

MODIFYING SOYBEANS TO MITIGATE FOOD ALLERGY

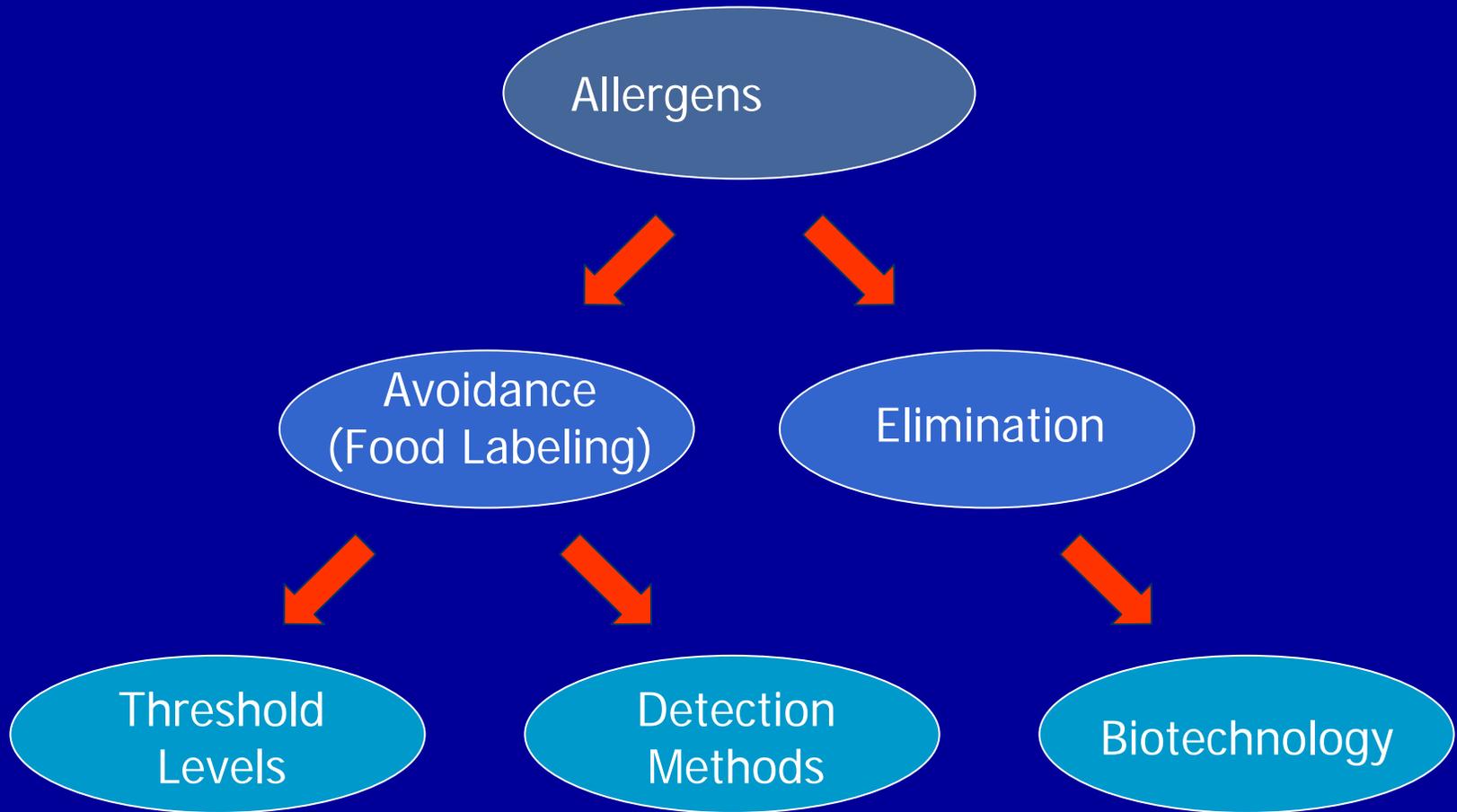
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Strategies for Managing Allergens



Lower Allergen Food Is A Variation Of Avoidance, Avoiding The Allergen Not The Primary Food

Characterize and identify offending proteins.

Determine variability of allergen abundance in food due to genetics or plasticity of expression due to growth conditions.

Potential mitigation methods include selecting low allergen varieties or nulls, controlling growth conditions that may reduce specific allergen expression, or suppressing allergen genes.

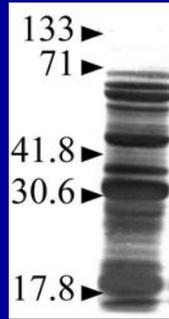
Relationship of Food Allergens To Plant Biology

Most plant food allergens are vacuolar proteins (storage proteins, trypsin inhibitors, lectins and enzymes) and many have a plant defense function.

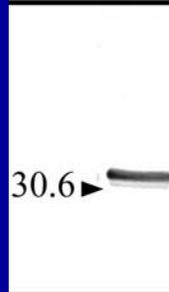
The implications of this is that these proteins may be essential for crop health (growth and development as well as pest resistance), they are products of gene families, and the relative abundance may be regulated by crop growth conditions, crop pedigree and/or pest challenge. This means there is not necessarily a standard allergenic level, allergens can vary due to biology.

