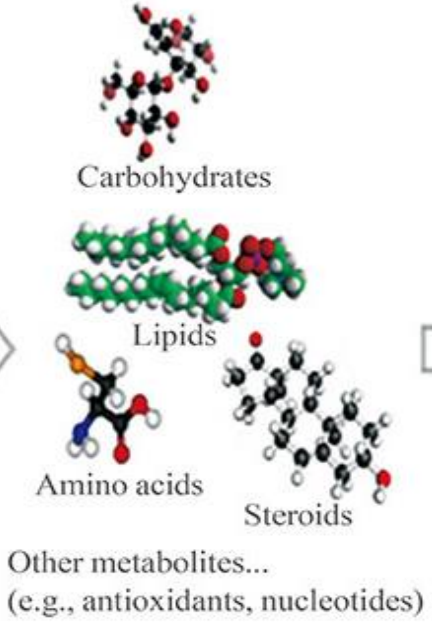
Hypothesis-driven approach

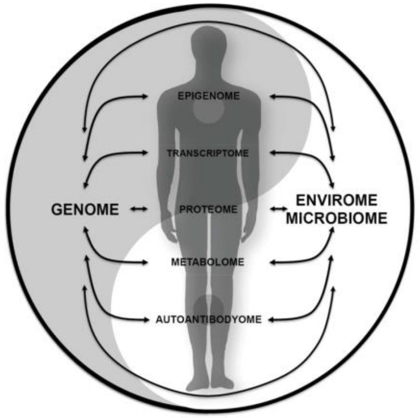
Based on the introduced trait, is there a reasonable hypothesis for compositional change?

Overview of omics in agriculture

Omics technologies provide a global analysis of a biological system

DNA | **RNA** | **Protein** | **Biological molecules** | **Observable Characteristics/ traits**





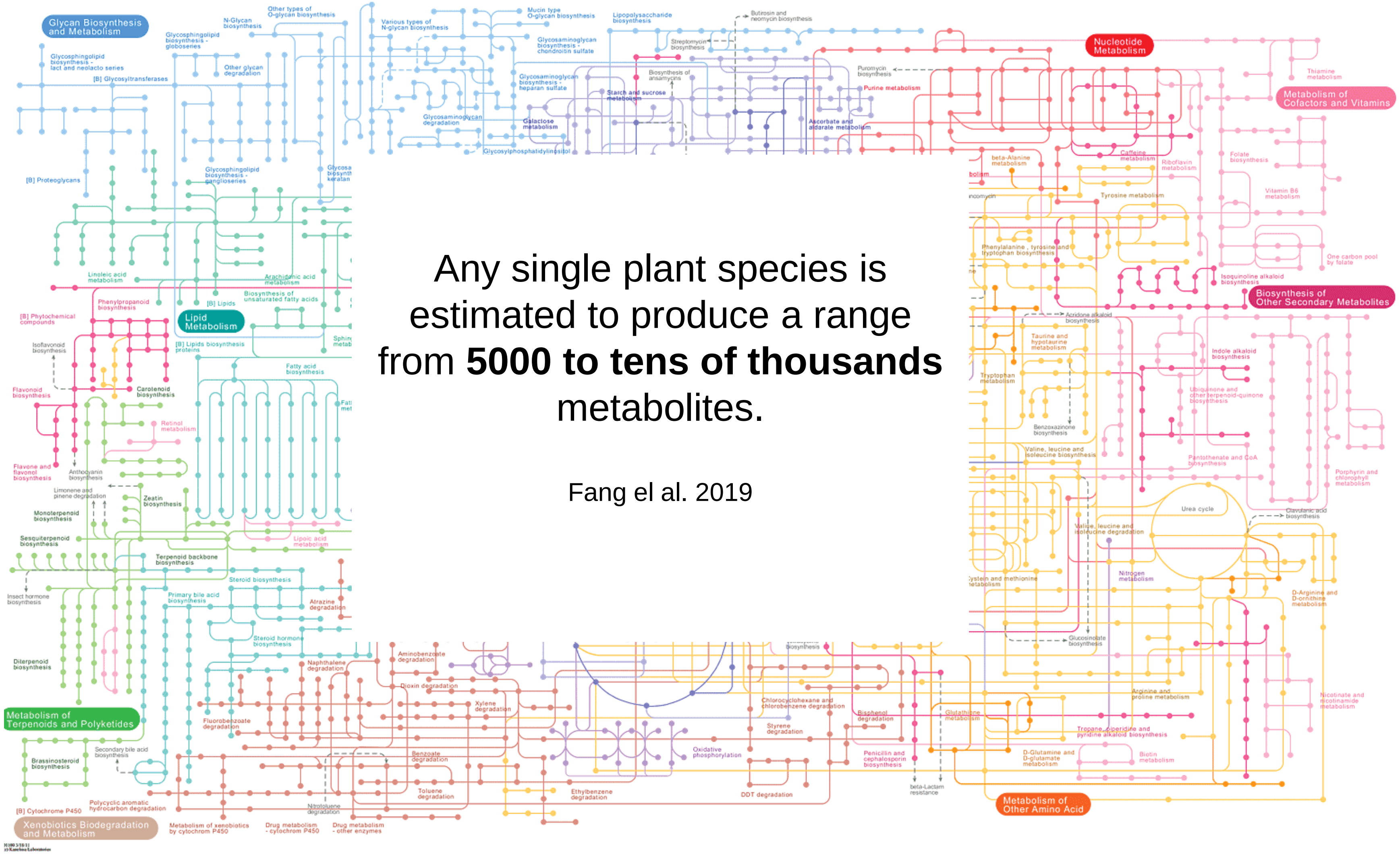
Genomics | Transcriptomics | Proteomics | Metabolomics | Phenotype

