

‘Omics’ technologies of GM Crop

National GMO Awareness Seminar

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Presentation overview

- Argument issues in GM crops
- What is 'omics' technologies?
- Why 'omics' technologies?
- The technologies
 - Transcriptomics
 - Proteomics
 - Metabolomics
- Findings
- Challenges

Argument issues in GM crops

- Possible occurrence of unintended changes resulting from transgene
- Unpredictable alterations that might have an impact on human health or/and environment
- Common used substantial equivalence evaluation by targeted approach (predefined compounds) is biased and unintended effects might escape detection

Intended effect

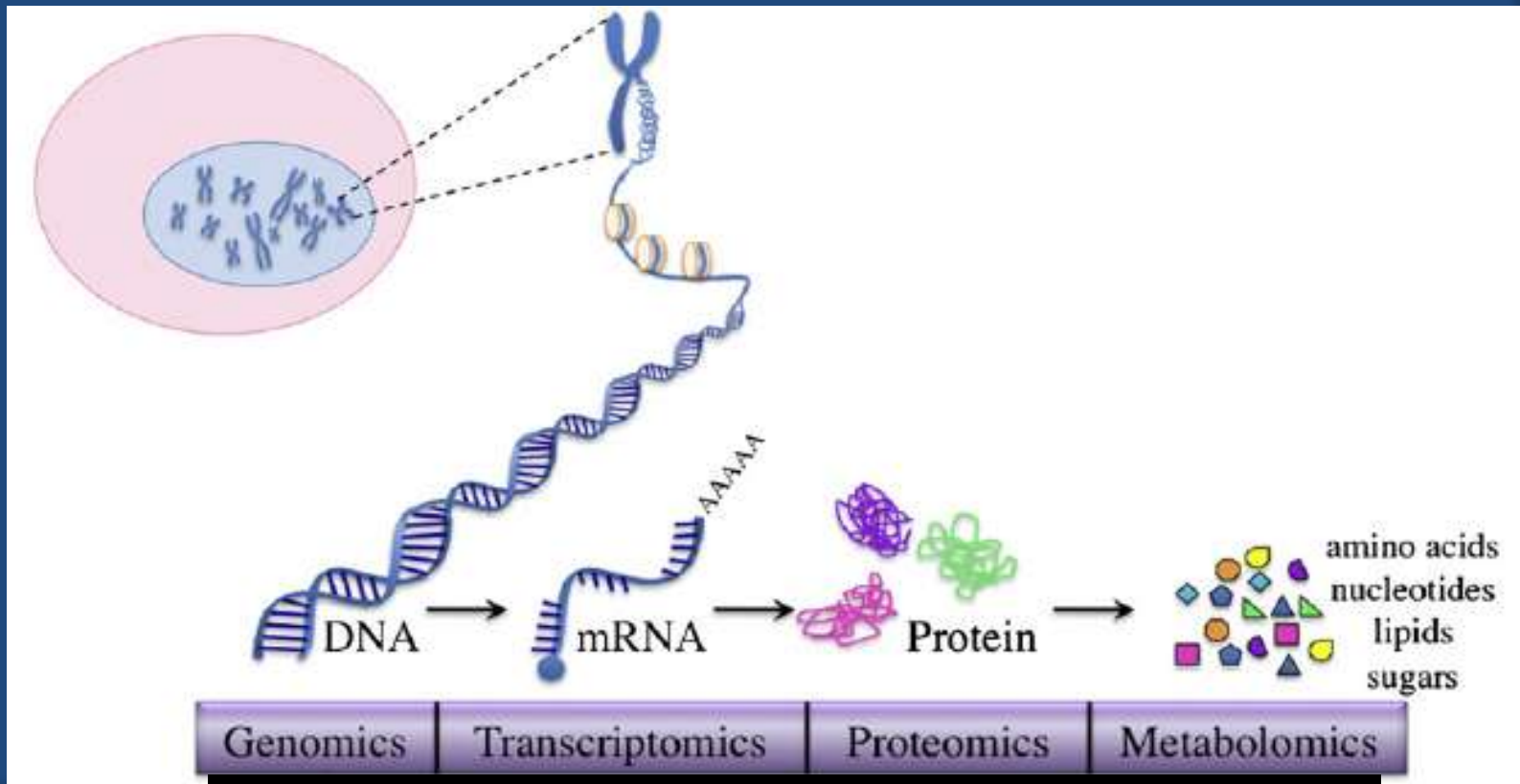
- Targeted to occur after gene transformation
- Lead to improvement in crops
- Could be analyzed by targeted quantitative method

