Trait stacking

and the likelihood of trait interactions in stacked GM crops

1785
The University of Georgia

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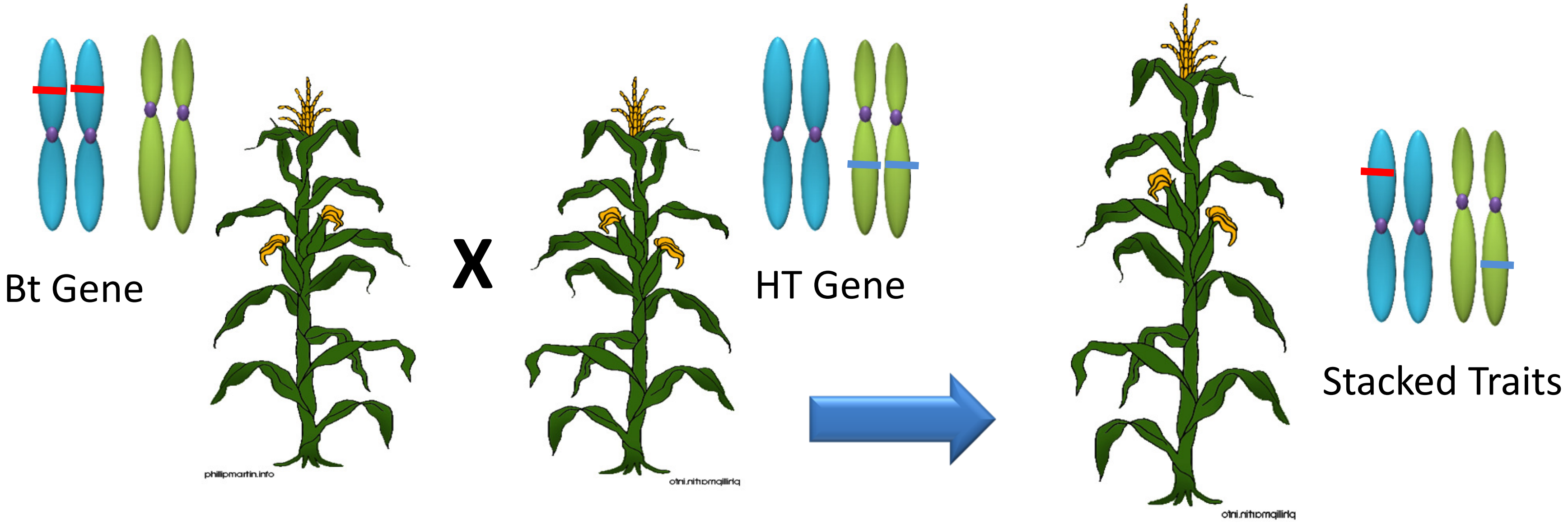
What is a breeding stack?

- Transgenic events combined by conventional crossing
  - Each transgenic event has been highly selected and received prior regulatory approval
    - Intended effects are safe
    - No unintended effects



Bt Gene

X

HT Gene

Stacked Traits

Two concerns about stacks

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Herbicide-tolerant Bt maize, Colombia

- Does stacking transgenes by crossing affect DNA stability?
- How can potential interactions between products of transgenes be assessed in a crop with stacked events?

ILSI-IFBiC Tripartite Task Force

Peer-reviewed by 20 experts around the world

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- **Academia:**
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    - University of Dundee, UK
  - Curt Hannah
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- **Industry:**
  - BASF
  - Bayer CropScience
  - Dow AgroScience
  - Monsanto Company
  - Pioneer, A DuPont Business
  - Syngenta Biotechnology


Conventional & IR/HT cotton

Plant breeding as a guide

What do we know about plant breeding & domestication?

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University of Kentucky wheat variety trials
http://www.uky.edu/Ag/GrainCrops/ID125Section3.html

Agricultural pests

Control with chemicals or with genetic resistance

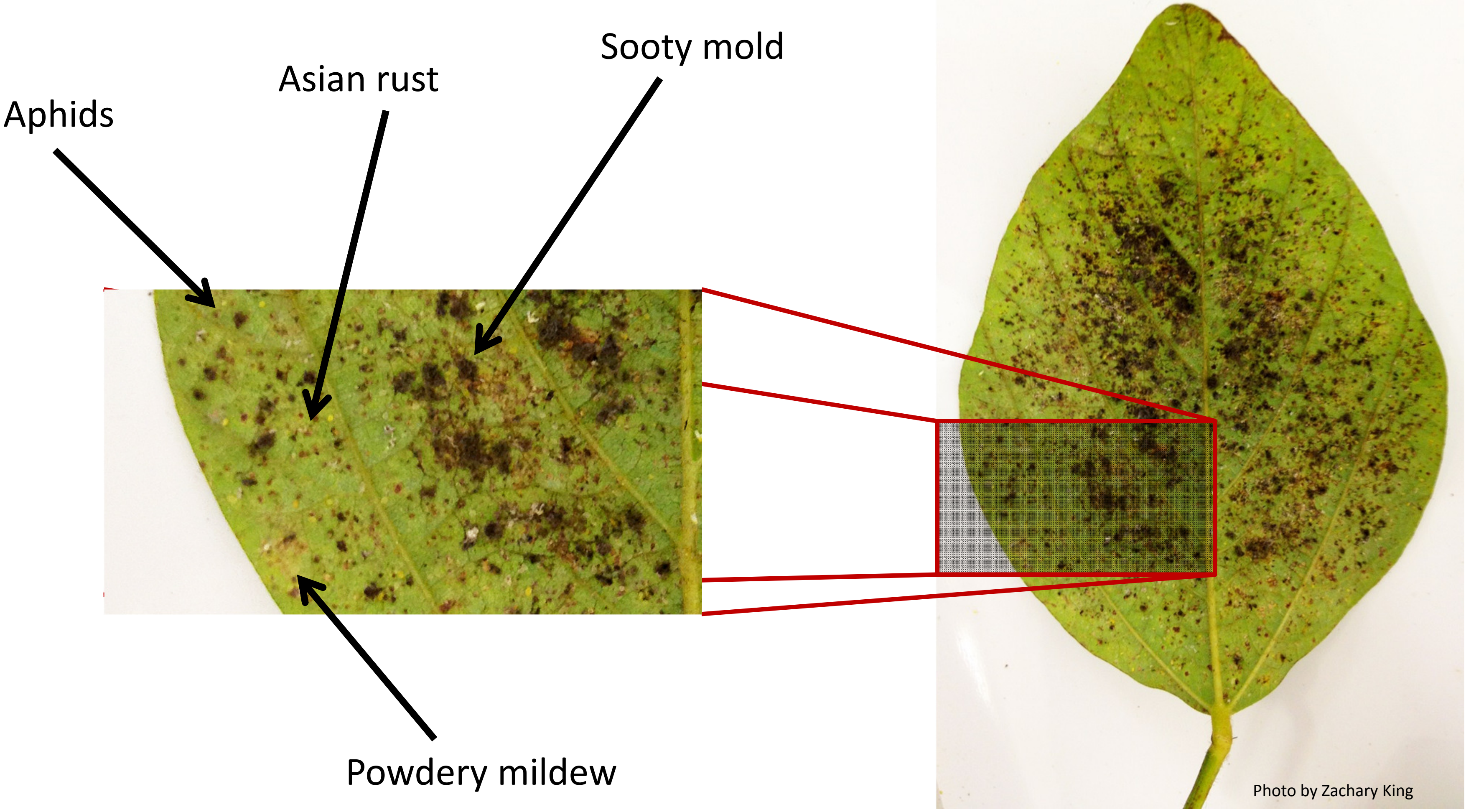

Photo by Zachary King

Conventional Plant Breeding
Stacks genes for desirable traits

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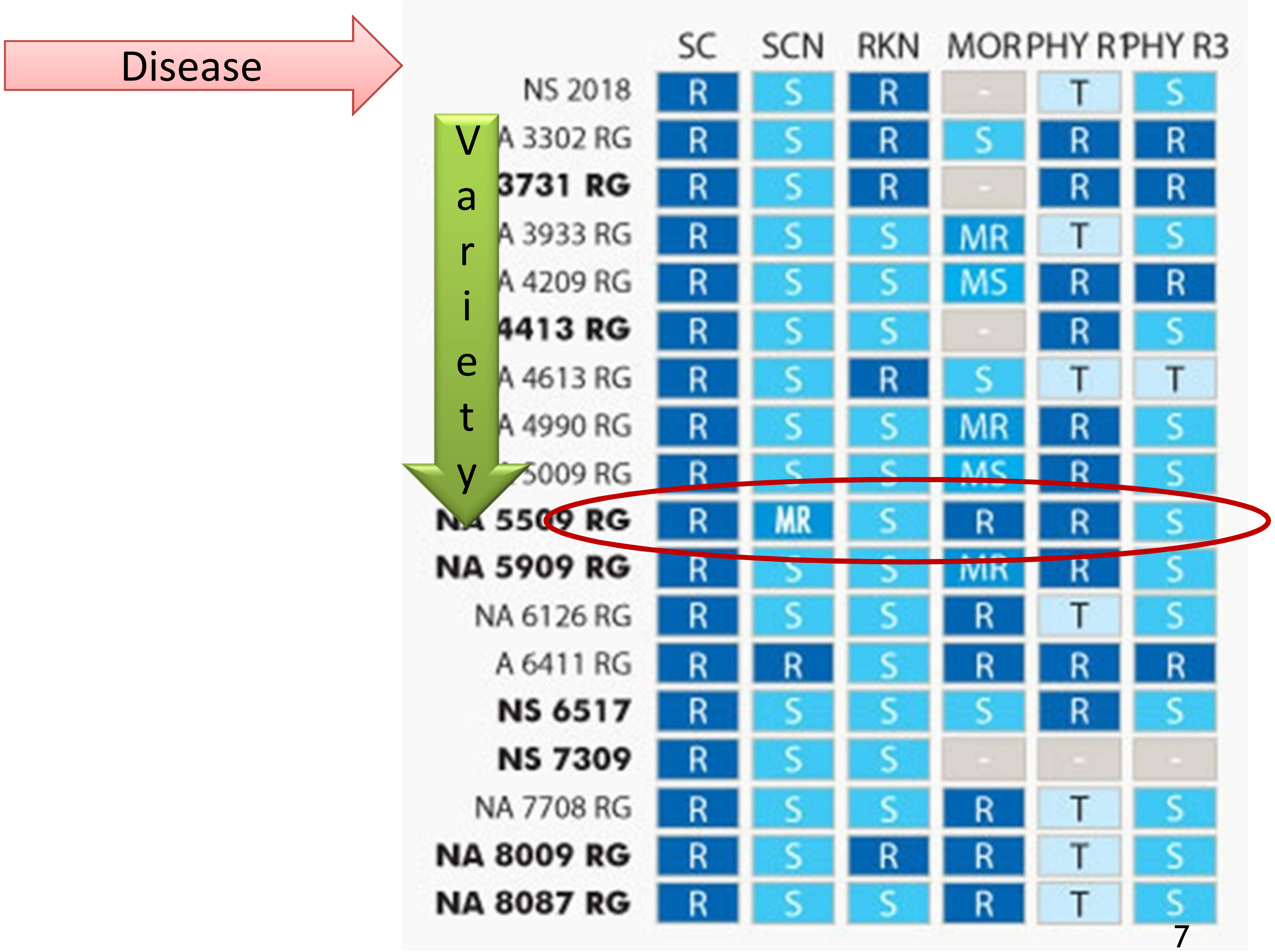

