UTILITY OF PROTEOMIC TECHNIQUES FOR ASSESSING PROTEIN EXPRESSION

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Soybean Genomics and Improvement Laboratory

Mission: To improve the quality of Soybean and to reduce the costs of production.

Research Goals:

1) Define the Function and Interaction of Genes and Metabolites Controlling Soybean Resistance to Disease

2) Develop Genetic Markers and Genome Maps to Expedite the Deployment of Genes

3) Determine and Define Unintended Effects Associated with Transgenic Soybean

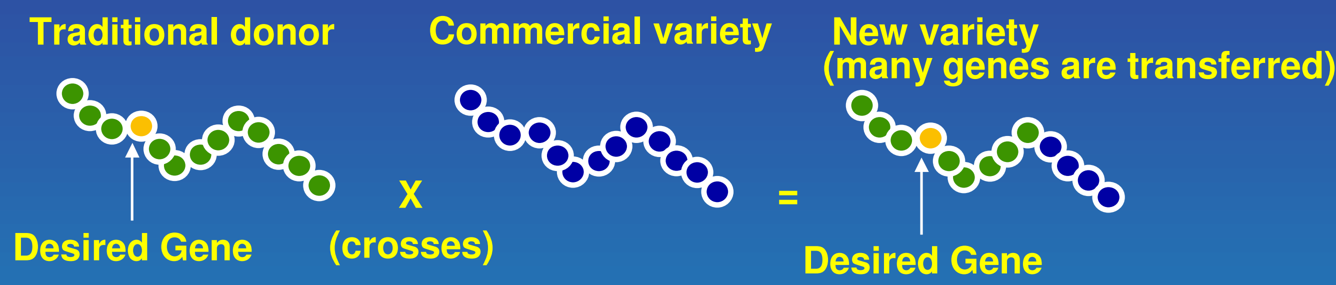
Outline

- Genetic Modification and Benefits
- Evaluations of GMO Crops
- Proteomics Technologies and its Applications

Plant Breeding Vs. Modern Biotechnology

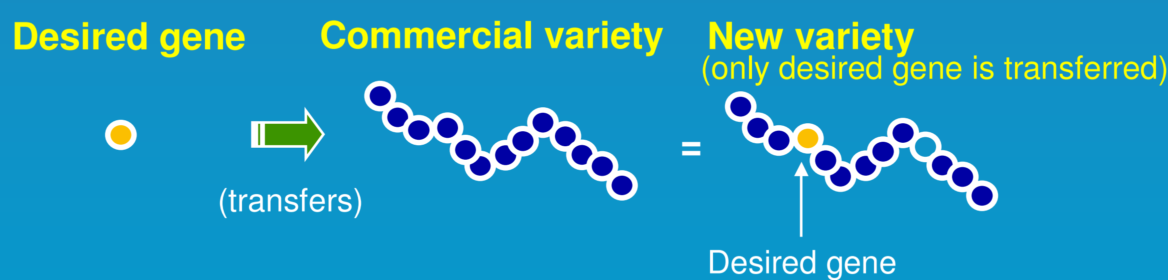
Plant Breeding

Traditional plant breeding combines many genes at once.



Modern Plant Biotechnology

Using plant biotechnology, a single gene may be added to the strand.



Source: Council for Biotech. Inf.

