SAFETY OF NOVEL FOOD AND GENETICALLY ALTERED CROPS - WHAT WOULD SCIENCE-BASED REGULATION LOOK LIKE?

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&

School of Pharmacy, University of Canberra,

[General Manager Risk Assessment, FSANZ – retired]

Teosinte (ssp *parviglumis*)

Landraces

Inbred Lines

Artificial Selection

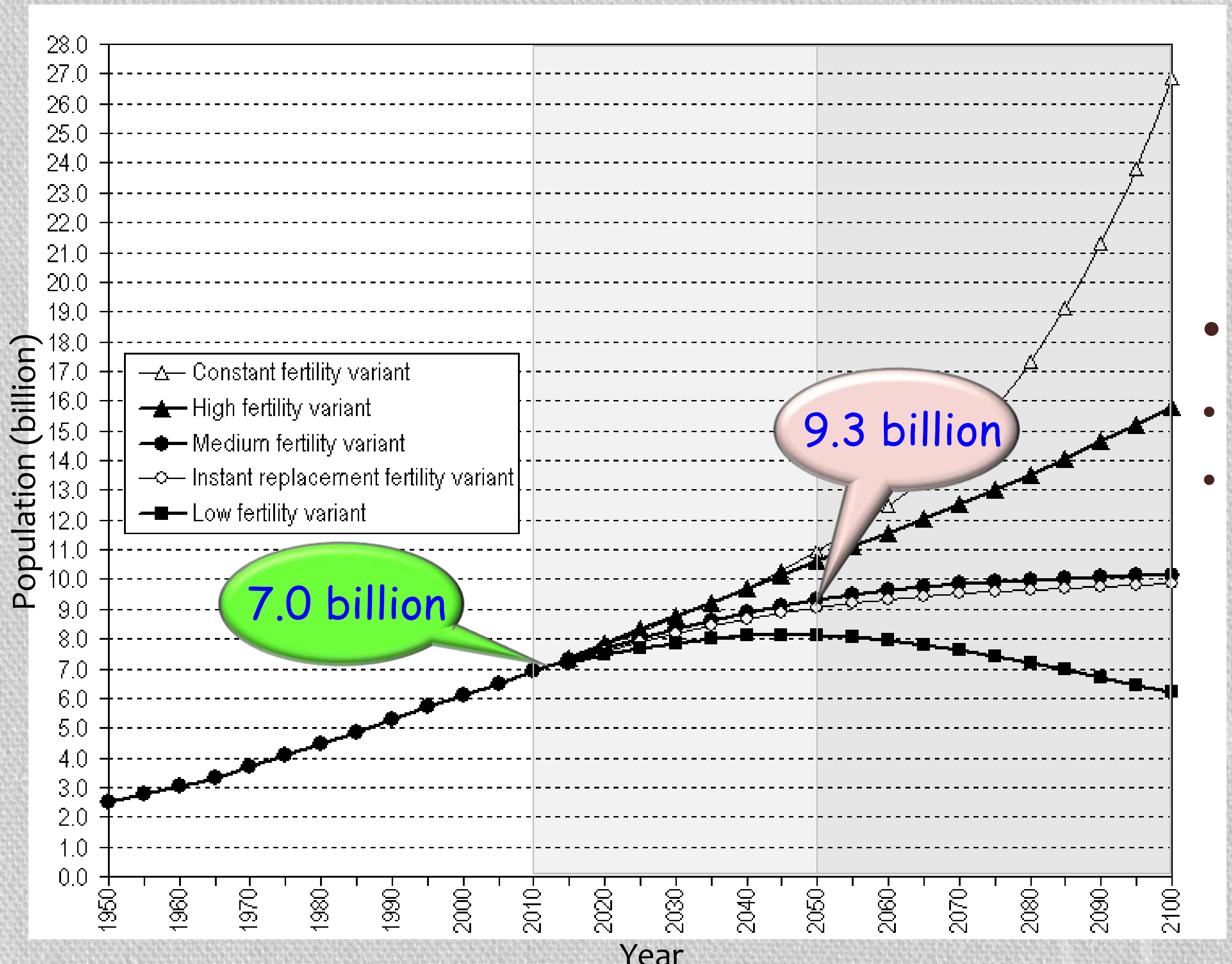
Overview

- Basic principles of ethical regulation
- What are the concerns of new and existing biotechnologies ?
- Relevance of genome plasticity
- Sources of Variability – Genotype & phenotype
- So what does the data tell us ?

General Principles of ethical regulation

- Ethical regulation is proportionate to risk
  - For new technologies in general risk derives from
    - The nature of the applications of that technology (what it is used to achieve) and
    - **The novelty arising from those applications against the context of natural and pre-existing analogues of those outcome**
- Data requirements should address sources of risk and viable risk management options
  - Value of information (VOI)
    - Data is necessary only where the information has a material influence on regulatory outcome (risk management strategies)
- A "Precautionary approach" is **NOT** necessarily precautionary
  - All actions and inactions have consequences
  - The consequences of blocking or restricting new technologies on the basis of vague hypothesised harms may result in greater harm than those being avoided
- The Objective is - Balance, proportionality, pragmatism, cost effectiveness, impartiality, & most importantly - **scientific integrity**

Estimated and projected world population according to different variants (1950-2100)



- Kazumi KITTA, Ph.D.
- National Food Research Institute
- National Agriculture and Food Research Organization

Source: UN, Department of Economic and Social Affairs, Population Division(2011)

Technologies and Outputs

- New and existing biotechnology is used to alter the genome of plants to achieve new traits
- Assessing the safety of the new (inserted or induced) trait is not controversial and requires only an understanding of the trait itself
- **The regulatory debate revolves around unintended, unpredictable consequences of gene insertion or induced random mutation.**
- Genome plasticity and the consequences of this plasticity provides the context for addressing these issues

THE CONCERNS

What is the Concern

- ***De novo* production of High potency protein toxins**
  - Very small amounts of an extreme toxin such as botulinum toxin in food presents an immediate, high risk if present, **BUT**
  - Is this plausible accidentally just from transgene insertion.
    - **Systemic** toxicity of an ingested protein requires at least three highly specific**, and separate**, structural characteristics
      - Resistance to digestion
      - Ligand for specific, and species specific, gut uptake transporters
      - Ligand for site and species specific receptor mediating toxicity
- ***De novo* generation of biochemical machinery** to produce a novel toxic secondary metabolite unrelated to the parent plant or the source or function of the transgene itself
  - as for protein toxins, multiple co-ordinated alterations would be required - is this plausible
- **Reactivation of dormant pathways**
  - Tissue dormancy/inactivation versus species/strain dormancy

What is the concern?

- "Additionally, plants, …, have metabolic pathways that no longer function because of mutations that occurred during evolution."
  - "Products or intermediates of some of these pathways may include toxicants."
- "…, such silent pathways may be activated by the introduction or rearrangement of regulatory elements, or by the inactivation of repressor genes by point mutations, insertional mutations, or chromosomal rearrangements."

The Safety of Foods Developed by Biotechnology

David A. Kessler, Michael R. Taylor, James H. Maryanski, Eric L. Flamm, Linda S. Kahl

Science, 256, (Jun. 26, 1992), pp. 1747-1749

Relevance of genome plasticity

- 2 principle sources of "risk" in plant breeding (both for conventional and new technologies)
  - The expression of an Inserted/introduced gene(s) – or modification of the expression of an endogenous gene
  - Random genetic variability arising from the process (whether natural, conventional, biotech, NBT),
- Variability in nature is the norm
  - Both phenotypic and genotypic variability in food crops is much greater than previously recognised
  - The potential risks arising from variability secondary to NBT and recombinant DNA techniques is related to the magnitude of that variability in comparison to that occurring naturally or in conventional breeding

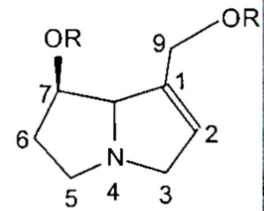
Formulation of the problem

- How variable is the phenotype without genome plasticity
- How common are toxins in nature and how variable are they
- What is their purpose in nature
- How common are gene insertions in nature
  - What are the sources of the inserted genes
  - What are the consequences of those insertion
- How common are point mutations in nature
  - What are the consequences
- Is there evidence for activation of dormant pathways from either
  - Tissue versus species specific dormancy
- Is there evidence for ***de novo*** generation of toxins from mutations or insertions