Disease

Variety

	SC	SCN	RKN	MOR	PHY R1	PHY R3
NS 2018	R	S	R	-	T	S
A 3302 RG	R	S	R	S	R	R
3731 RG	R	S	R	-	R	R
A 3933 RG	R	S	S	MR	T	S
A 4209 RG	R	S	S	MS	R	R
4413 RG	R	S	S	-	R	S
A 4613 RG	R	S	R	S	T	T
A 4990 RG	R	S	S	MR	R	S
5009 RG	R	S	S	MS	R	S
NA 5509 RG	R	MR	S	R	R	S
NA 5909 RG	R	S	S	MR	R	S
NA 6126 RG	R	S	S	R	T	S
A 6411 RG	R	R	S	R	R	R
NS 6517	R	S	S	S	R	S
NS 7309	R	S	S	-	-	-
NA 7708 RG	R	S	S	R	T	S
NA 8009 RG	R	S	R	R	T	S
NA 8087 RG	R	S	S	R	T	S

SC: Stem canker
SCN: Cyst nematdoe
RKN: Root-knot nematode
MOR: Frogeye leaf spot
PHY: Phytophthora races1, 3 and 4

Plant Breeding for disease resistance

Frogeye leafspot

R. Boerma

Conventional breeding

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1,000 F1

2,000,000 F2 - Disease & habit

50,000 F3

4,000 F4 - Quality

1,000 F5

500 F6 - Yield

50 F7

8 F8 – Regional trials

1 Variety

Elimination of undesired types

Soybean variety trial

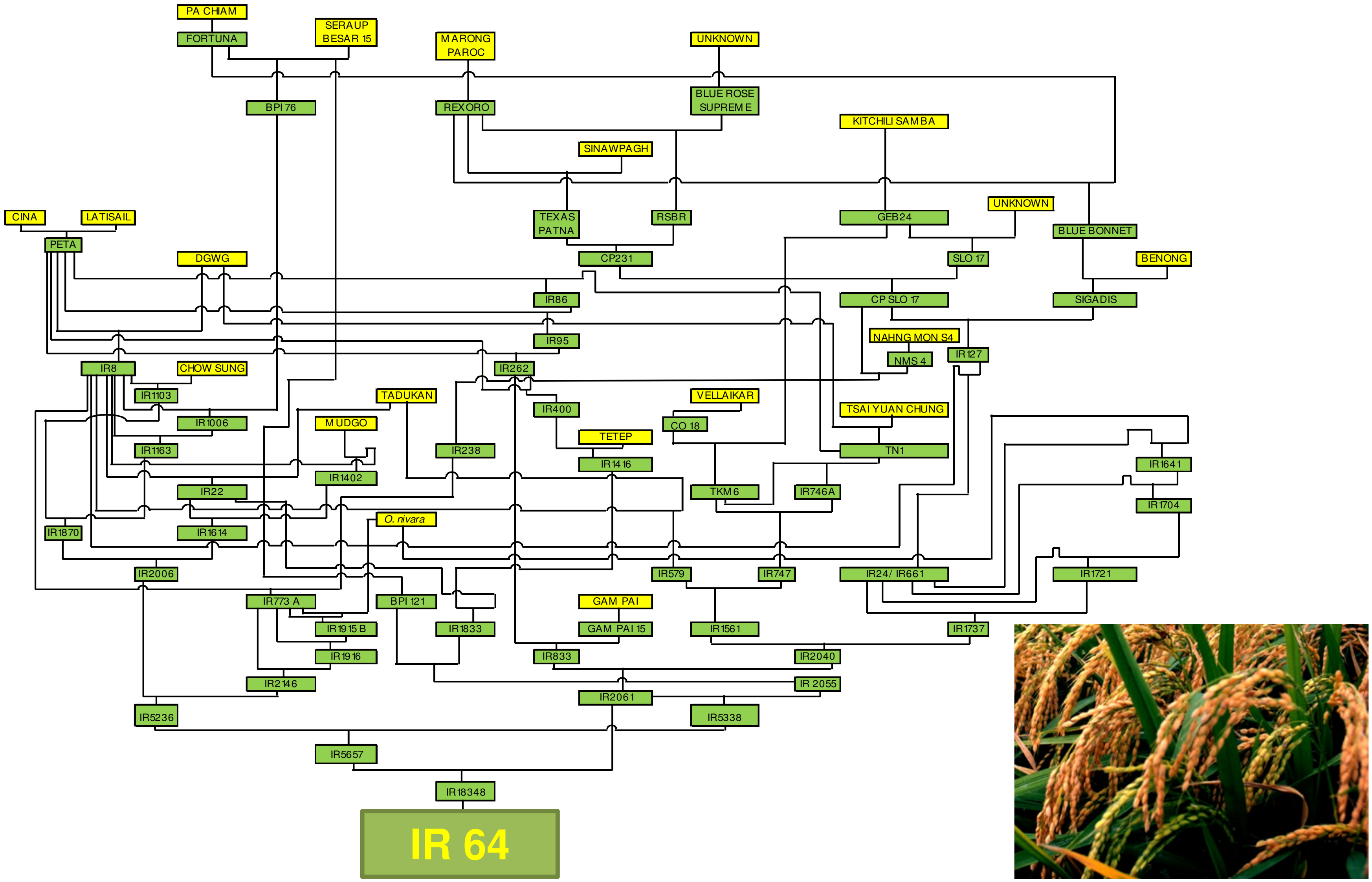
http://www.plantpath.wisc.edu/soyhealth/bsr/bsrvar.htm

Modified from: http://www.generationcp.org/plantbreeding/index.php?id=052

What happens during selection

- Select for desirable traits
  - Intended
  - Unintended
- Discard undesirable traits
  - Expected
  - Unintended

E.g., IR64 stacks traits from 20 different landraces



Question 1:
Does stacking affect DNA stability?

- How “stable” is the plant genome?
- Stability affected by stacking events?
  - I.e., are there DNA-DNA interactions that are a safety issue?

B. Rambo-Martin

What is genetic diversity like at the DNA level?

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- Changes in appearance or behavior caused by changes at DNA level
- Changes caused by breeding & domestication can be used to predict safety of transgenes
- First step is to understand what happens at the DNA-level


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Tools from genomics

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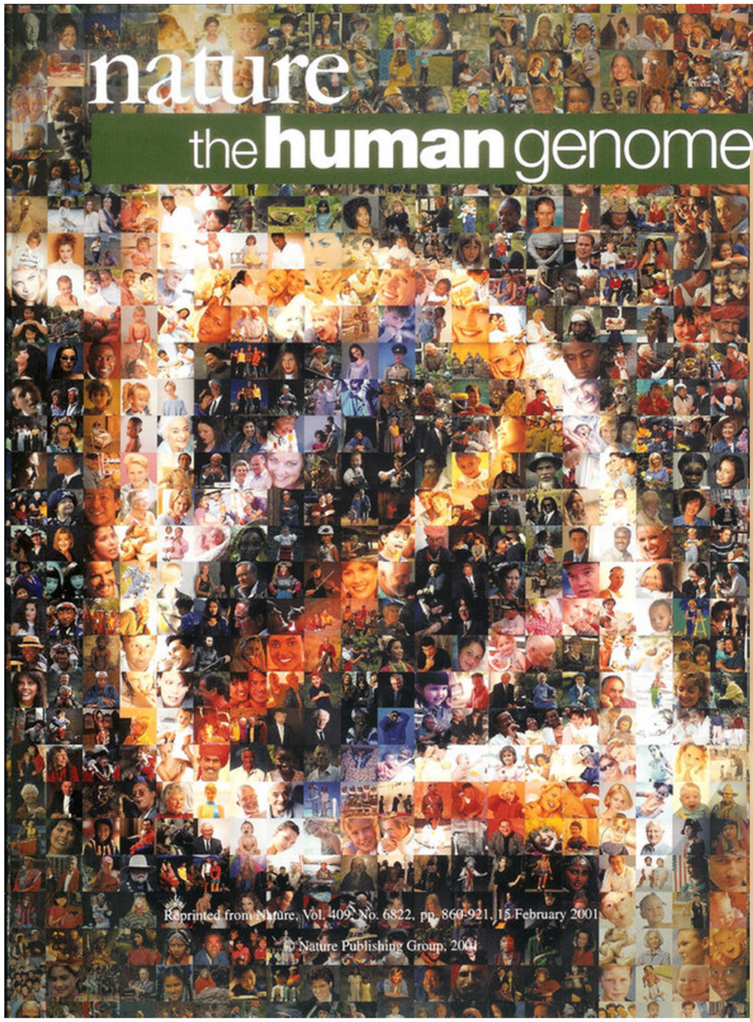
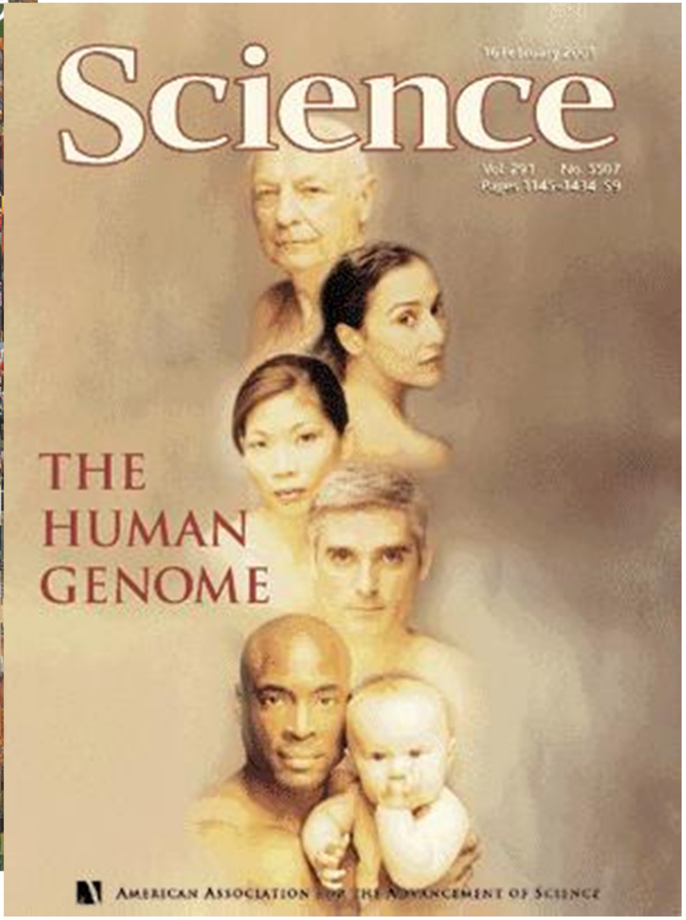
Have given a new view of the plant genome