Unintended effects:

- Untargeted effect represent statistically significant difference
- Biological variation of the comparator is included
- Changes might impact on agronomic performance but not necessarily pose threats for human health or environment

What is 'omics' technologies?

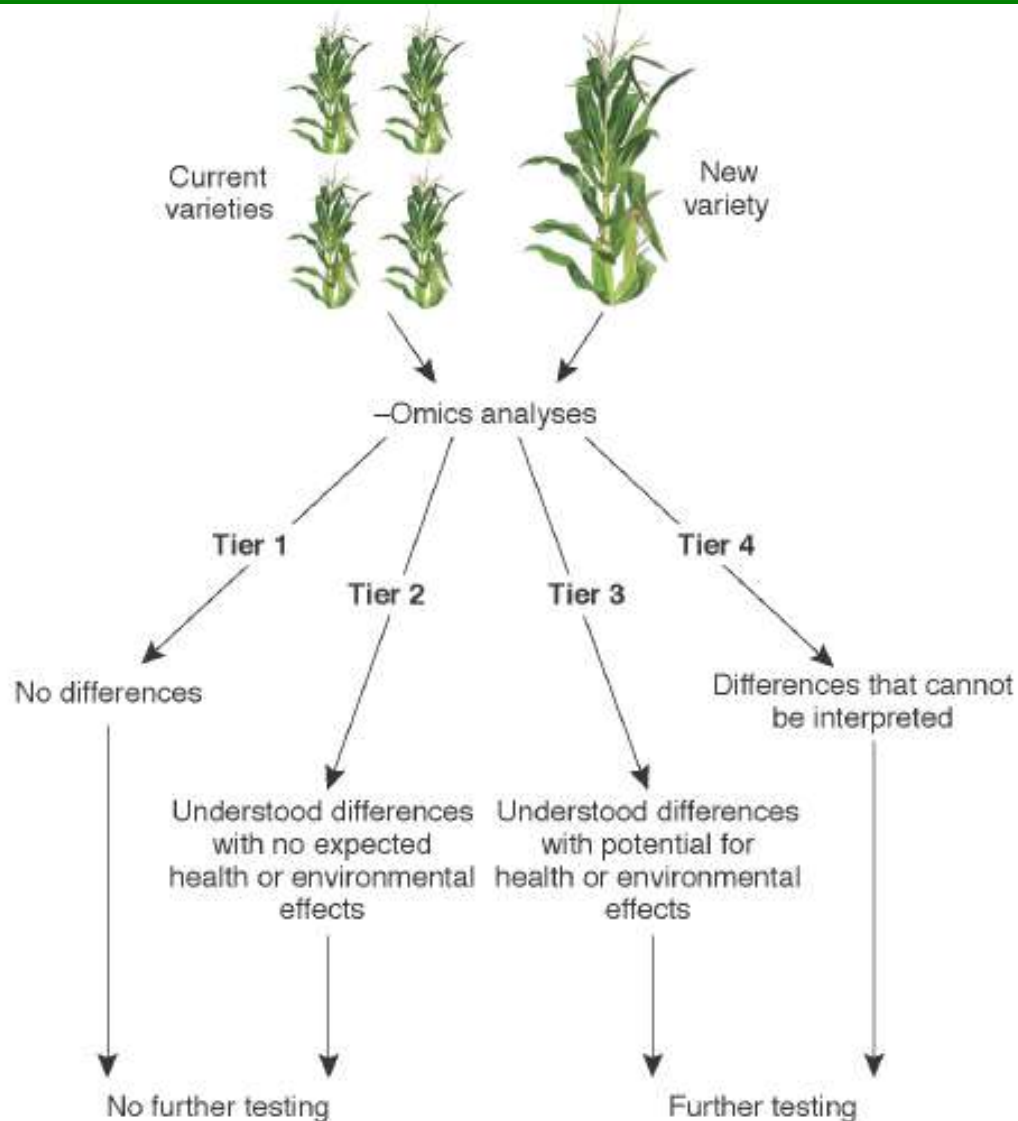
- 'Omics' – collective technologies allow simultaneous analysis of large amounts of data with high-throughput techniques, analytical tools and data mining to extract information.