CROP VARIABILITY

Potential Candidates

- Secondary metabolites
  - Endogenous pesticides
  - Herbivore anti-feedants
  - Stress response
- Nutrients, trace elements
  - Macro
    - Lipid profiles
  - Micro
    - Minerals & vitamins
    - Heavy metals
- In humans these may be
  - Contact allergens
    - Psoralens in celery
  - Carcinogens
    - Aristolochic acid
  - Specific organ toxins
    - Pyrrolizidine liver toxins
  - Nutritional
    - Lipid profile



Pyrrolizidine alkaloid

2 ■ Biochemistry of Plant Secondary Metabolism

Table 1.1 Number of known secondary metabolites from higher plants

Type of secondary metabolite	Number[a]
Nitrogen-containing	
Alkaloids	21 000
Non-protein amino acids (NPAAs)	700
Amines	100
Cyanogenic glycosides	60
Glucosinolates	100
Alkamides	150
Lectins, peptides, polypeptides	2000
Without nitrogen	
Monoterpenes (C10)[b]	2500
Sesquiterpenes C15)[b]	5000
Diterpenes (C20)[b]	2500
Triterpenes, steroids, saponins (C30, C27)[b]	5000
Tetraterpenes (C40)[b]	500
Flavonoids, tannins	5000
Phenylpropanoids, lignin, coumarins, lignans	2000
Polyacetylenes, fatty acids, waxes	1500
Polyketides	750
Carbohydrates, organic acids	200

Secondary metabolite toxicity not random

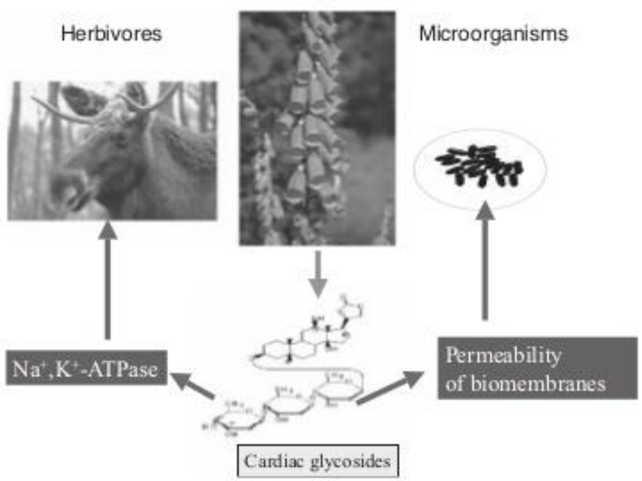


Figure 1.8 Schematic view of the ecological roles of plant SM. Foxglove (*Digitalis purpurea*) produces cardiac glycosides, which are very toxic to animals (vertebrates, insects) because they inhibit Na^+, K^+-ATPase, one of the most important transporters in animal cells. Cardiac glycosides are additionally toxic to microbes because the molecules have detergent properties and disturb membrane fluidity. (See Plate 7 in colour plate section.)

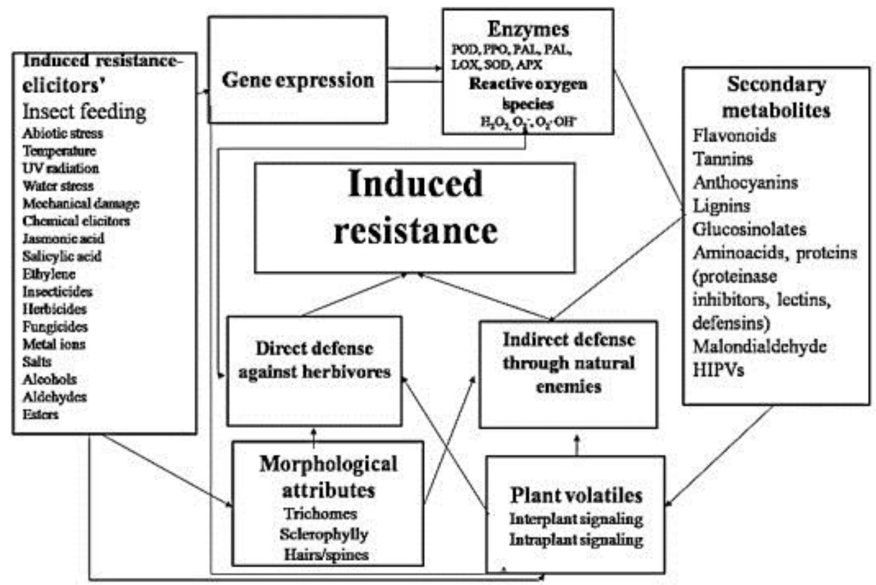


Figure 1. Mechanism of induced resistance in plants. POD, peroxidase; PPO, polyphenol oxidase; PAL, phenylalanine ammonia lyase; TAL, tyrosine alanine ammonia lyase; LOX, lipoxygenase; SOD, superoxide dismutase; APX, ascorbate peroxidase; HIPVs, Herbivore induced plant volatiles

Plant Signal Behav. 2012 October 1; 7(10): 1306–1320.

Biochemistry of plant secondary metabolism. 2nd edition, Annual Plant Reviews volume 40 Michael Wink ed. 2010. Wiley-Blackwell

Determinants of Secondary Metabolite levels - Psoralens

Table 1
Effect of environmental conditions and elicitor treatments on furocoumarin content in higher plants

Environmental factor or elicitor	Plant species	Effect on furocoumarin content
Plant diseases		
Ceratocystis fimbriata	*Pastinaca sativa* (root apex)	×20 (8-MOP)
Sclerotinia sclerotiorum	*Apium graveolens* (stalks)	×235 (Psoralen) ×24 (Total furocoumarins)
Unknown	*Daucus carota* (roots)	×77 (8-MOP)
Erwinia carotovora	*Apium graveolens* (stalks)	×24 (Total furocoumarins)
Rhodotula rubra	*Ruta graveolens* (hydroponic)	No modification
Phoma companata	*Pastinaca sativa* (leaves)	×5 (Total furocoumarins)
Pseudomonas cichorii	*Glehnia littoralis* (roots)	×9 (psoralen)
Insect damages	*Pastinaca sativa* (leaves)	×2.2 (8-MOP) ×1.8 (Psoralen)
Effect of light		
UV	*Apium graveolens* (stalks)	×3.4 (Linear furocoumarin)
UV	*Ruta graveolens* (leaves)	×2.5–10 (Total furocoumarin)
UV	*Glehnia littoralis* (roots)	×2 (Psoralen)
Air quality		
Acidic fog	*Apium graveolens* (leaves)	×5.4 (Linear furocoumarins)
Ozone	*Petroselinum crispum* (leaves)	×2 (Total furocoumarins)
Temperatures		
Cold (−15° C, control 26° C)	*Apium graveolens* (leaves)	×8.8 (Linear furocoumarins)
Hot (32° C, 21° C) control 21° C)	*Psoralea cinerea* (leaves)	×11 (Psoralen)
Chemicals		
$CuSO_4$	*Apium graveolens* (leaves)	×2.2 (Linear furocoumarins)
$CuSO_4$	*Psoralea cinerea* (fruits)	×2.8 (Psoralen)
NaCl	*Ruta graveolens* (leaves)	Decrease but higher percentage on leaf surface
NaCl	*Psoralea cinerea* (fruits)	×2 (Psoralen)
H_2SO_4	*Ruta graveolens* (leaves)	Decrease but higher percentage on leaf surface
$Ca(OCl)_2$	*Psoralea cinerea* (fruits)	×1.5 (Psoralen)

