ALLERGENICITY ASSESSMENT OF GENETICALLY MODIFIED FOODS

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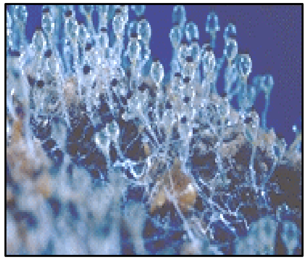
(EU-India S & T Cooperation Days)

Allergy

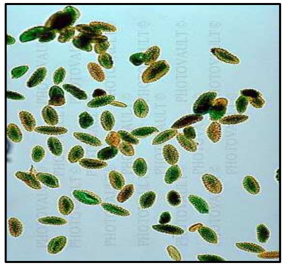
Allergy is a hypersensitivity reaction initiated by immunologic mechanisms caused by specific substances called allergens

Foods

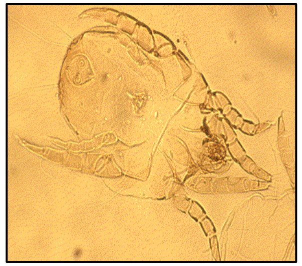


Fungi

Insects



Pollens



Dust mites

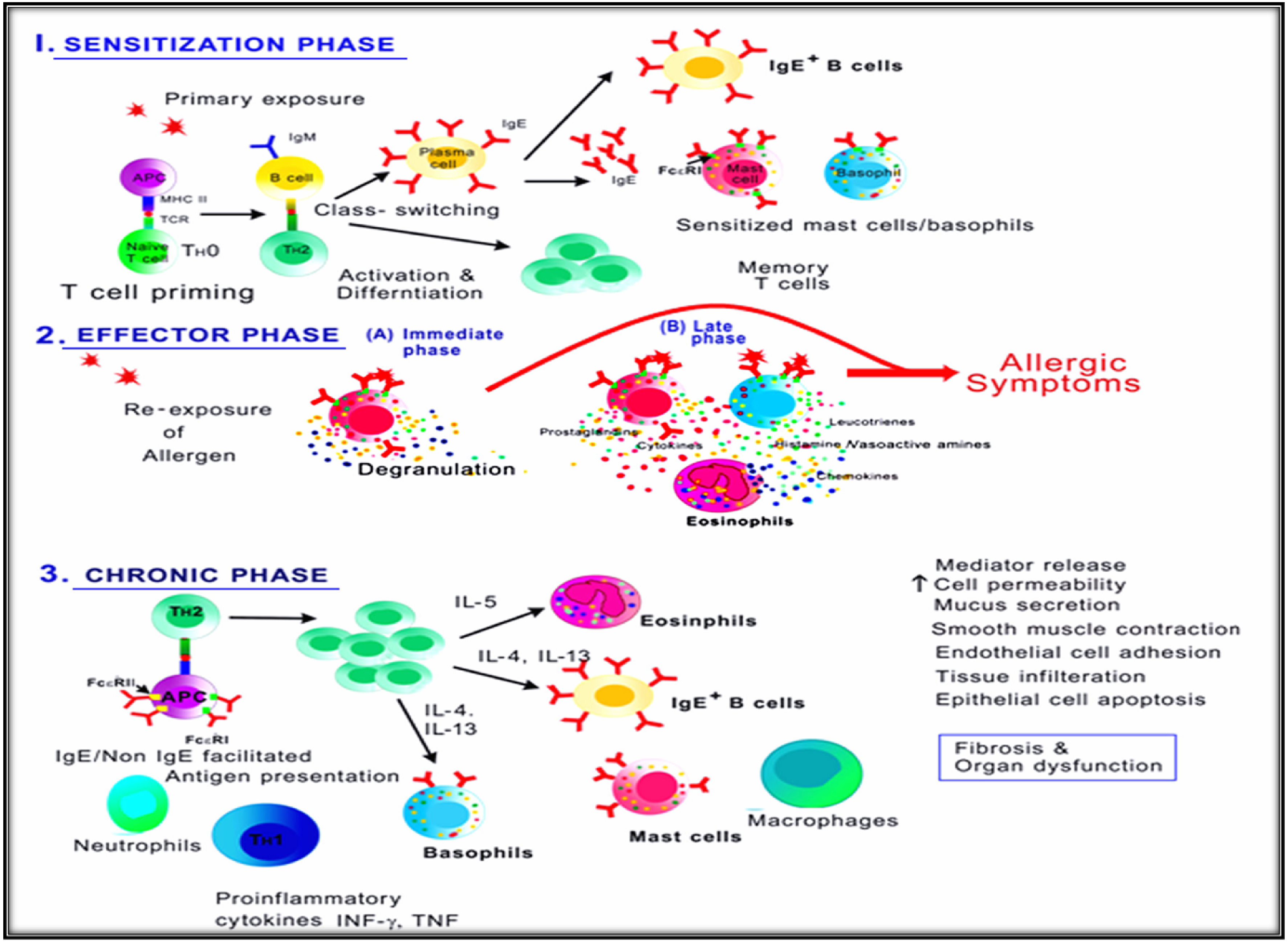
What is food allergy?

Food allergies are adverse reaction to a food or food component involving the body's immune system

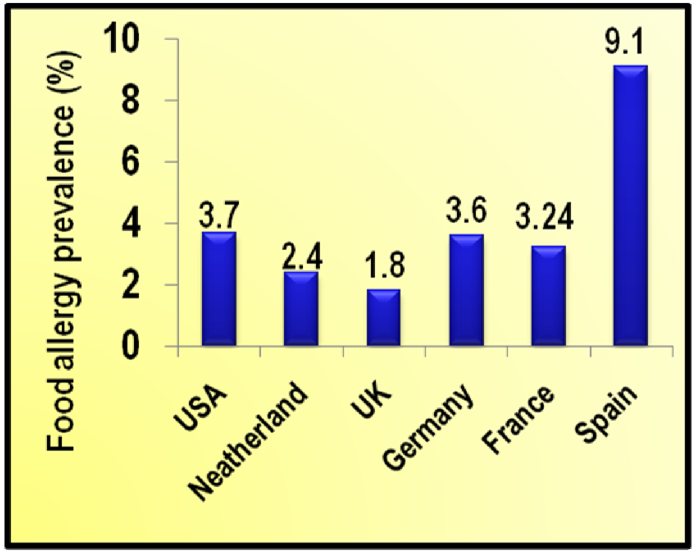
It involves two primary components:

- **Contact** with food **allergens**
- **Immunoglobulin E** (**IgE**: an **antibody** in the immune system that reacts with **allergens** present on **mast cells** (tissue cells) or **basophils** (blood cells), which release **histamine** and other mediators causing allergic symptoms.

Phases of Allergic Reaction



Food allergy: Epidemiology & Impact



World wide prevalence:

Perception :2-65.5%

Actual rate: 6-8 % (infants)

3-4 % (adult)

Doubled within few years in some countries!!!!!!!

Impact

Quality of life

Morbidity

Mortality

Clinical, Social & Economic Burden

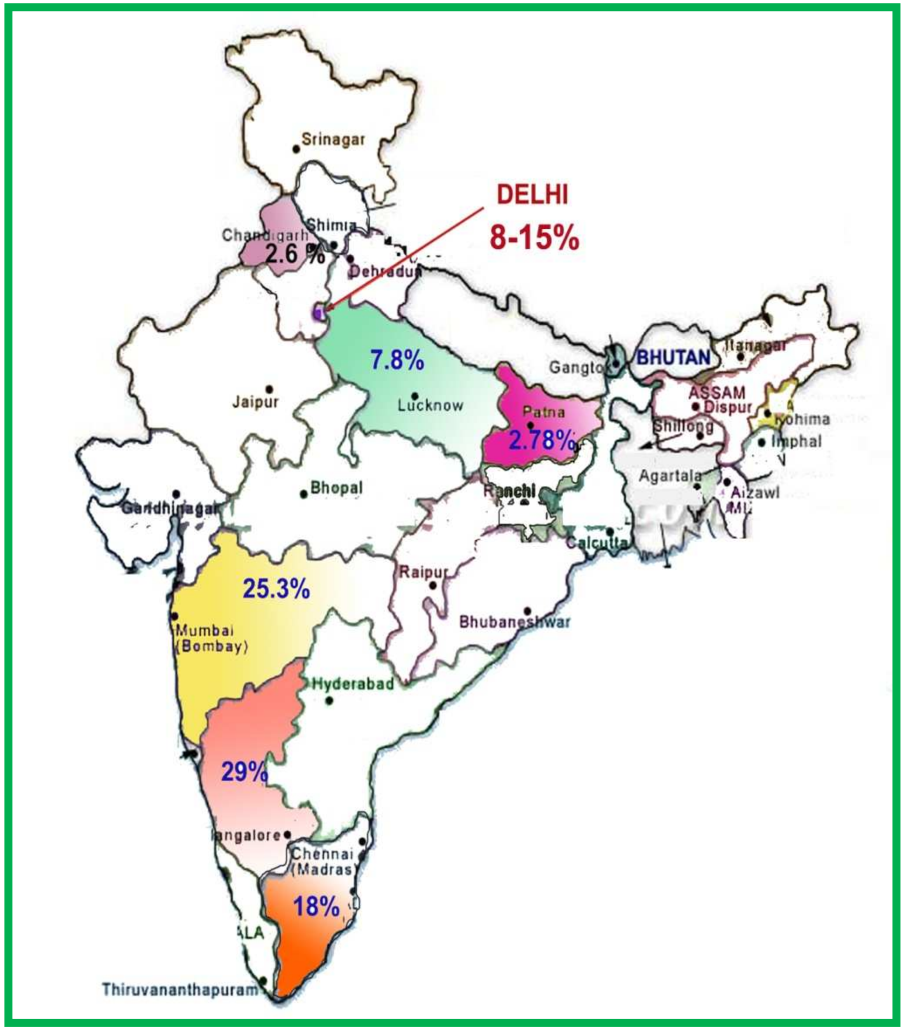
29000 episodes of anaphylaxis- 120-150 death /year (U.S)

200 deaths /year (Europe)

Prevalence of allergic diseases in Asian countries

Allergy in India

- 1/7th of the world population
- diverse culture and dietary habits
- Rising prevalence of allergic diseases



Food Allergy

- Some work done on inhalant allergens
- Food allergy attracted little attention

Preliminary studies:

- Common food allergens:
- Barley, Mustard, Pea & Corn: 30%(n=64 asthmatics)
- Egg, milk, cereals, **Legumes**
- **Rice, banana**, colocasia, radish and citrus fruits
- fish allergy
- Chickpea allergy:2.7%(n=1400)

Food intolerance vs. allergy

A food intolerance is an adverse food-induced reaction that does not involve the immune system.

e.g. Lactose intolerance.

