Workshop on the

Allergenicity Assessment of GM Plants

EFSA, Brussels, 17 June 2015

Endogenous Allergenicity

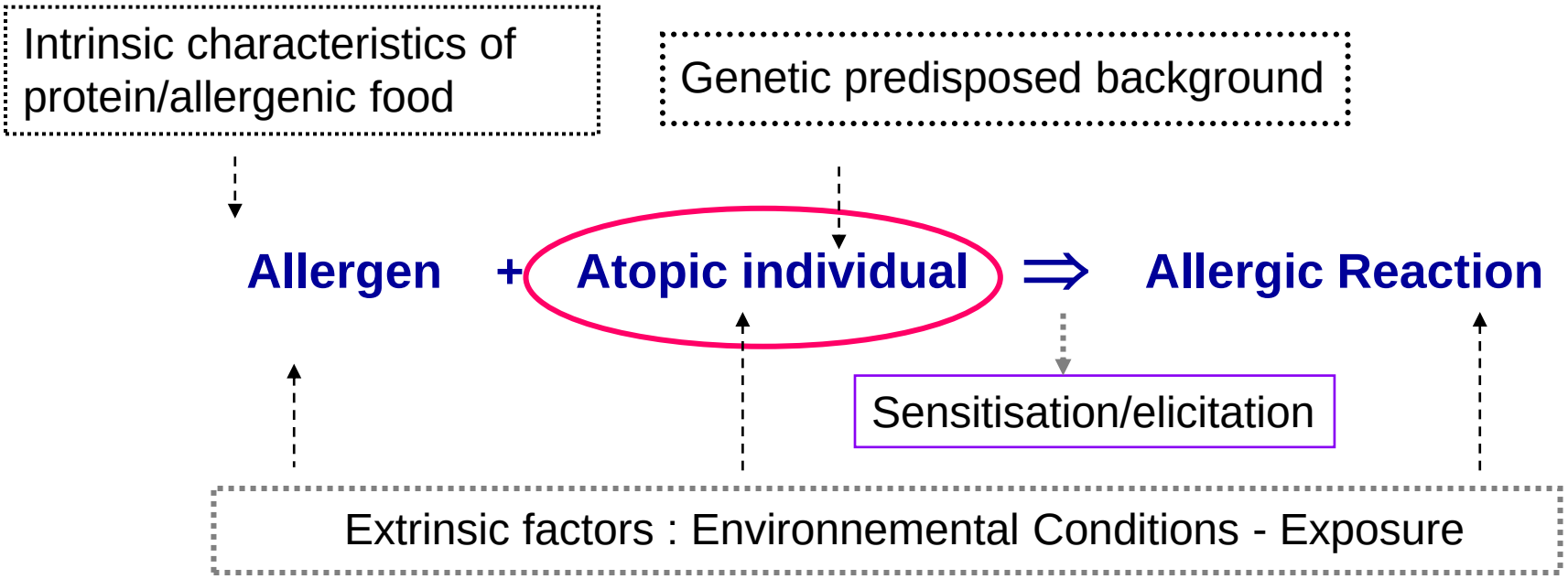
Jean-Michel Wal

INRA, AgroParisTech, Paris, France

jean-michel.wal@agroparistech.fr

Assessment of allergenicity of GM Plants

“Allergenicity is not an intrinsic, fully predictable, characteristic property of a given food/protein but is a biological property requiring an interaction with individuals with a pre-disposed genetic background.”



Allergenicity of GM foods : What are the issues ?

Two major (independent) issues associated

1. **with the source of the transgene**
2. **with the recipient of the genetic modification**

1. **Allergenicity of the newly expressed protein(s) :**

   Is the newly expressed protein likely to be allergenic ?

2. **Increased allergenicity of the whole GM crop, e.g. by alteration of the expression of endogenous natural allergenic proteins or occurrence of novel allergens**

⇒ **Is the GMP more allergenic than its conventional counterpart ?**

***Besides food intake, also other routes of exposure are to be considered. Particularly in the case of application of a GMP for cultivation, the respiratory allergy risk due to pollen should also be assessed*.**

Assessment of Endogenous allergenicity : Why ?

Possible unintended/unexpected effect of the genetic modification (e.g. pleiotropic effect of the transgene) may result in possible deregulation of genes encoding endogenous allergenic proteins and thus modifying the natural allergen qualitative and quantitative composition of the plant. This may modify the exposure of at risk consumers and impact on the risk of sensitization and/or elicitation of an allergic reaction.

Assessment of Endogenous Allergenicity of GMP

"When the recipient plant is known to be allergenic, the applicant shall assess any potential change in the allergenicity of the genetically modified food or feed by comparison of the allergen repertoire with that of its conventional counterpart. The potential over-expression of natural endogeneous allergen(s) in the GMP shall, in particular, be investigated"

(EC, 2013)

- ✓ **It is a mandatory part of the risk assessment and not only a risk management issue.**
- ✓ **To be performed on a case by case basis**

⇨ **Compare qualitative and quantitative composition of endogenous allergens in the GMP and its non GM comparator(s)**

Assessment of Endogenous allergenicity : Why ?
Clinical relevance

An increased expression of particular endogenous allergens may increase the allergenicity and increase the risk for at risk (allergic) individual.