Stability of the plant genome?

- The plant genome is NOT a fixed entity
- Plant genomes are highly variable
  - Natural mutation rate
  - Transposons & retrotransposons
    - "Jumping genes"
    - Insertions
  - Copy Number Variation
    - Duplications

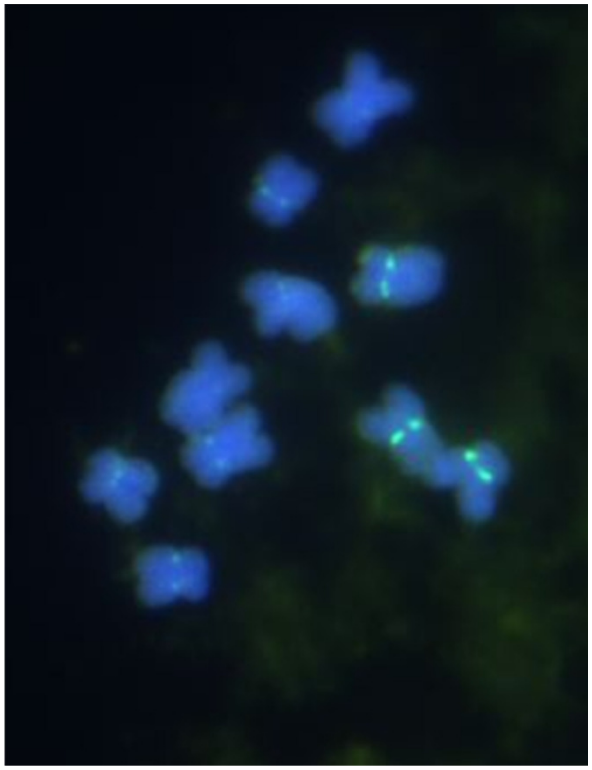


Photo by Benjamin Rambo-Martin

A. The effect of insertions

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- "Jumping genes"
  - **DNA sections** that move naturally move around the genome

Pariti island, Lake Titicaca

© Eduardo Forno

Transposable elements

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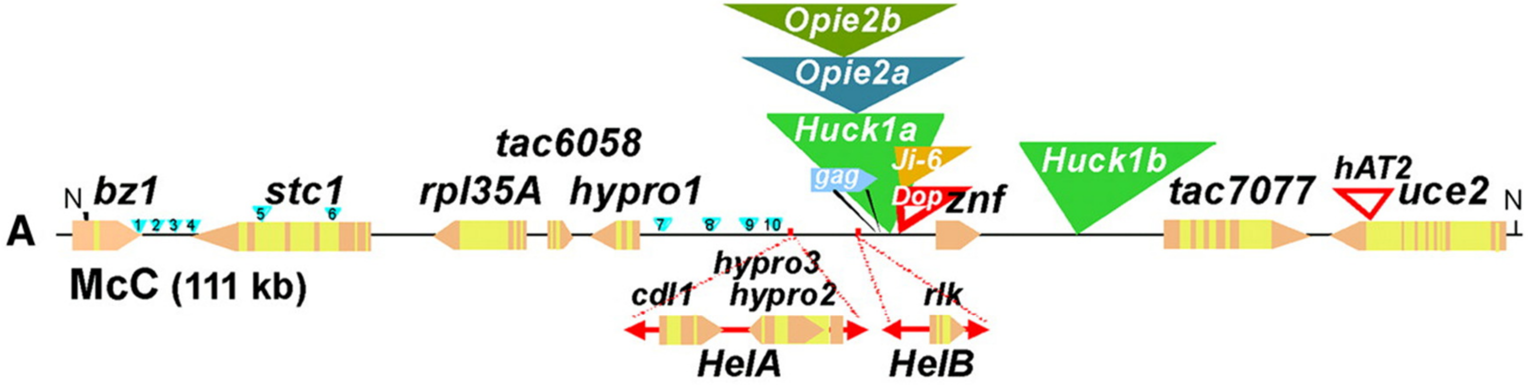
- Cacao
  - 28,798 protein-coding genes
  - 552 RNA-coding genes
  - 67,575 transposons

Argout X, Salse J, Aury J-M, Guiltinan MJ, Droc G, Gouzy J, Allegre M, Chaparro C, Legavre T, Maximova SN, et al (2011). The genome of *Theobroma cacao*. Nat Genet **43**: 101–108

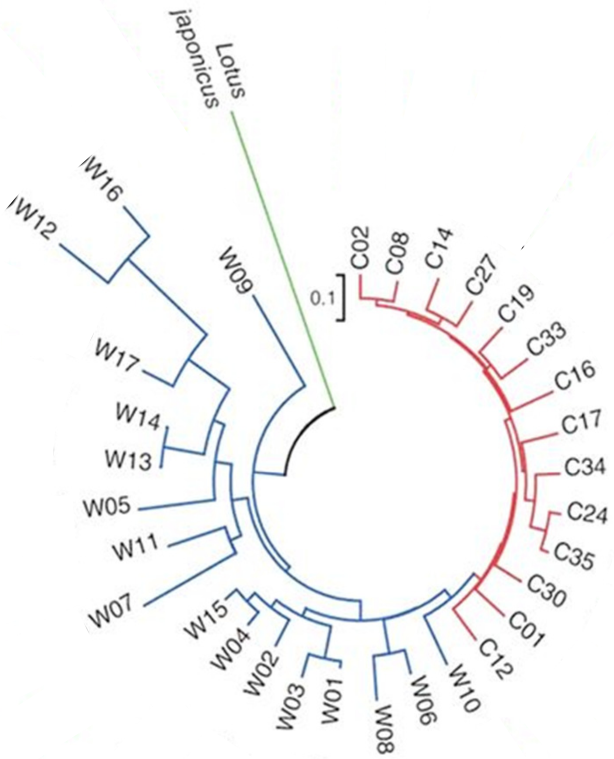
Jumping genes are common



Dooner & He. 2009. Plant Cell 20:249-258

How common are insertions?

Unique jumping gene insertions in soybean compared to reference genome



N = 25,628 unique insertions

Tian et al. 2012. Nonreference TE insertions identified in the 31 wild and cultivated soybean genomes. Plant Cell 24:4422-4436