Review

Production of plant secondary metabolites: a historical perspective

F. Bourgaud *, A. Gravot, S. Milesi, E. Gontier

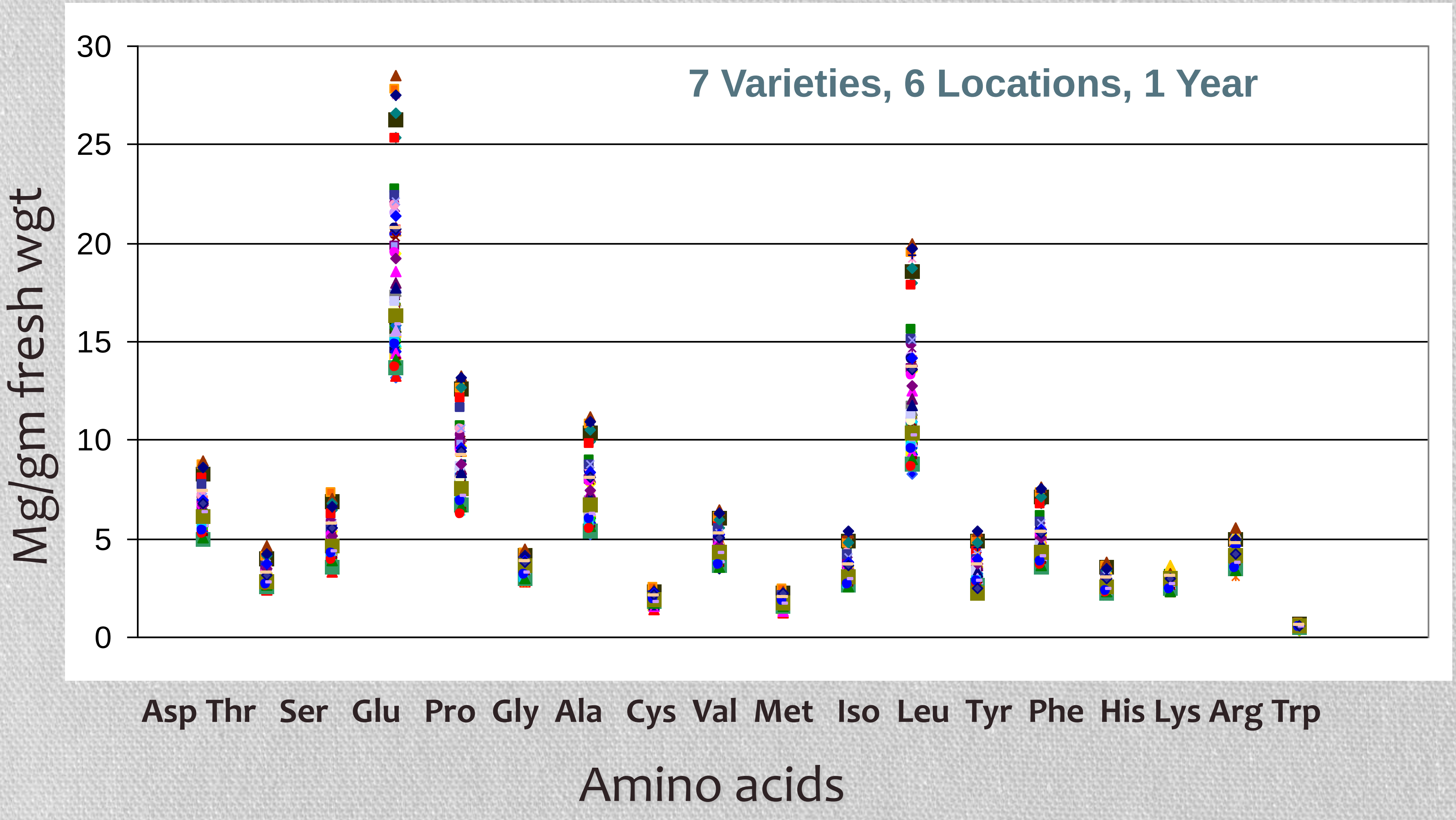
Plant Science 161 (2001) 839–851

- A response to
  - Disease
  - Heat/cold
  - Light
  - Air quality
  - Insect damage
  - Agronomic practices
- Highly variable 2 orders of magnitude

Variability –Composition

- Product of the interaction of
  - Pest pressure
  - Climate, micro climate – water, temperature, light duration & intensity, humidity, wind stress etc
  - Soil variability – macro & micro across regions and within a crop
  - Agronomic practices
- Compositional variability due to environmental variation is greater than that secondary to gene insertion or alteration (ie other than the variation specifically expressed by the inserted or modified gene)

Compositional Variability – Conventional Maize Hybrids



(Reynolds *et al.*, 2005).

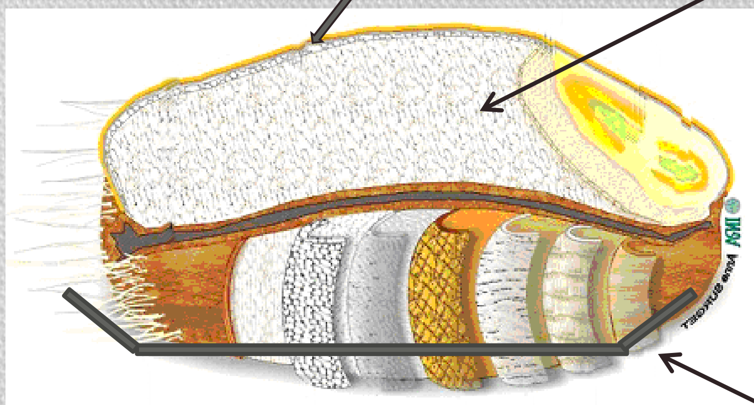
Grain tissues vary in composition

Starchy endosperm
(white flour)

Starch
70–80%

Protein
10–15%

Cell walls
(dietary fibre)
2–3%



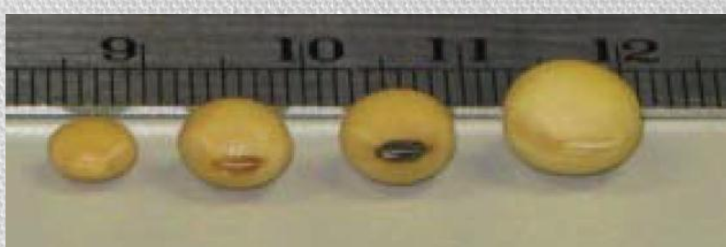
Cell walls
(dietary fibre)
20–30%

Phytochemicals

Minerals

B vitamins

Outer layers (bran)
aleurone, pericarp, testa



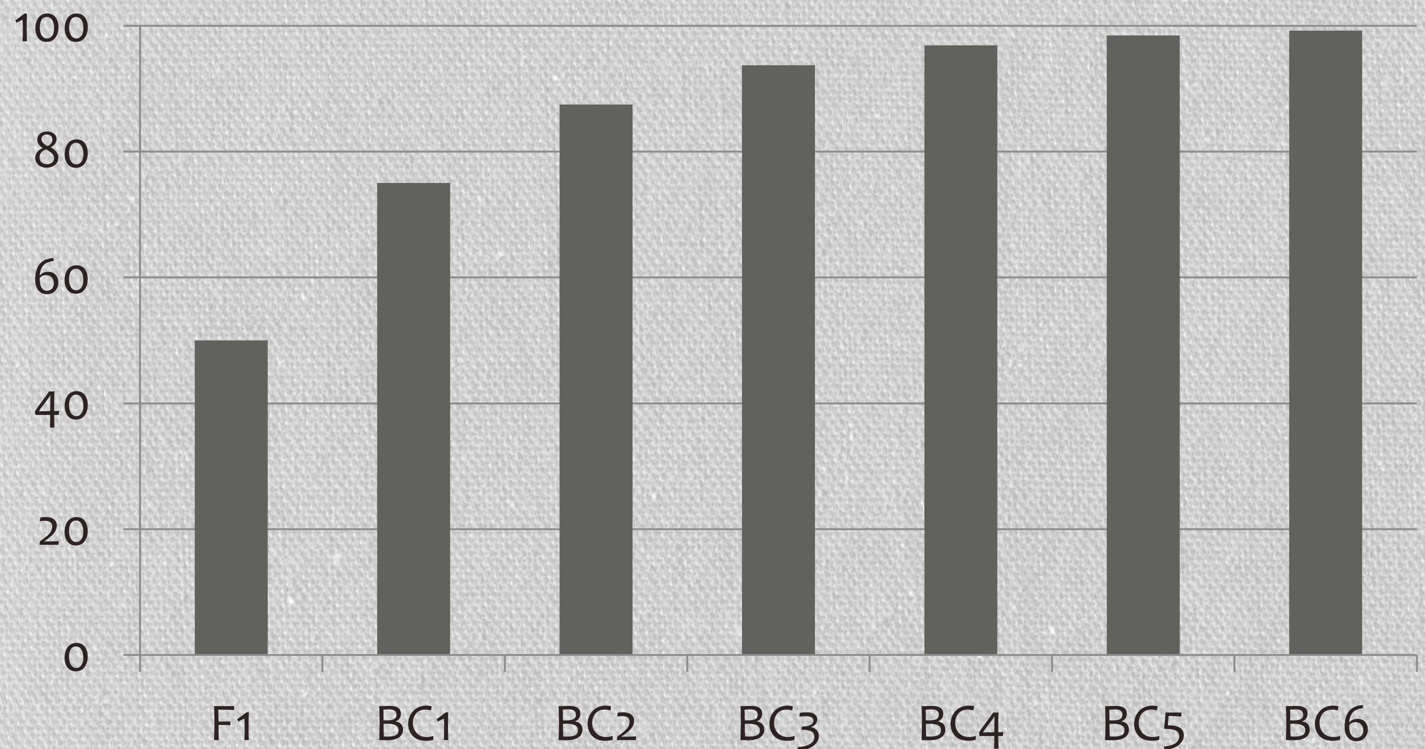
Bigger seeds: *Bunya*[Φ] *soybean seeds are larger than other varieties, which increases yield and makes them popular with soy manufacturers.*