Why 'omics' technologies?

- High-throughput screening – advantages over targeted analysis
- Offering comparative approach on a broad screening - non-selective and unbiased manner
- Provide complementary or additional tools to study potential unintended effects of GM crops

Proposed strategy for evaluating crops using -omics technologies

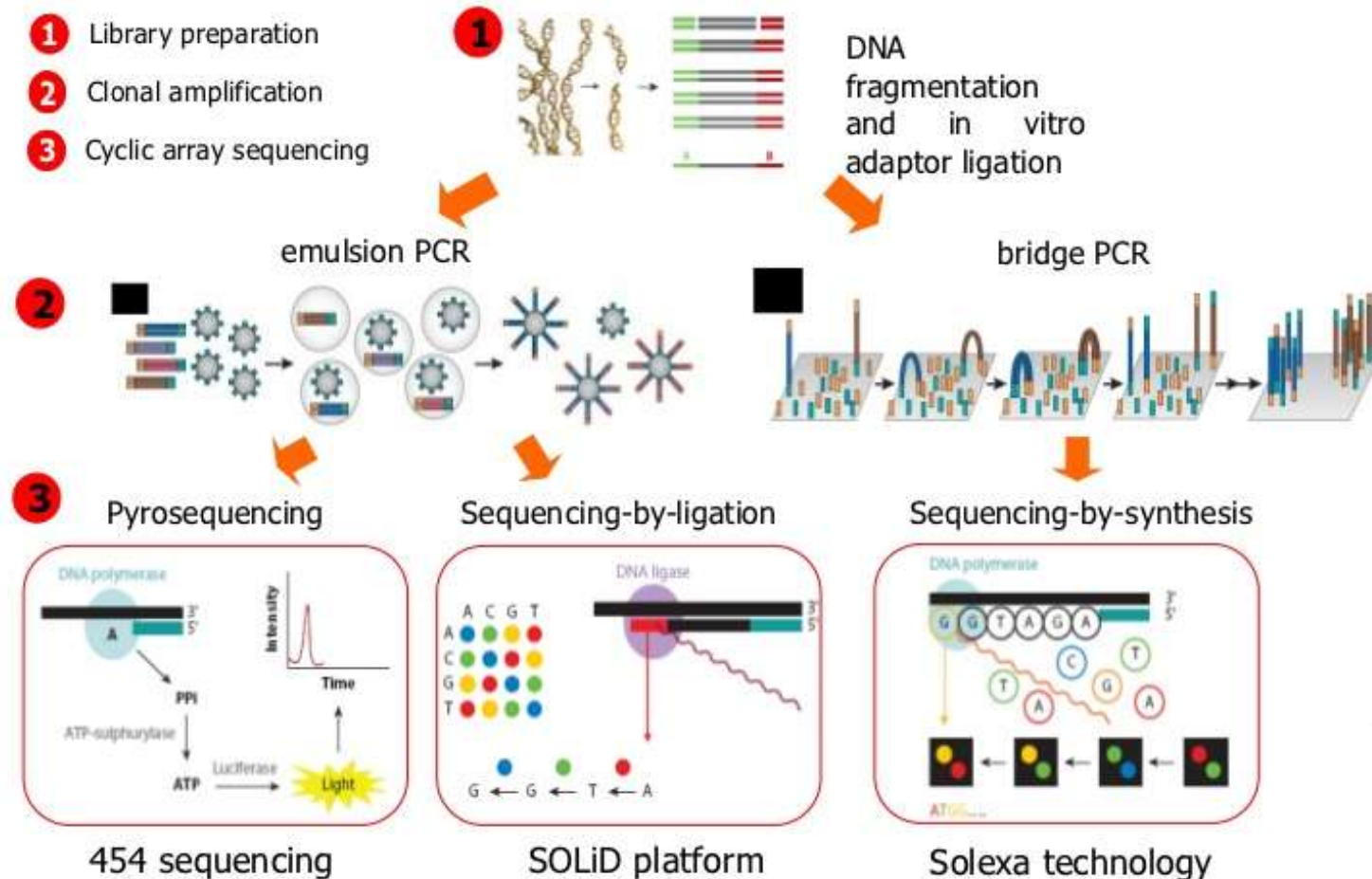


Transcriptomics

- Profiling tool to identify differences in the transcription of genes representing a portion of genome between two or more plant samples
- Difference in transcriptome does not necessarily reflect change in the profile of 'end-products'

Transcriptomic work flow

Next-generation DNA sequencing