Insertions on the farm

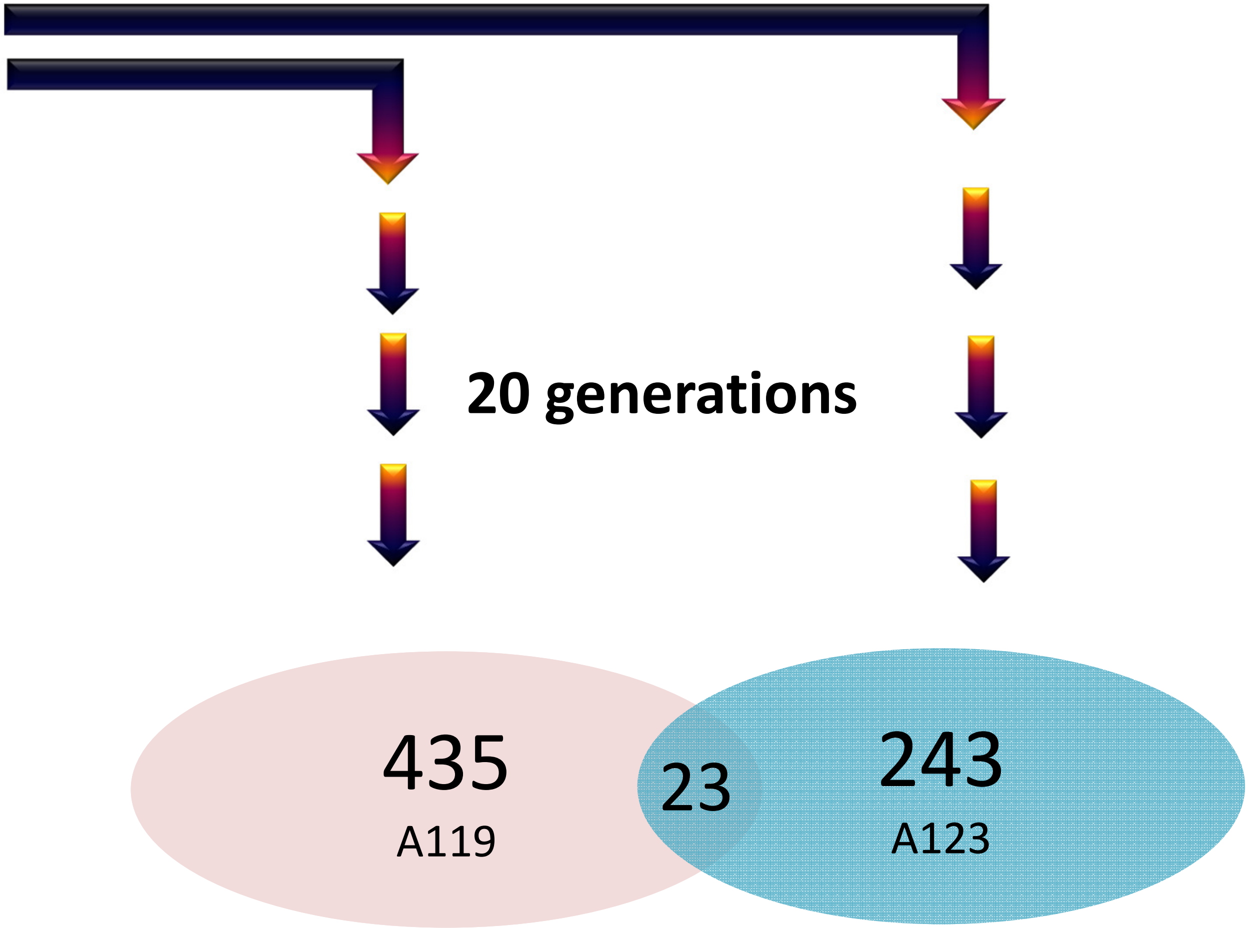

Commons.wikipedia.org

- Gimbozu
  - Ancestor to modern varieties
  - 49 to 63 new insertions per plant per generation
- Nipponbare & TN67
  - ~ 1 new insertion per 3 plants per generation

- "… our results demonstrate that *mPing* was also activated in the farmer's field."