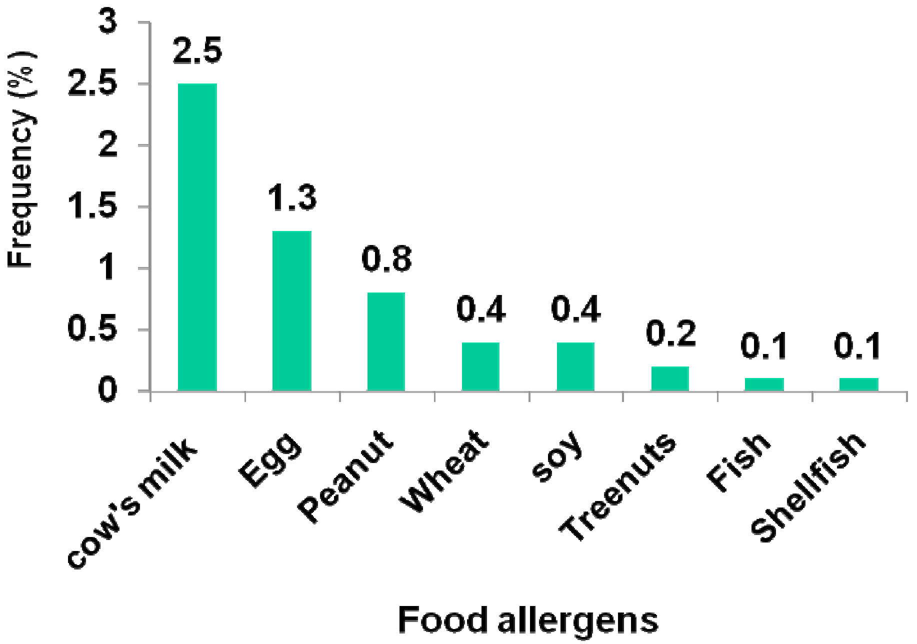
- Person lacks an enzyme needed to digest milk sugar.
- When eats milk products, symptoms such as gas, bloating, and abdominal pain may occur.

A food allergy occurs when the immune system reacts to a certain food.

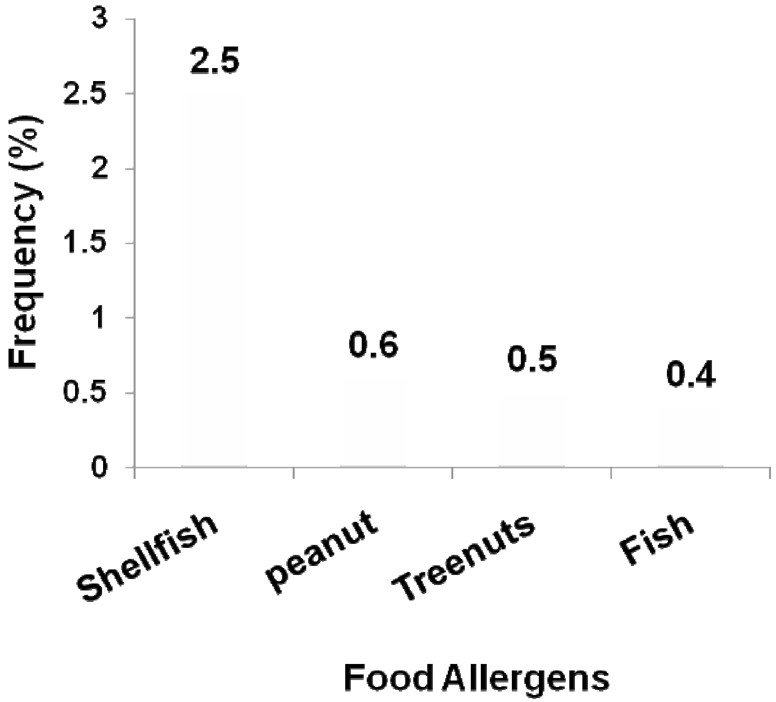
- The body synthesizes IgE antibodies to the food.
- On cross linking of these IgE antibodies, histamine and other chemicals (called mediators) cause hives, asthma, or symptoms of an allergic reaction.

Food intolerance involves the body's metabolism but not the immune system

Common Food Allergens



In children



In adults

Food Allergens

(Based on sensitization)

Class 1 food allergens

(Complete allergens)

- Primary sensitizers
- Mainly affect young children
- Sensitization usually occur through the GI tract.
- Water-soluble glycoproteins of 10 to 70 kDa
- Stable to heat, acid and proteases
- Also termed as 'complete food allergens'.
- E.g. Milk, Egg , Peanut

Class 2 food allergens

(cross-reactive/ Incomplete/ Non-sensitizing Elicitors)

- Plant-derived proteins.
- Sensitization usually occurs in adults.
- Sensitive to heat and digestive enzymes
- Cannot cause per-oral sensitizations,
- Provoke allergic reactions in sensitized patients.
- Often called 'incomplete food allergens' or non-sensitizing elicitors.
- Major culprits of adult onset of food allergy.

PLANT FOOD ALLERGENS

Proteases

Papain like cysteine proteases

Substilin like serine proteases

Prolamins

Cereal prolamins

2S albumins

Nonspecific lipid transfer proteins

α-amylase and protease inhibitors

Pathogenesis related proteins

PR-1:

PR-2: Endo b1 3 glucanase

PR-3: class I chitinases

PR-4: Win like proteins

PR-5 : Thaumatin-like proteins (TLPs)

PR-9: lignin-forming peroxidases

PR-10: Bet v1 homologues

PR-14: lipid transfer proteins

Cupins

Vicilins (7SSeed storage pr.)

Legumins 11SSeed storage pr.)

Protease inhibitors

Kunitz type protease inhibitors

Cereal α- amylase/protease inhibitor

Common food allergens: world-wide

- Legumes (Peanuts and Soybeans)
- Milk
- Eggs
- Fish (cod, salmon, haddock, etc)
- Crustacea (shrimp, crawfish, lobster,etc.)
- Wheat
- Tree nuts (almonds, walnuts, Brazil nuts, etc)
- Mollusks (snails, mussels, oysters, scallops, clams, squid)
- Selected food additives

Common food allergens in Indian Population

Top 10 allergic foods from a survey of 2000 patients

Rice
Blackgram
Lentil
Citrus fruits
Pea
Maize
Banana
Lima bean
Peanut
Fish

Cross-Reactions: Food and pollens

- **Ragweed-** Watermelon, cantaloupe, honeydew, bananas
- **Mugwort-** Celery
- **Birch pollen-** Carrots, apples, hazelnuts, potatoes
- **Banana –** Latex

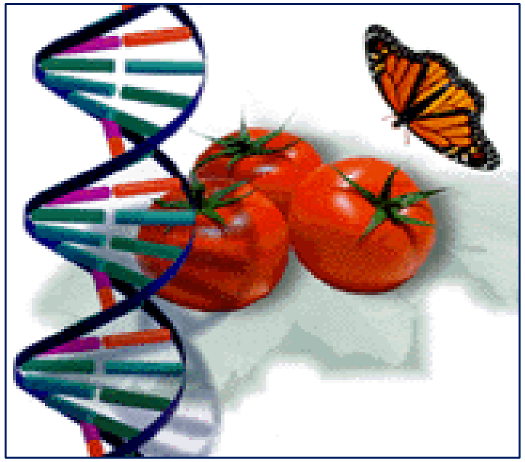
National prevalence of allergic diseases

- Allergic Rhinitis 20%
- Allergic Asthma 15%
- Atopic Dermatitis 5%
- Insect Allergy 2%
- Food Allergy 2%

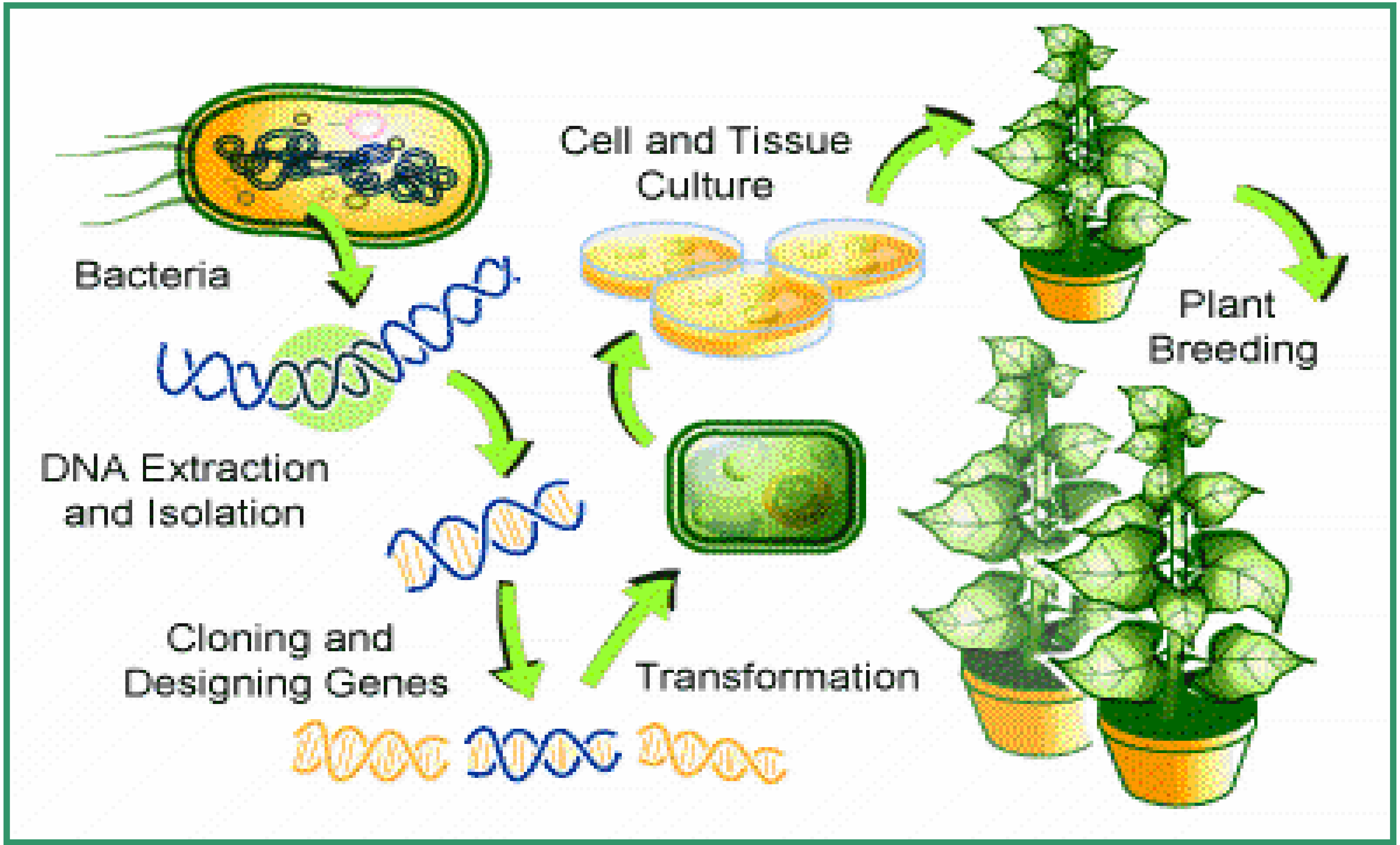
http://www.worldallergy.org/wad2005/national_data.pdf.