The primary soybean seed allergen is P34/Gly m Bd 30k

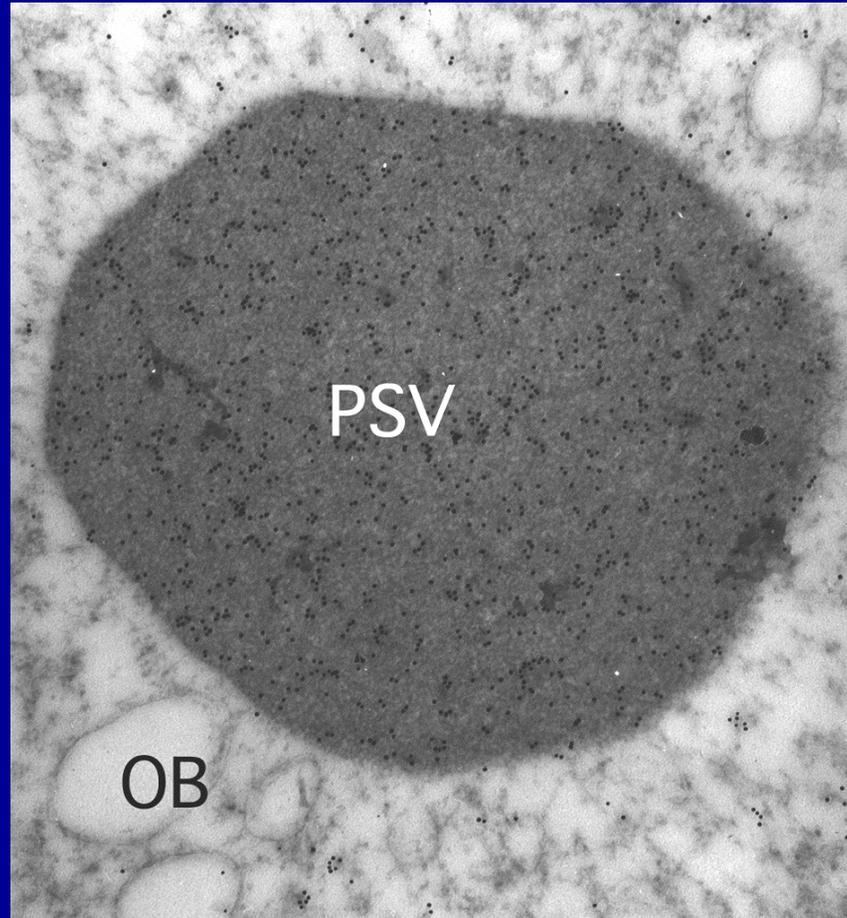
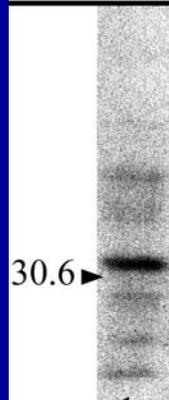
Total protein



Anti-P34
monoclonal
assay



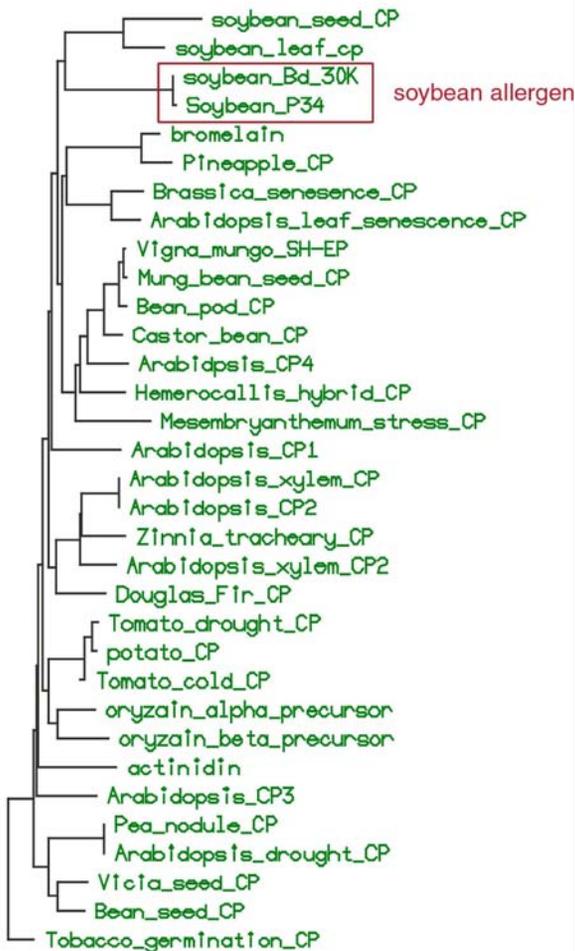
Soy-sensitive
human IgE assay



Immunogold localization of IgE cross-reactive sites showing that most cross-reactive epitopes are in the protein storage vacuoles