Although research and clinical investigations still needed to establish a quantitative (causal) relationship, this concern has already been taken into consideration for a long time by e.g. :

- **ILSI-IFBC, 1996**
- **FAO-WHO, 2001**
- **Codex Alimentarius, 2003-2009**
- **EFSA 2006, 2011**
- **OECD (e.g. revised Consensus Document on Soybean, 2012)**
- **EU Implementing Regulation 503/2013**

Crit Rev Food Sci Nutr. 1996;36 Suppl:S165-86.

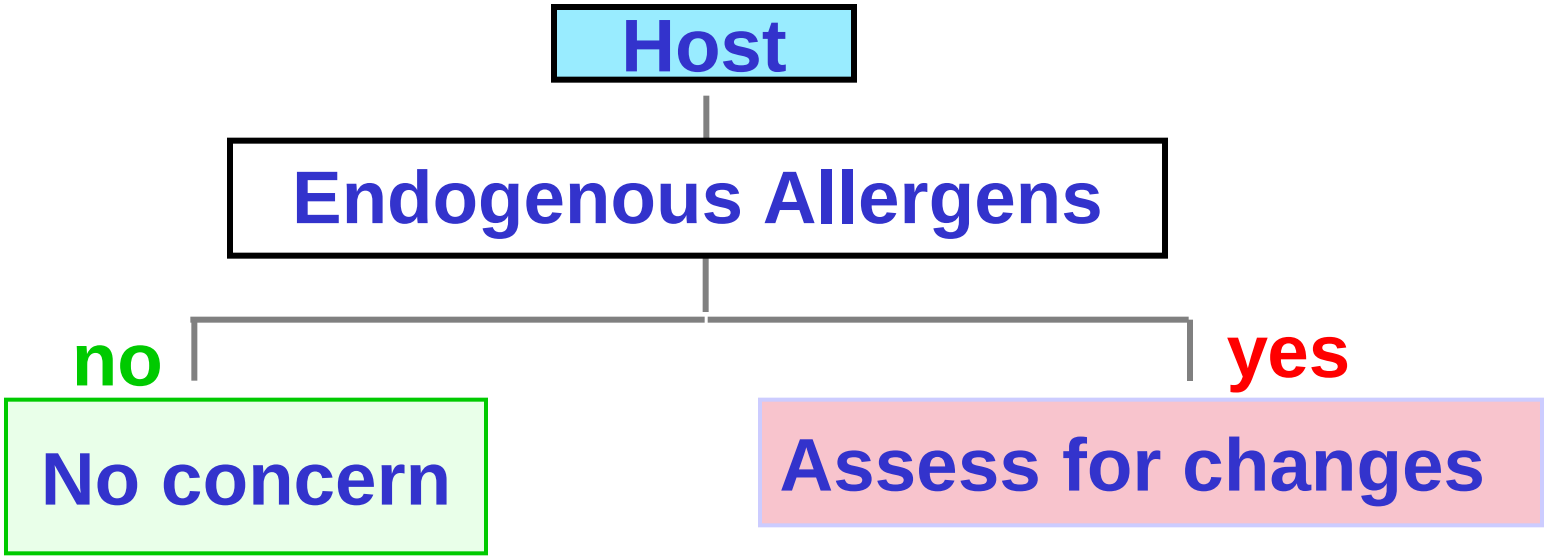
Assessment of the allergenic potential of foods derived from genetically engineered crop plants.

Metcalfe DD[1], Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL.

Report of an ILSI-IFBC workshop

Abstract

This article provides a science-based, decision tree approach to assess the allergenic concerns associated with the introduction of gene products into new plant varieties. The assessment focuses on the source from which the transferred gene was derived. Sources fall into three general categories: common allergenic food proteins; less common allergenic foods or other known allergen sources; and sources with no history of allergenicity. Information concerning the amino acid sequence identity to known allergenic proteins, in vitro and/or in vivo immunologic assays, and assessment of key physiochemical properties are included in reaching a recommendation on whether food derived from the genetically modified plant variety should be labeled as to the source of the transferred gene. In the end, a balanced judgement of all the available data generated during allergenicity assessment will assure the safety of foods derived from genetically engineered crops. Using the approaches described here, new plant varieties generated by genetic modification should be introduced into the marketplace with the same confidence that new plant varieties developed by traditional breeding have been introduced for decades.



J Allergy Clin Immunol. 1995 Dec;96(6 Pt 1):1008-10.

Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties.

Burks AW[1], Fuchs RL.