Metabolomics diversity requires multiple techniques for analysis

Primary Metabolites
- Amino acids
- Organic acids
- Sugars
- Amines
- Alcohols

Secondary Metabolites
- Phenypropanoids
- Flavonoids
- Glucosinolates
- Terpenoids
- Saponins
- Lignin
- Tannins
- Steroids

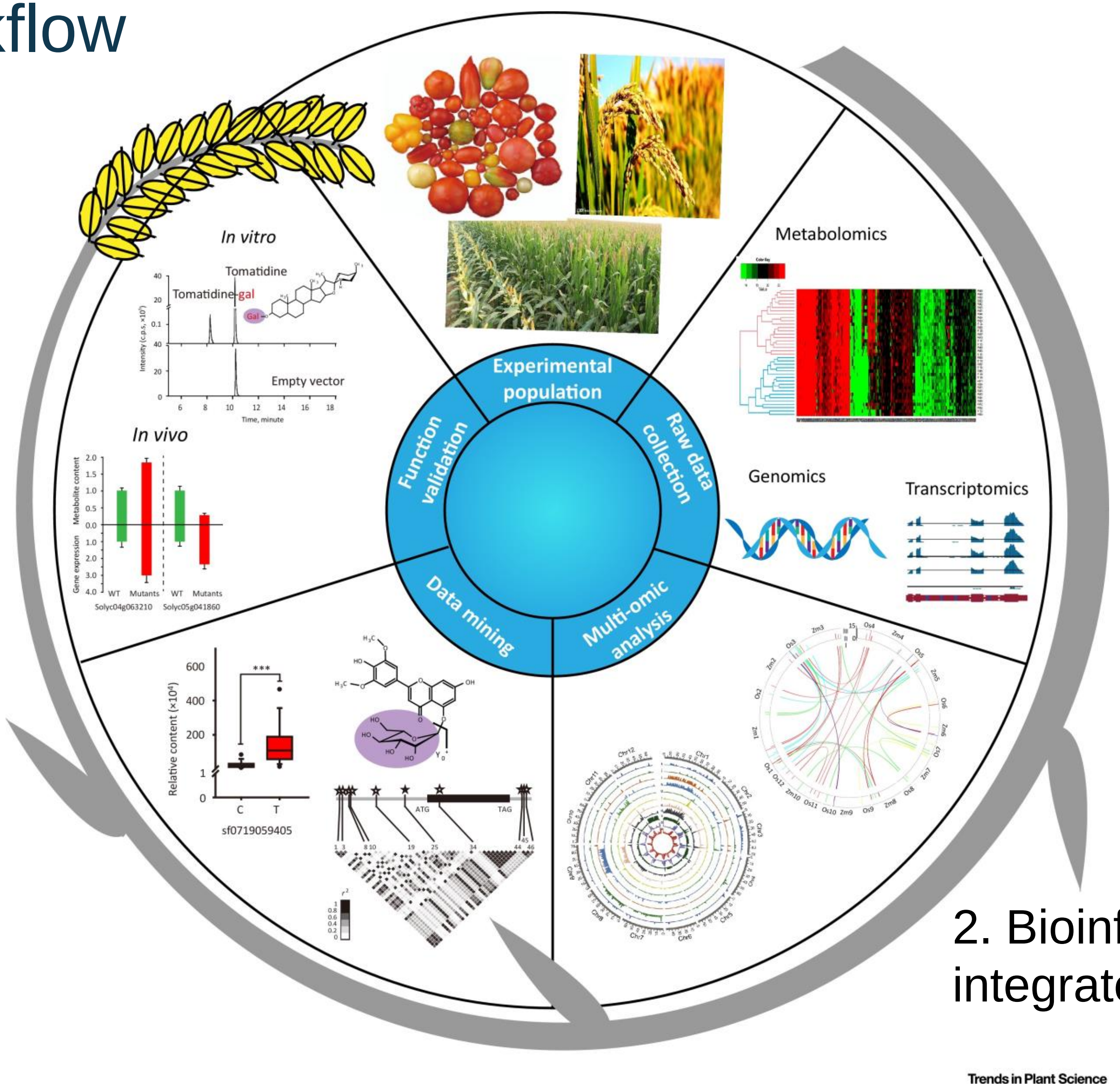
Lipids
- Fatty Acids
- Glycerophospholipids
- Glycerolipids
- Sphingolipids
- Sterols
- TGAs



Any single plant species is estimated to produce a range from **5000 to tens of thousands** metabolites.