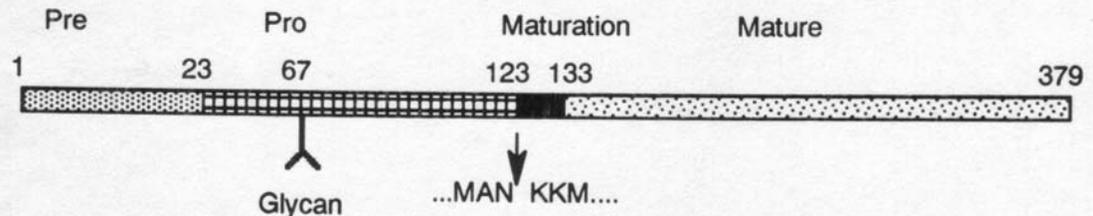
P34/Gly m Bd 30k Is A Seed Specific Cysteine Protease

Cysteine Protease Tree

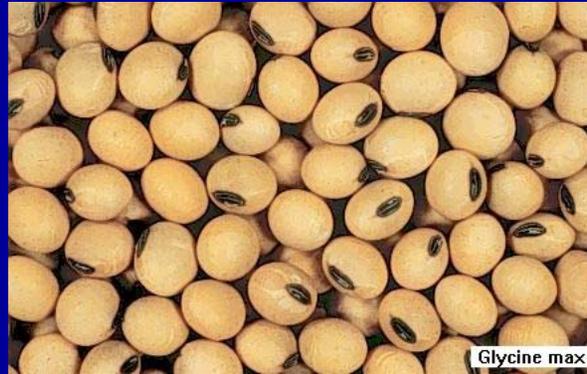


VKYQG GCGR **GW** AFSAT P34
 VKDQG GCGS **CW** S FSTT leaf

Structure of P34 Primary Translation Product



P34/Gly m Bd 30k Is A Member Of Cysteine Protease Family That Contains Major Allergens From Several Sources



Soybean

(P34/Gly m Bd 30k)



Dust Mite

(Der 1p)



Kiwi Fruit (Actinidin)



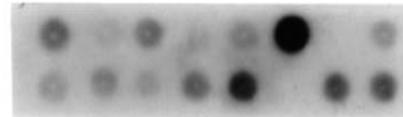
Papaya Fruit (Papain)

The Human IgE Epitopes Are Mapped With Overlapping Peptides Covering The Entire P34/Gly m Bd 30k Sequence

Sequence Alignment of Soybean Vegetative Cysteine Protease With The Soybean P34 And The Human IgE Epitopes That Induce The Allergic Response