Case study

GM maize MON810 (Insect resistance BT type) and non-GM

Analysis

- Transcriptomes profile was compared between GM and non-GM in *in vitro* culture, field and in real agriculture condition

Results

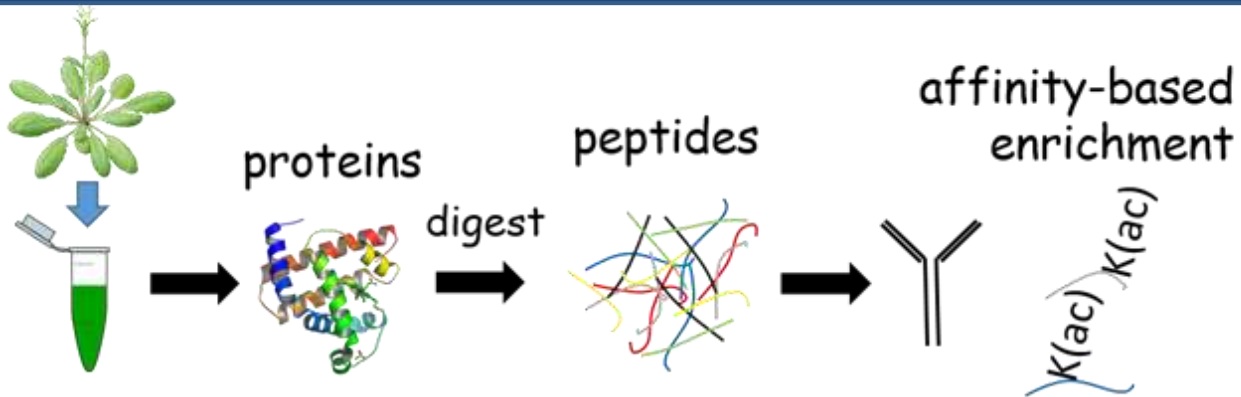
- Minor difference was observed in the field and *in vitro*
- Differential expression gene - only 0.14% in agriculture condition
- Varietal differences had the highest impact on gene expression patterns
- Transgenes had the lowest impact

Coll *et al.* (2010) Natural variation explains most transcriptomic changes among maize plants of MON810 and comparable non-GM varieties subjected to N-fertilization farming practices. *Plant Mol Biol* 73:349-362