What are GM Foods ?

Plants in which genetic structure is manipulated by inserting a foreign gene from another biological organism or synthesized in lab to improve crops characteristics.



Overview of how transgenic crops are created



Associated Benefits of GM food crops

Human health Benefits:	Ecological Benefits:	Economic Benefits:
➢Nutritionally improved foods ➢More food security ➢Plant-produced vaccines	➢Reduced need for agro-chemicals ➢Soil conservation / improved soil quality ➢More efficient production (less land needed for food)	➢Welfare of Human health and Ecological benefits. ➢Benefits of trade, to sellers and consumers

Associated Risks of GM food crops

Human health risks:	Ecological Risks:	Economic Risks:
➢ Allergy / Toxicity	➢ Gene Flow	➢ Costs of health
➢ Changes in nutritional composition	➢ Effects on non-target species	➢ Ecological damage
➢ Cumulative effects on many new foods	➢ Effects on ecological balances	➢ Major economic displacements
		➢ Loss of business (consumer choice)

Transgenic crops under development and field trials in India

Crop	Organisation	Gene
Brinjal	IARI, New Delhi MAHYCO, Mumbai	*cry1Ab* *cry1Ac*
Cauliflower	MAHYCO, Mumbai Sungrow Seeds Ltd., New Delhi	*cry1Ac* *cry1Ac*
Cabbage	Sungrow Seeds Ltd., New Delhi	*cry1Ac*
Chickpea	ICRISAT, Hyderabad	*cry1Ac, cry1Ab*
Groundnut	ICRISAT, Hyderabad	IPCVcp, IPCV replicase
Maize	Monsanto, Mumbai	CP4 EPSPS
Mustard	IARI, New Delhi NRCWS, Jabalpur TERI, New Delhi UDSC, New Delhi	Cod A, Osmotin Bar, barnase, barstar Ssu-maize Psy, Ssu-tp Crtl Bar, barnase, barstar
Okra	MAHYCO, Mumbai	*cry1Ac*
Pigeonpea	ICRISAT, Hyderabad MAHYCO, Mumbai	*cry1Ab+SBTI* *cry1Ac*
Potato	CPRI, Simla NCPGR, New Delhi	*cry1Ab* Ama-1
Rice	Directorate of Rice Research, Hyderabad Osmania University, Hyderabad IARI, New Delhi MAHYCO, Mumbai MKU, Madurai MSSRF, Madurai TNAU, Coimbatore	*Bacterial blight resistant,Xa-21,cry1Ab,gna gene, sheath blight resistant* gna Bt,chitinase,cry1Ac and cry1B-cry1Aa *Cry1Ac* *Chitinase,B-1,3-glucanase,osmotin* *Genes from mangrove species, chitinaqse*
Sorghum	MAHYCO, Mumbai	*cry1Ac*
Tomato	MAHYCO, Mumbai NCPGR, New Delhi	*cry1Ac* *OXDC*

• Source: Department of Biotechnology, Government of

Global adoption of biotech crops in 2007: by Country

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Rank	Country	Biotech Crops
1	USA	Soybean, maize, cotton, canola, squash, papaya, alfalfa
2	Argentina	Soybean, maize, cotton
3	Brazil	Soybean, cotton
4	Canada	Canola, maize, soybean
5	India	Cotton
6	China	Cotton, tomato, poplar, petunia, papaya, sweet pepper
7	Paraguay	Soybean
8	South Africa	Maize, soybean, cotton
9	Uruguay	Soybean, maize
10	Philippines	Maize
11	Australia	Cotton
12	Spain	Maize
13	Mexico	Cotton, soybean
14	Colombia	Cotton, carnation
15	Chile	Maize, soybean, canola
16	France	Maize
17	Honduras	Maize
18	Czech Republic	Maize
19	Portugal	Maize
20	Germany	Maize
21	Slovakia	Maize
22	Romania	Maize
23	Poland	Maize

Source: Clive James, 2007

International Guidelines for GM food Safety Assessment

- **WHO/FAO Decision tree for allergenicity assessment**

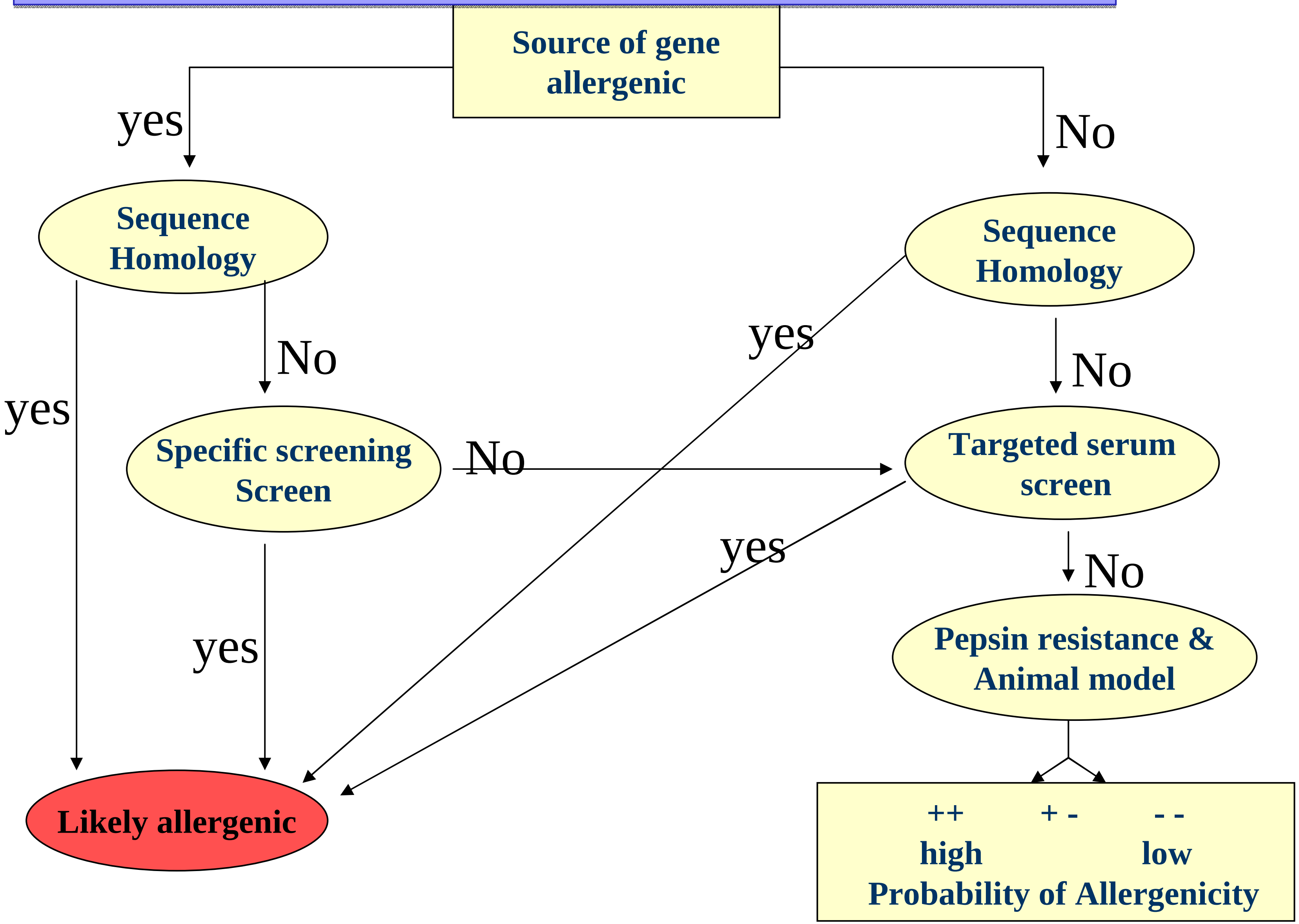
  1997 **First report for allergenicity assessment**

  2001 **Addition of animal model studies**

- **Codex guidelines** **2001/2003**

  Safety assessment by substantial equivalence between GM and Native crop.

Allergenicity assessment of GM foods FAO/WHO Guidelines 2001



Substantial Equivalence – CODEX 2003

PHENOTYPE

Morphology

Agronomic

- disease resistance
- drought resistance
- yields

Organoleptic

COMPOSITION

Macronutrients

AA composition

FA composition

Anti-nutrients

Toxic substances

Allergens

Specific constituents

SAFETY ASSESSMENT

Toxicity

Allergenic potential

Nutritional

FEED EQUIVALENCE

Performance

Indian guidelines for allergenicity assessment of GM foods (Recommended by ICMR/DBT)

Assessment of Possible Allergenicity (Proteins)

- Indicate if the donor organism(s) is a known source of allergens (defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available).
- Amino acid sequence homology comparison of the newly expressed protein and known allergens.
- Demonstrate the susceptibility of each newly expressed protein to pepsin digestion.
- Where a host other than the transgenic plant is used to produce sufficient quantities of the newly expressed protein for toxicological analyses, demonstrate the structural, functional and biochemical equivalence of the non-plant expressed protein with the plant expressed protein.
- For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays is to be performed where sera are available.

Genetically Modified Mustard

Plant : *Brassica juncea**

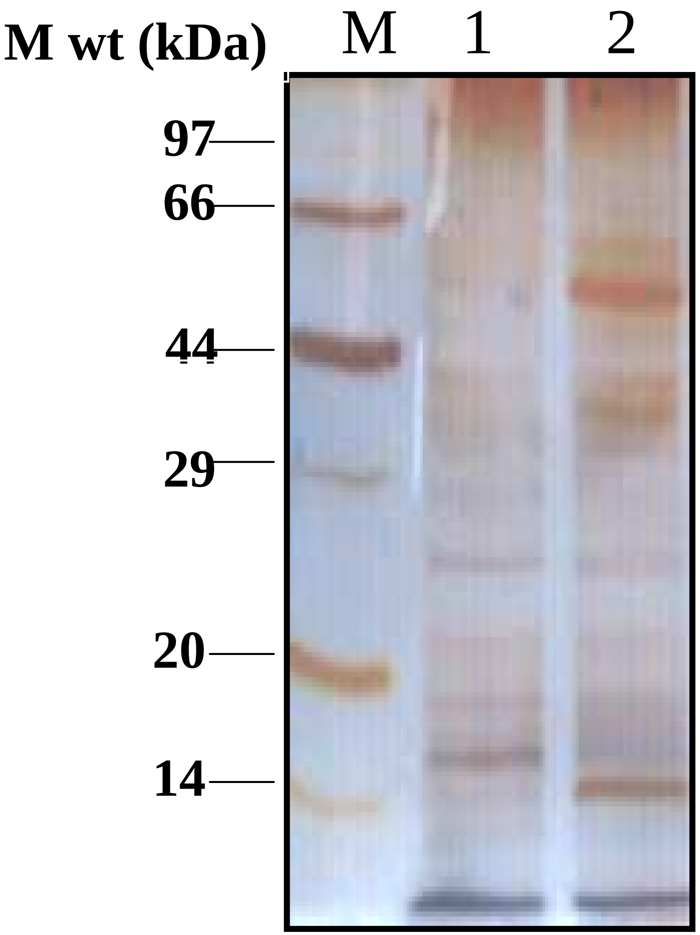
Gene introduced: *codA* gene from *Arthrobacter globiformis*