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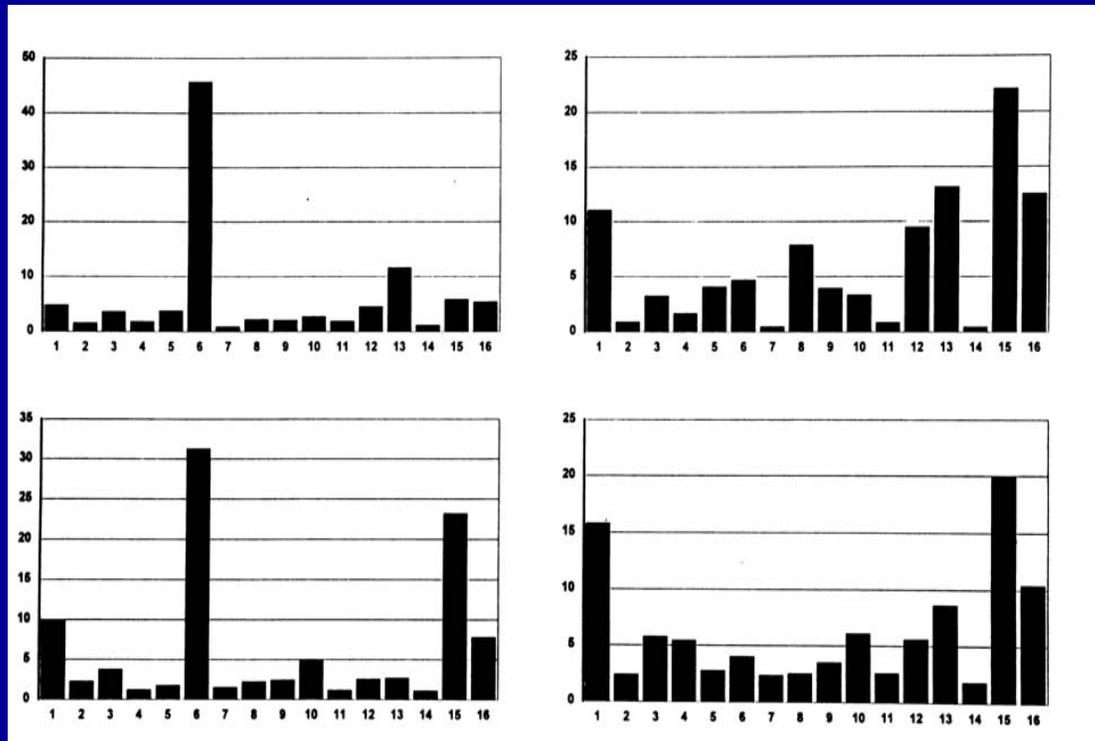
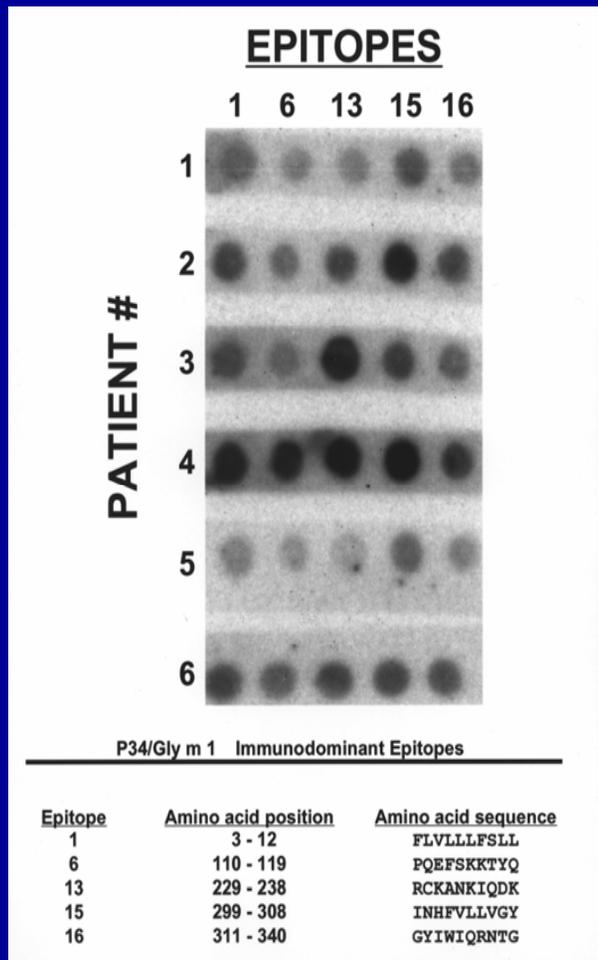
1 .....NSARGELSRPK 11 Vegetative
1 MGFLVLLLFSLLGLSSSSSISTHRSIILDLTKFTTQKQVSSLFQLWKSE 50 P34
12 FRPRPYATKEEHDHRFGVFK..SNLRRASCTPSSTPRVH..GVTKFSDLT 57
51 ..HGRVYHNHEEEAKRLEIFKNNSNYIRDMIANRKS PHSRLGLNKFADIT 99
58 PAEFRRQFLGL....KAVRFPAHAQKAPILPTKDLPKDFDWRDKGAVTN 102
100 PQEFSKTYLQAPKDVSSQIKMANKMKKEQYSCDHPPASWDWRKKGVITQ 149
103 VKDQGGCGSCWFSSTGGALEGAYLATGELVSLSEQQLVDCDHVCDPEEY 152
150 VKYQGGCGRGWAFSATGAIEPAHAIAATGDLVSLSEQELVDCVEESE... 195
153 GACDSGCGGLMNAFEYILQSGGVQKEKDYPTGRDGTCKFDKTKVAAT 202
196 ....GCYNGWHYQSFEWVLEHGGIATDDDDYPYRAKEGRCKANKIQDKVT 240
203 VSNYSVVCLEDEEQIAAN.....LVKNGPLAVAINAVFMQTYVGGV..S 243
241 IDGYETVIMSDESTESETEQAFLSAILLEQPISVSIDAKDFHLYTGGIYDG 290
244 CPYICGKHLHDHGVLVGVYEGAYAPIRFNKPKYWIKNKSWGESWGENGYD 293
291 ENCTSPYGINHFFVLLVGYGSA.....DGVDYWIAKNSWGEDWGEDGYI 333
294 EICRGR...NVCGVDSMVS.....TVAAIYPSH*..... 320
334 WIQRNTGNLLGVCGMNYFASYPTKEESETLVSARVKGHRRVDHSPL* 380
    
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IgE-BINDING EPITOPES

FLVLLLFSLL	RSILDLDLTK	SSLFQLWKSE	EAKRLEIFKN
IFKNNSNYIR	PQEFSKTTYQ	YLQAPKDVSSQ	ASWDWRKKGV
KKGVITQVKY	NGWHYQSFEW	TDDDDYPYRAK	YRAKEGRCKA
RCKANKIQDK	ISVSIDAKDF	INHFFVLLVGY	GYIWIQRNTG