There is nothing homogenous about "conventionally" developed crops – even grain size affects composition

Backcross theory

Rita H. Mumm, PhD
University of Illinois at Urbana-Champaign
ritamumm@illinois.edu

Expected %RP germplasm recovered



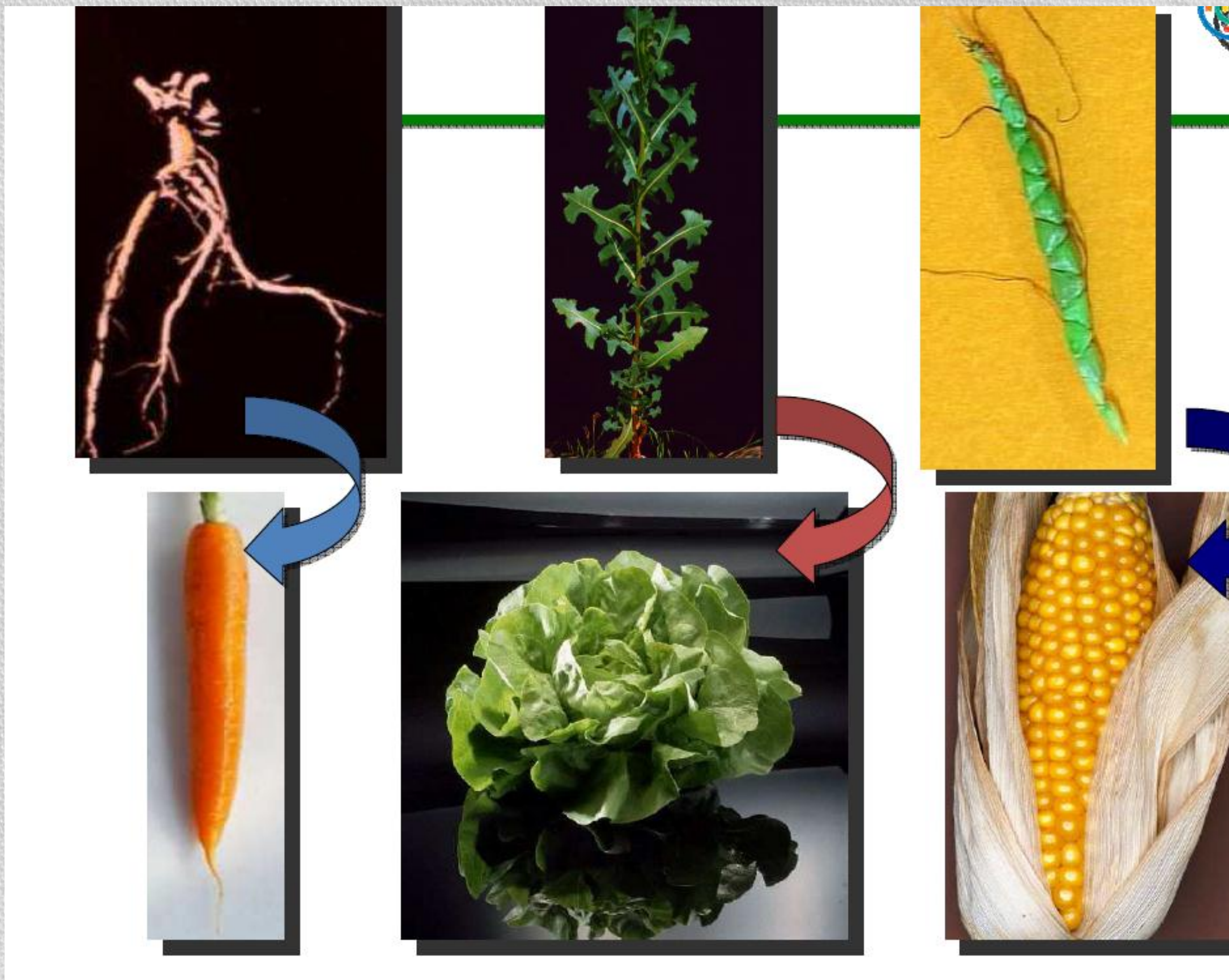
50	75	87.5	93.75	96.88	98.44	99.22

$$RP\%_{(n)} = 1 - \left(\frac{1}{2}\right)^{n+1}$$

RP%: percentage of recurrent parent germplasm recovered
n: backcross generation number

GENOTYPE VARIABILITY

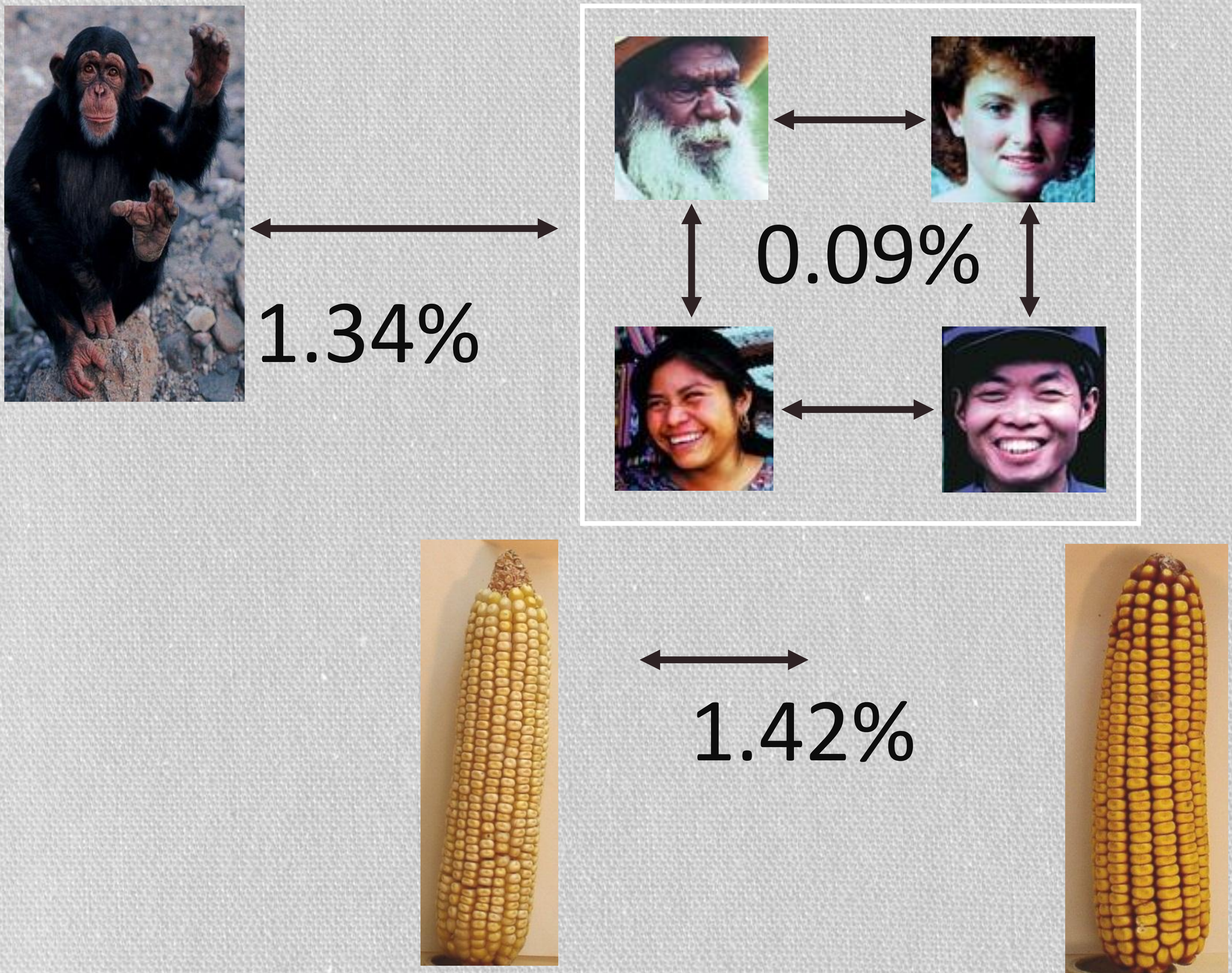
"Natural Food" is a Myth



Acknowledgements: top Peggy Lemax, John Meade, Raul Coronado
Bottom : Corbis
From a slide deck by Wayne Parrot

