Measurement of endogenous allergens in genetically modified soybeans

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ILSI HESI Protein Allergenicity Technical Committee

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ILSI Health and Environmental Sciences Institute

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Endogenous allergen assessments are required for GM soybean varieties

EU Regulated Food Allergens


Molluscan


Crustacean


Cereals


Eggs


Milk


Tree Nuts


Peanuts


Celery


Mustard


Sulfites


Soybean


Fish


Sesame


Lupin

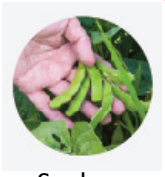
GM crops


Canola


Cotton

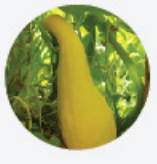

Alfalfa


Sugar Beet

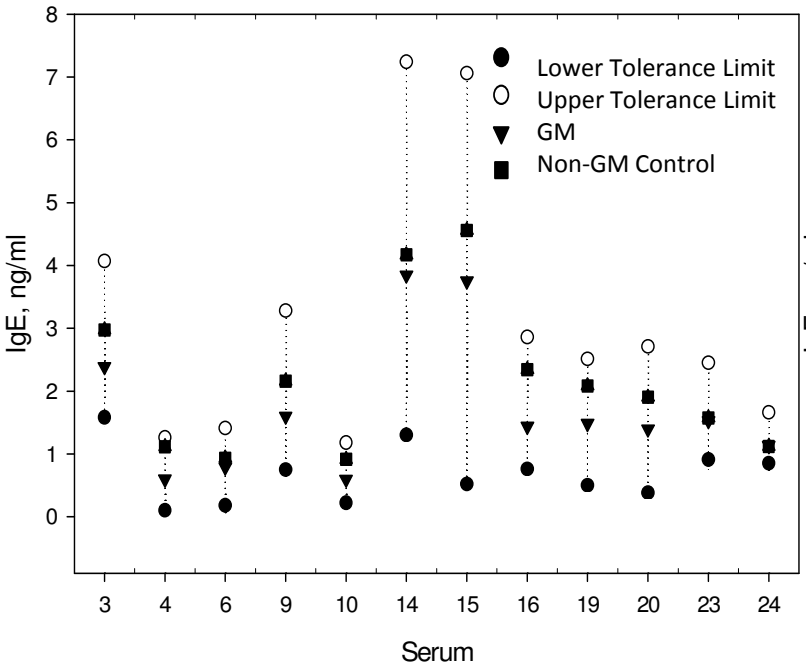

Soybean


Corn


Papaya


Summer Squash

New EU regulations are changing the methodology used to assess potential changes in endogenous allergens



New regulatory requirements

- Require information on individual allergens
- Require inclusion of allergens in the comparative composition assessment
  - Which allergens?
  - What methods?
  - Should allergens be measured?

*Ladics et al., (2014). Reg, Tox, & Pharm 70(1): 75-9.

Soybean contains eight proteins that have some evidence that they may cause allergic disease

- Many allergen lists exist
- CLI-allergy technical committee performed a rigorous assessment of scientific literature*
  - Criteria used:
    - Clear evidence of IgE binding using sera from soybean allergic individuals
    - Patients were food challenged

Soybean Allergens
Gly m 3
Gly m 4
Gly m 5
Gly m 6
Gly m 8
Gly m Bd 28K
Gly m Bd 30K
Trypsin Inhibitor