The P34/Gly m Bd 30k allergenic epitopes of unrelated people is different

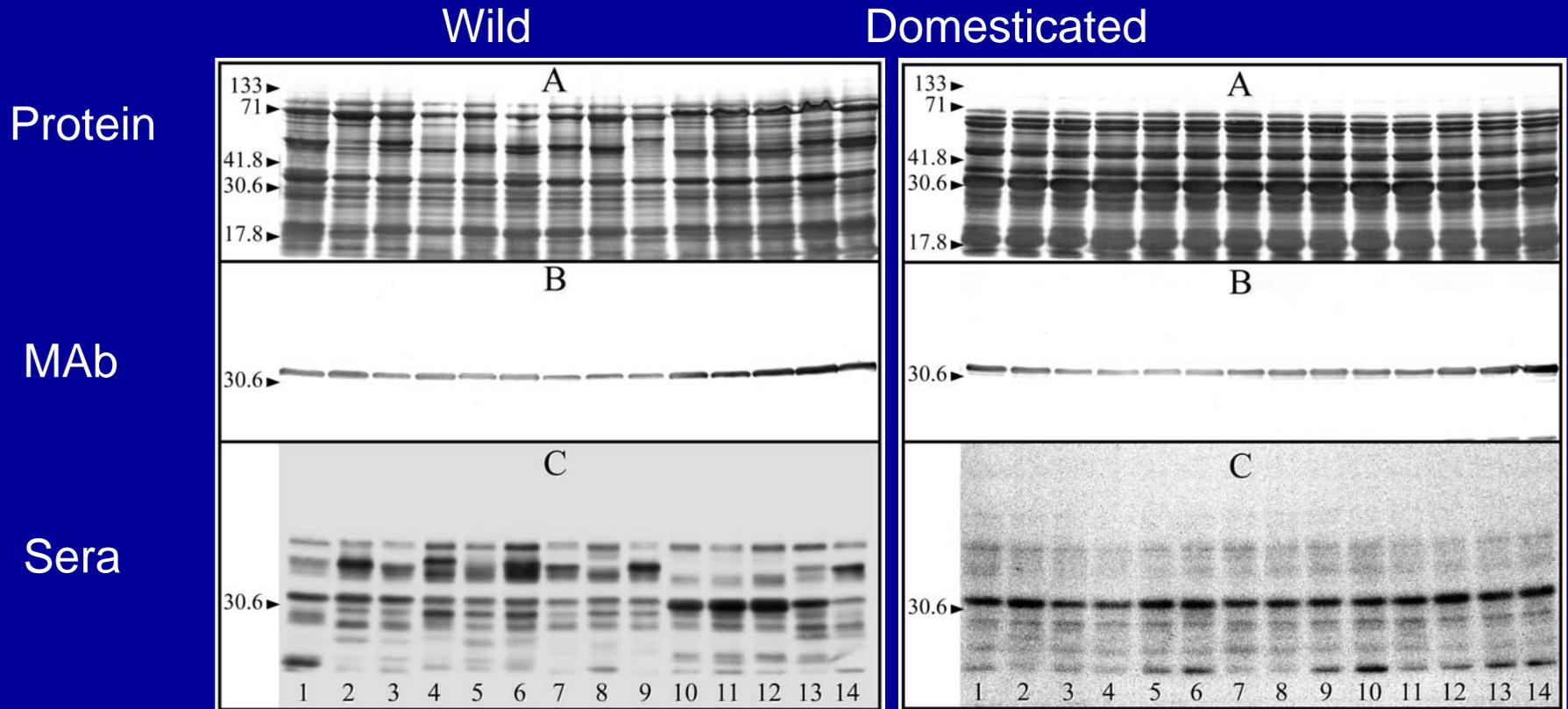


The USDA Collection Of Soybeans Is A Diverse Collection



How variable is allergen content among diverse soybeans ?

Screening The Soybean Collection For Allergen Content Variants

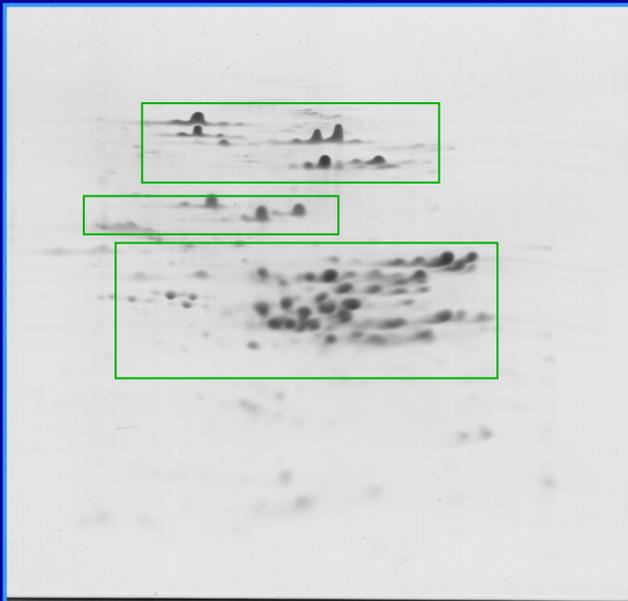


Wheat Proteins in Allergies & Celiac Disease

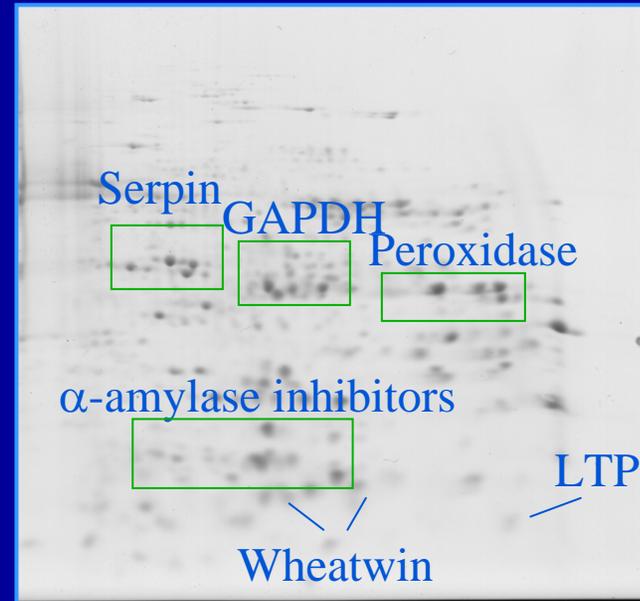
Celiac Disease
Exercise-induced Anaphylaxis
Urticaria