Humans Have Limited Molecular Diversity

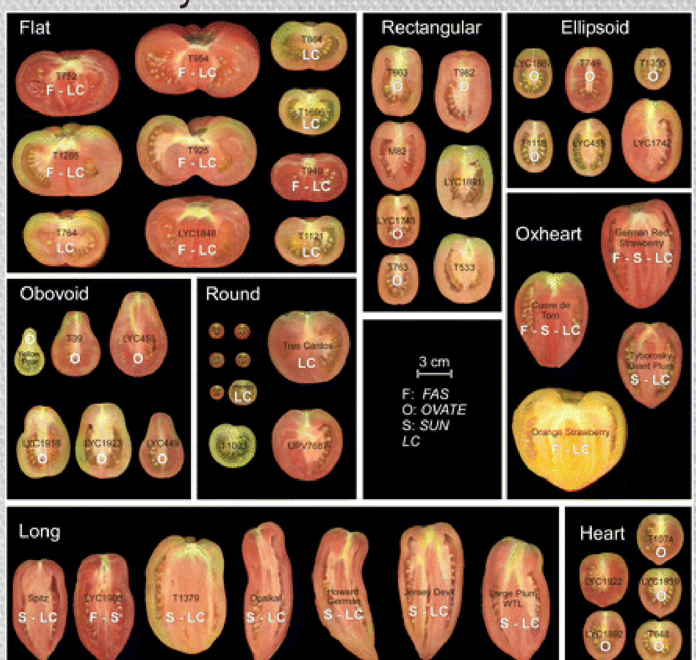
Maize diversity is greater than the difference between humans and chimps



Silent Diversity; Zhao, et al. (2001) *PNAS*

Genotypic variability

Barley

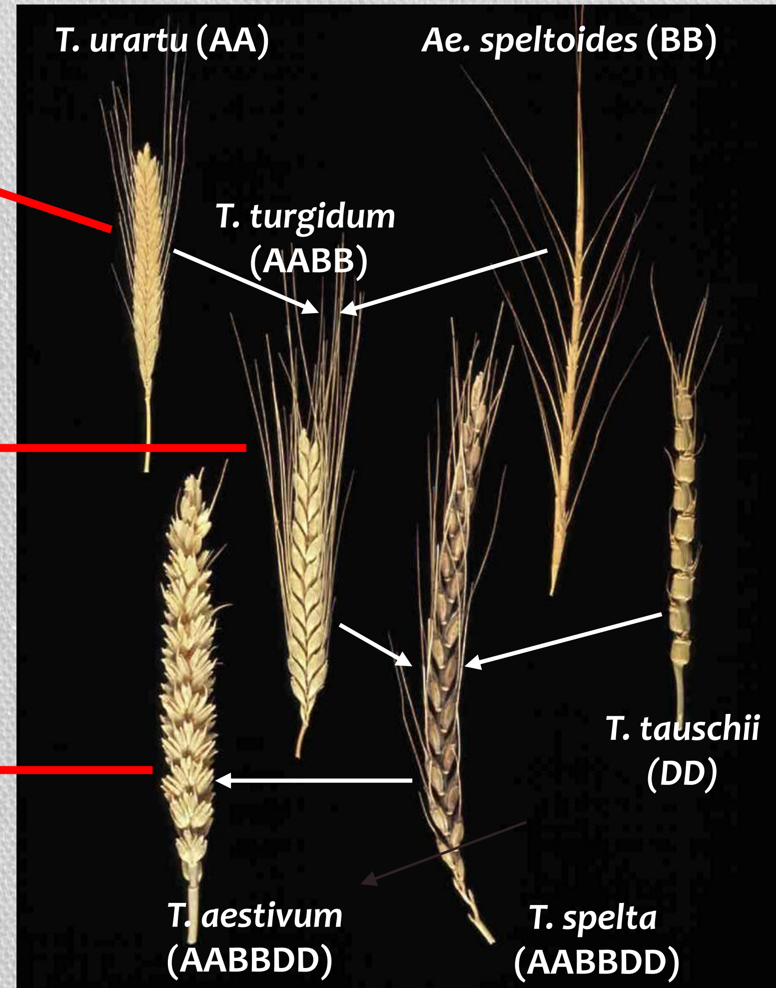


Einkorn wheat

Emmer wheat

Durum (pasta) wheat

Bread wheat



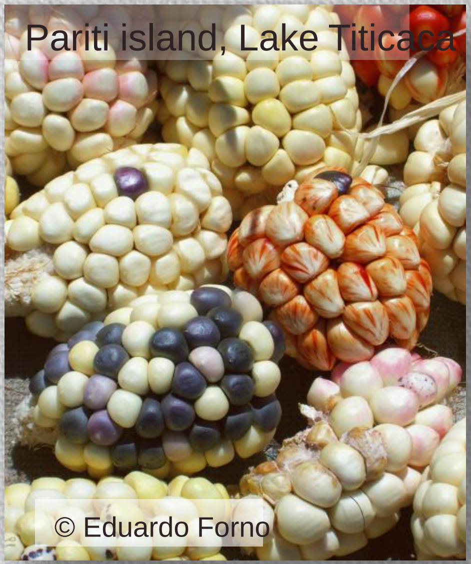
Wheat

Slide modified from Peter Shewry, Rothamsted Research & Sherry Flint-Garcia, USDA-ARS Columbia.

Rodríguez, et al. (2011) *Plant Physiol.*

Maize

- Maize
  - More than 50 million identified Single Nucleotide Polymorphisms (SNPs) – ie point mutations – catalogued from 103 lines (Soybean 5 million so far)
    - Same protein (300-400 aa) from 2 corn lines will differ on average by 3-4 aa due to SNPs
  - 85% of the genome sequence of the reference inbred (B73) is identified as transposable elements – jumping genes
  - Yellow maize is the result of A 382-bp *Ins2* into phytoene synthase promoter region
    - Prevents the carotenoid pathway shutdown in the seed
    - ***Ie activation of a tissue dormant pathway***



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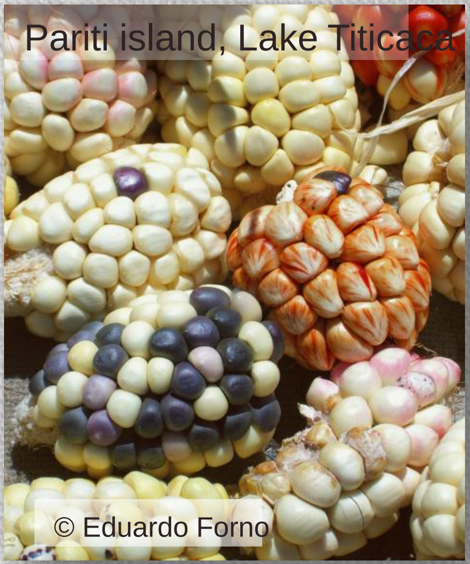
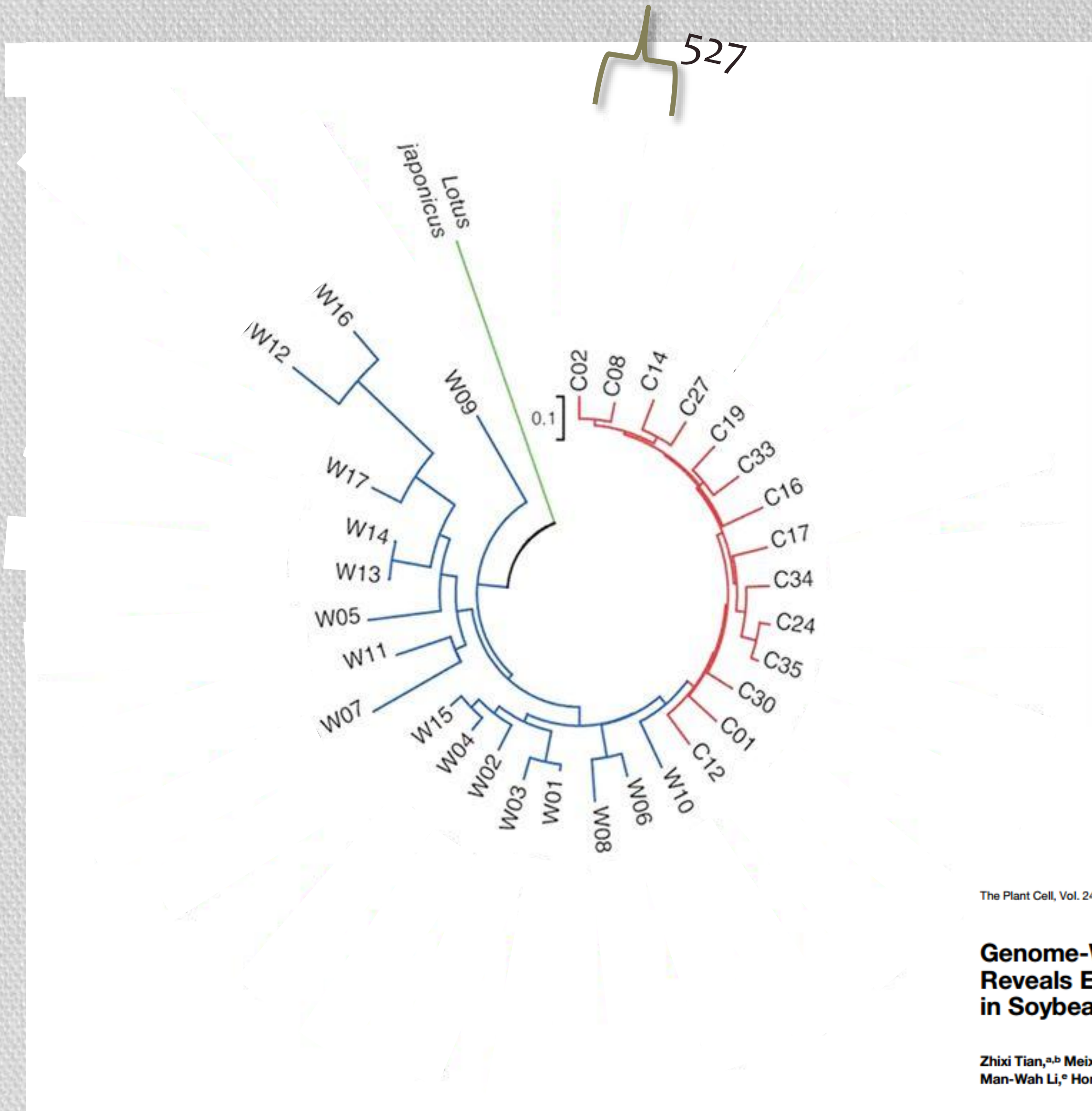