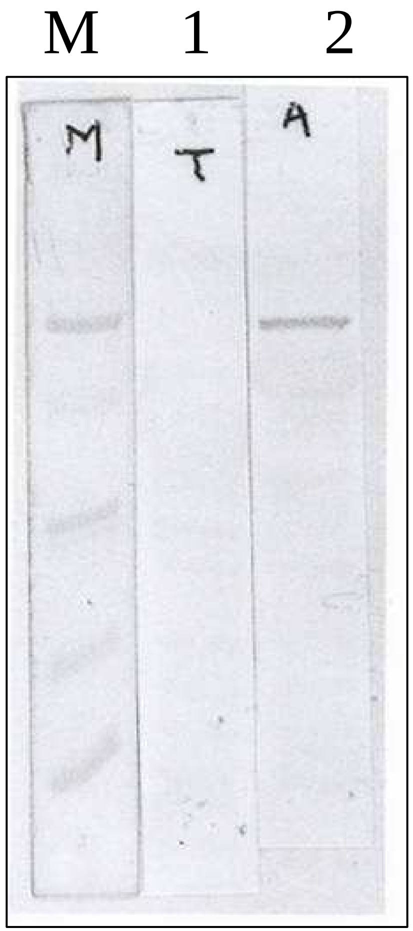
Protein expressed: Choline Oxidase

Mol wt.: ~60 kDa

Function: Choline oxidase provides resistance to plants against abiotic stresses such as high salinity, frost, etc.

Prasad et al., 2001





Lane 1: Native Mustard (Leaf)

Lane 2: GM Mustard (Leaf)

SDS-PAGE showing Silver stained bands

Western blot with antibodies raised in rabbit against choline oxidase

Computational Analysis for allergenicity

Sequence similarity with known Allergens

Homology studies

Structural database of allergenic proteins (SDAP):

>35% similarity to known allergens.

Food Allergy Research and Resource Program (Farrp):

E-Value < 0.02

Swissprot database: To study cross-reactive epitopes

Identical six aa stretch

Bioinformatic studies with Choline oxidase

% aa identity with allergens using SDAP database

Allergen	Accession No.	% identity
Cand a 1	P43067	7.69 (42/546)
Cry j 1	BAA05543	6.96 (38/546

Sequence homology using Farrp database

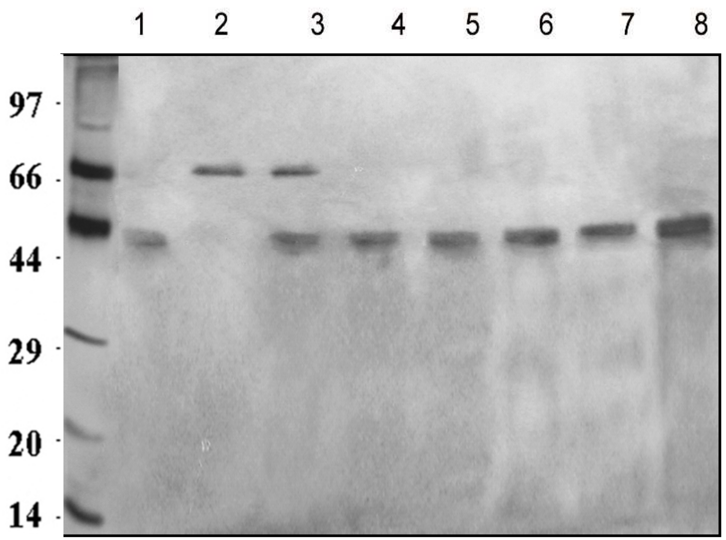
Allergen	Accession No.	E- value
Glycinin, Soyabean	AAA33964	0.49
Tri r 2, *T. ruburum*	AAD52013	1.4

Identical 6 aa match using Swissprot database

Allergen	Accession No.	Species	Amino acid Match
Hev b 6	P02877	*Hevea brasiliensis*	VGGGSA

Digestibility Studies

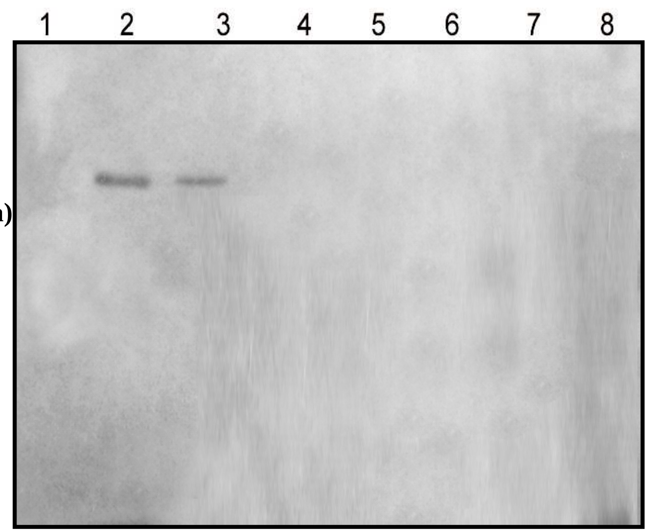
Choline oxidase degradation by Simulated Gastric Fluid (pepsin)



Choline Oxidase

Pepsin (43.5 kDa)

SDS-PAGE



Choline Oxidase

(60kDa)

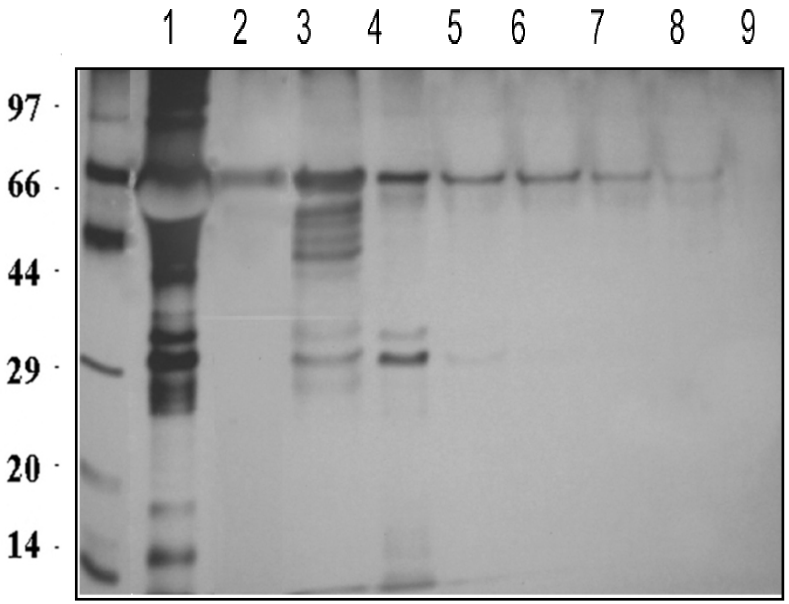
Western blot with choline oxidase antibodies

Lane 1 : Pepsin

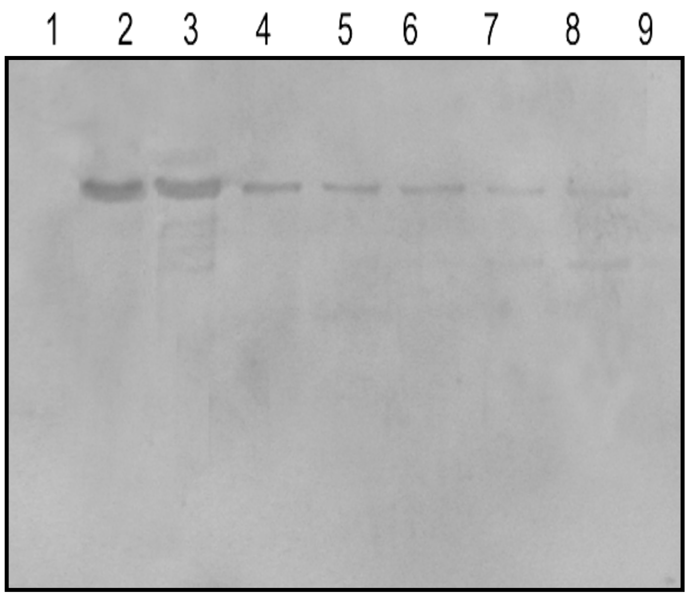
2 : Choline oxidase pure

3-8 : Choline oxidase + SGF for 0, 5, 30, 60,120 and 300 sec.

Choline oxidase degradation by Simulated Intestinal Fluid (pancreatin)



SDS-PAGE



Choline Oxidase

(60kDa)

Western blot with choline oxidase antibodies

Lane 1 : Pancreatin

2 : Choline oxidase pure

3-8 : Choline oxidase + Pancreatin for 0, 0.5, 1, 2, 4, 6 and 8 hr.