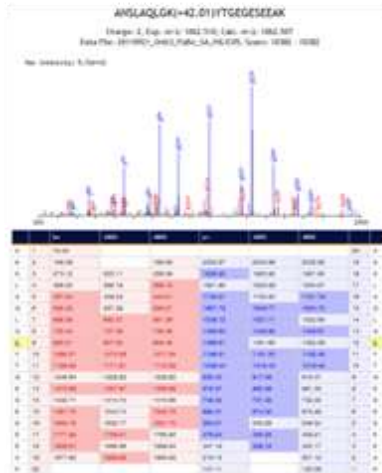
Proteomics

- An analysis of systematic identification and quantification of the complete synthesized protein by a particular cell at a particular time
- Proteins are key player in gene function, directly involved in metabolism and cellular development (e.g: toxins, allergens)

Proteomic work flow



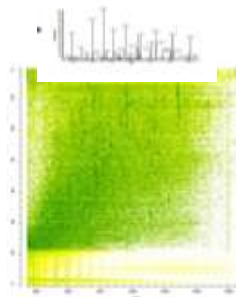
Protein
identification



affinity-based
enrichment

LC-MS/MS

Data analysis



Case study

Cotton leaves: transgenic Bt-cotton and non-transgenic

Analysis:

- ELISA :58 differentially expressed proteins (DEPs)
- MS: 35 proteins identified
- Pathway : carbon fixation and photosynthesis; glyoxylate and dicarboxylate metabolism; oxidative pentose phosphate

Results

- Exogenous DNA can affect plant growth and photosynthesis
- There were some unintended variation but no toxic proteins or allergens detected
- GM operation did not alter cotton leaf proteomic
- Target protein were hardly checked by traditional proteomic analysis

Wang *et al.* (2015) Comparative proteomics of Bt-transgenic and non-transgenic cotton leaves. *Proteome Science* 13:15. Doi:10.1186/s12953-015-0071-8