How common are insertions?

Unique transposon insertions in soybean compared to reference genome



N = 25,628 unique insertions

The Plant Cell, Vol. 24: 4422–4436, November 2012, www.plantcell.org

Genome-Wide Characterization of Nonreference Transposons Reveals Evolutionary Propensities of Transposons in Soybean

Zhixi Tian,[a,b] Meixia Zhao,[a] Maoyun She,[a] Jianchang Du,[a,1] Steven B. Cannon,[c] Xin Liu,[d] Xun Xu,[d] Xinpeng Qi,[e] Man-Wah Li,[e] Hon-Ming Lam,[e] and Jianxin Ma[a,2]

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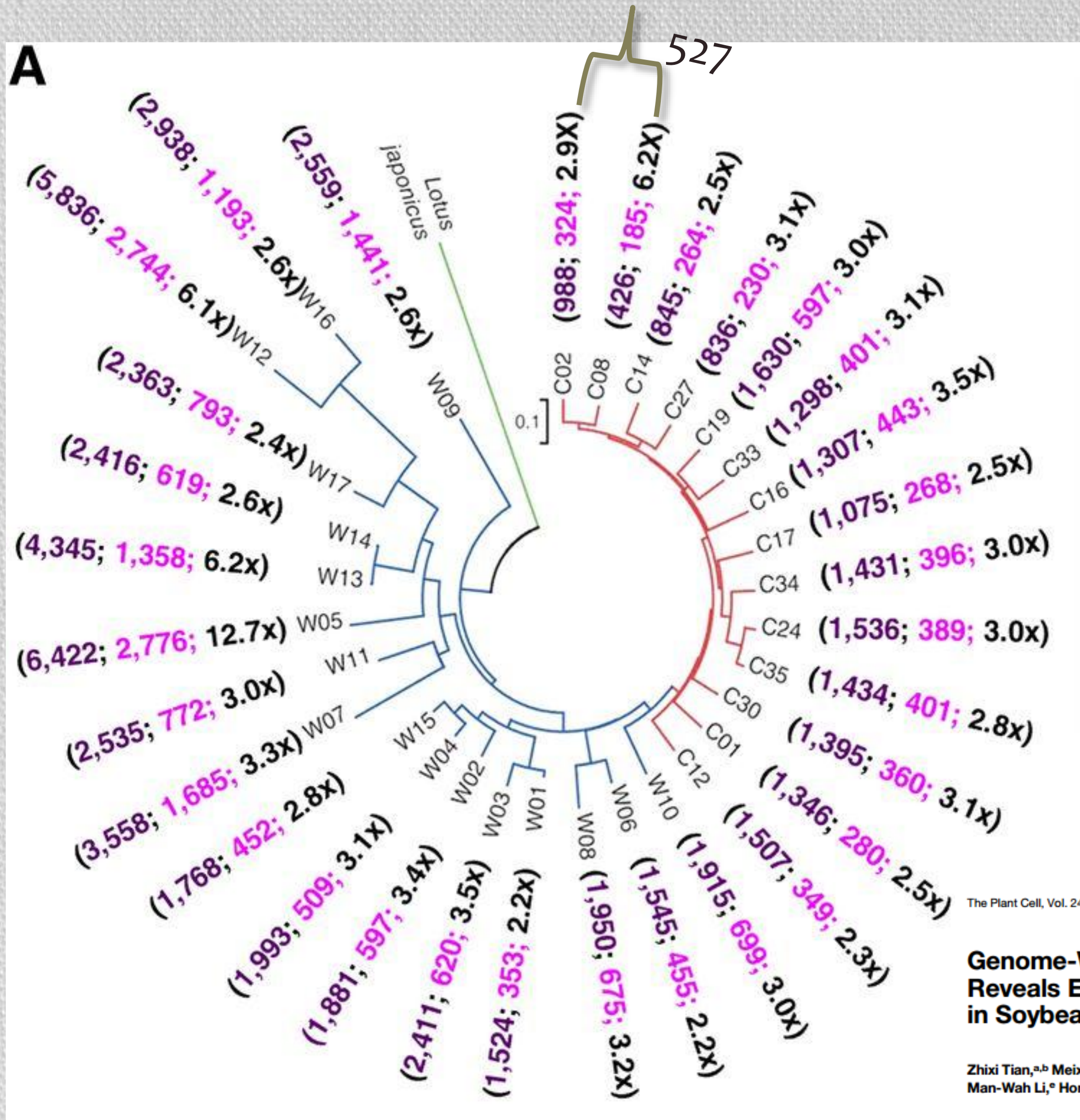

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Other common insertions

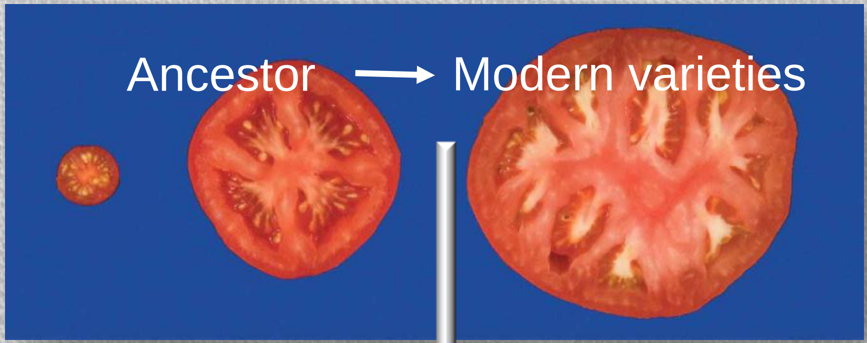
Rice

- Gimbozu
  - Ancestor to modern rice varieties
  - 49 to 63 new insertions per plant per generation
- Modern rice varieties have more stable genomes eg Nipponbare & TN67
  - ~ 1 new insertion per 3 plants per generation

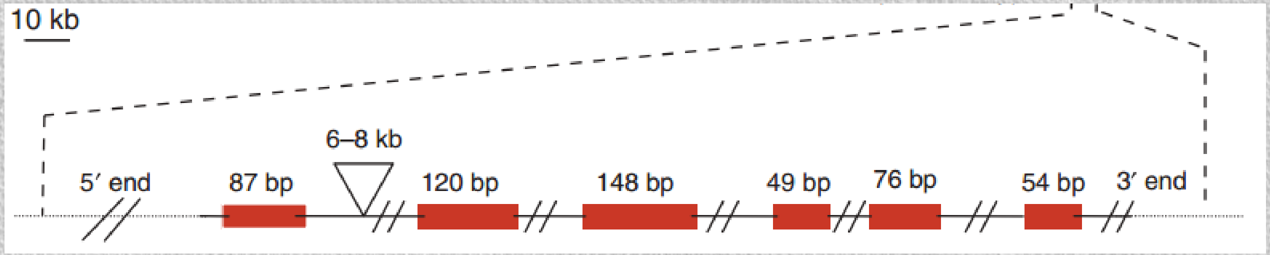
Naito et al. 2006. Dramatic amplification of a rice transposable element during recent domestication.
Proc. Natl. Acad. Sci. 47:17620-17625.