Approved GMO Crops

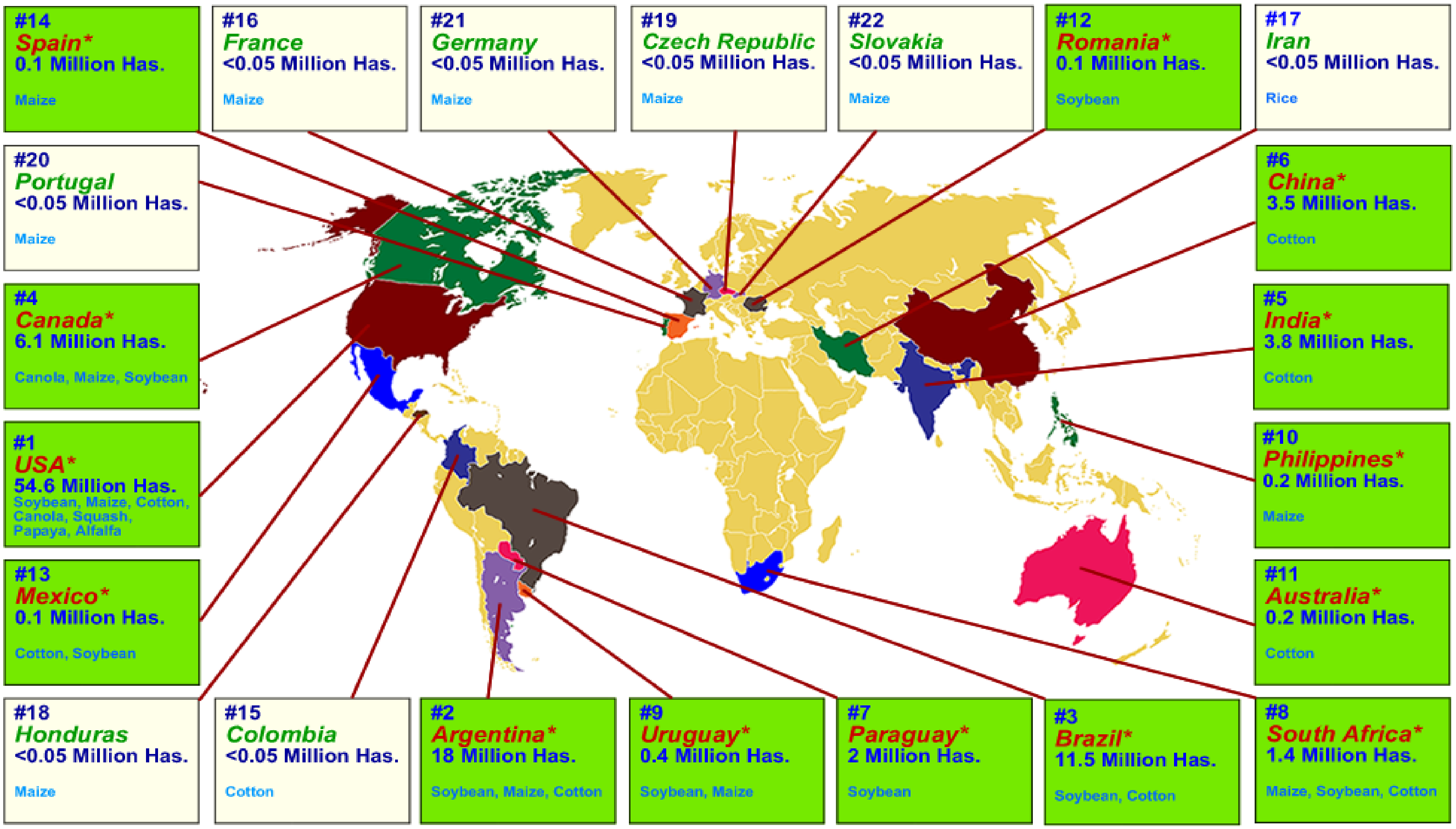
More than 60 biotech food products have been assessed for safety and approved for commercial use. The safety assessment has been conducted from guidance of a variety of expert scientific bodies, including US FDA, EPA, USDA (1992, 1994), The European Commission (1995), ILSI AII/IFBC (1996), FAO/WHO (1996, 2000), and Codex (2000, 2002, 2003)

Examples…

- Canola
- Corn
- Cotton
- Papaya
- Potato
- Soybeans
- Squash
- Sugar beets
- Sweet corn
- Tomato

Source: Council for Biotech. Inf.