Fang el al. 2019

An omics workflow



1. Omics methodology

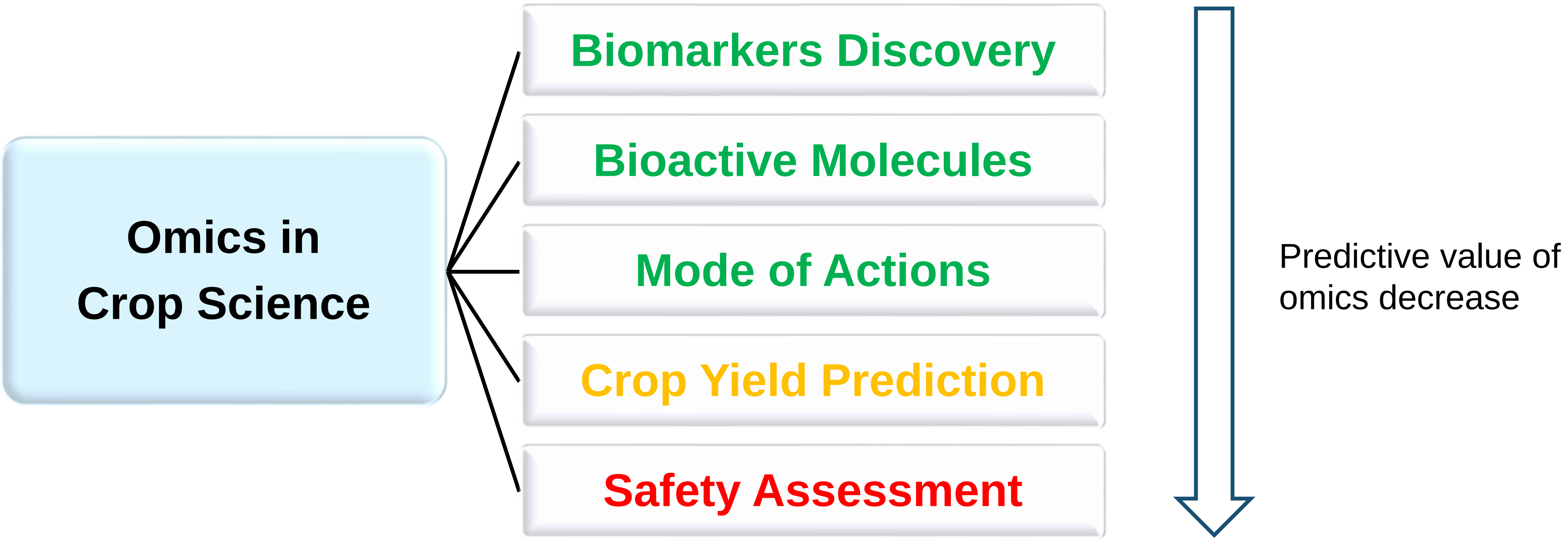
2. Bioinformatic to analyze, integrate large data sets

3. Discovery

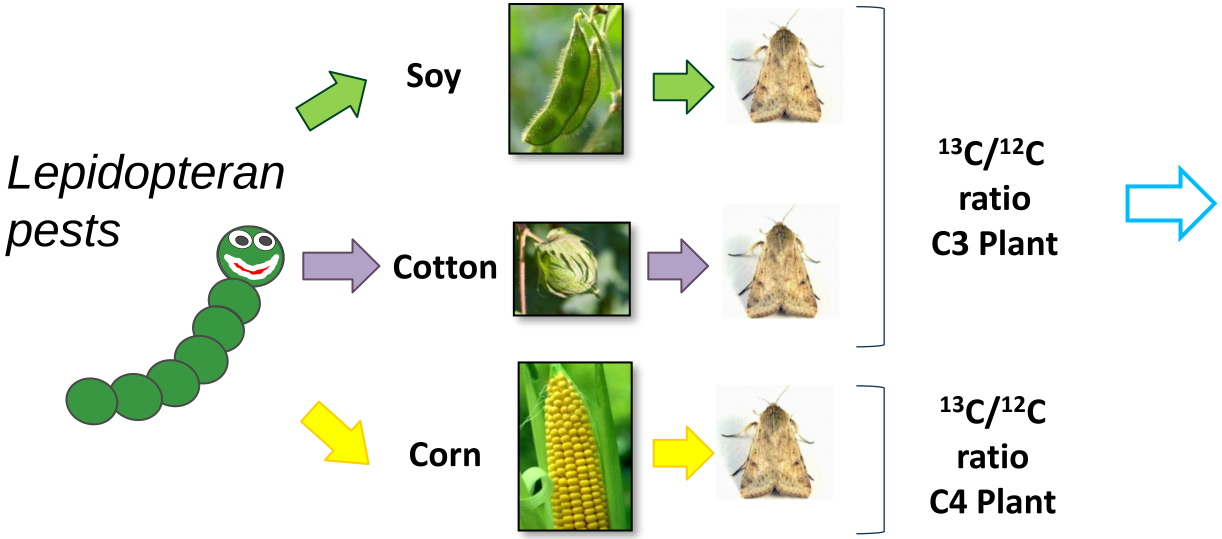
4. Validate results

Fang et al., Trends in Plant Sci. (2018) 24:83-98

Omics can be valuable tools for generating hypothesis in discovery research



Metabolomics can help identify a “biomarker” that can be tracked to see what an insect pest had for dinner