Animal Model Studies

DEVELOPMENT OF ANIMAL MODEL

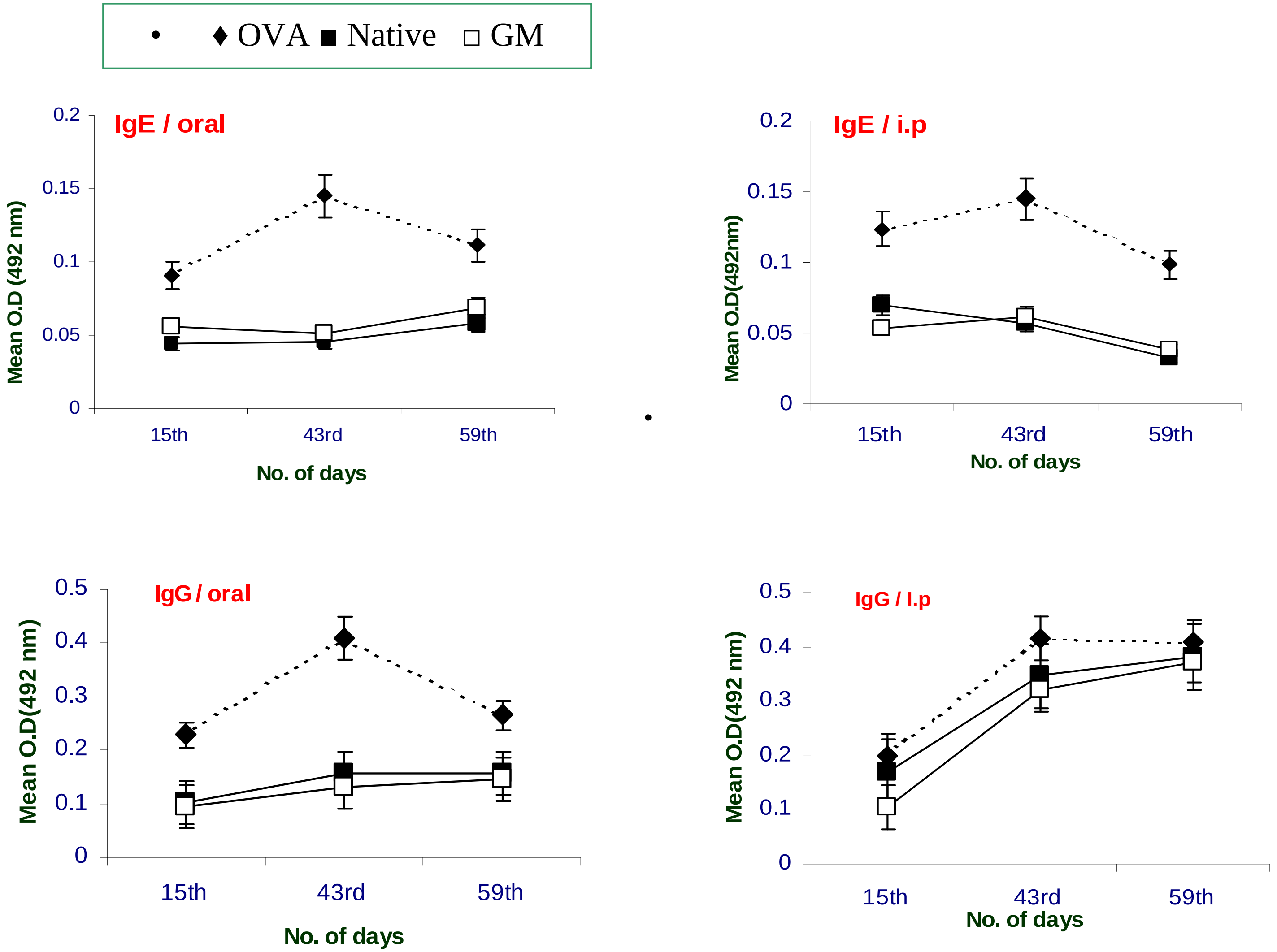
Balb/c mice (8 weeks)

Control group	Ovalbumin gp. (*i.p* and oral)	GM mustard gp. (*i.p* and oral)	Native mustard gp (*i.p* and oral)
•PBS orally •	• 0.1mg protein •in PBS	•0.1 mg protein •in PBS	•0.1 mg protein •in PBS

***i.p.* dosage**: 0.1 mg protein on each 7th day for 6 weeks

oral dosage: 0.1 mg protein daily for 6 weeks

- Blood taken on day 15, 43 and 59 for estimating specific IgE and IgG antibodies.
- After sensitization mice were challenged with antigen.



•IgE

•IgG

Singh et al., Allergy 61:491-497, 2006

Sensitized mice challenged with the respective proteins

Mice No./dose Groups	Ovalbumin Oral	Ovalbumin i.p.	Native Mustard Oral	Native Mustard i.p.	GM mustard Oral	GM mustard i.p.
1. 12 mg	1,2,4	1,2,3	0	0	0	0
2. 12 mg	1,2,3,4	1,2,4	0	0	1	0
3. 6 mg	1,3,	2,3	0	1	0	0
4. 6 mg	1,2,3,4	1,2,4	0	1	0	1
5 3 mg	1,2,3	1,3	-	-	-	-
6. 3 mg	1,3	1 ,2,3,4	-	-	-	-

Control group challenged with 12 mg protein shows no symptom score.

Symptoms score Card

0 : No Symptoms

1 : Scratching and rubbing around nose, head & ear

2 : Labored respiration, heart beat fast and cyanosis around mouth and tail.

3 : Tremor and convulsions

4 : Death

•**Singh et al., Allergy 61:491-497, 2006**

Histological analysis of mice lungs after challenging with presensitized protein

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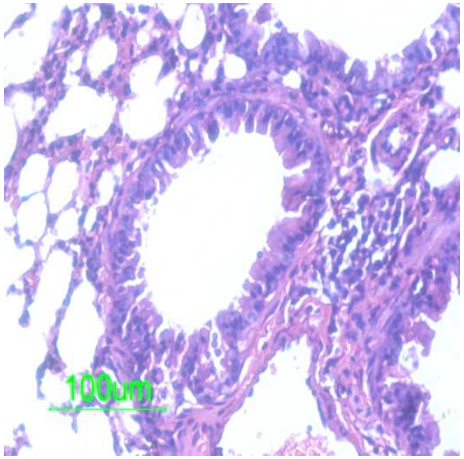
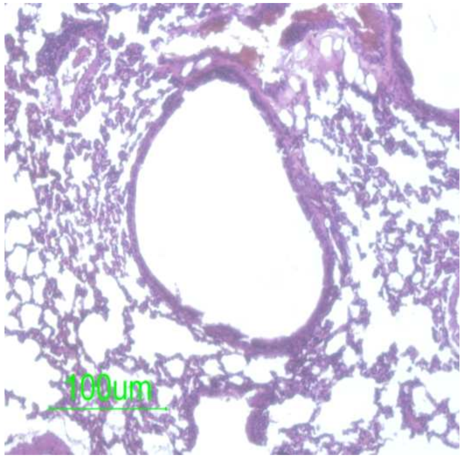
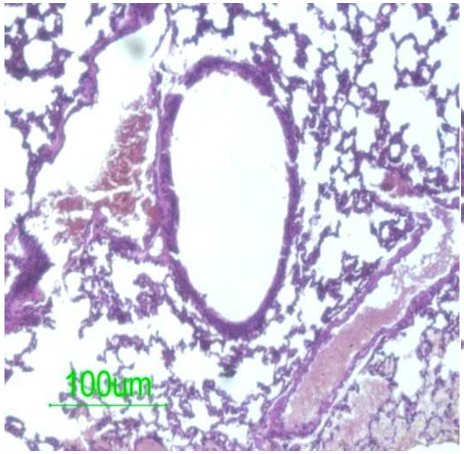


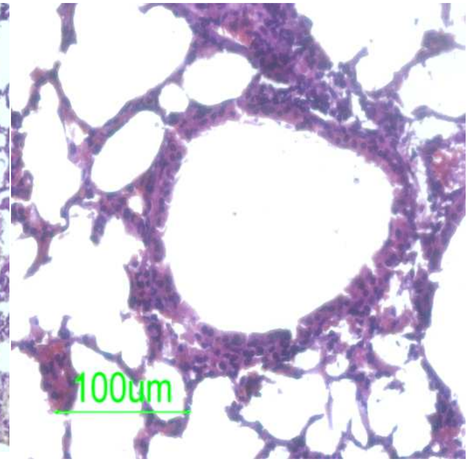
Ovalbumin oral



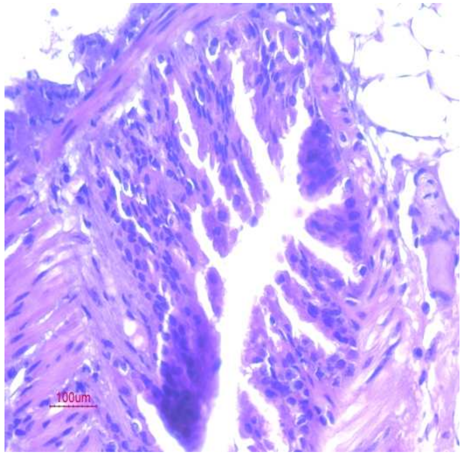
Native oral

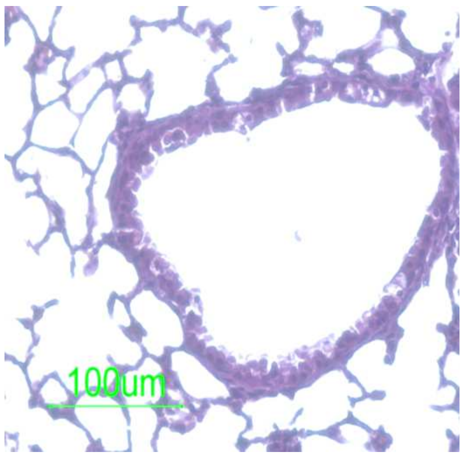


Native I.p.

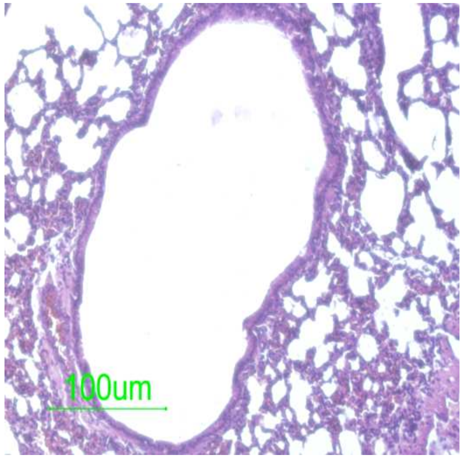


Control


Ovalbumin i.p



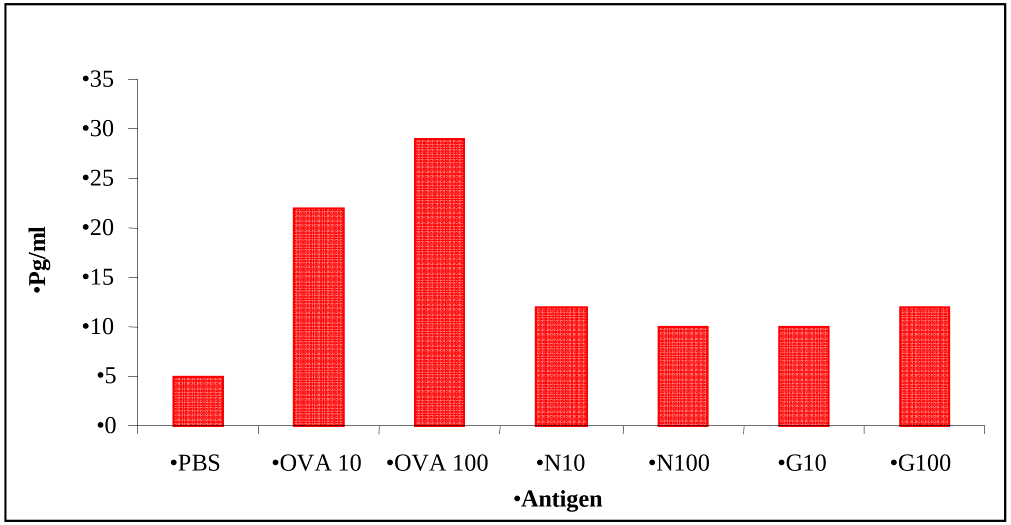
GM oral



GM i.p.