Biotech Crop Countries and Mega-Countries*,2006

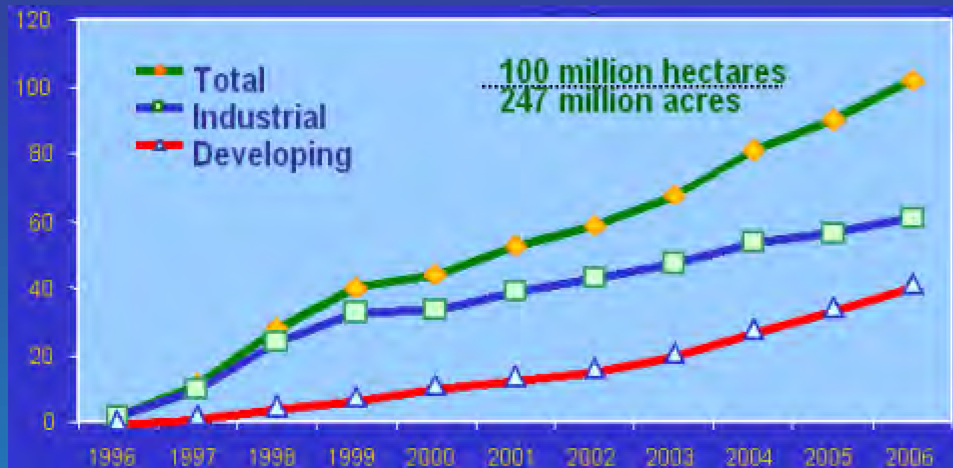


* 14 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.