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

Comparison of Jumping Genes after 20 generations in rice



20 generations

435

A119

23

243

A123

mPing insertions

S Wessler, L Lu, S Robb, J Stajich, unpublished

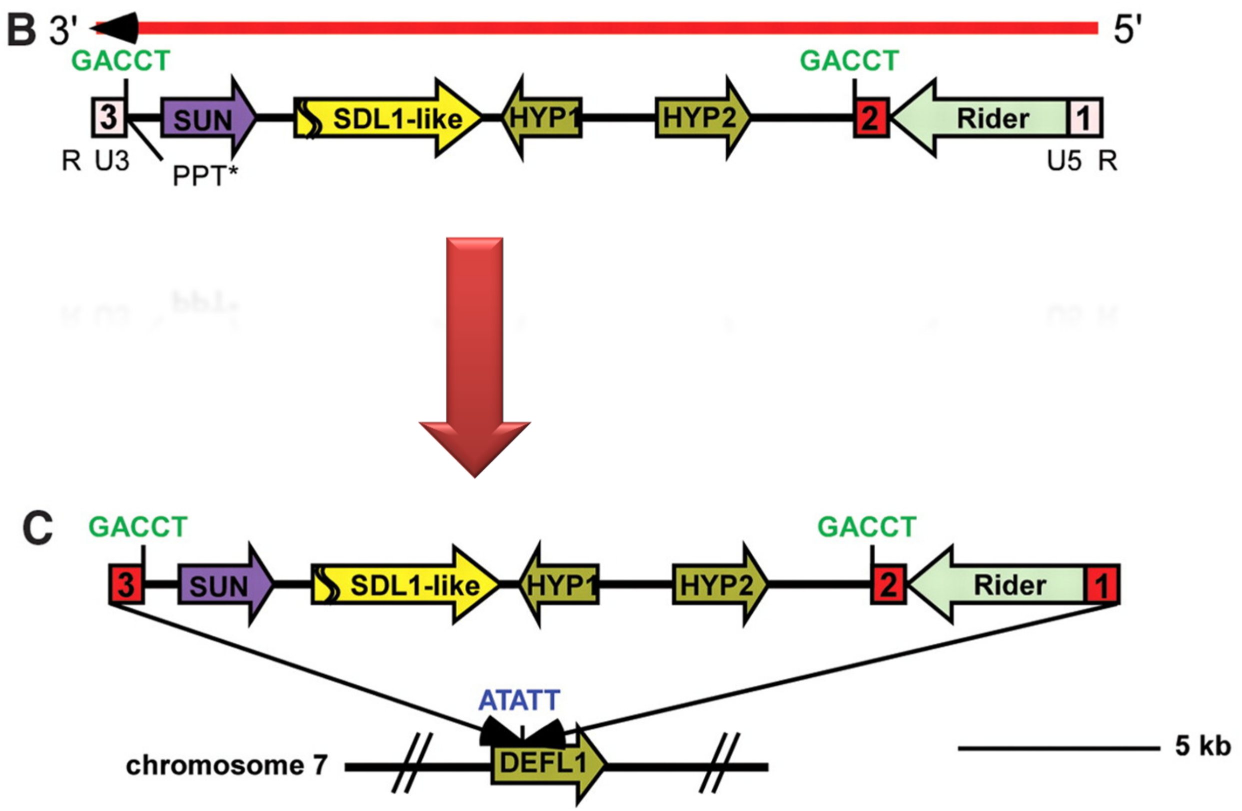
Many traits appeared in recent history

- E.g., the elongated tomato
  - Probably Spain

I. Paran, E. van der Knaap, 2007. *J. Exp. Bot.* **58**, 3841

Photo: Corbis

Elongated fruit in tomato

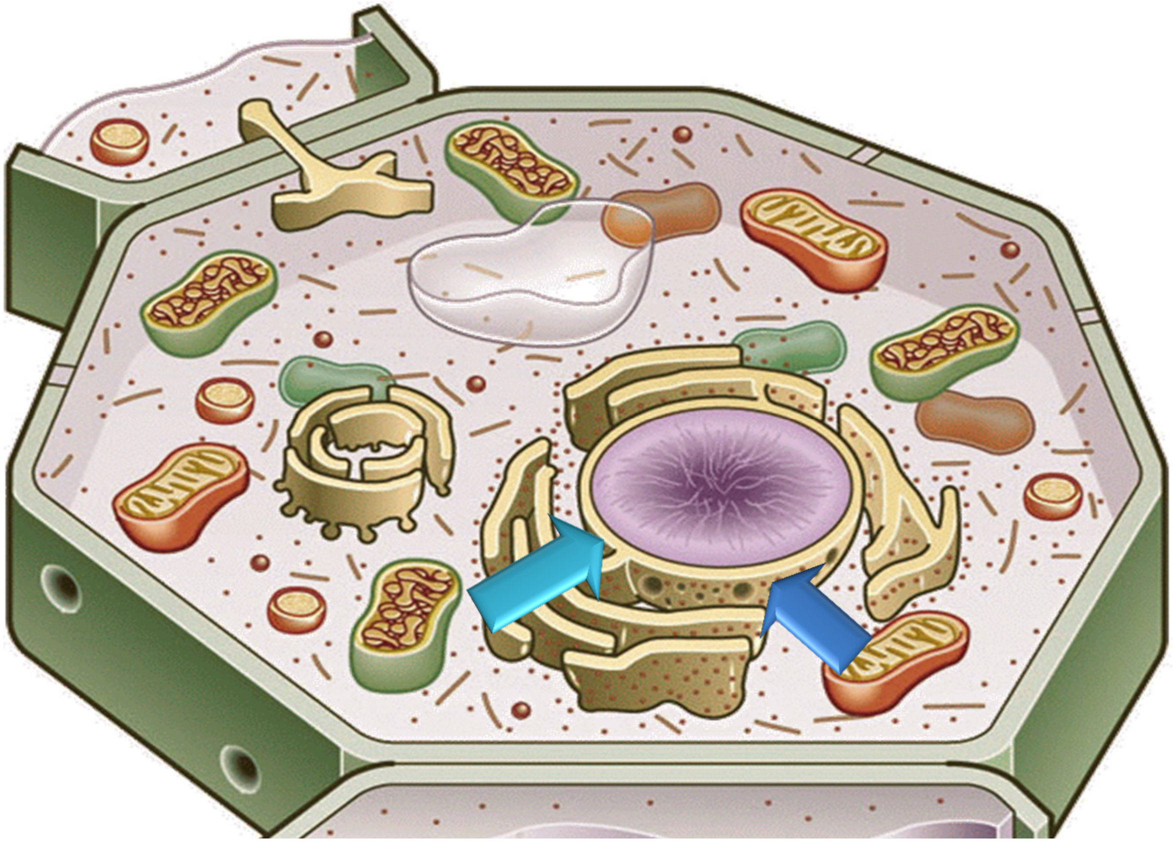


24.7 kb duplication on Chromosome 10

Movement of duplicated segment onto chromosome 7

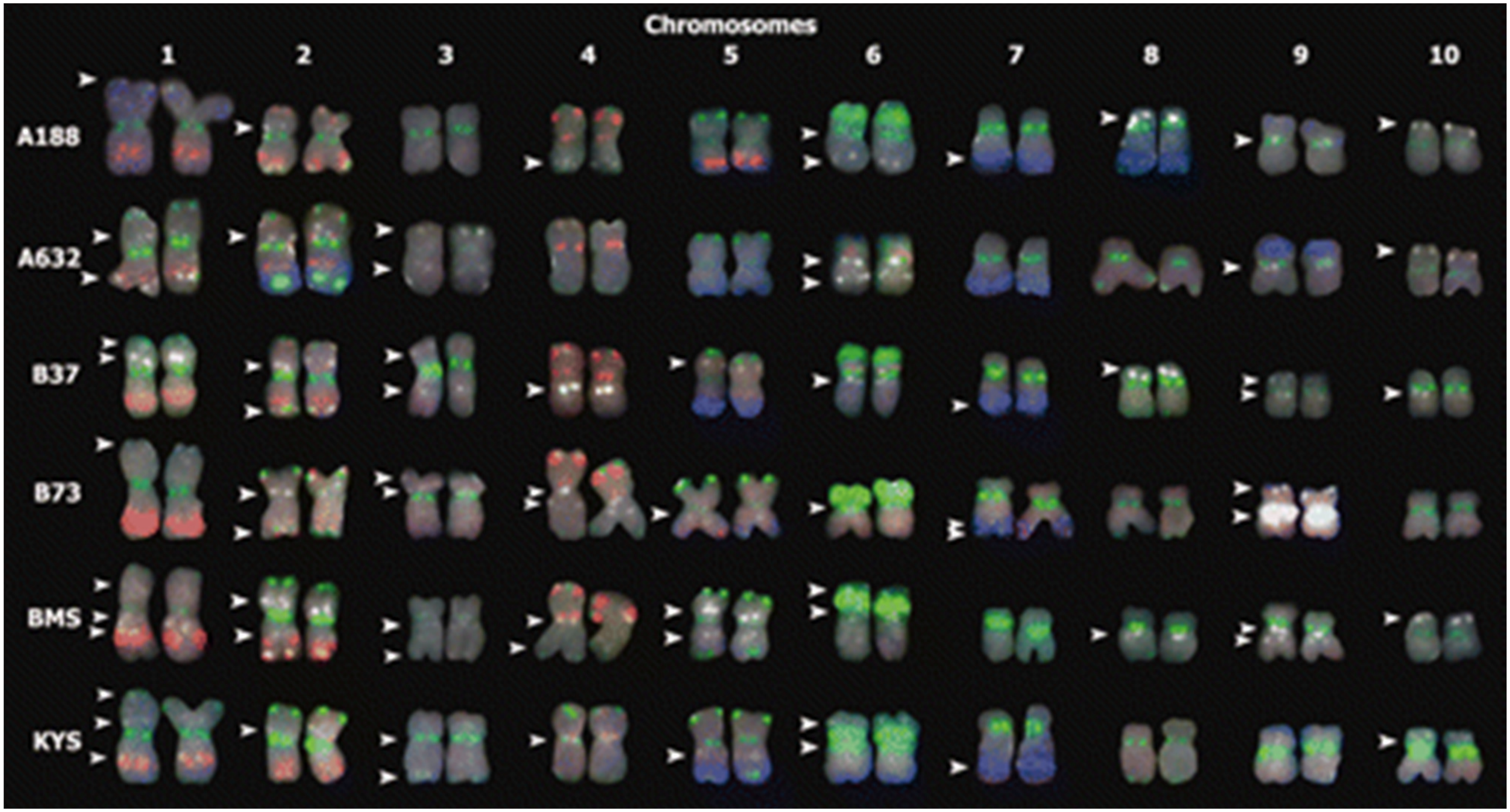
Xiao H, Jiang N, Schaffner E, Stockinger EJ, van der Knaap E. 2008. A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science* **319**: 1527-1530.

Movement of genes to the nucleus



Buchanan et al. 2000
Biochemistry & Molecular Biology of Plants
American Society of Plant Physiologists

Mitochondrial DNA in the nucleus of maize inbreds



Lough, A. N. et al. Genetics 2008; 178:47-55

Examples of natural gene transfer

- Entire genome of banana streak virus

- Rice tungro bacilliform virus

- Tobacco vein clearing virus

Review: Harper et al., 2002. Annu. Rev. Phytopathol. 40:119-136.

Photos by Corbis

B. The effect of gene duplication

rDNA copies in maize

W23	5,000 copies
B14	8,500 copies
W117	12,000 copies
"Reverse high protein"	23,100 copies

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Phillips, 1978

High level of natural duplication

- Polyploidy
- Gene families
- Transposable elements
  - BARE1 - barley
    - 50,000 copies per genome
  - Bis-1 – wheat
    - 5% of genome
  - Ping/Pong – rice
    - >98,000 copies per genome

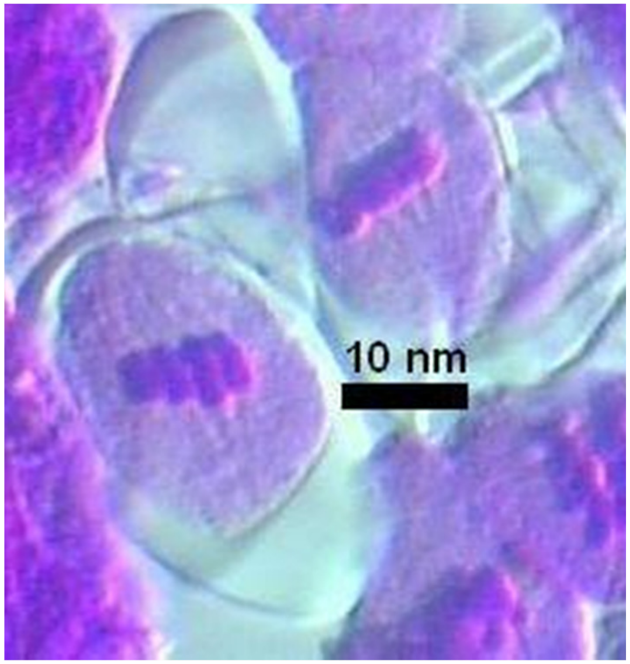
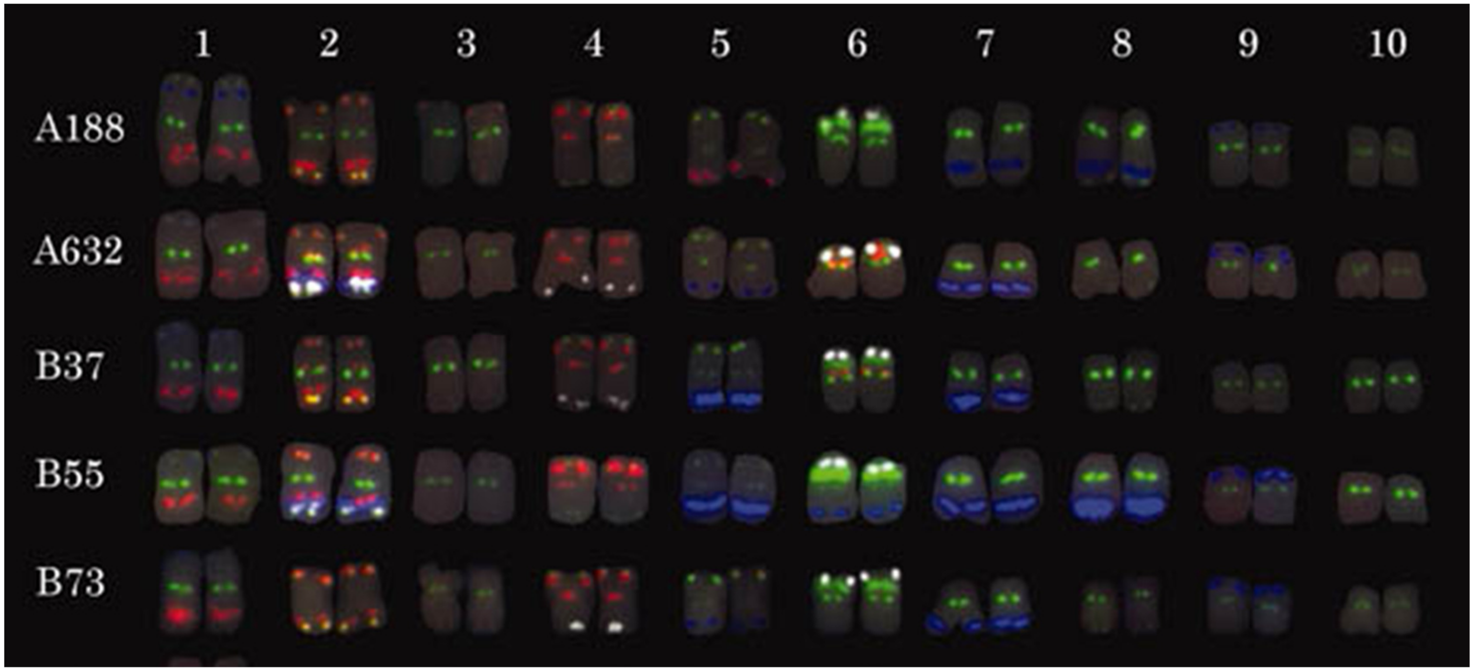


Photo by Aaron Hoskings

Genome variability

- Random transposon movement and imperfect replication of repeats results in intraspecific genomic differences



Kato et al., 2004. PNAS 101:13554-9

Differences in DNA (pg/2C)

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Soybean (4%)

= 34 million base pair difference in DNA

Hardee	2.86
Jupiter	2.83
Aojia	2.79
Pando	2.71
McCall	2.68
Maple Presto	2.51

Maize

- Palomero toluqueño has 22% less DNA than B73

- Graham et al., 1994. Theor. Appl. Genet. 88:429-432
- Vielle-Calzada et al., 2009. Science 326:1078

The worst that could happen due to genomic instability

- Loss of transgene expression
  - Commercial issue, not safety issue
- Should become apparent in seed production fields

http://www2.dupont.com/Media_Center/en_US/assets/images/releases/nr_Pioneer081109_Utica_IL_0009.jpg

Genomic Stability of Stacked Events

- Does the stacking of events alter DNA in a way that would impact safety?
  - Every situation that causes concerns in stacks happens in nature
- Conclusions:
  - There is no novel concern
  - Genomic analysis of stacked event products does not contribute to safety
- Focus on possible interactions between transgene products

Editor's Choice

Editor's Choice: Crop Genome Plasticity and Its Relevance to Food and Feed Safety of Genetically Engineered Breeding Stacks[1]

Genetically engineered (GE) stacks, combinations of two or more single transgenic events (i.e. single-locus insertions) that have been produced by crossing sexually compatible parents, are an important and growing sector of the crop seed market. Stacked traits covered 26% of the global transgenic crop area in 2011 and were the fastest growing trait group, with a 31% increase in the area planted compared with 2010 (James, 2011). Stacked traits already dominate the market in some regions. For example, 95% of the cotton (*Gossypium* spp.) grown in Australia during 2011 had both herbicide tolerance and insect resistance traits (James, 2011). Worldwide, at least 12 countries are now growing stacked varieties, of which nine are developing countries (James, 2011). The rapid adoption of GE stacks has focused attention on whether the safety of such products differs from that of the individual events.