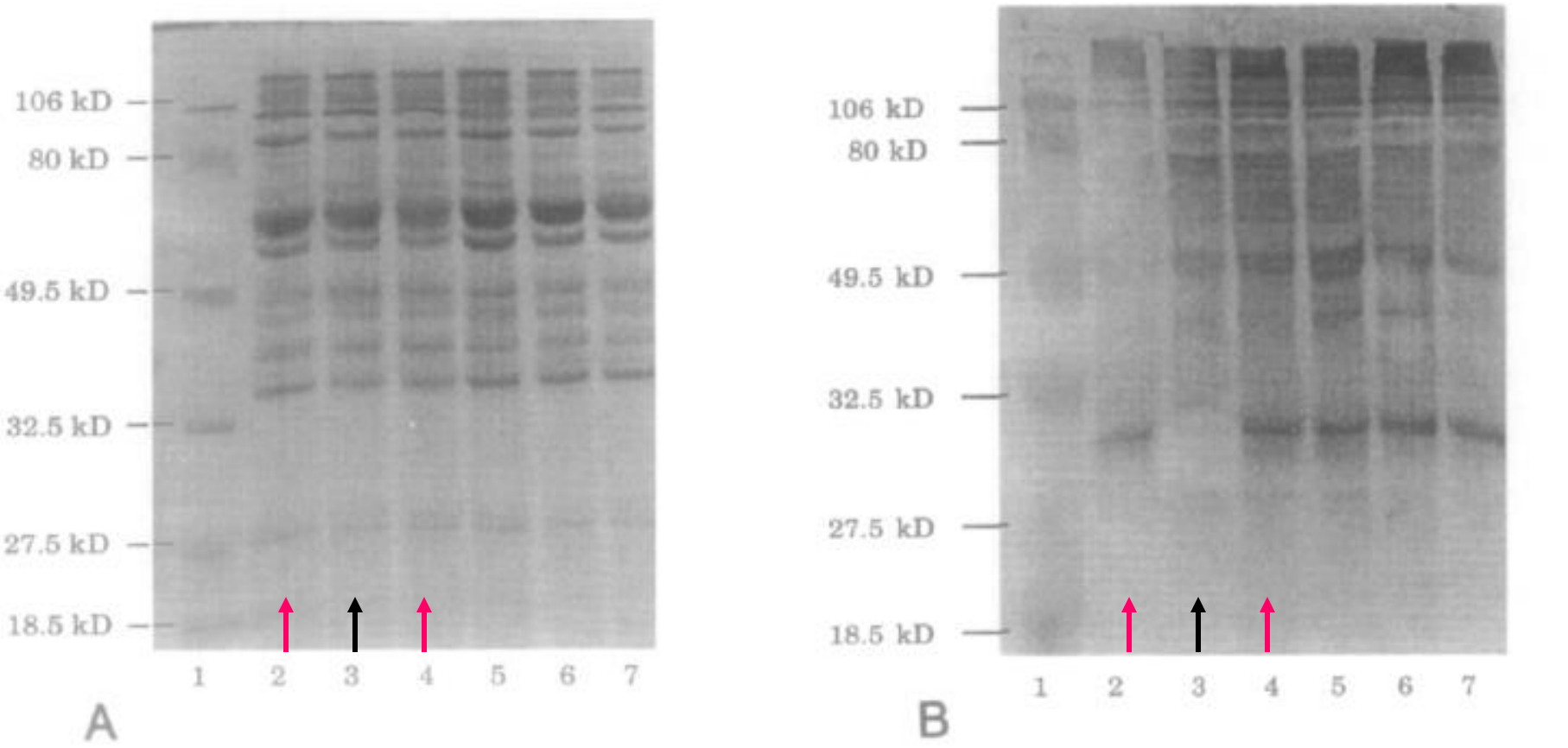


FIG. 1. A, Coomassie blue–stained SDS-PAGE gel with soybean extracts. *Lane 1,* Prestained molecular weight standards; *lane 2,* extract from GTS line 61-67-1 (30 μg); *lane 3,* extract from parental line A5403 (30 μg); *lane 4,* extract from GTS line 40-3-2 (30 μg); *lane 5,* extract from Cargill control 1 (30 μg); *lane 6,* extract from Cargill control 2 (30 μg); *lane 7,* extract from ADM control (30 μg). **B,** Immunoblot with serum containing IgE antibodies from soybean-sensitive patients. *Lane 1,* Prestained molecular weight standards; *lane 2,* extract from GTS line 61-67-1 (30 μg); *lane 3,* extract from parental line A5403 (30 μg); *lane 4,* extract from GTS line 40-3-2 (30 μg); *lane 5,* extract from Cargill control 1 (30 μg); *lane 6,* extract from Cargill control 2 (30 μg); *lane 7,* extract from ADM control (30 μg).

Prevalence estimate of food allergies in different geographic areas

- **The most prevalent/severe food allergies vary worlwide (e.g. depending upon the traditional diet).**
- **There are important individual and geographical differences in the pattern of sensitizations to foods depending upon genetic background, age and environmental conditions (e.g. exposure).**

Prevalence of sensitization to oilseed rape and maize pollens and seeds in France

Eur Ann Allergy Clin Immunol. 2012 Dec;44(6):225-35.

Prevalence of sensitisation to oilseed rape and maize pollens in France: a multi-center study carried out by the Allergo-Vigilance Network.

Moneret-Vautrin DA1, Peltre G, Gayraud J, Morisset M, Renaudin JM, Martin A.

Conclusions :

- **Frequent sensitizations observed in atopic patients (adults) living in regions that contain a high density of rapeseed and maize fields.**
- **The prevalence of sensitization to rapeseed and maize pollen/seeds (as measured by SPT) is positively correlated to the level of exposure (i.e. crop density).**
- **This prevalence is higher in patients with actual atopic disease as compared to those with asymptomatic atopy.**
- **Cross-reactivities between pollens and seeds could potentially elicit cross-reacting food allergies.**

Assessmemnt of Endogenous Allergenicity : When ? What allergens ?