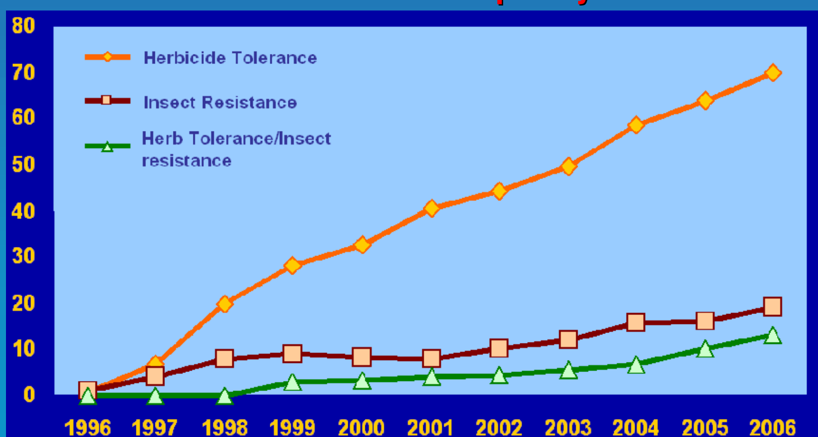
Source: Clive James, 2006

Global Cultivation of GMO Crops

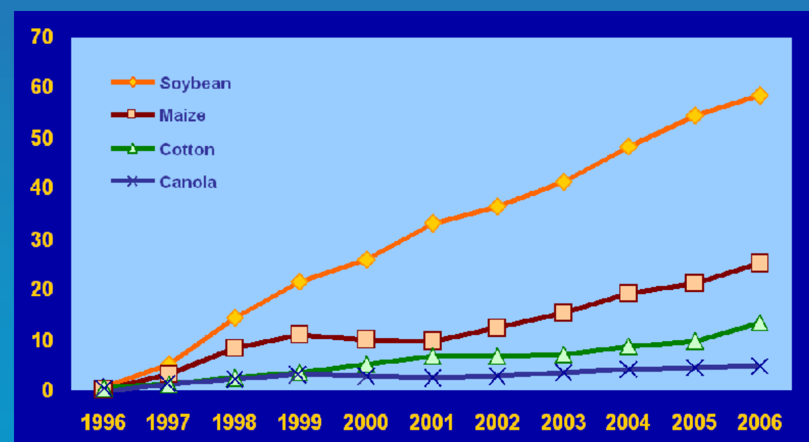
Industrial and Developing Countries



Global Area of Biotech Crops: By Trait



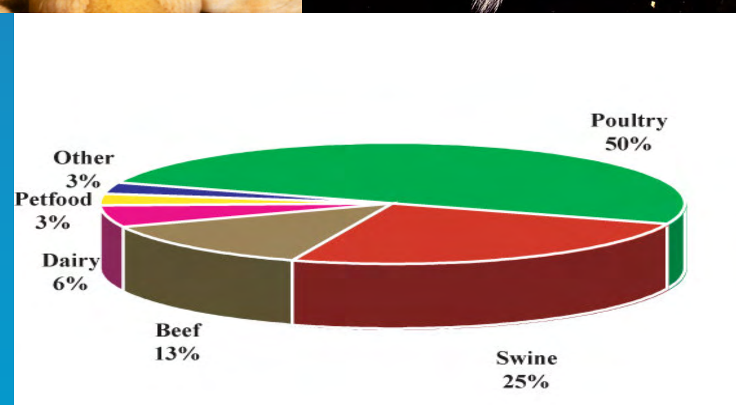
Global Area of Biotech Crops: By Crop



ISAAA

Soybeans

- Second most valuable crop in the US (Annual value $19.8 Billion)
- Inexpensive source of proteins
- About 75 % of soybean planted in the US is GMO
- Health issues-allergens and anti-nutrients
- Transgenic technology has emerged as a solution to improve deficiencies
- Critical to feeding future world populations



Source: Council for Biotech. Inf. ; USDA

Benefits of GMO

- **Nutritious and healthier foods**
  - Manipulate fatty acids and
  - Reduce or eliminate naturally occurring allergen proteins
- **Better crop production**
  - Tolerate herbicide
  - Disease resistance
- **Overproduction to meet the**
  - global demand
  - Enhance revenue
- **Suitable for Livestock**
  - Provide nutritious food
  - Meet diary/meat supplies

GMO SOYBEANS

Commercialized Transgenic Soybeans

Roundup Ready Soybean (RR)-Monsanto

- Glyphosate herbicide tolerance
- Improved weed control

High-Oleic soybean (HO)-DuPont

- Low in saturated fat with no trans-fatty acids
- Enhanced thermostability of the oil for cooking purposes

Transgenic Soybeans in R&D

- Allergen suppression (P34)
- Milk protein (Bovine casein)
- Virus resistance (Bean pod mottle)
- Production of Monoclonal antibody
- White mold resistance (oxalate oxidase)
- High Phytase content

Source: Monsanto

National Center for Food and Agricultural Policy

Coordinated Framework - 1986

- Crops produced using genetic engineering pose the same kinds of risks as crops produced by conventional breeding for similar traits

- Regulation should be science-based and should be conducted on a case-by-case basis

- The existing laws provide adequate authority for regulation of the products of biotechnology

Regulatory Agencies

USDA UNITED STATES DEPARTMENT OF AGRICULTURE → *Is it safe to grow?*

U.S. Department of Health and Human Services
Food and Drug Administration → *Is it safe to eat?*

EPA United States Environmental Protection Agency → *Is it safe for the environment?*

Source: Council for Biotech. Inf.

Substantial Equivalence

"Concerning food safety, every biotech food crop on the market has been evaluated by the FDA. The FDA has determined that every biotech crop on the market today is 'substantially equivalent' and therefore as safe as its traditional counterpart"

Source: Council for Biotech. Inf.

The Concept of Substantial Equivalence

- The concept of substantial equivalence is widely accepted by international & national agencies as the best available guidance for the safety assessment of new GMO crops

- The guiding principle in the evaluation of GMO foods by regulatory agencies in Europe and the U.S. is that their human and environmental safety is most effectively considered, relative to comparable products and processes currently in use

- If a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as being as safe as the conventional food and does not require extensive safety testing

(FDA, 1992; Kuiper et al., 2001; Maryanski, 1995; OECD, 1993; WHO, 1995; FAO, 1996; Konig et al., 2004. Food & Chem. Toxicol. 42: 1047-1088)

Proteomics

- Proteomics can be a useful tool for the identification of precursors, post-translational modifications and degradation products of proteins
- The evaluation of substantial equivalence requires the availability of database based on natural variation of proteins
- Such a database is important for determining if the new biotech product falls within or outside the range of natural variation
- Proteomics allows us to build such a database for protein variation