At issue is whether combining two or more events via conventional breeding creates changes that require additional safety assessment, even though the safety of each event in the stack has been assessed previously. The two main concerns are (1) whether combining two or more events into a plant by conventional breeding increases genomic instability and (2) whether potential interactions between the products of the transgenes in GE stacks impact safety. This paper draws on insights from plant breeding, describes the plasticity of conventional plant genomes over generations of crossing and selection, and considers the implications of event stacking on food and feed safety in the context of the normal plant genome.

The term GE is used here to distinguish the process of specific, intentional, and directed physical modification of the genome of a plant from random genetic modifications that occur in conventional breeding or by mutagenesis. The term GE is preferred over the term "genetically modified" (commonly referred to as GM) for these reasons. The term "event" refers to a single-locus insertion of recombinant DNA into the host genome regardless of the number of genes contained on the inserted piece of DNA. The term "conventional breeding" refers to methods of crossing plants with desired characteristics to generate offspring combining those desirable characteristics. These characteristics may include both non-GE and GE traits.

SCOPE

This paper focuses on (1) the potential of transgenes to alter genome stability and (2) the potential risks to food and feed safety associated with genome instability. A companion paper focuses on potential interactions that can occur from transgene stacking (H.Y. Steiner, C. Halpin, J.M. Jez, J. Kough, W. Parrott, L. Underhill, N. Weber, and L.C. Hannah, unpublished data). The potential environmental impact that may arise from the cultivation of crops with GE stacks is outside the scope of these articles. Crops containing a single event, that carry multiple traits that are introduced simultaneously (i.e. molecular stacks resulting from cotransformation), or that are produced by retransformation of an event require a de novo safety assessment, as is customary for all new events, and, hence, are also outside the scope of this paper.

STACKING OF ENDOGENOUS GENES IS COMMON IN PLANT BREEDING PROGRAMS

Plant breeding is a major underpinning of modern agriculture, as it creates varieties containing multiple desirable traits through the stacking of both known and many unknown genes. While increasing yield potential is a major objective, protecting yield potential (i.e. breeding for resistance to biotic and abiotic stresses) is also critical. Many stress resistance genes have come from related species such as wild relatives of crop plants. Hajjar and Hodgkin (2007) reported that conventional breeding efforts in 19 of the world's major crops had incorporated 111 genes from wild relatives into new varieties over the previous 20 years. Eighty percent of these genes confer disease resistance; the remainder control abiotic stress resistance or quality traits (Hajjar and Hodgkin, 2007).

Modern non-GE crop varieties differ mainly from their predecessors by the incorporation and stacking of genes from distant relatives. For example, IR8 rice (*Oryza sativa*), released in 1966, is resistant to the green leafhopper (*Nephotettix* spp.) and moderately resistant to salinity, rice blast, and phosphorus deficiency. Just 11 years later, IR42 was released, which possessed resistance to multiple diseases (rice blast, *Grassy stunt virus, Rice tungro virus, Ragged stunt virus*, and bacterial blight), pests (brown planthopper [*Nilaparvata lugens*], stem borer), and abiotic stresses (nitrogen deficiency, zinc deficiency, iron deficiency, alkalinity, and iron toxicity; International Rice Research Institute, 1981). Today, many seed catalogs list the multiple resistance traits present in each variety. Although the genetic and biochemical bases for these traits

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[1] This work was supported by the members of a task force of the International Life Sciences Institute International Food Biotechnology Committee: BASF Plant Science, Bayer CropScience, Dow AgroSciences, Monsanto Company, Pioneer Hi-Bred International, and Syngenta Biotechnology.

www.plantphysiol.org/cgi/doi/10.1104/pp.112.204271

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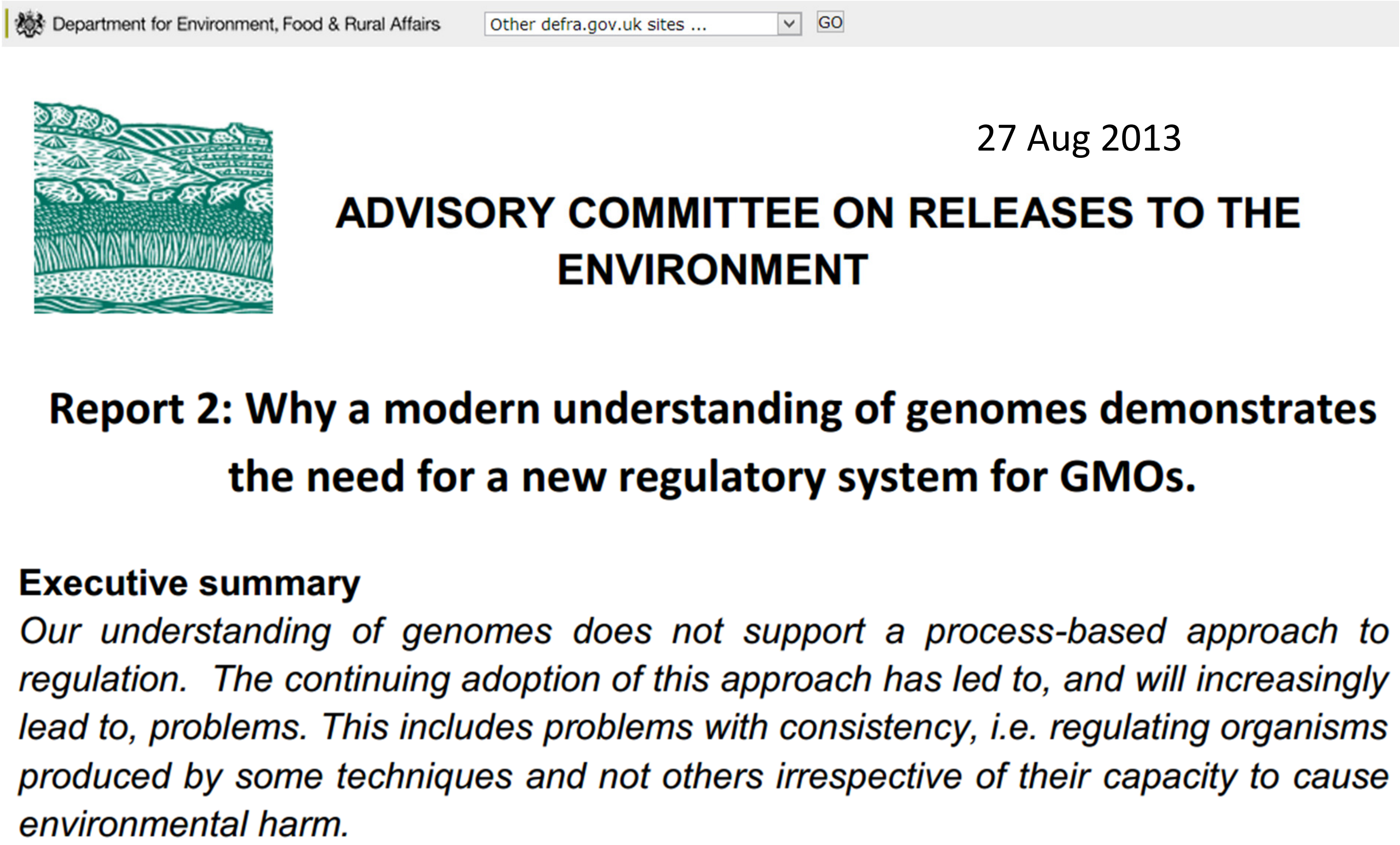
Plant Physiology December 2012. 160:1-12



27 Aug 2013

ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Report 2: Why a modern understanding of genomes demonstrates the need for a new regulatory system for GMOs.

Executive summary

Our understanding of genomes does not support a process-based approach to regulation. The continuing adoption of this approach has led to, and will increasingly lead to, problems. This includes problems with consistency, i.e. regulating organisms produced by some techniques and not others irrespective of their capacity to cause environmental harm.

Question 2:
Interactions in Stacked Events

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- Interactions of transgene products
- Biochemical and metabolic changes caused by the different transgenes are known
  - It is possible to make predictions on possible interactions between traits in the stacked event
  - Hypothesis-driven assessment
    - Interaction does not immediately mean a safety risk
  - Case-by-case approach

➢ **Is it expected or probable that the products of the transgenic events will interact?**

➢ **Could such interaction cause a safety risk?**

Another look at traditional plant breeding

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- Provides a baseline from which to evaluate interactions in transgenics

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Presence Absence Variation

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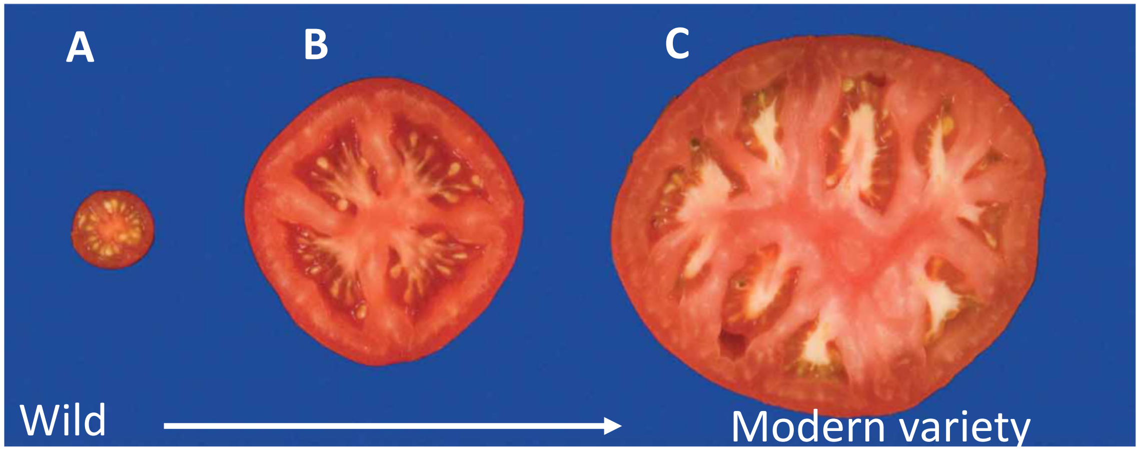
Crop	Genes present or absent	Reference
 Maize	1000's genes different between B73 & Mo17	Lai et al., 2010 E Buckner, PC
 Potato	2 genotypes sequenced differ by 275 genes	Potato Genome Consortium 2011
 Soybean	856 genes in wild soybean that are not in domesticated soybean	Lam et al., 2010
 Soybean	4 Varieties: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

- Crossing a variety without a gene to one with a gene
  - Creates the same type of interactions as adding a transgene

Changes in transcription factors



- Progression of fruit size increase during tomato domestication.
  - Due to YABBY- like transcription factor
  - 50% increase in fruit size

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

Changes in transcription factors

- The dwarf plants of the Green Revolution were based on plants that had a mutation in a TF
  - Better fertilizer response
  - Less lodging

The Harvest, by Pieter Bruegel, 1565

Peng et al. 1999. ‘Green Revolution’ genes encode mutant gibberellin response modulators. Nature 400, 256–261.