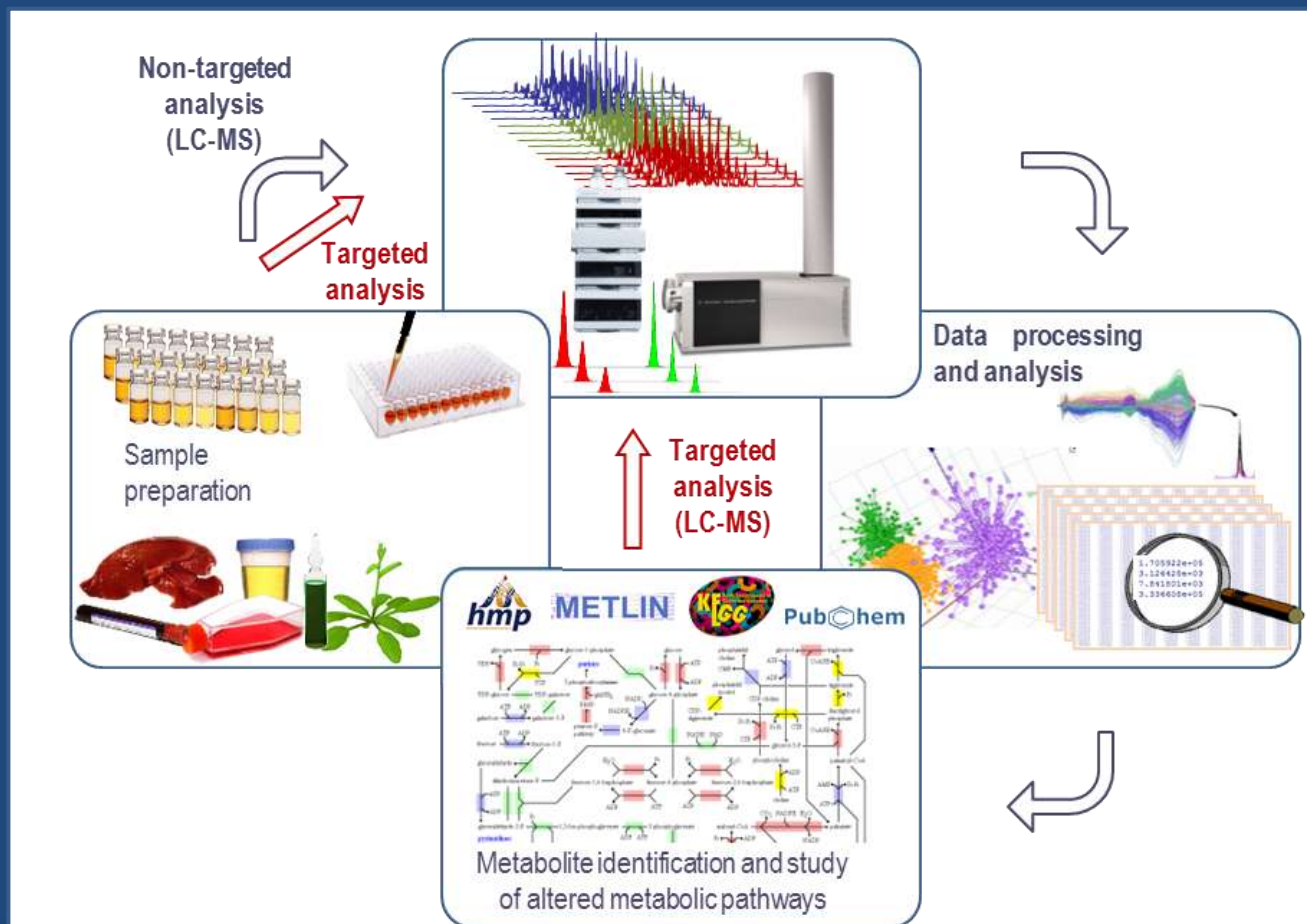
Metabolomics

- Compilation of all primary and secondary metabolites – final recipient of genetic information
- Data-driven approach aims to assess all measurable metabolites without any pre-conception or pre-selection
- Provide new dimension in study of system biology

Metabolomics work flow

Three main experimental stage:

- 1) Preparation of sample
- 2) Acquisition of data - analytical methods
- 3) Data mining - chemometric methods and compound identification



Analytical platforms:

- GC-MS
- LC-MS
- NMR
- FT-IR

Case study

Soybean seed – 8 replicates from 49 conventional accessions and GM-herbicide tolerance

Analysis:

- UHLC/MS/MS and GC/MS

Results:

- Identify 169 known structure compounds, covering 51 biochemical pathways and compounds types –represent natural variation range
- Herbicide tolerance line could not be distinguished from conventional line
- Comparing GM line to the range of performance in a crops' native germplasm pool by metabolomics analysis – proper standard as part of safety and equivalence assessment program

Clarke et al (2013) Assessment of Genetically Modified Soybean in relation to natural variation in the soybean seed metabolome. *Scientific Reports* 3. Doi:10.1038/srep03082

Major findings of 'omics' technologies

- In general – GM crops showed no significant differences from natural counterpart except of changes in the targeted engineered genes, protein and metabolites
- Majority of differences were related to environmental factors rather than genetic modifications
- Few studies showed variation in level of several metabolites and alteration of some metabolic pathways in GM crops – no direct association to genetic modification

Challenges for 'omics' technologies

- Outputs are huge – advance data mining and reliable comparative analysis are required
- Reproducibility and standards for methodology, data handling and analysis
- Knowledge of many metabolic pathways in plants and inability to identify majority of metabolites synthesized in the plant kingdom – limit the beneficial use of 'omics' data

**THANK YOU
FOR YOUR ATTENTION**