Metabolomics of moths eating different plants

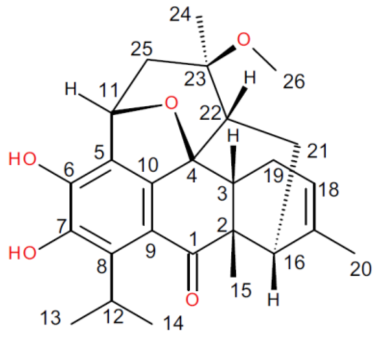
Identification of an unknown biomarker

Validation of the biomarker

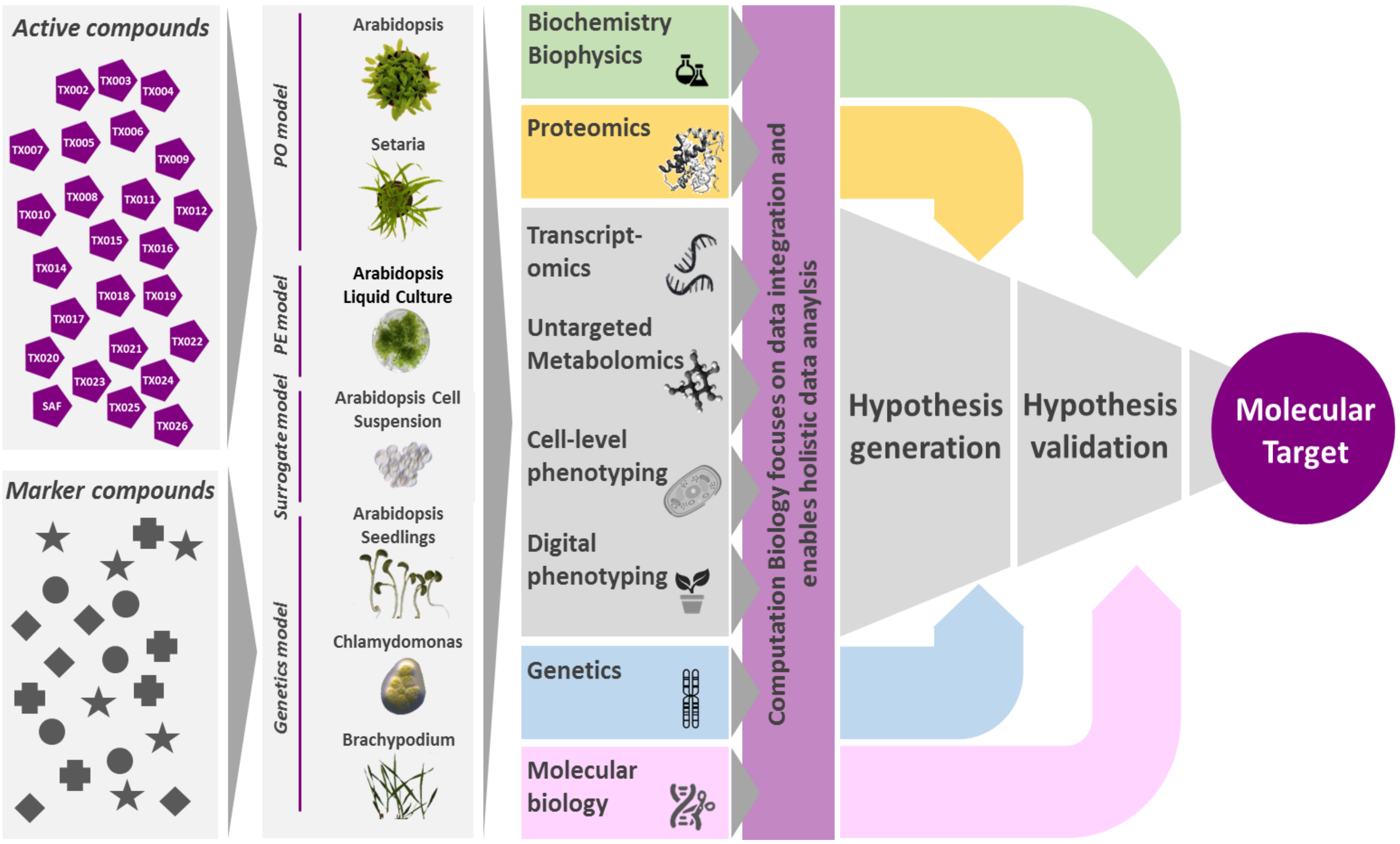
Kim et al. (2020) J. Chem. Ecol. 46: 956

Cotton biomarker:

Tricycloheliocide H_4



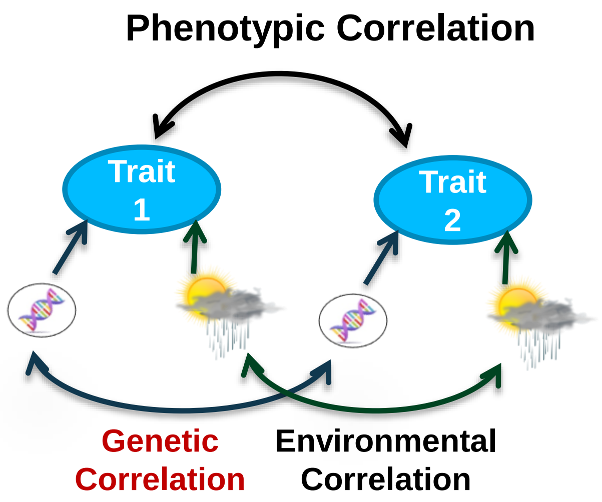
Omics is a key tool for herbicide mechanism of action (MoA) elucidation