Normal and dwarf wheat

Conventional plant breeding

Base line for interactions -

- Interactions always occur in conventionally bred crops
  - – Eg, hybrid vigor (heterosis)
  - – Basis not known and, therefore, not testable

- Genetic changes from transgenes are known
  - It is possible to make hypotheses on possible interactions

plantandsoil.unl.edu

Interactions in Stacked Events

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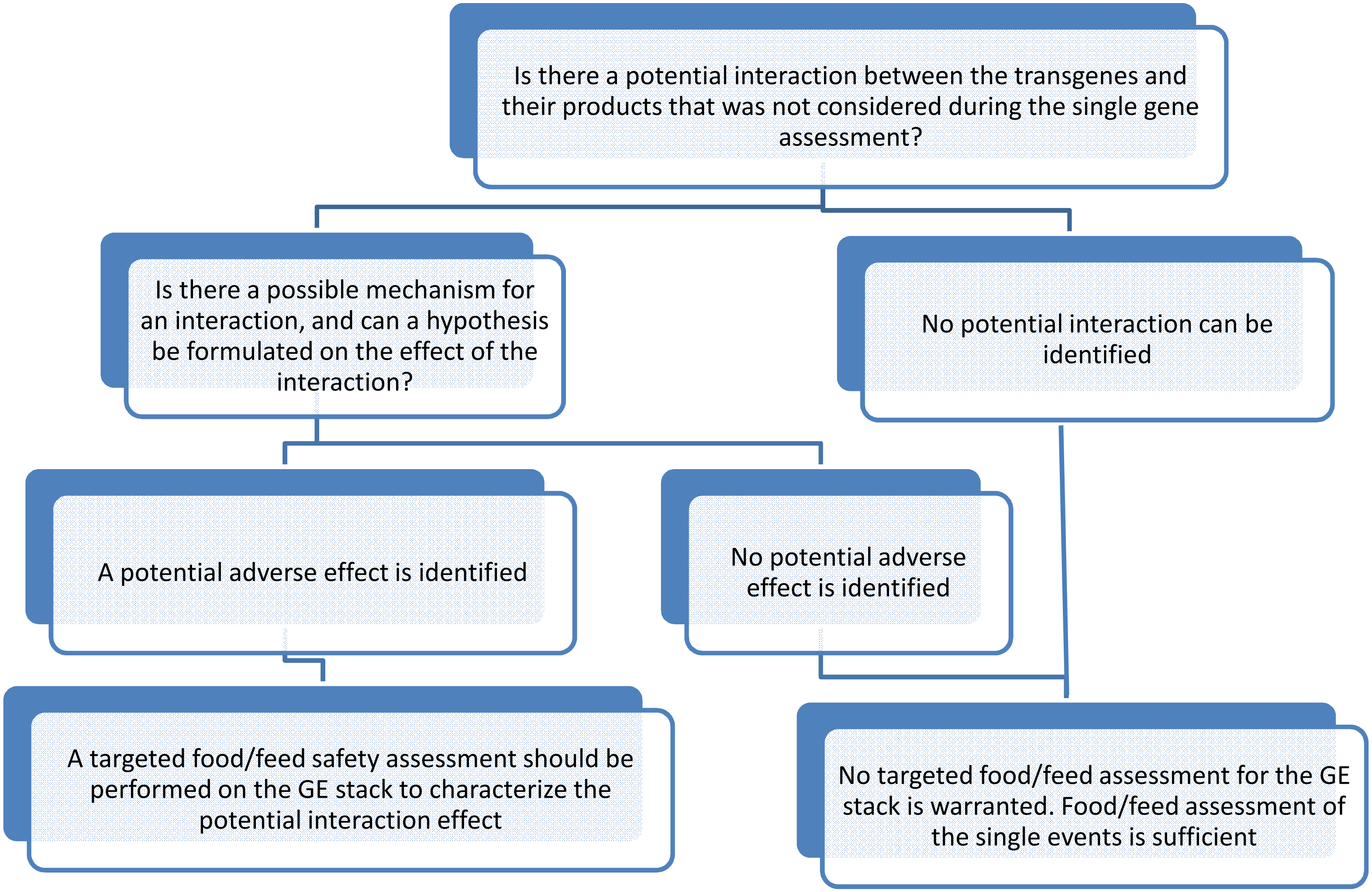
- Guiding questions:
  - Is a protein formed?
  - Can proteins interact?
  - Cell compartment?
  - Are the proteins enzymes?
  - Affect same metabolic pathway?
  - Are gene products translocated?
  - Do gene expression patterns overlap?


IR/HT maize in Honduras, 2011

Putting it all together



Is there a potential interaction between the transgenes and their products that was not considered during the single gene assessment?

Is there a possible mechanism for an interaction, and can a hypothesis be formulated on the effect of the interaction?

No potential interaction can be identified

A potential adverse effect is identified

No potential adverse effect is identified

A targeted food/feed safety assessment should be performed on the GE stack to characterize the potential interaction effect

No targeted food/feed assessment for the GE stack is warranted. Food/feed assessment of the single events is sufficient

Example 1:
Insect resistance and herbicide tolerance

- Different biochemical pathways; located in different cellular locations
  - Low probability of interaction
- Prior safety assessments sufficient
- Additional safety assessments not warranted

Example 2:
Enzymes or substrates in same metabolic pathway

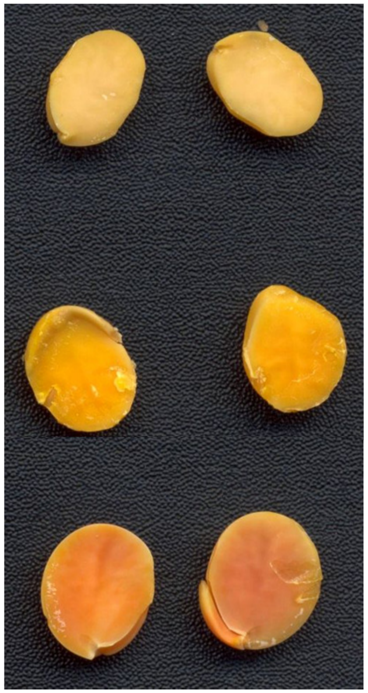
- Yes = Possibility of interaction
- Hypothesis-based information to characterize the nature of any potential hazard from the interaction
- Depending on the possible hazard, may need targeted assessment of the stack
  - If product is well known, no new assessment needed
  - Eg, carotenoids for aquaculture

Carotenoid biosynthesis

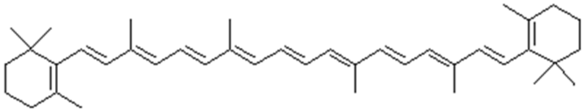
CrtB from *Pantoea ananatis*

Endogenous enzymes in soybean embryo

geranylgeranyl diphosphate *crtB* → phytoene → ε -carotene → lycopene → **β carotene**



β carotene

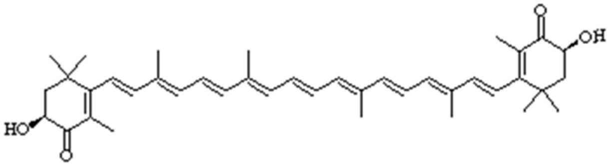


CrtS frm *Xanthophyllomyces dendrorhous*

or

Adketo1 from *Adonis aestivus*

Astaxanthin



Presence of an interaction is not an automatic safety issue

- Does the interaction result in a novel product?
  - Not a safety issue if it does not

Example 3:

Subunits of same enzyme

- ATP + glucose-1-phosphate => ADP-glucose + PP
- ADP-glucose is substrate for starch synthesis
- Enzyme is a heterotetramer, two different subunits
- Insertion of two transgenes for two subunits leads to a protein-protein interaction
- More starch, no safety issue

Example 4:

Broad plant responses and transcription factors

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- Events previously assessed as safe
- Traits mirror traditionally bred traits
- No reasonable expectation for interaction
- No hypothesis for hazard exists
- Prior safety assessments sufficient
- Additional safety assessments not warranted

Example 5 (*hypothetical*): An interaction with safety concerns

- Events previously assessed as safe
- Transgene 1:
  - Elevates levels of pre-existing cyanogenic glycosides for pest resistance
  - Levels not of toxicological concern
- Transgene 2:
  - B-glucosidase targeted to vacuole
  - Normally not toxic due to the lack of substrate in the vacuole
- Upon chewing, the two get mixed and cyanide is formed
- Additional safety assessment required

Trait interactions and safety

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- If there is a potential for gene products to interact based on prior trait knowledge:
  - And if the interaction lead to a potential adverse effect on safety (case-by-case)
    - Eg. Novel metabolic pathways
  - Might require targeted food/feed assessment of the stack
- If no reasonable expectation for interaction:
  - No hypothesis for hazard exists
  - Food/feed safety assessments of the single events are sufficient

Plant Physiology 2013. 161:1587–1594

Editor's Choice

Editor's Choice: Evaluating the Potential for Adverse Interactions within Genetically Engineered Breeding Stacks[1]

Plant breeding has a long history of developing varieties with desirable traits in response to the needs of both growers and consumers. Although the bases for most of these traits are not known genetically or biochemically, conventional breeding combines these multiple traits to create new hybrids and stable varieties that are safe and not generally subject to safety assessment. With the advent of genetic engineering, a tool for incorporating additional traits has become available to plant breeders. The safe application of genetic engineering to food and feed crops is widely acknowledged as a useful tool in addressing global agricultural challenges, including population growth and climate change. As used here, the term genetically engineered (GE) stack refers to a plant in which two or more transgenic events (i.e. single-locus insertions) that have been separately assessed for safety have been combined by conventional breeding (Table I). In recent years, increasing numbers of GE stacks have been planted, the first of which offered combinations of insect and herbicide tolerance genes to combat a wider range of pests and weeds than covered by the single events (Que et al., 2010; James, 2011).