Tomatoes

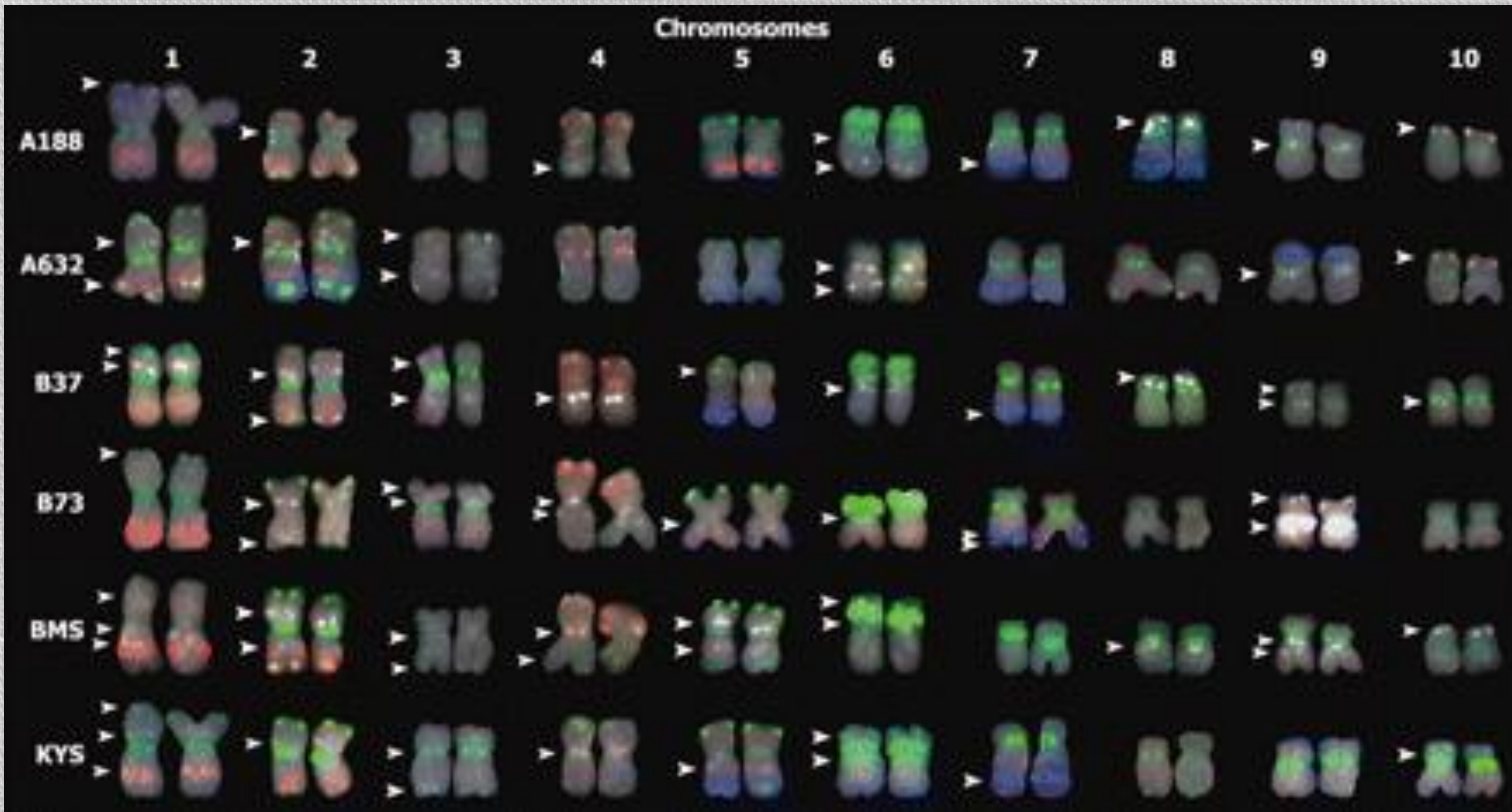
- A 6-8 kb insertion responsible for the multi-lobe structure of ox heart tomato



Cong et al., 2008. Nature Genetics 40: 800-804



Mitochondrial DNA in the nucleus of maize inbreds



Genetics 178: 47–55 (January 2008)

Mitochondrial DNA Transfer to the Nucleus Generates Extensive Insertion Site Variation in Maize

Ashley N. Lough,*,1 Leah M. Roark,*,1 Akio Kato,† Thomas S. Ream,‡ Jonathan C. Lamb,§ James A. Birchler* and Kathleen J. Newton*,2

Pararetroviruses

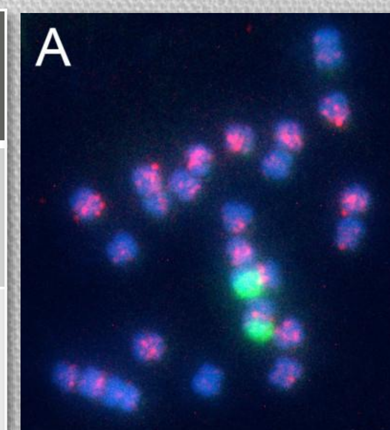
Transient integration

- Days to thousands of years

Stable integration

	Viral insert number	Virus
Rice, Indica (93-11)	74	rice tungro bacilliform
Rice, Japonica (Nipponbare)	88	rice tungro bacilliform

Tomato



- Liu et al 2012. Evolutionary force of AT-rich repeats to trap genomic and episomal DNAs into the rice genome: lessons from endogenous pararetrovirus. Plant Journal 72:817-828
- Staginnus et al. 2007 Endogenous pararetroviral sequences in tomato *(Solanum lycopersicum)* and related species *BMC Plant Biology* 2007 **7**:24

Changes in transcription factors due to SNPs

- The dwarf wheat of the Green Revolution
  - Due to a SNP in 2 TFs

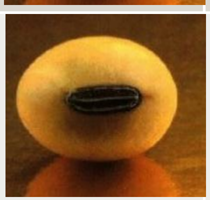
The Harvest, by Pieter Bruegel, 1565

'Green revolution' genes encode mutant gibberellin response modulators

Jinrong Peng*, Donald E. Richards*, Nigel M. Hartley, George P. Murphy, Katrien M. Devos, John E. Flintham, James Beales, Leslie J. Fish, Anthony J. Worland, Fatima Pelica, Duraialagaraja Sudhakar†, Paul Christou, John W. Snape, Michael D. Gale & Nicholas P. Harberd

256
NATURE | VOL 400 | 15 JULY 1999 | www.nature.com

Presence Absence Variation

Crop	Genes present or absent	Reference
 Maize	1000's genes different between B73 & Mo17	Lai et al., 2010 E Buckner, PC
 Potato	2 genotypes sequenced differ by 275 genes	Potato Genome Consortium 2011
 Soybean	856 genes in wild soybean that are not in domesticated soybean	Lam et al., 2010
 Soybean	4 Varieties: 133 genes found only in 1 variety and not others	McHale et al., 2012

When DNA changes were not feared

Source: *Popular Mechanics* 1961

“Conventional” Mutation breeding in changes DNA - “Gamma Gardens”

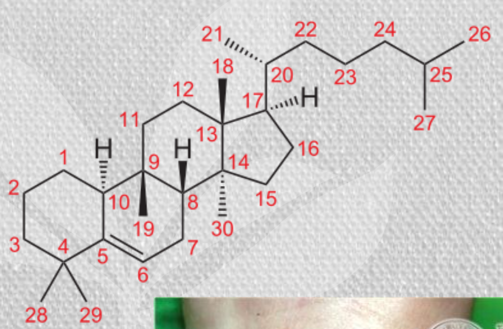
- DNA changes - mostly not characterised but include
  - 4 bp to 8 kb deletions
  - Inversions of up to 1.5 kb
  - Insertions ~200 bp
  - Frame-shift mutations
  - Premature stop codons

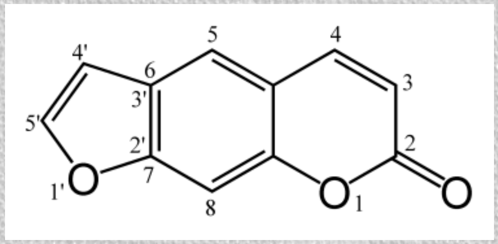
- radiation breeding has produced thousands of useful mutants and a sizable fraction of the world’s crops, including varieties of rice, wheat, barley, pears, peas, cotton, peppermint, sunflowers, peanuts, grapefruit, sesame, bananas, cassava and sorghum………..