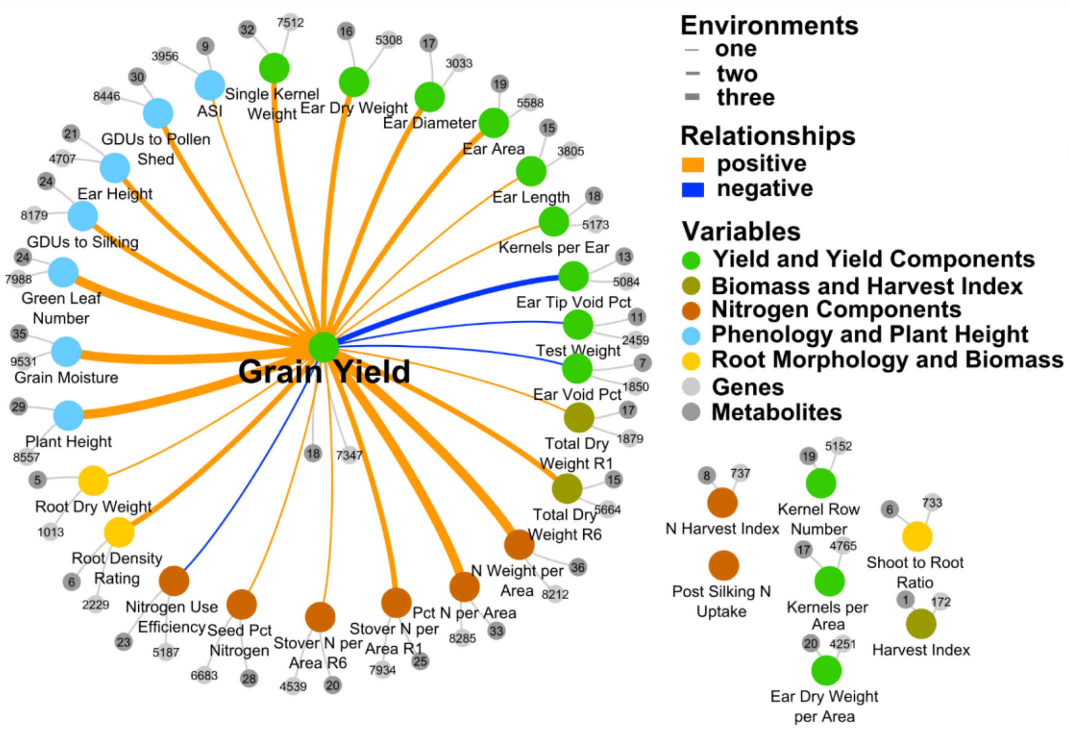


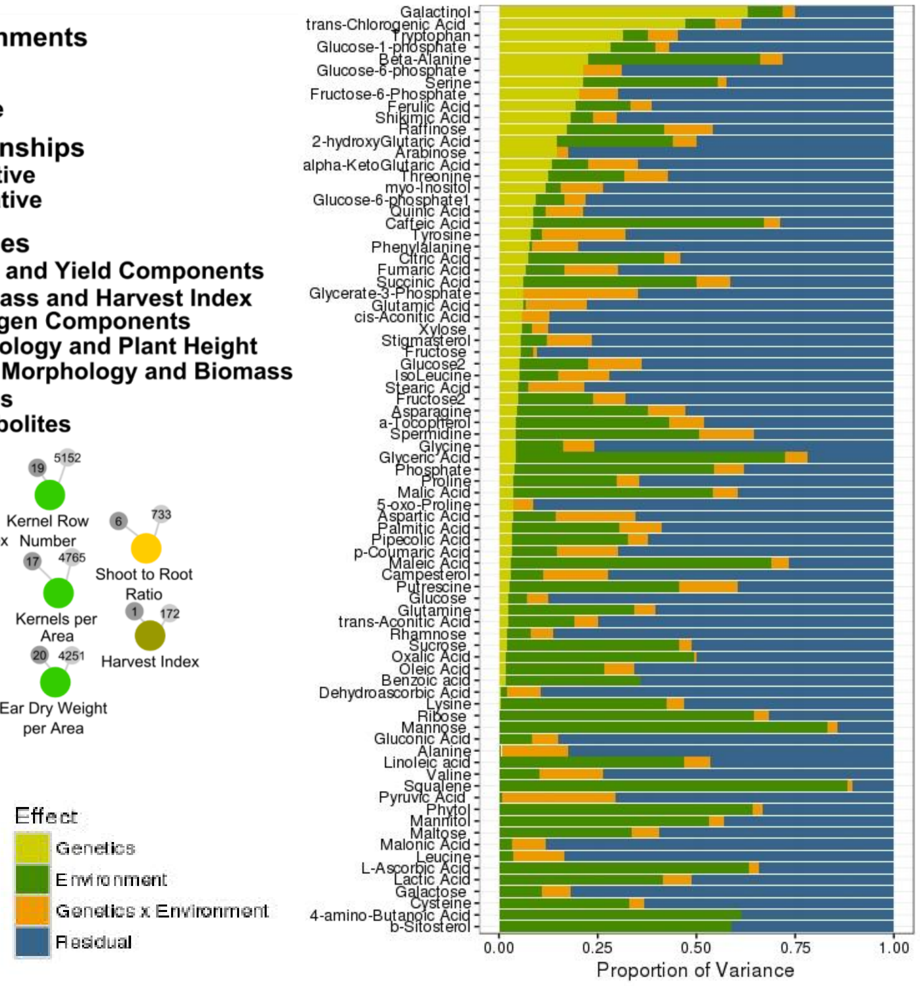
targenomix
the target identification company