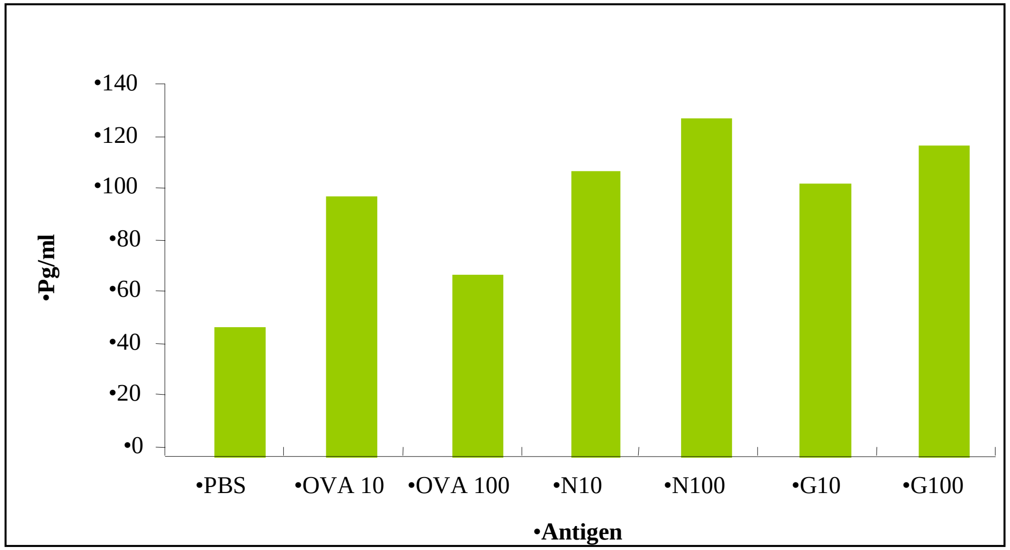
Singh et al., Allergy 61:491-497, 2006

Cytokines analysis for Th1/Th2 response



•OVA = ovalbumin,
•N = Native mustard,
•G = GM mustard

•IL-4 analysis of OVA,GM and native proteins sensitized mice

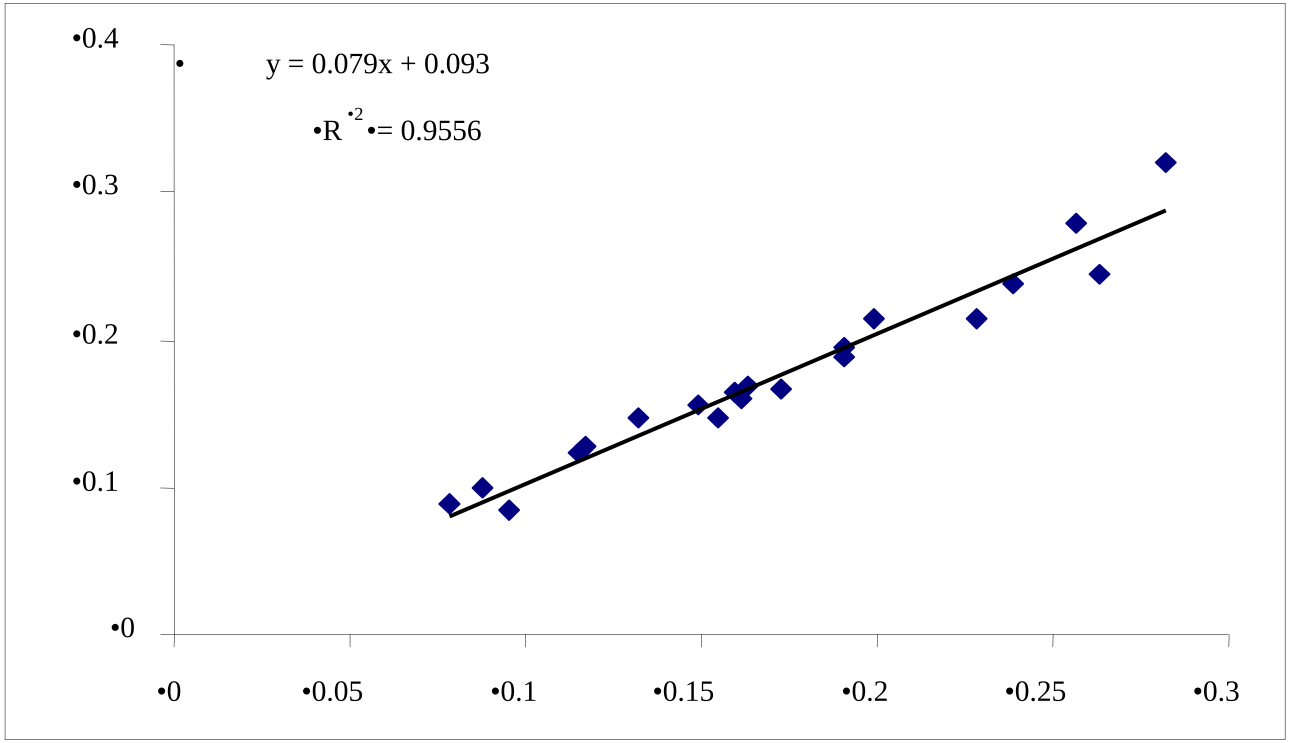


•OVA = ovalbumin,
•N = Native mustard,
•G = GM mustard

•IFN-γ analysis of OVA,GM and native proteins sensitized mice.

Specific sera screening

•Correlation between IgE binding of GM and Native mustard proteins in hypersensitive patient's sera

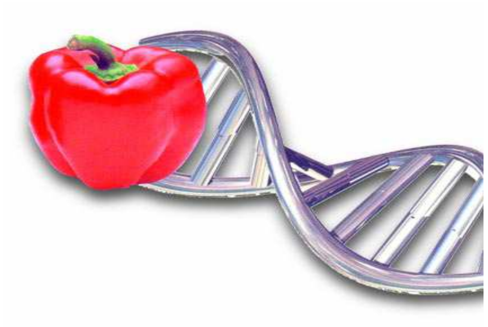


Summary

- GM mustard expressing choline oxidase have similar reactivity to that of native mustard.

- The protocols developed for comparative proteomics analysis can be used to study other GM crops.

- Animal model developed can be used for allergenicity assessment of other GM crops.



OSMOTIN

- **Basic ~25-kDa protein (246 amino acids) belonging to the PR-5 family.**

By interaction with G proteins, it permeabilizes fungal cell wall and induces spore lysis, thus protecting the plant from such pathogens

Implicated in tolerance against abiotic stresses.

Induced in tobacco leaves, stems and roots by drought, high salt

Enhanced tolerance to infection by *Phytophthora infestans*

Osmotin gene from *Nicotiana tobacum* (common tobacco) was thus introduced in tomato/mustard

Allergenicity Assessment of

Osmotin

using bioinformatics

Sequence homology using Farrp allergen database

Allergen	Accession No.	% similarity
Actinidia deliciosa (kiwifruit)	CAI38795	84.375
Cryptomeria japonica (Japanese cedar)	BAF51970	74.766
Jun a 3	AAF31759	72.174
Jun r 3	AAR21072	75.120
Cup s 3	AAR21074	74.641
Malus x domestica	AAX19848	63.306
Mal d 2(apple)	Q9FSG7	63.710
Act c 2	P83958	89.474
Olea europaea	AAK58515	52.577
Act d 5	P84527	45.763
Dermatophagoides pteronyssinus	CAD38366	45.918

•11 allergens have 35%or more homology with osmotin protein

Amino acid identity with allergens using SDAP database

Allergen	Accession No.	% identity
Lyc e NP24(tomato)	P12670	89.80(220/245)
Cap a 1w(bell pepper)	CAC34055	86.53(212/245)
Cap a1(bell pepper)	AAG34078	57.96(142/245)
Jun a3(mountain cedar)	P81295	46.53(114/245)
Cup a 3(cyprus)	CAC05258	42.45(104/245)
Pru av 2(sweet cherry)	P50694	39.59(97/245)
Mal d 2(apple)	CAC10270	39.18(96/245)
Act c 2(kiwi)	P81370	8.57(21/245)

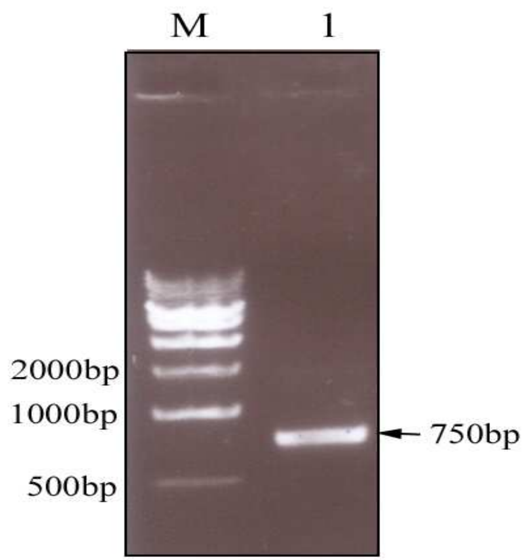
7 allergens showed 35% or more homology with osmotin protein