- When the host (recipient) of the GM is a common allergenic food

- "Common allergenic foods" usually refers to Annex IIIa of Directive 2007/68/EC for labelling purpose.
  For the present time this pertains to GM soybean only.

➡ **Q. Are other crops likely to be concerned in the future ?**

- Endogenous allergens are those listed in OECD Consensus Documents. They are identified and recorded in recognized allergen data bases

Soybean allergens listed in the OECD consensus document (2012)

Table 20. Potential soybean allergens

IgE-binding proteins	Allergen nomenclature	Molecular weight (kDa)	Family
Hydrophobic proteins	Gly m 1[1]:	7.0-7.5	Lipid transfer protein
Defensin	Gly m 2[1]	8.0	Storage protein
Profilin	Gly m 3[1]	14	Profilin
SAM22	Gly m 4[1]	16.6	Pathogenesis related protein PR-10
P34	Gly m Bd 30 K	34	Protease
Unknown Asn-linked glycoprotein	Gly m Bd 28 K	26	Unknown
β-Conglycinin (vicilin, 7S globulin)	Gly m 5[1]	140–170	Storage protein (with subunits)
Glycinin (legumin, 11S globulin)	Gly m 6[1]	320–360	Storage protein (with subunits)
2S albumin	Not assigned	12	Prolamin
Lectin	Not assigned	120	Lectin
Lipoxygenase	Not assigned	102	Enzyme
Kunitz trypsin inhibitor	Not assigned	21	Protease inhibitor
Unknown	Not assigned	39	Unknown
Unknown	Not assigned	50	Homology to chlorophyll A-B binding protein
P22-25	Not assigned	22–25	Unknown

Source: adapted from L'Hocine and Boye, (2007); updated with information from WHO/IUIS (2011)

[1] WHO/IUIS (2011) Allergen nomenclature recognized by WHO and IUIS

Assessment of Endogenous Allergenicity : When ? - What allergens ?

- "Common food allergens" usually refers to Annex IIIa of Directive 2007/68/EC for labelling purpose.

  For the present time this pertains to GM soybean only.

Q. Are other crops likely to be concerned in the future ?

- Endogenous allergens are listed in OECD Consensus Documents. They are identified and recorded in recognized allergen data bases.

Q. Can we identify most "relevant" allergens to be selected for inclusion in the comparative analysis ?

What criteria may account for their importance for Public Health ? (e.g. potency, abundance, major *vs* minor allergens, …)

Assessment of Endogenous Allergenicity : How ?

Targeted analyses, e.g. :

- 2D electrophoresis + western blotting (WB)

⇨ Requires specific allergic human sera

- Specific determination of known allergens,

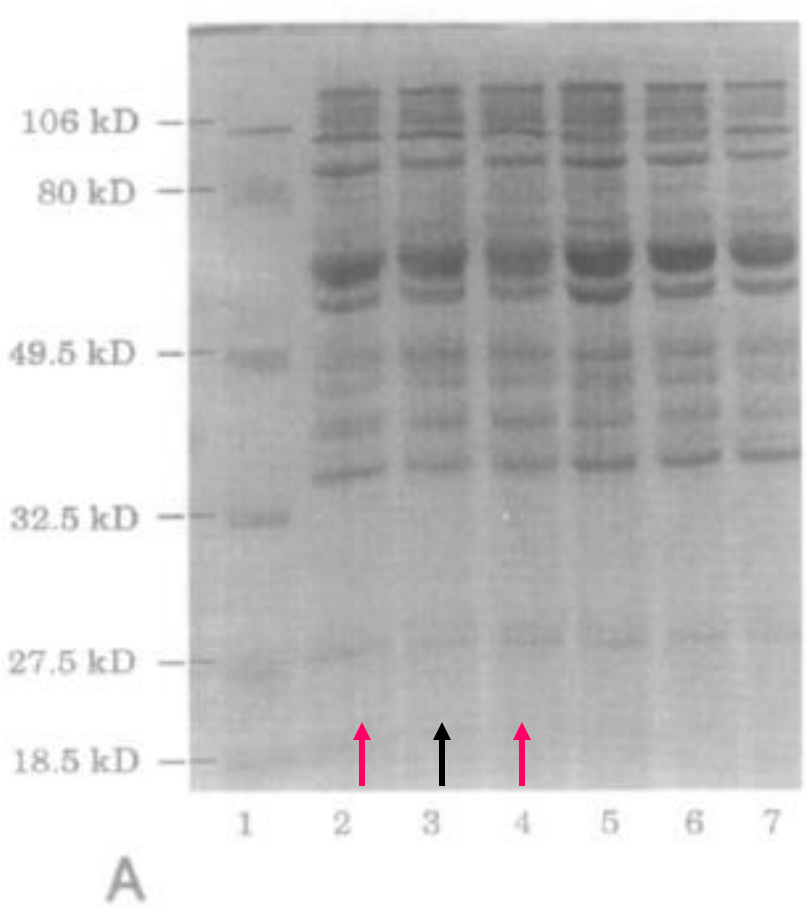
Non targeted analyses, e.g. :