Proteomics Applications

- Valuable for the detection of food/pollen allergens by using patients' immune sera
- 2D followed by immunoblotting was used to characterize and identify the IgE-reactive proteins of *Hevea* latex, which is the main cause of the latex type I allergy & the major latex allergens have been localized on 2D-maps
- By 2D blotting using patients' IgE and monoclonal antibodies, Petersen et al., detected IgE –reactive isoforms and found single amino acid substitutions in different- sized group I grass pollen

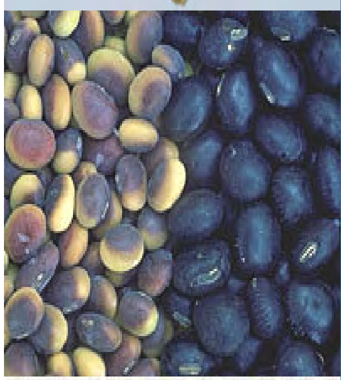
Petersen et al.,1997. Electrophoresis18: 819-825
Posch et al., 1997. Electrophoresis18: 2803-2810
Cellini et al., 2004. Food & Chem. Toxicol.42: 1089-1125

Research Challenges

- Analytical challenges for better separations and accurate identification of proteins
- Limitations in detecting very large/small acidic/ basic proteins and less abundant proteins
- The sensitivity of staining procedures sets some limits to the amount of protein needed for loading, identification and quantification
- The comparisons of datasets between different laboratory requires a standardized method to be developed for sample isolation and electrophoresis
- More background data on natural variation are needed, because the environment greatly influences protein abundance