Baker's Asthma
Urticaria

HMW-GS
 ω -Gliadins
 α -, γ -Gliadins
LMW-GS



Gliadins & Glutenins



Albumins & Globulins

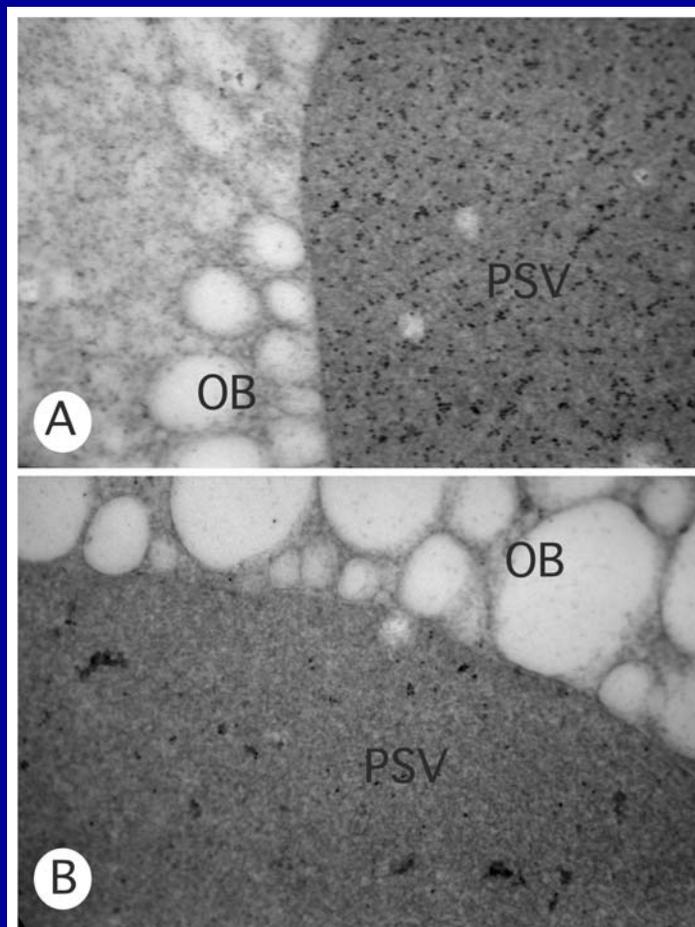
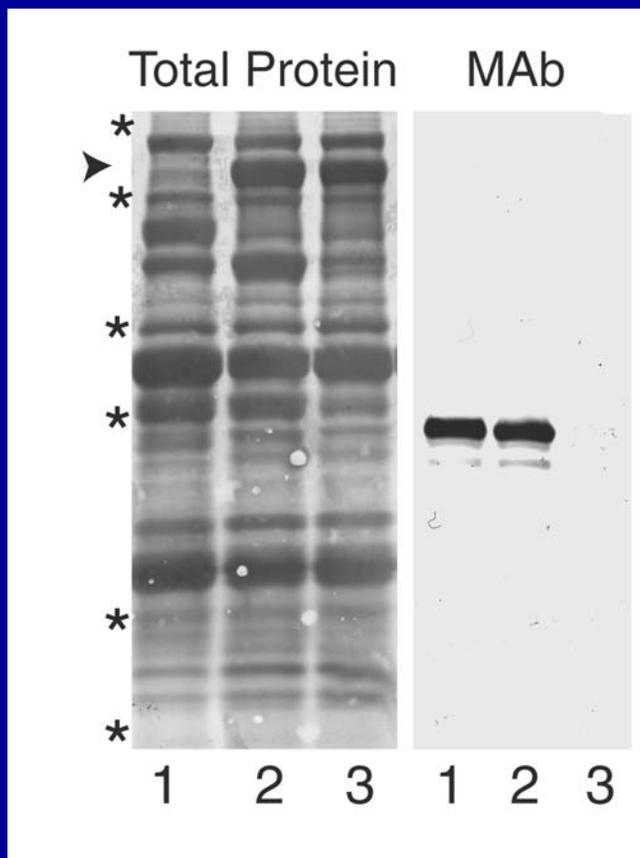
An important finding is that some potential allergens increase in amount in grain exposed to high temperatures during grain fill.

BIOTECHNOLOGY APPROACH TO SUPPRESS THE SOYBEAN ALLERGEN



We have used sense suppression of P34/Gly m Bd 30k to produce an allergen null seed by transferring the P34 gene to soybeans controlled by the conglycinin promoter.