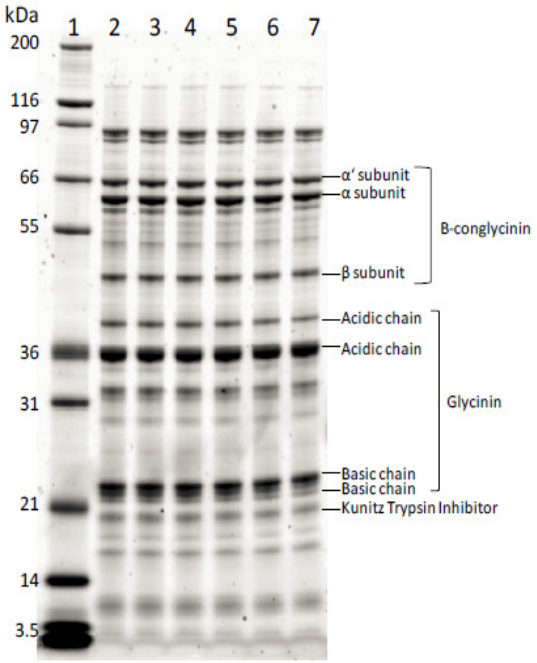
*Ladics et al., (2014). Reg, Tox, & Pharm 70(1): 75-9.

No other soybean proteins have evidence of allergenicity

- **Gly m 1, Gly m 2, Unknown 50kDa:** Proteins not found in seed; Allergic reactions caused by contaminating molds, not soybean
- **Lipoxygenase:** IgE reactivity to contaminants in protein extract
- **Unknown 39kDa, P22-25, lectin**: No reported IgE reactivity with soybean allergic patients

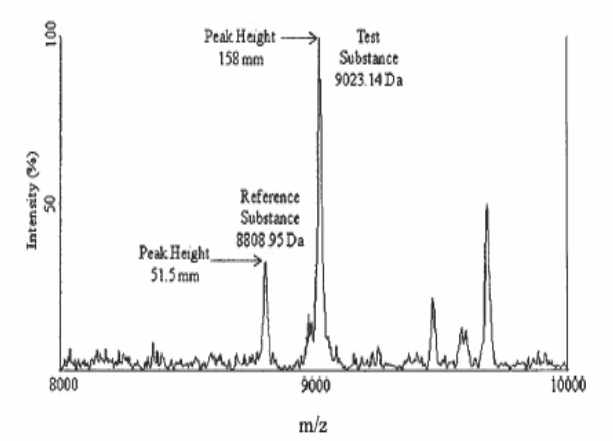
There are several methods that can be used to measure endogenous allergens

-Gel Separation-
Visualize individual proteins using electrophoresis



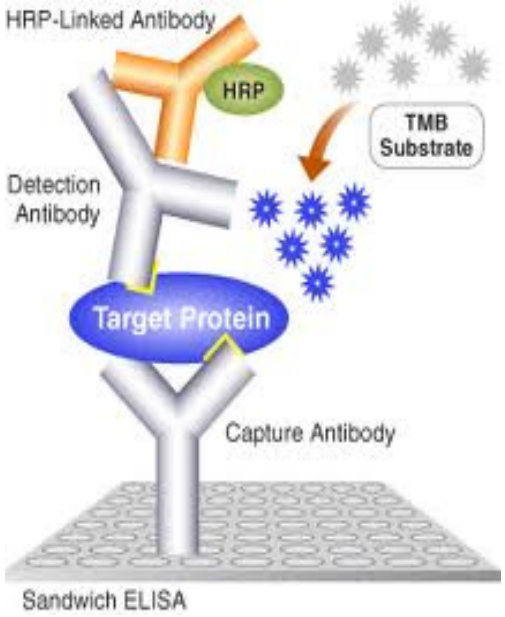
Rouquié D et al. (2010). Regul Toxicol Pharmacol.58(3 Suppl):S47-53.