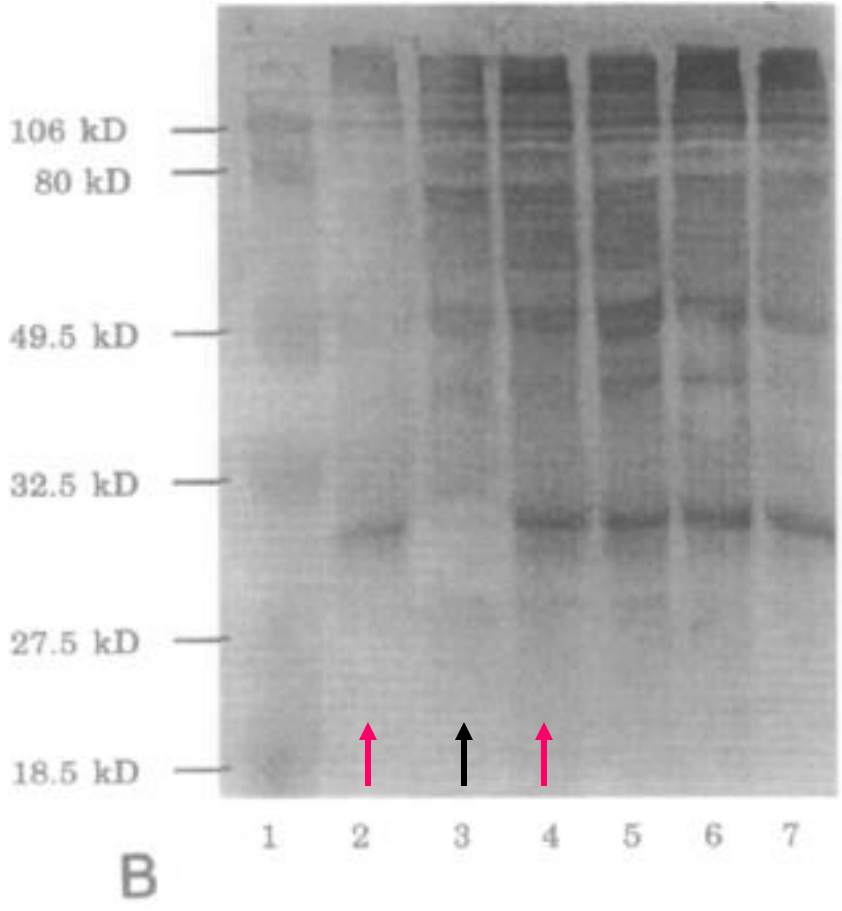
Proteomics and high throughput analytical methods using mass spectrometry

J Allergy Clin Immunol. 1995 Dec;96(6 Pt 1):1008-10.

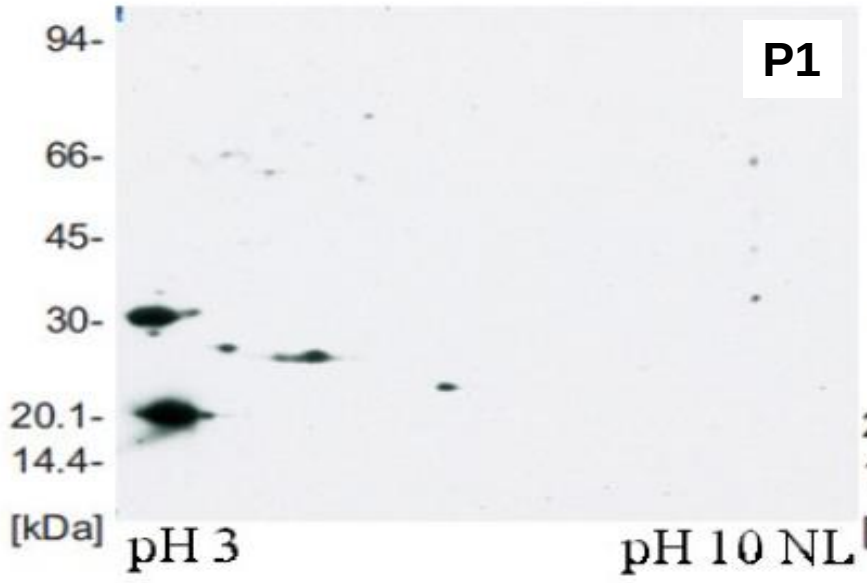
Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties.

Burks AW[1], Fuchs RL.