Gene suppression can reduce the P34/Gly m Bd 30k to minimal levels without collateral consequences



PROBLEMS IN ACCEPTANCE OF GENETIC MODIFICATION FOR MITIGATION OF ALLERGENS HAS LED TO USING PARALLEL APPROACHES

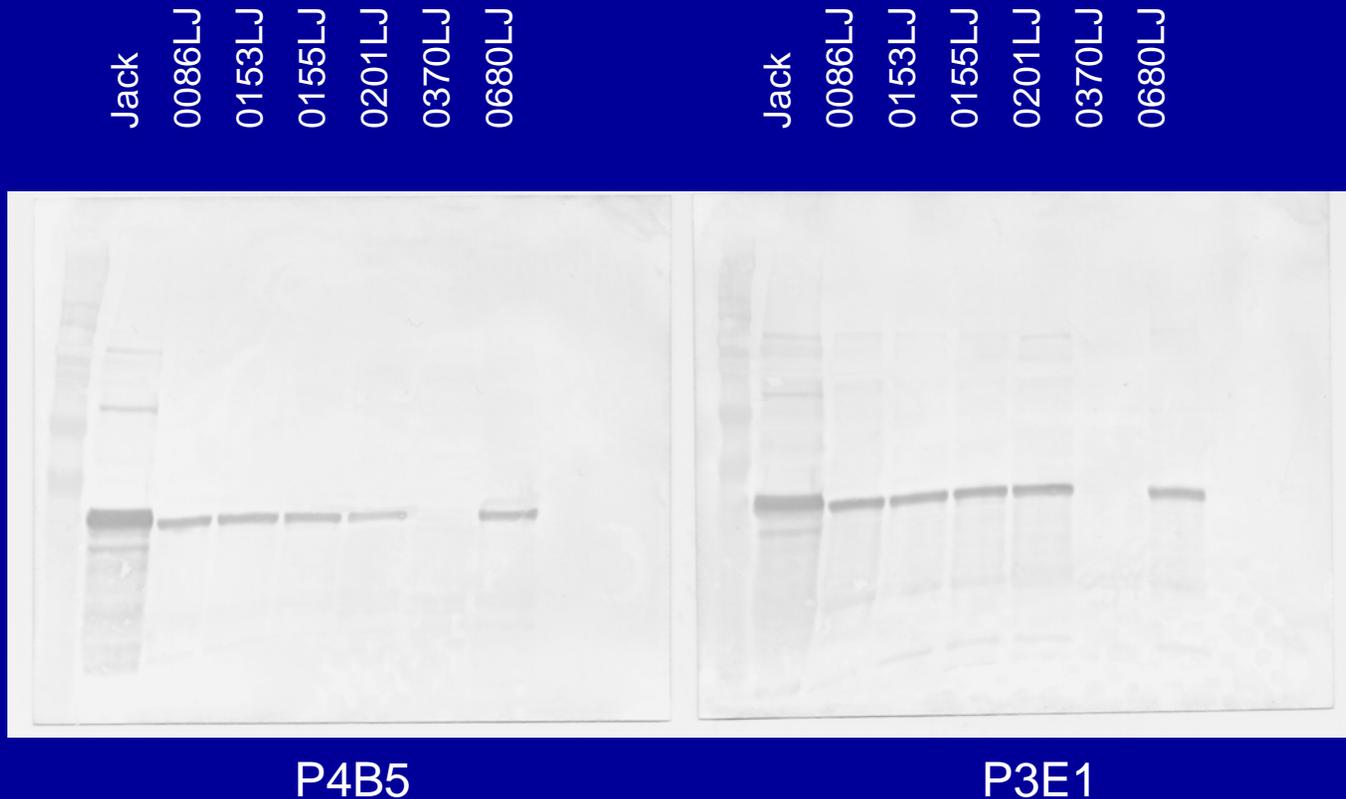


The USDA Collection Of Soybeans Is A Diverse Collection

Of 20,000 Members That Can Be Searched For Traits Such As Low Allergen. This Is Not Genetically Engineered.