Institute of Radiation Breeding
Ibaraki-ken, JAPAN
www.irb.affrc.go.jp/

Crop toxicity reports

- Individual fruits within a variety
  - Squash & zucchini
    - 1981 & 1982 – 22 people got ill after eating bitter zucchini
      - Plants from 2 widely grown cultivars in Australia
    - 1981 – 1 California field and 2 home gardens in Alabama reported bitter fruit
    - Due to known toxins occurring in cucurbits – cucurbitacin – an herbivore anti-feedant
- Late harvest, pest pressure, altered agronomics
  - Celery – increased furanocoumarins – contact photodermatitis
- Unusual weather conditions
  - Magnum Bonum potato in Sweden
    - 3rd most widely grown potato for 50 years
    - In 1986, turned bitter and caused gastric distress
- At least 2 toxic varieties have been bred but turn out to be known toxins pre-existing in the species





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- Rymal K.S., Chambliss O.L., Bond M.D., Smith D.A. (1984) Squash containing toxic cucurbitacin compounds occurring in California and Alabama. Journal of Food Protection 47:270-271

Toxic varieties

- Of millions of conventional varieties
  - 2 have been reported to have unintended effects from toxins
  - Dermatitis and stomach aches
- All involved elevated levels of **known** toxins
  - Part of OECD list of known plant toxins
  - When crops have known toxins, testing of new varieties has become customary
- What about unknown toxins?
- In all the history of breeding
  - A toxin that did not exist at the genus level has NEVER been reported
- Previous report that a novel toxin was found in a potato somatic hybrid (noted in an EFSA review)
  - Laurila et al., 1996. Plant Sci 118:145-155
  - Missed the fact that same toxin was previously described in some genotypes of potato
    - Jadhav et al. 1981. CRC Critical Reviews in Toxicology pp 21-104.

- Anon (1970) Name of potato variety Lenape withdrawn. Am J Potato Res 47: 103.
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- Jadhav SJ RP Sharma and DK Salunkhe. 1981. Naturally occurring toxic alkaloids in foods. Crit Rev Toxicol 9:12-104
- Laurila J, Laakso I, Valkonen JPT, Hiltunen R, Pehu E (1996) Formation of parental-type and novel glycoalkaloids in somatic hybrids between *Solanum brevidens* and S. tuberosum. Plant Sci 118: 145-155
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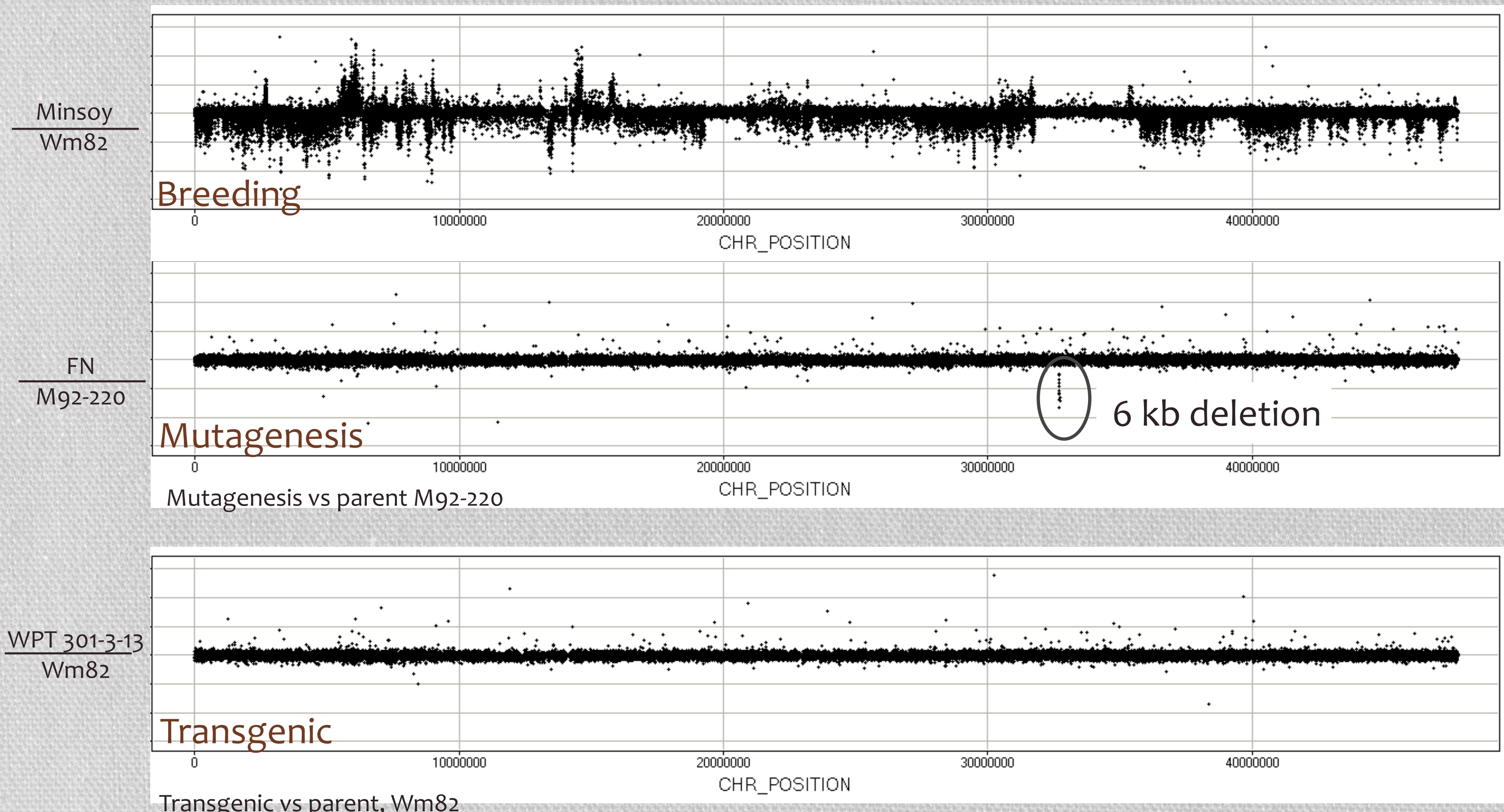
http://www.dermis.net/dermisroot/en/13121/image.htm

In all the history of breeding there is not a single report of a new toxin coming into existence.

GMO < variable than breeding
Soybean comparisons



Slide by Robert Stupar, University of Minnesota

We can now answer our original questions

- How variable is the phenotype without genome plasticity
  - Highly variability due to agronomic, climate and environment factors
- How common are gene insertions in nature
  - Found in every plant examined
    - Very common, both endogenous and exogenous genes
  - What are the consequences of those insertion
    - Have never resulted in ***de novo*** toxicity
      - May up regulate existing pathways
      - May activate pathways in a specific tissue when already present and active in the whole plant
- How common are point mutations in nature
  - Exceptionally common
  - What are the consequences
    - Despite tens of millions of known SNPs in corn there has never been a toxic corn
    - Radiation mutation of plants under conventional breeding has never produced a ***de novo*** toxin
- Is there evidence for activation of dormant pathways
  - Tissue specific activation of pathways active in the plant but dormant in the tissue has been observed.
- Is there evidence for *de novo* generation of toxins from mutations or insertions
  - Not ever despite frequent, wide spread mutation and transposition of genes

Conclusions

- The plant genome is highly plastic
- **Crops are genetically unstable**
- Variability seen in both phenotype and genotype is considerable and normal
- Wide spread insertions, deletions or SNPs in a non toxic crop have never produced a ***de novo*** toxin but may up regulate known toxins already present in a crop – as can stress related to pest pressure, climate, environment, agronomic practices
- From a purist scientific perspective, the need to regulate NBTs and the extent of any regulation considered necessary should be weighed against the now substantial body of evidence of highly plastic plant genomes and the absence of consequent hazard beyond the up regulation of production of known toxins for that genus.

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  - Sherry Flint Garcia of the USDA
  - Peter Shewry, University of Reading
  - Videos of the presentations that have provided the background material for todays talk can be found at http://www.hesiglobal.org/i4a/pages/index.cfm?pageID=3654
  - http://www.ilsi.org/FoodBioTech/Pages/2012PlantCompositionWorkshop.aspx