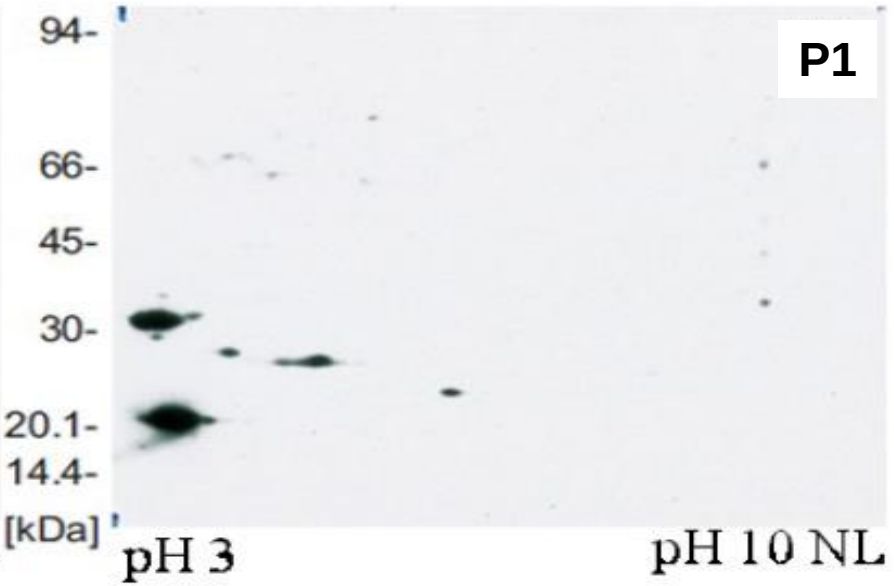




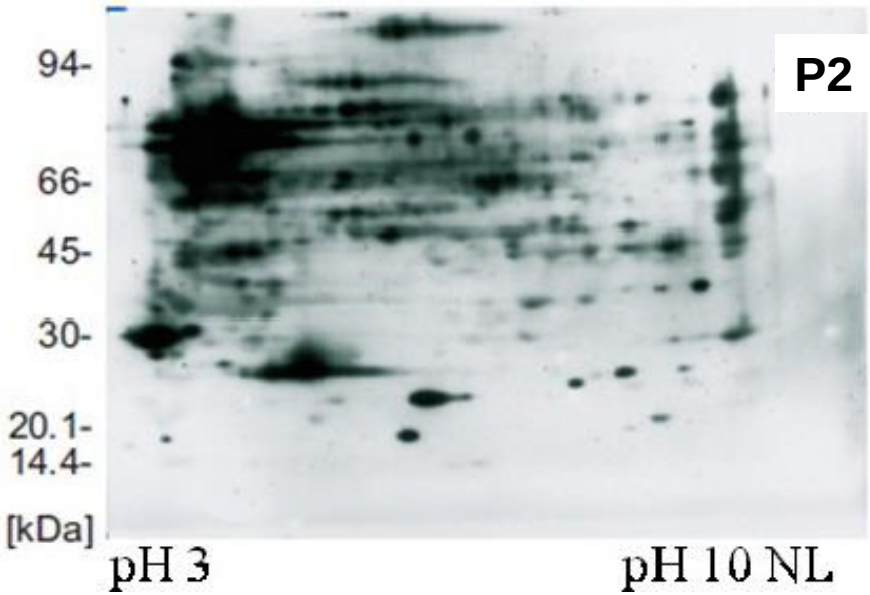
2D immunoblots of seed protein extracts from a GM soybean and its non GM counterpart