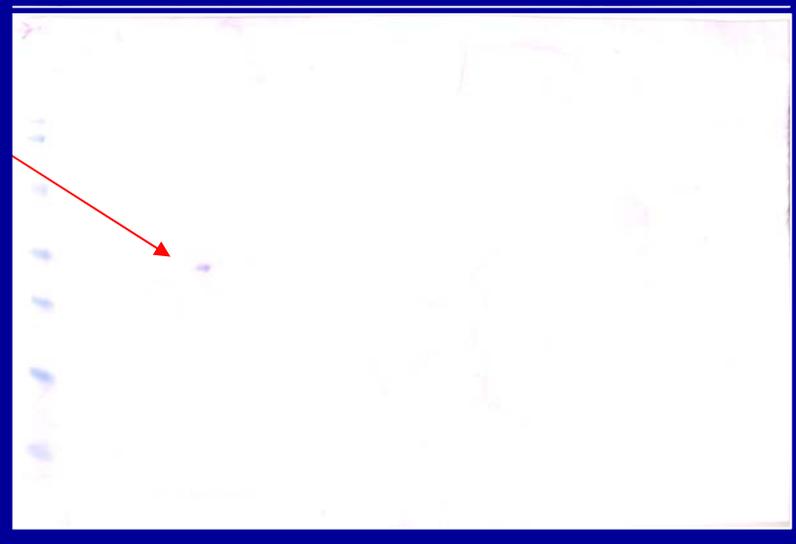
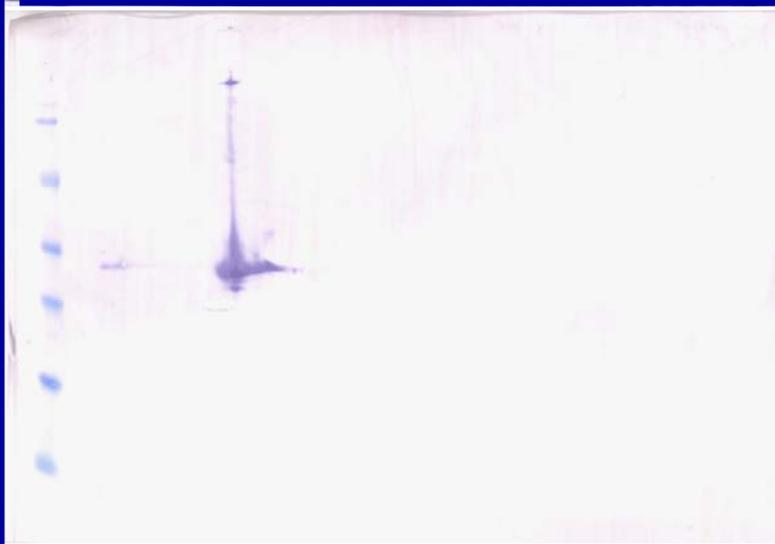
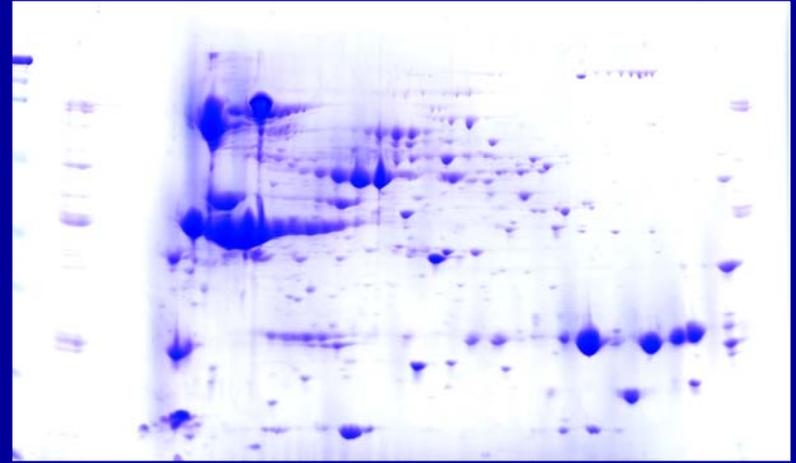
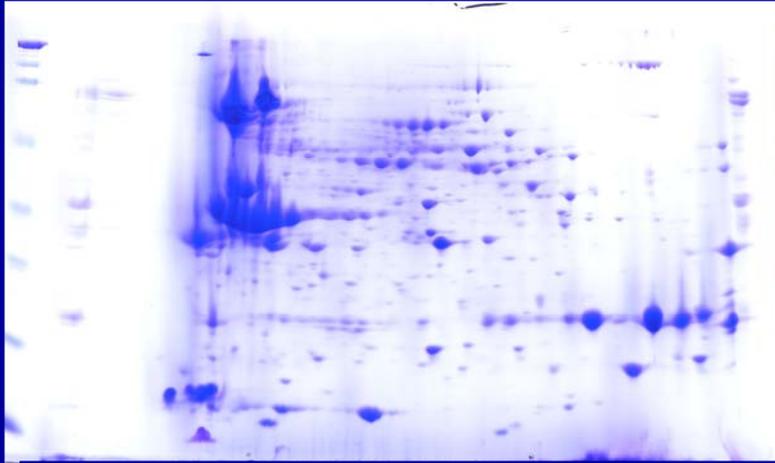
This type of project requires the tool box of molecular biology that is the only way to analyze such a large sample.

A portion of the survey of soybean accessions from the USDA collection screened with Mabs against two distinct P34/Gly Bd m 30k epitopes.



A putative null that needs to be characterized !

We have found TWO out of the entire collection that have nearly no allergen. These are being crossed into other soybean lines in an attempt to transfer the trait.



Control (Kunitz null)

P34-suppressed from collection

MODIFYING PLANTS IS A USEFUL APPROACH TO MITIGATE FOOD ALLERGIES

By either producing (GE) or finding (soy collection) lines that lack the allergenic protein(s) we can reduce causes of food intolerance.

With identity preservation it should be feasible to develop specific lines optimized for formula, food, feed aimed at a soy-sensitive population (whether human or animal) market.

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