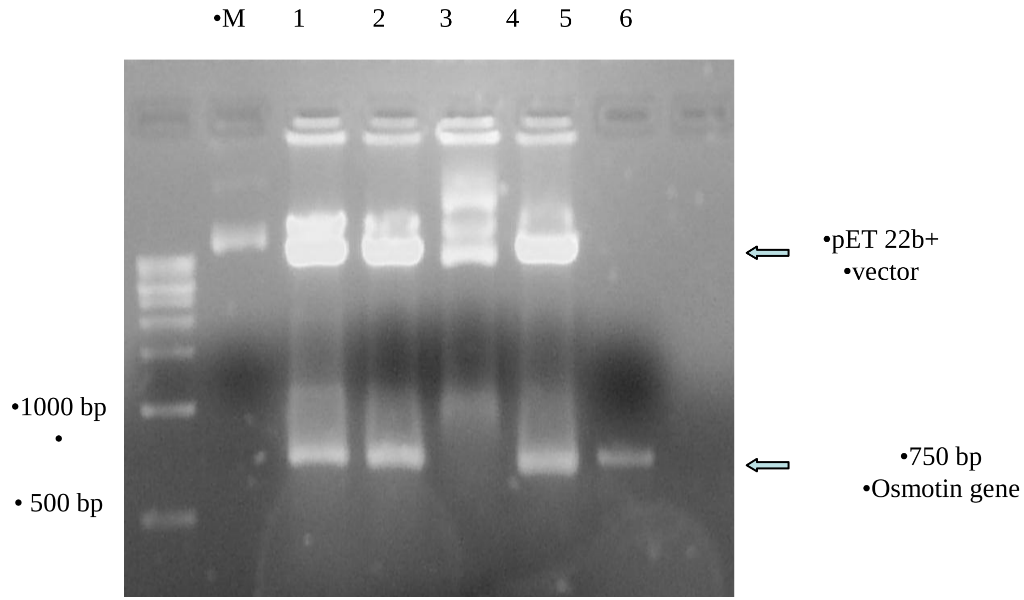
Summary

•**Osmotin protein has homology with certain allergens.**

•**Hence, studies are required to confirm this observation.**

Cloning of osmotin gene

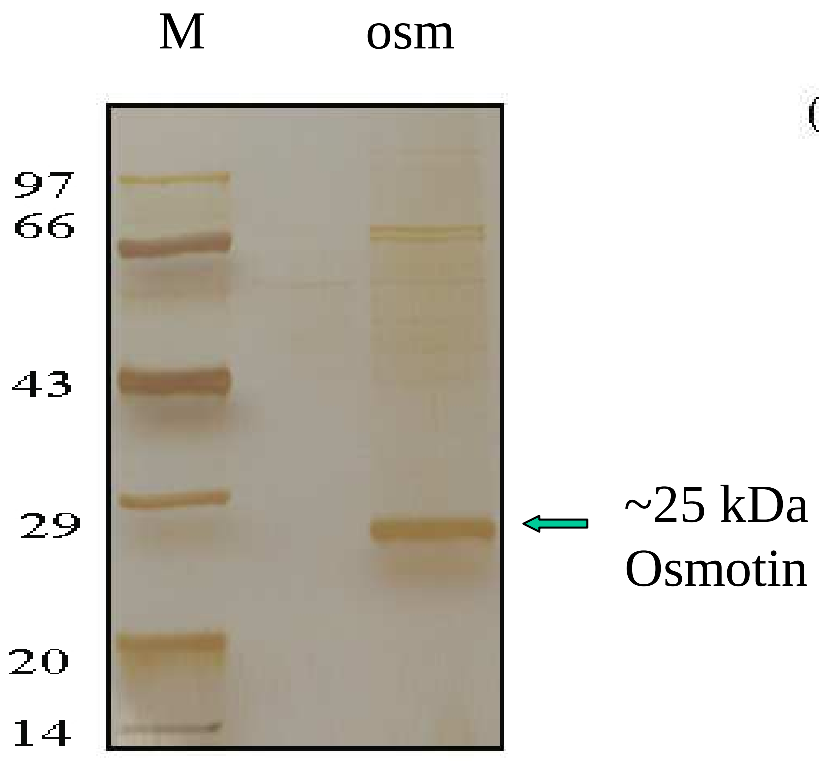




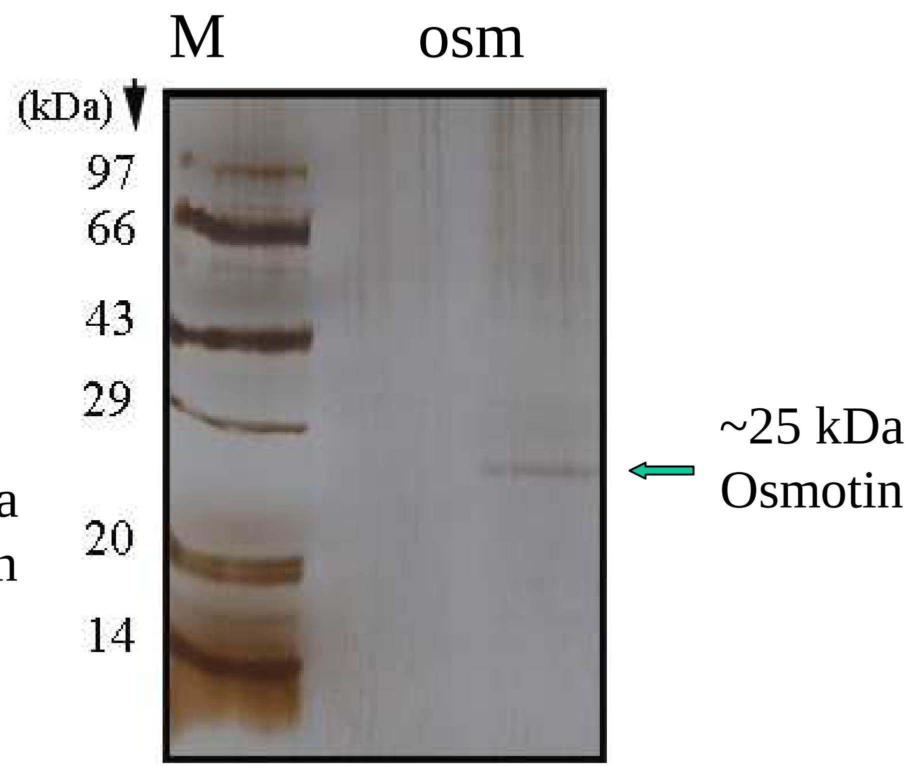
•PCR amplified Osmotin gene
•Source : *Nicotiana tabaccum*

- M) marker
  1) pET 22b+ vector
  2) clone 1 digested vector
  3) clone 2 digested vector
  4) clone 3 digested vector
  5) clone 4 digested vector
  6) Osmotin DNA

Expression and purification of Osmotin protein



Purification under Native condition

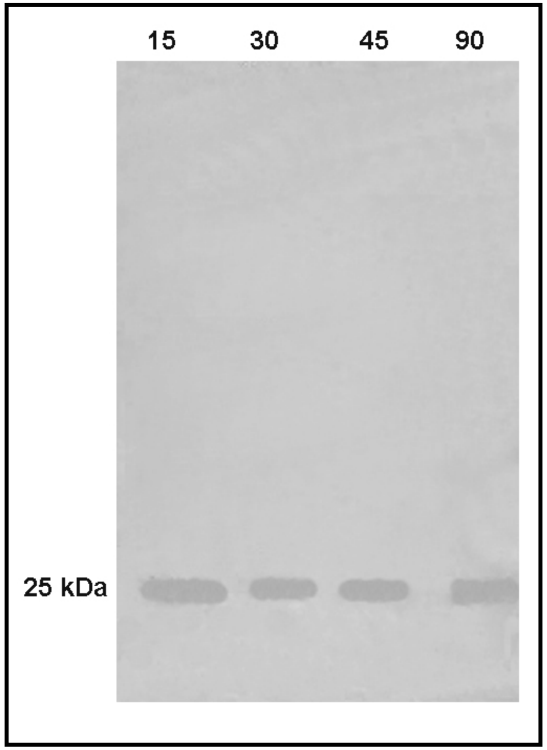


Purification under denaturing condition

In-vitro studies with purified Osmotin protein

Heat treatment

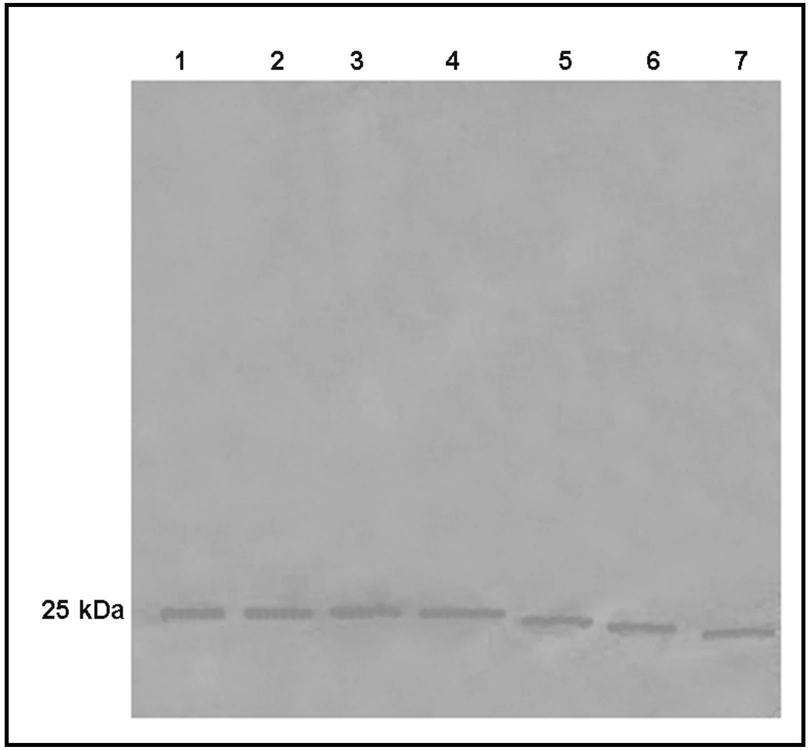
Time in min.



90°C

Simulated Gastric Fluid Treatment

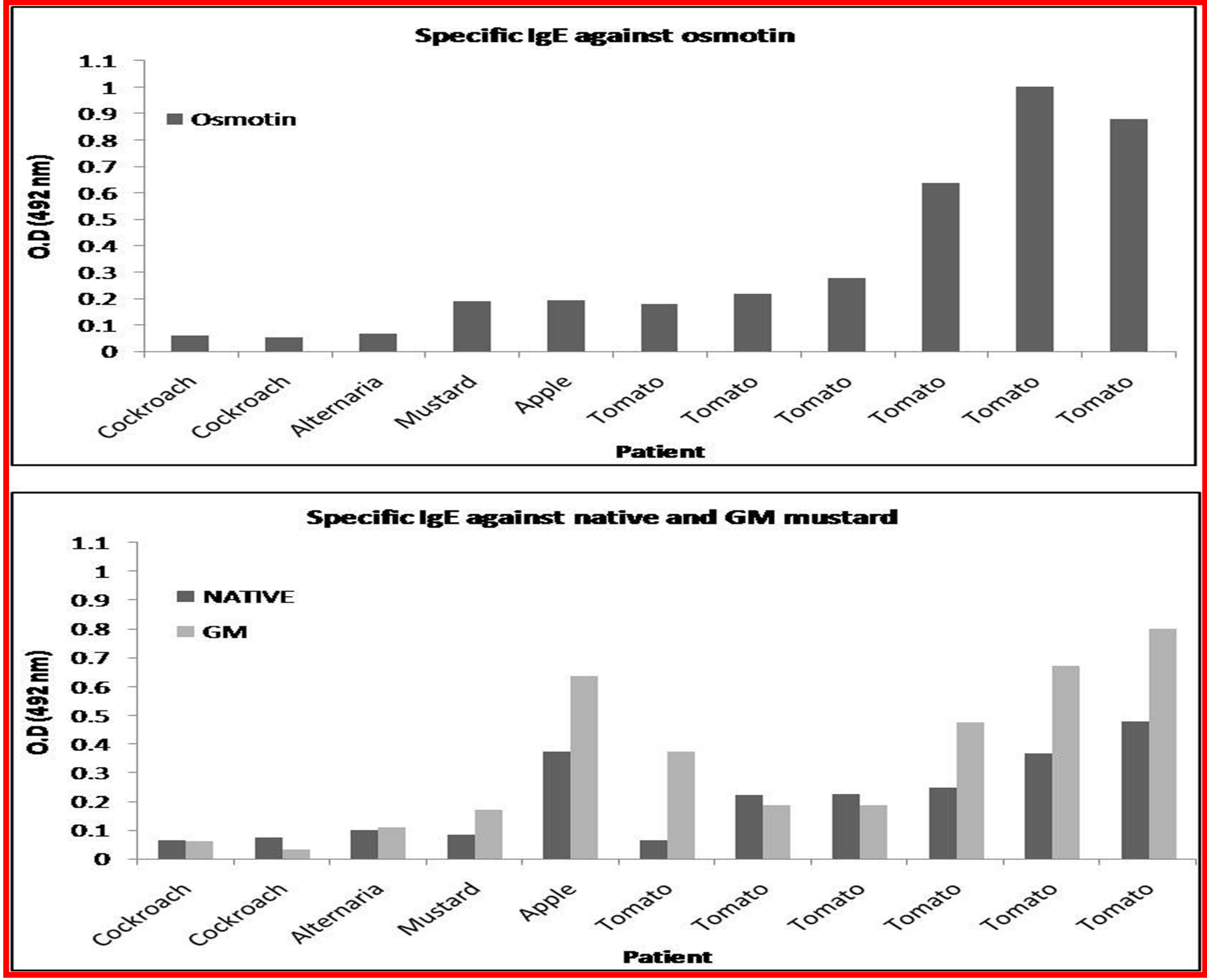
•Time in min.