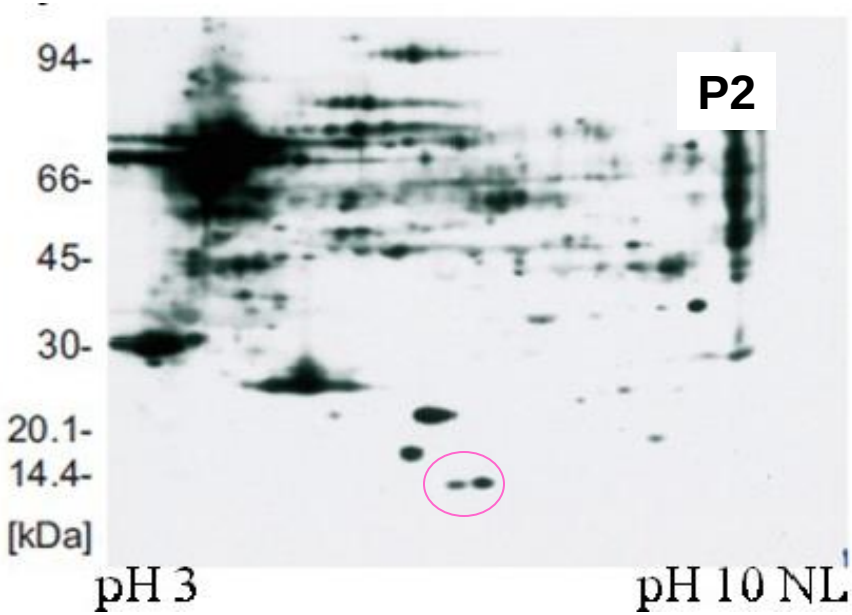


Conventional soybean

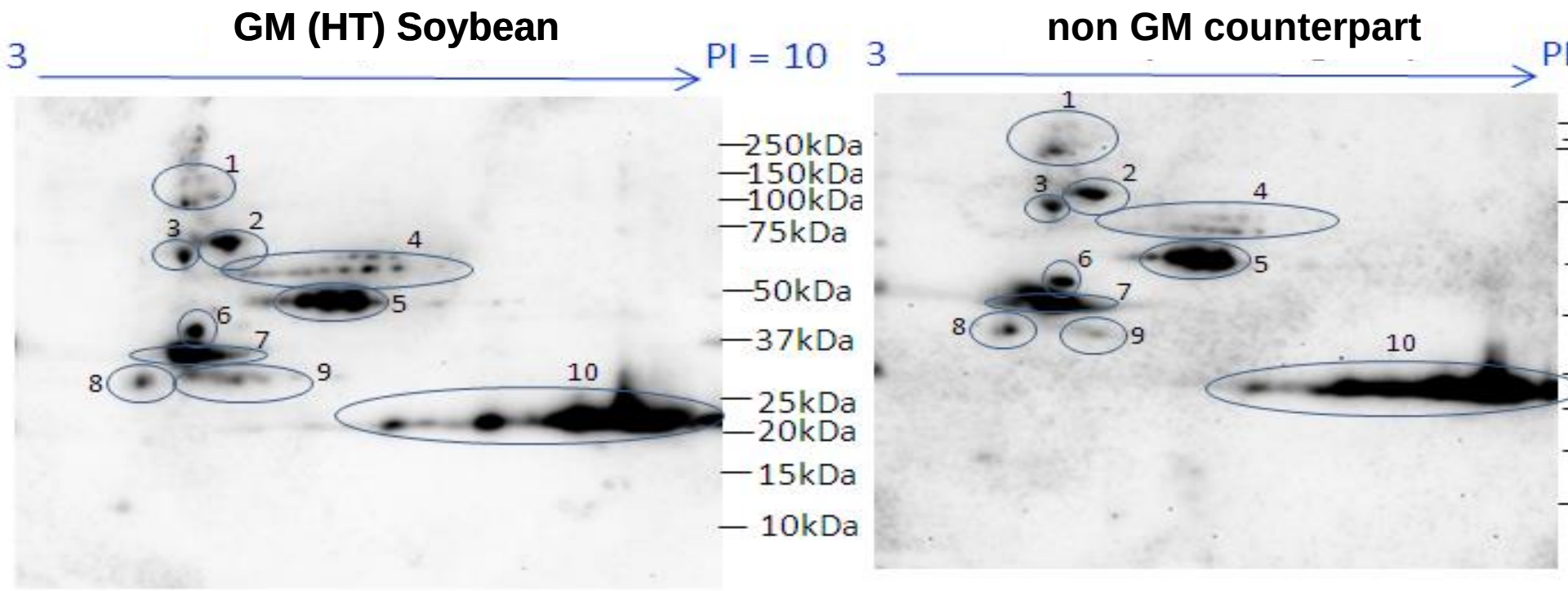


GM soybean





2D immunoblots of seed protein extracts of a GM-HT soybean and its non GM counterpart



Probable spot identities

1. Unknown
2. β-Conglycinin (α' subunit, 72 kDa/5.2)
3. β-conglycinin (α subunit, 70 kDa/4.9
4. Unknown
5. β-conglycinin (β subunit, 52 kDa/5.6-6.0)
6. Glycinin (acidic polypeptide, 31-45 kDa/4.8-5.5)
7. Glycinin (acidic polypeptide, 31-45 kDa/4.8-5.5)
8. Glycinin (acidic polypeptide, 31-45 kDa/4.8-5.5)
9. Glycinin (acidic polypeptide, 31-45 kDa/4.8-5.5)
10. Glycinin (basic polypeptides, 18-20 kDa/6.5-8.5)
18 β-Conglycinin (α' subunit, 72 kDa/5.2)
19. Glycinin (acidic polypeptide, 31-45 kDa/4.8-5.5)

From R. Goodman et al., J Agric Food Chem, 2013

Targeted analysis of the whole GM crop : drawback

- **Serum screening should be carried out with sera from well-characterised allergic humans.**
- **Each serum should be tested individually in order to reflect the variability and wide pattern of specificity of the IgE response and to evidence potential IgE binding to minor allergens.**
- **A major drawback is that relevant human sera may be difficult to obtain. They are often limited in number and quantity ; their affinity/avidity and specificity are variable which makes it difficult to standardize IgE binding tests and derived immunoassays.**

Fernandez et al., 2013

Assessment of Endogenous Allergenicity : How ?

Targeted analyses, e.g. :

- 2D electrophoresis + western blotting (WB)