Targenomix developed & implemented numerous **omics platforms** to elucidate molecular targets of small molecules

Prediction of crop yield by metabolomics – not that easy







Environmental effect is higher than genetic effect on metabolism

Tucker et al. (2020) Plant Cell Environ. 43: 880

Challenges of Omics in Regulatory Studies

Metabolomics in safety assessment is a recurring topic

- EFSA 2014 - Modern methodologies and tools for human hazard assessment of chemicals.
- NAS 2016 - Genetically Engineered Crops: Experiences and Prospects
- EFSA 2017 - Annual meeting of GMO Network
- EFSA 2018 - EFSA scientific colloquium: Omics in risk assessment: State of the art and next steps
- Christ et al 2018 - Contribution of Untargeted Metabolomics for Future Assessment of Biotech Crops
- Fraser et al 2020 - Metabolomics should be deployed in the identification and characterization of gene-edited crops
- Enfissi et al 2021 - New plant breeding techniques and their regulatory implications: An opportunity to advance metabolomics approaches
- Gould et al. 2022 - Toward product-based regulation of crops

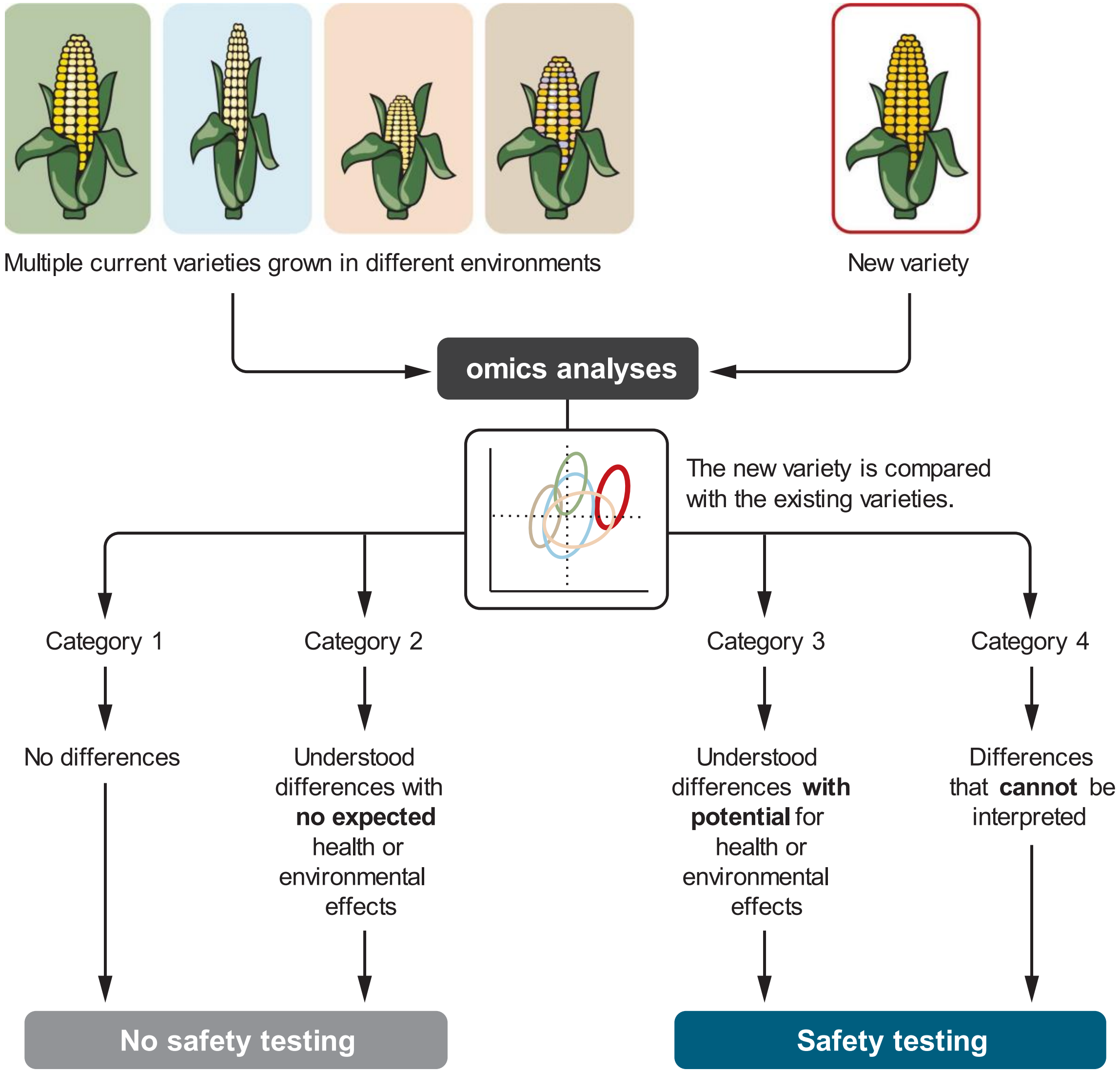
2018 EFSA scientific colloquium outcome:

Omics can be integrated into risk assessment even though there are development needs

- Metabolomics could either:
  - Fully substitute the existing end-point approach (opinion split 50:50)
  - Complement existing approach on case-by-case basis (supported by majority)
- Advantages:
  - More compounds can be analyzed, increased level of information
  - Focus on pathways rather than individual endpoints, providing a more holistic picture of the metabolism
- Development needs:
  - Reference data sets to inform on baseline variability
  - Standardization of experimental protocols and data analysis
  - Global regulatory harmonization and frame for interpretation in the risk assessment

A tiered based strategy to evaluate crop varieties using Omics technologies

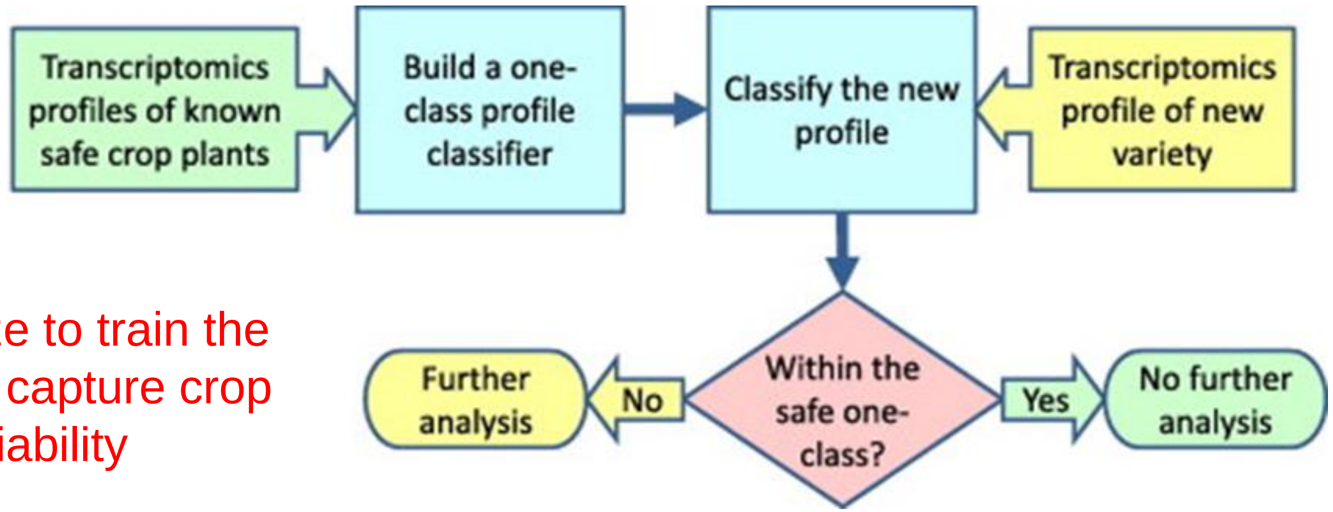
- Current risk assessment systems that use size and source of inserted genetic material is not fit for purpose
- Test a new variety against all current varieties
- Size and kind of differences?
  - International workshops of breeders, chemists, and molecular biologists to provide a range of potential options and costs.
  - Subsequent workshops that involve policy-makers, regulators, developers, public stakeholders, and social scientists.



Genetically Engineered Crops (2016): Experiences and Prospects
Gould et al. (2022) Toward product-based regulation of crops

Development of “One Class” model to evaluate safety assessment of plant varieties

- Use omics of commercial varieties to establish a safe one class model
- Test GM plant profile against the model



Sample size to train the model and capture crop natural variability

How to set the threshold to accept or reject a new variety?