-Mass Spectrometry (MS)-
Quantify allergens through detection of peptides



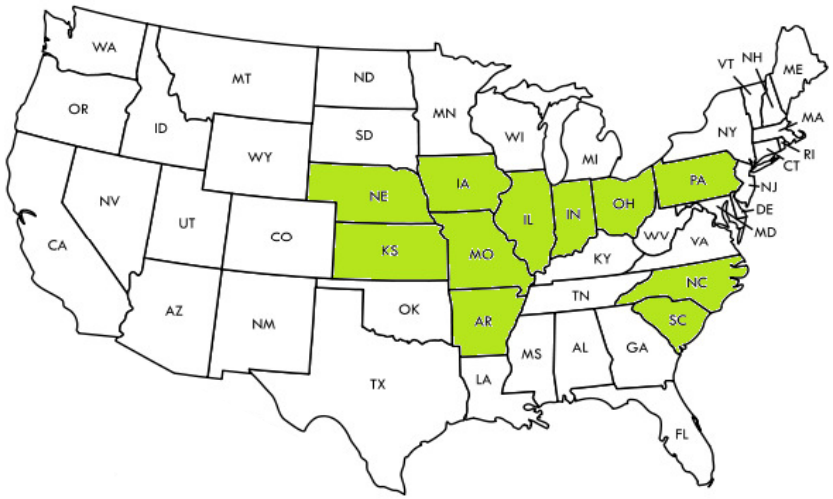
Houston NL, et al. (2011). J Proteome Res. 10(2):763-73.

- ELISA-
Quantify allergens using antibodies



Geng, T, et al. (2015). J Agric Food Chem. 63(20):4947-53.

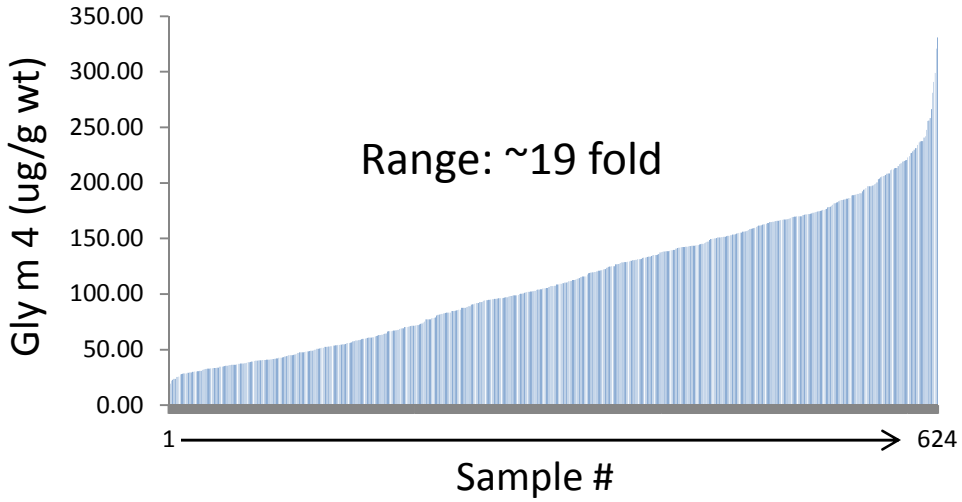
ELISA methods were used to understand the natural variability in allergen levels



ELISA methods were used to measure allergen levels in:

- 624 soybean seed samples
- 41 different varieties
- Grown over 5 different years
- 26 different field locations
  - 11 states in United States
  - 6 sites in South America

Allergen levels in conventional soybean varieties are highly variable



	Range (Fold change)
Gly m 3	9
Gly m 4	19
Gly m 5	16
Gly m 6	5
Gly m 8	19
Gly m Bd 28k	5
Gly m Bd 30k	6
Trypsin Inhibitor	40*

* Data from ILSI crop composition database

The non-allergic population safely consumes a LARGE range of allergen levels

The GM event selection process ensures that changes in allergen levels are unlikely

- 1000s of events → 1 commercial event
- Event selection based on phenotype → insertional effects eliminated
- Insertion not in or near an endogenous gene
- Environment and genotype are main source of unintended effects, not GM insertion*

- Summary of Monsanto's endogenous allergen assessments:
  - Sera: 7 events
  - Gel separation: 6 events
  - Allergen ELISAs: 1 event

* Venkatesh et al. (2015). Compositional differences between near-isogenic GM and conventional maize hybrids are associated with backcrossing practices in conventional breeding. Plant Biotechnol J. 13(2):200-10.