1 : Osmotin pure;
2-7 : Osmotin + SGF for 0,10, 15, 30, 45 and 60 min.

Western blot with antibodies against osmotin

IgE reactivity of Osmotin against food allergic patients' sera by ELISA



Specific IgE reactivity against osmotin protein in food allergic patients' sera by ELISA.

S.No.	Sample No.	Avergae O.D.(492 nm)
1	U G	0.4325
2	N	0.4855
3	182	0.6065
4	834	0.5265
5	482	0.4985
6	375	0.7805
7	1386	0.5405
8	1406	0.487
9	1328	0.5235
10	1176	0.4665
11	540	0.458
12	300	0.495
13	340(1:5)	0.785
14	1526	0.623
15	1040	0.6875
16	316	0.515
17	464	0.4385
18	343	0.439
19	As	0.302
20	284	0.4515
21	578	0.4515
22	552	0.53
23	Control (-ve)	0.138
24	Control (-ve)	0.175

•117 food allergic patient's sera were screened for specific IgE reactivity by ELISA and 22 patient's sera showed positive reaction against osmotin.

Summary

Osmotin has the properties of an allergen

but more studies are

required to confirm this observation

Health Risks of Genetically Modified Foods

It directly enters Human system

The GM crops give us a way to counter Global Food problem/nutrition

All these demands a proper assessment

Health Came First

Health Came First

I dont understand! i planted carrots!!

•Acknowledgements

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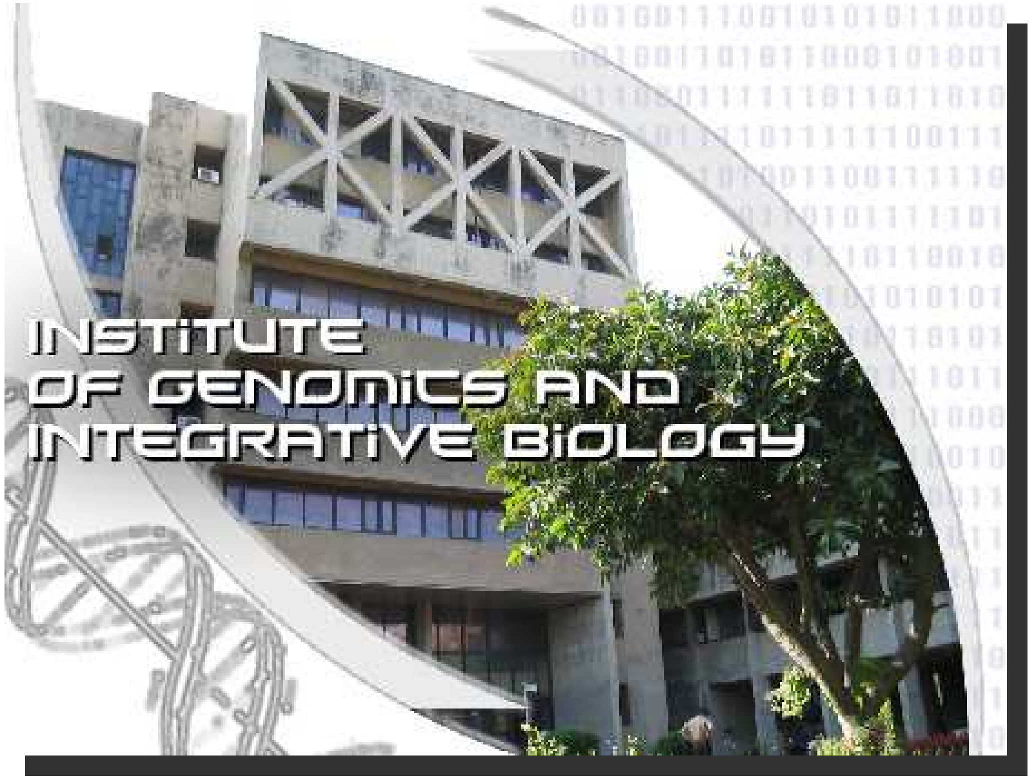


•**IGIB mission** ***" To translate concepts developed in basic biological research to commercially viable technologies for health care ".***

•Thank You

Genetically modified crops under field trial in India

Crop	Trait	Organization
Brinjal	Insect resistance	IARI,MAHYCO ,Sungro seeds Pvt. Ltd.
Cabbage	Insect resistance	Sungro seeds Pvt. Ltd.
Cauliflower	Insect resistance	Sungro seeds Pvt. Ltd.
Corn	Insect resistance	Monsanto (India), Metahelix Life science
Cotton	Insect resistance	IARI, MAHYCO along with 20 different Pvt. groups
Ground nut	Virus resistance	ICRISAT
Mustard	Cytoplasmic male sterility	University of Delhi
Okra	Insect resistance	MAHYCO
Pigeon pea	Fungal resistance	ICRISAT
Rice	Fungal, insect resistance	IARI,MAHYCO
Tomato	Insect and viral resistance	MAHYCO,IARI

Source: Department of Biotechnology ,INDIA

The Star link Episode

- Starlink corn developed by Aventis Cropsciences, USA
- contained a variant of BT toxin called Cry9C
- many people become ill from eating corn products containing the Cry9C protein.
- resistant to digestion in a laboratory test
- potential for allergenicity

"Star Link Corn is eventually out"

Monarch butterfly death from GM Pollen

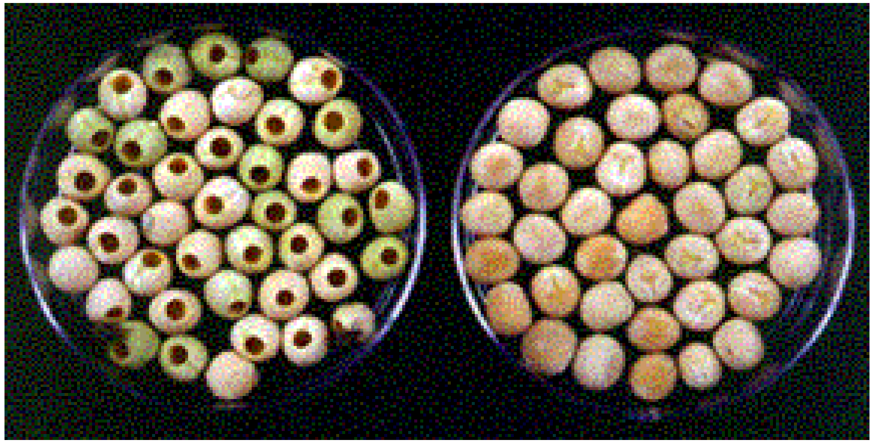
- The Monarch Butterfly is the "Bambi of insects"
- GM plants having *cry* gene produce a pollen containing crystalline endotoxin
- Pollens through air reaches milkweed that monarchs larvae feed
- Most of monarch butterflies died and the rest grew to only 50% normal size

“Monarch Butterfly are endangered Now”

GM peas expressing a bean gene

• **Developed by CSIRO Australia**

• **Protection against Pea weevil**



Non GM Pea (Weevil infected) **GM Pea**

➢ **bean α-amylase inhibitor gene is expressed in peas.**
➢ **Mice exposed to α-amylase inhibitor GM-peas showed immune response.**
➢ **inflammation in the lungs and increased serum antibody levels.**

The development of GM peas is now stopped

Major uncertainties over the safety of the GM process

- Arpad Pusztai found that **GM potatoes** with snowdrop lectin adversely affected every organ system of young rats & small intestine lining grew up to twice the thickness of controls

- Scientists in Egypt found similar results in the gastrointestinal tract of mice fed **GM potato** with Bt toxin.

- US FDA showed rats fed **GM tomatoes** with antisense gene to delay ripening developed small holes in their stomach.

- Aventis (now Bayer) found 100% increase in deaths of broiler chickens fed **glufosinate-tolerant GM maize** T25 compared to controls.

- Numerous anecdotes indicating that livestock wildlife and lab animals avoid GM feed, and fail to thrive or die when forced to eat it .

•Major uncertainties over the safety of the GM process

•Between 2001 and 2002, twelve dairy cows died on a farm in Hesse, Germany, after eating Syngenta's Bt176 GM maize, and others in the herd had to be slaughtered on account of mysterious illnesses [21]. To-date, there has been no detailed autopsy reports available, even though the company claims the deaths and illnesses were unrelated to Bt176. Nevertheless the Spanish Food Safety Authority has just withdrawn authorisation for Bt176 cultivation in Spain [22] after it had occupied almost all of the 20 000 hectares of GM maize grown in Spain since 1998 [23]. The decision was taken following an EFSA recommendation that GMOs containing antibiotic resistance marker genes such as that found in Bt 176, be restricted to field trials.

•Arpad Pusztai and colleagues found that GM potatoes with snowdrop lectin adversely affected every organ system of young rats, and the stomach and small intestine lining grew up to twice the thickness of controls [24].

•Scientists in Egypt found similar results in the gastrointestinal tract of mice fed GM potato with Bt toxin [25].

•US Food and Drug Administration had data since the early 1990s showing that rats fed GM tomatoes with antisense gene to delay ripening developed small holes in their stomach [24].

•Aventis (now Bayer) found 100% increase in deaths of broiler chickens fed glufosinate-tolerant GM maize T25 compared to controls [26].

•Numerous anecdotes from farmers and others indicating that livestock, wildlife and lab animals avoid GM feed, and fail to thrive or die when forced to eat it [26, 27].

Major GM foods in the market

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Crop	Year	Gene Introduced	Intended Effect
Mustard	1992 1997 2001	*acyl carrier protein thioesterase* PAT *epsps*	High laurate canola oil Glufosinate herbicide resistance Glyphosate herbicide resistance
Corn	1995 1998 1998 1998 2003	*CryIAb* DAM PAT *Cry 9C* *Cry 1F*	Resistance to European corn borer insect Male sterility Glufosinate herbicide resistance Resistance to lepidopteron insect Resistance to lepidopteron insect
Soybean	1994 1996	*epsps* GmFad2-1 gene	Glyphosate herbicide resistance High oleic acid