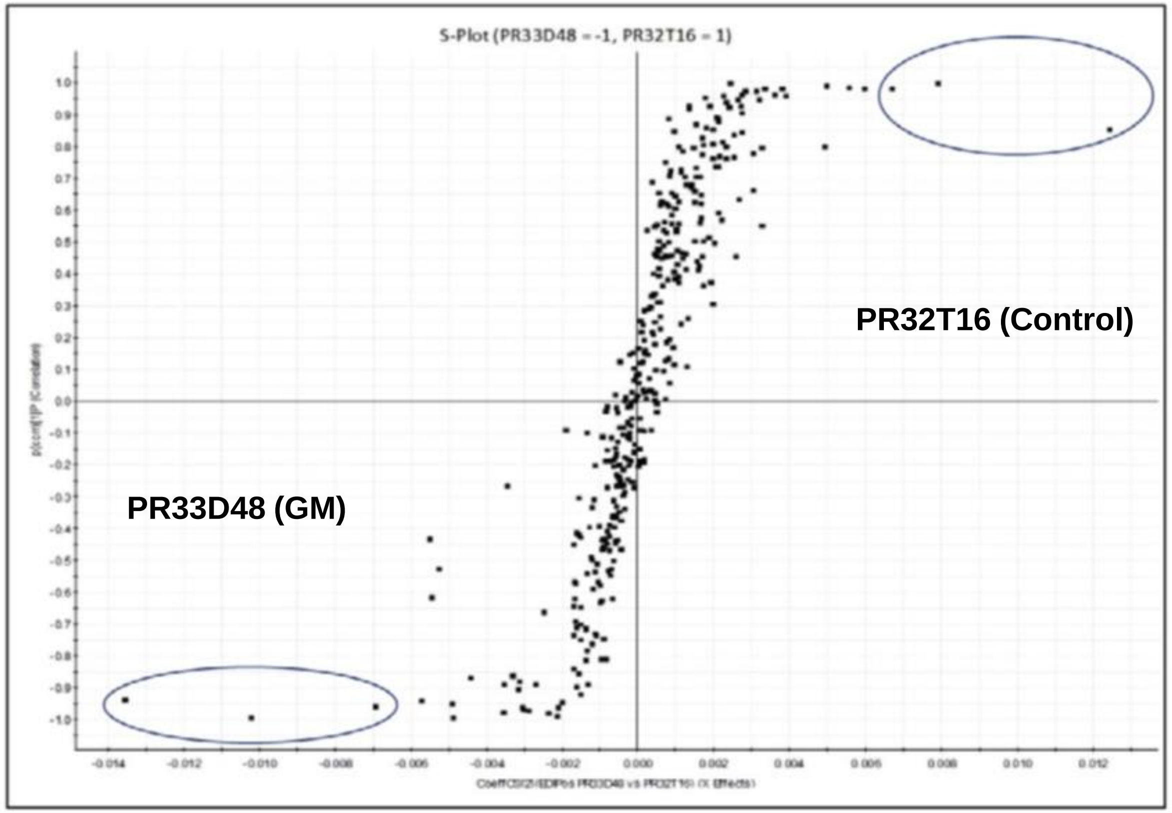
- van Dijk et al. (2014) Regulatory Toxicology and Pharmacology 70: 297–303
- Kok et al. (2019) Food Chemistry 292: 350-358
- Corujo et al. (2019) Food Chemistry 292: 359-371

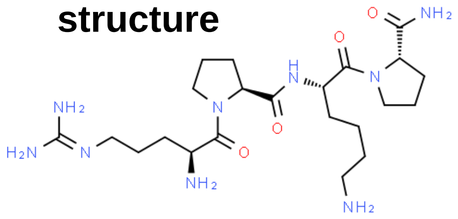
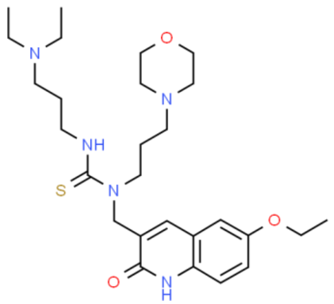
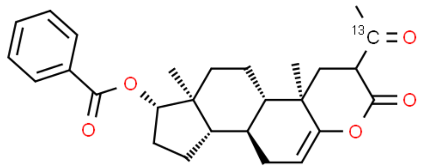
The “one-class” model has limitations

	Replicates	Total classifications	Classified 'in'	Percentage 'in'	By majority vote	By test set threshold
RIKILT						
Total test set		198	127	64.1		
DKC6667YG (GM)	2	84	50	59.5	in	out
PR33D48 (GM)	3	126	107	84.9	in	in
Fungal infected sample 1	2	84	0	0	out	out
Fungal infected sample 2	2	84	0	0	out	out
CSIR						
Total test set		120	97	80.8		
DKC6667YG (GM)	MA4 a	24	24	100	in	in
	MA4 b	24	24	100	in	in
	MA4 c	24	24	100	in	in
PR33D48 (GM)	MB8 a	24	24	100	in	in
	MB8 b	24	24	100	in	in
	MB8 c	24	24	100	in	in

Corujo et al. (2019) Food Chem. 292: 359-371

Structural elucidation is a major challenge for the interpretation of metabolomics data to understand biological systems



RT min & m/z	Fold change	Molecular formula	ChemSpider structure
25.48 496.3357	2.9	$C_{22}H_{41}N_9O_4$	
25.48 518.3156	3.1	$C_{27}H_{43}N_5O_3S$	
8.67 438.2361	1.6	$C_{26}{}^{13}CH_{32}O_5$	

Corujo et al. (2019) Food Chem. 292: 359-371

What is needed for metabolomics to be ready for risk assessment of GM crops

- Tools to interpret metabolomics data for GM comparative safety assessments
- Major advancement in mass spectrometry signal identification
- Alignment on the metabolomics method that captures the diversity of the plant metabolome
- Standardization of the metabolomics method

Evaluation of the use of untargeted metabolomics in the safety assessment of genetically modified crops
Bedair M. and Glenn, K. Metabolomics (2020) 16:111

Take home message:
Omics for discovery, Targeted Assays for Regulatory

Omics	Targeted Assays
Discovery	**Validated**
Hypothesis generating	Hypothesis driven
Relative quantitation	Absolute quantitation
Databases and libraries for identity	Authentic reference standards
Putative identification	Confirmed compound identification
Depends on acquisition platform	Reproducible

Thank you

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