Cellini et al., 2004. Food & Chem. Toxicol. 42: 1089-1125

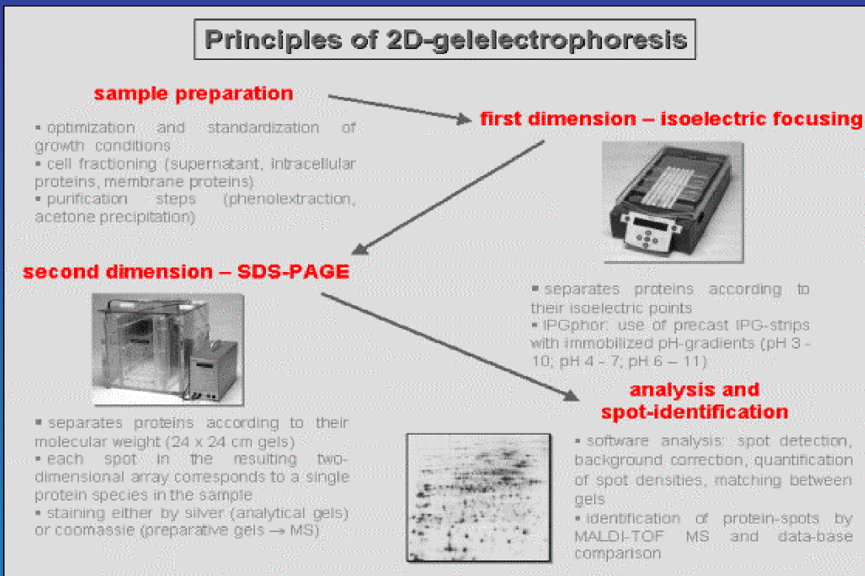
Research Objectives

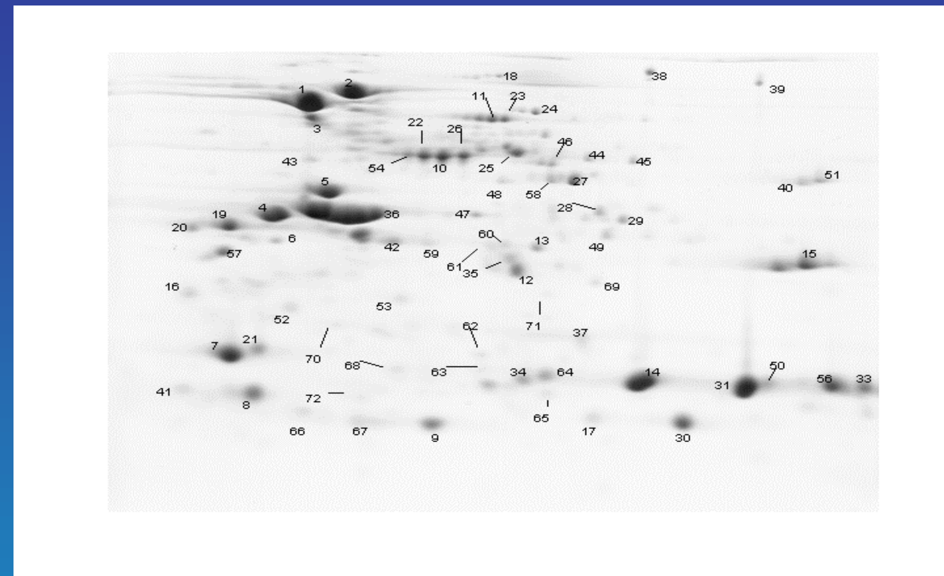

- Develop and standardize effective proteomic methodologies and establish a database of soybean seed proteins
- Determine the natural variation of seed proteins of a wide range of soybeans to establish a baseline
- Determine unintended expression of protein in transgenic soybean by comparing with the natural variations

Proteomics Tools

2D gel method



2D gel electrophoresis



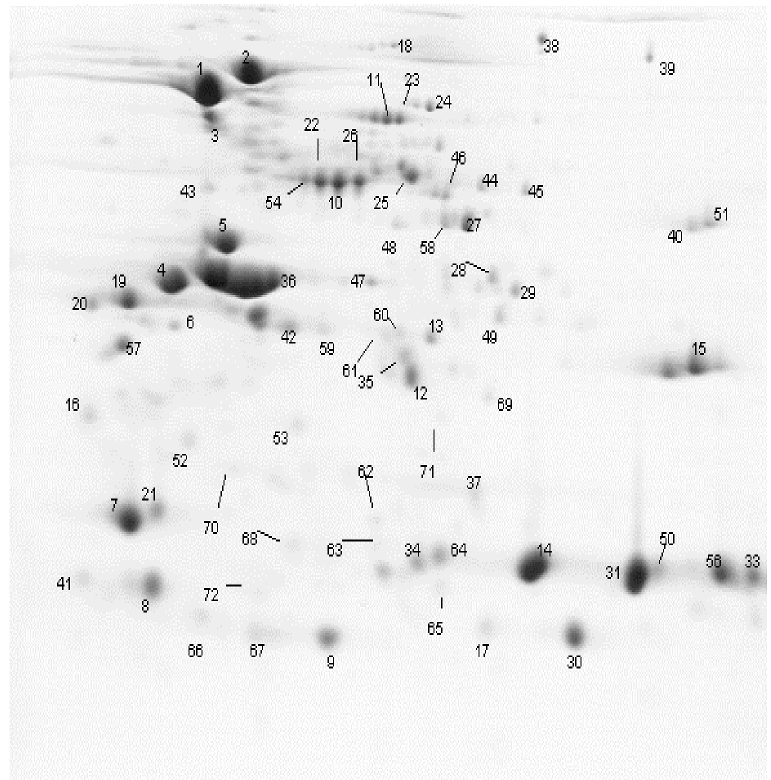
MALDI-TOF MS

LC-MS

Source: Waters Corporation

Soybean Seed Proteins

- Developed an efficient protein extraction method for soybean proteins
- Modified TCA/acetone extraction method is efficient and more reliable than Thiourea, Urea and Phenol extraction methods to solubilize abundant and less abundant proteins
- Identified storage, allergen and anti-nutritional proteins
- Partially developed Soybean Seed Protein Database (SSPD) that provides access to soybean seed proteins



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