The GM event selection process ensures that changes in allergen levels are unlikely

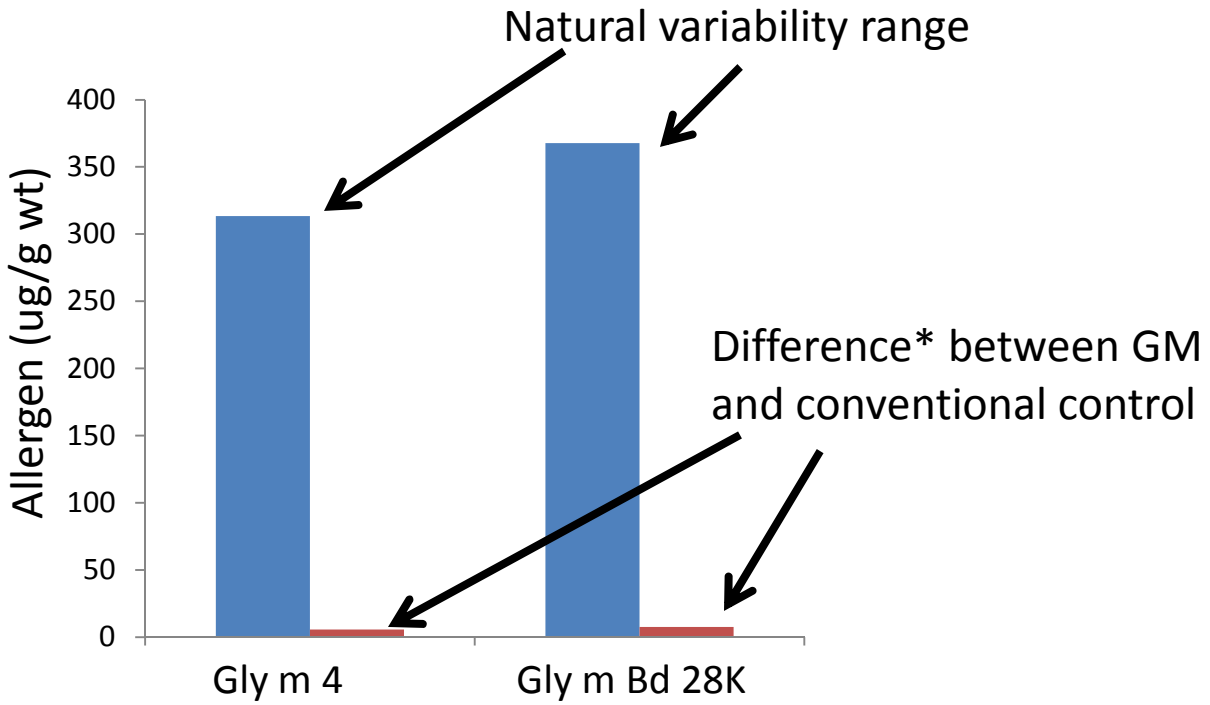
- 1000s of events → 1 commercial event
- [illegible]
- [illegible]
- Environment and genotype are main source of unintended effects, not GM insertion*

- Summary of Monsanto's

No significant change in allergen levels between GM and conventional varieties

* Venkatesh et al. (2015). Compositional differences between near-isogenic GM and conventional maize hybrids are associated with backcrossing practices in conventional breeding. Plant Biotechnol J. 13(2):200-10.

Difference in allergen levels between GM and conventional is much smaller than the natural variability range



*GM and conventional not statistically different

Does measurement of allergens provide information on the safety of the GM variety?

- Allergic individuals avoid the offending food
- Conventional soybeans have a large range of allergen levels
  - Environmental conditions are the largest factor affecting allergen levels
- The process of genetic modification does not result in relevant changes in allergen levels
  - Difference between GM and conventional control varieties is much less than natural variability of non-GM varieties
- No clear link between allergen amount and allergic disease
  - Timing of exposure is more critical*

*Du Toit et al., 2015. N Engl J Med. 372(9): 803-13.


THANK YOU