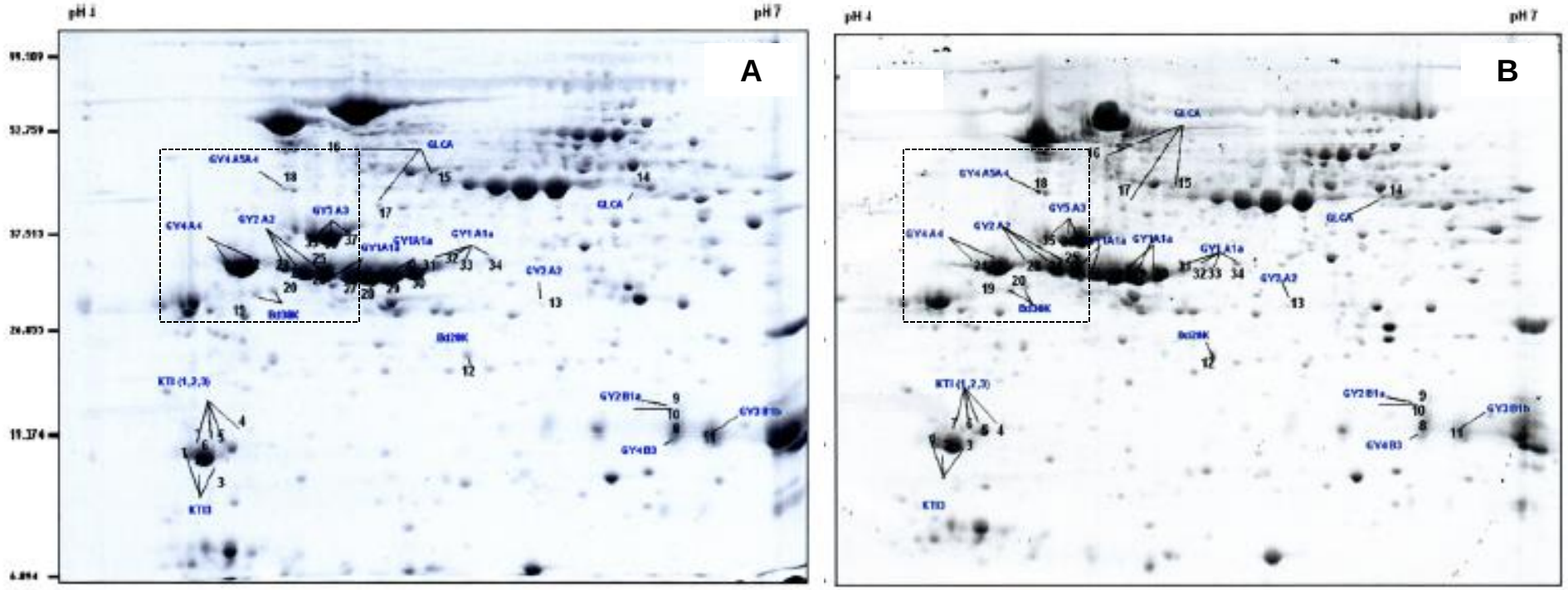
  ⇨ Requires specific allergic human sera

- Specific determination of known allergens,

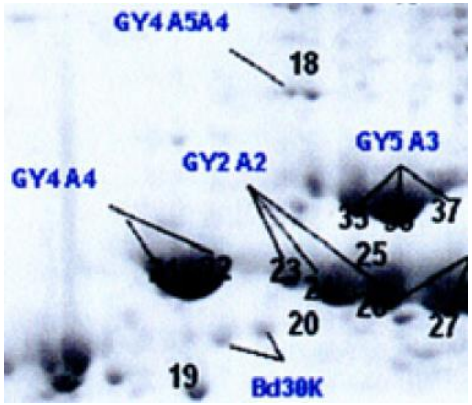
Non targeted analyses, e.g. :

Proteomics and high throughput analytical methods using mass spectrometry

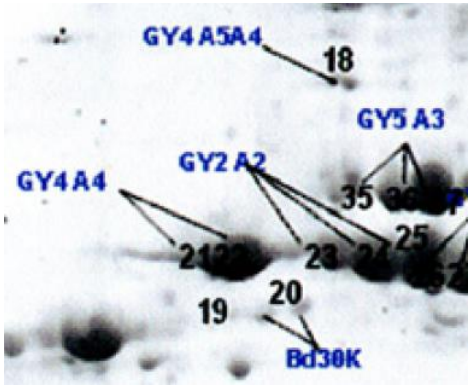
Proteome maps of seed protein extracts from a GM-HT soybean (A) and its non GM counterpart (B)



GM (HT) soybean



Non GM counterpart



From D. Rouquié et al.
Reg Toxicol Pharmacol 2010

Proteomics and high throughput analytical methods using mass spectrometry or other–OMICS technologies

- ⇨ Allow identification and quantification
- ⇨ May not require human sera
- ⇨ Require equipments and competence
- ⇨ Already informative although not fully standardized
- ⇨ Rapid developments to improve sensitivity and specificity and allow validation

Relevance of possible observation of unintended changes

Assessment of the relevance of a potential over expression of (some) endogenous allergens in GMP based on e.g. :

- **Information on the natural variability.**
- **Magnitude of the differences**

The specific quantification of individual endogenous allergens is part of the comparative compositional analyze and should include difference and equivalence tests.

- **Nature of concerned allergens**

➡ **May require additional information/investigations to further characterize the allergenicity (e.g. when difference and non equivalence), such as whether or not similar differences are observed in all patients when IgE binding tests (e.g. WB) are performed.**

Outcomes of allergenicity assessment of GM crops by the EFSA GMO Panel

- To date concerns only GM soybean.
- Technology used has continuously improved. Experiments appropriate, correctly performed and in line with requirements
- No evidence of significant differences between GM soybean and conventional comparator observed
- Generally differences much bigger between reference lines than between the GM plant and its non GM comparator

➡ No indication of significant unintended changes

➡ No indication of possible adverse effects.

➡ No safety concern

Thank you for your attention

Acknowledgements

EFSA GMO Panel "Allergenicity WGs" and particularly Dr Antonio Fernandez Dumont, EFSA Scientific Officer