Two main questions arise when considering the food and feed safety of GE stacks: (1) does incorporation of more than one event increase genomic instability, and (2) can potential interactions between the products of the combined events impact safety? A related paper considered the stacking of events in light of the plasticity of plant genomes and concluded that enhanced genetic instability from a transgene or from common sequences in two or more transgenes is remote (Weber et al., 2012). This paper addresses the second question of potential interactions between events and their products combined in a stack, reviews the basic principles of plant breeding and its history of safe use, and extends these principles to the feed and food safety of events combined through the same processes used in conventional breeding of non-GE plants. Potential environmental impacts are outside the scope of food and feed safety.

The new varieties developed through modern biotechnology are identified by a number of terms, including genetically modified (GM), GE, transgenic, biotech, recombinant, and plants with novel traits. The term GE is used here as defined by Weber et al. (2012). For these reasons, the term GE is preferred over the term GM.

There are many methods encompassed by the general term conventional breeding, including wide crosses and selection, mutagenesis, and somaclonal variation. When the parental species are not closely related, the cross may be facilitated by embryo rescue, somatic hybridization, or x-ray-induced translocations.

The term interaction, as used in this paper, refers to an effect, such as a new or modified metabolic activity, resulting from a combination of transgenes. An example of an interaction is protein-protein binding resulting in a novel effect only seen with a specific combination of proteins, for instance, protein cofactors or subunits for the same enzymatic complex or subcellular metabolic binding reaction. Examples can also include a direct metabolic interaction that would inhibit or activate components in a metabolic pathway shared by the proteins newly combined in the GE stack or components of independent metabolic pathways that indirectly interact by way of a common metabolite. Thus, interactions within GE stacks generally refer to the metabolic or physiochemical interplay between the products of transgenes or between the product of one transgene and the second gene, rather than between the two genes themselves.

Interactions and Plasticity Are Ubiquitous and Important Phenomena in Conventional Breeding

Conventional breeding has a long history of safe use despite the presence of antinutritional factors, toxins, and allergens in crops. There is no evidence that a random genomic change in a crop has resulted in a novel food or feed safety issue (Weber et al., 2012). Historically, humans have selected desirable traits that arise from the crop's genomic plasticity and interactions between genes. As breeding became more advanced, new methods were applied to select and combine desired traits, which also modify the genome as a consequence.

Plants produce a multitude of metabolites that provide various functions. These include signaling activities in response to environmental stress or attack from plant pathogens and pests. Some metabolites have beneficial effects, while others are toxic when fed at high levels to sensitive animal species (Ames et al., 1990). Although particular metabolites tend to be specific to some plant families, there are metabolites of concern in a number of common food and feed crops, including apple (*Malus domestica*), apricot (*Prunus armeniaca*), *Brassica* spp., celery (*Apium graveolens*), cucumber (*Cucumis sativus*), lima bean (*Phaseolus lunatus*), potato (*Solanum tuberosum*), cherry (*Prunus avium* or *Prunus cerasus*), and sorghum (*Sorghum bicolor*; Beier,

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Overall conclusions

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- Genome stability is not affected by the stacking of events and should not be assessed
  - DNA:DNA interactions
- The need to assess potential interactions from gene products between events depends on the type of traits combined
- Any assessment of gene product interactions should be targeted to the introduced traits and be hypothesis-driven

To say it more simply

- Stacking of most transgenes is as safe as stacking traits in conventional breeding
  - Only rare combinations need additional safety assessment


IR/HT cotton, Colombia 2011

GM events by region of origin of development

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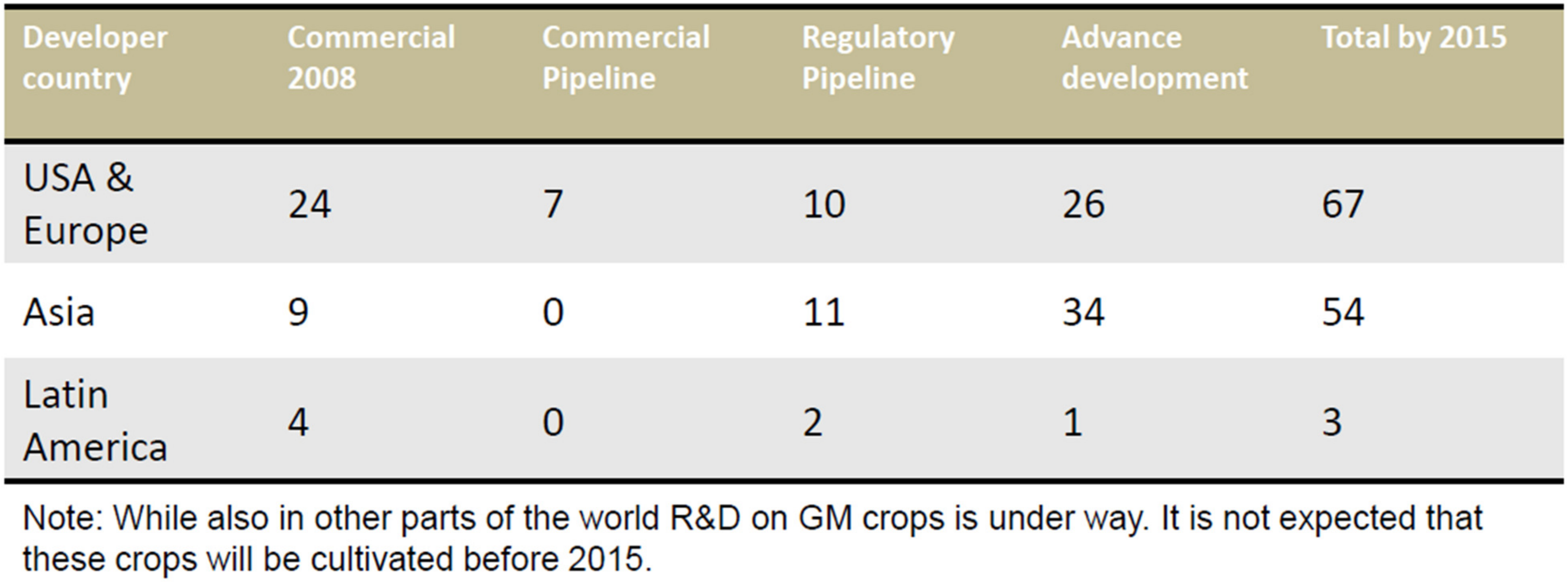

Developer country	Commercial 2008	Commercial Pipeline	Regulatory Pipeline	Advance development	Total by 2015
USA & Europe	24	7	10	26	67
Asia	9	0	11	34	54
Latin America	4	0	2	1	3

Note: While also in other parts of the world R&D on GM crops is under way. It is not expected that these crops will be cultivated before 2015.

- By 2016, almost 50% of commercial events will come from Asia and will be for domestic Asian markets/cultivation only

Source: CropLife

Trend in GM crop development

- In the first 13 years
  - 30 events were commercialized
- In the next 6 years
  - 90 events are expected to be commercialized
- By 2015
  - 24 corn events are expected to be marketed
    - If events are triple stacked this could equate to 2024 combinations
  - 17 soybean events are expected to be marketed
    - at double stacking this could equate to 136 different possible combinations
- Regulatory agencies that treat stacks like new events will be subject to an increasingly large workload
  - Most, except US, Canada, Australia
  - Brazil, Argentina just require bridging data

Stacking regulations in Asia

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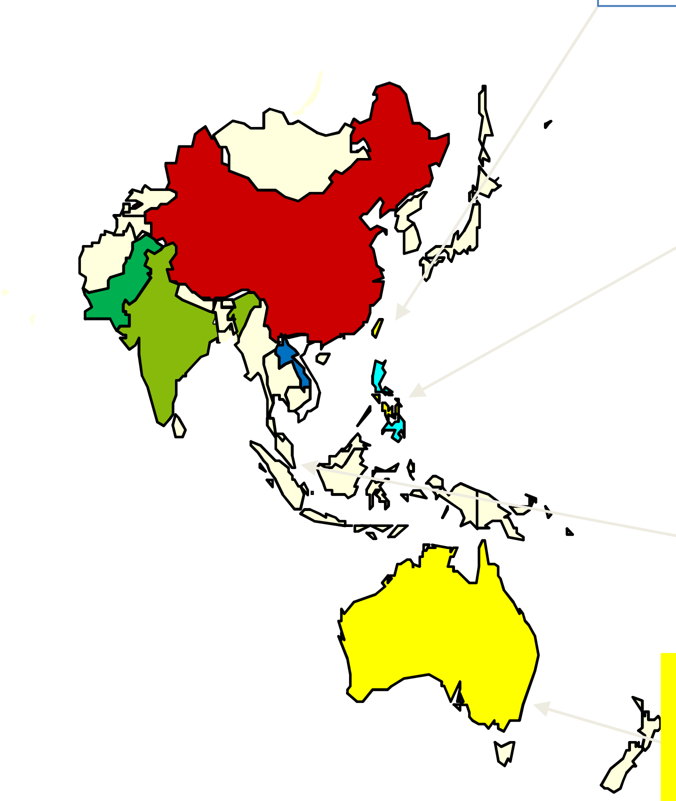

Taiwan: Guideline for food safety assessment of foods derived from genetically modified plants with stacked traits

Philippines: Risk Assessment of Plants Carrying Stacked Genes For Release Into the Environment

Risk Assessment for Stacked Gene Products Imported for Direct Use as Food and Feed or Processing

Singapore: Guidelines for the Risk Assessment of Foods/Crops in which Genetic Modifications have been Combined (or “Stacked”) by Conventional Breeding

Policy on Licensing of Plant GMOs in which different genetic modifications have been combined (or “Stacked”) by Conventional Breeding

- No additional review required

Proposed stacking regulations in Asia

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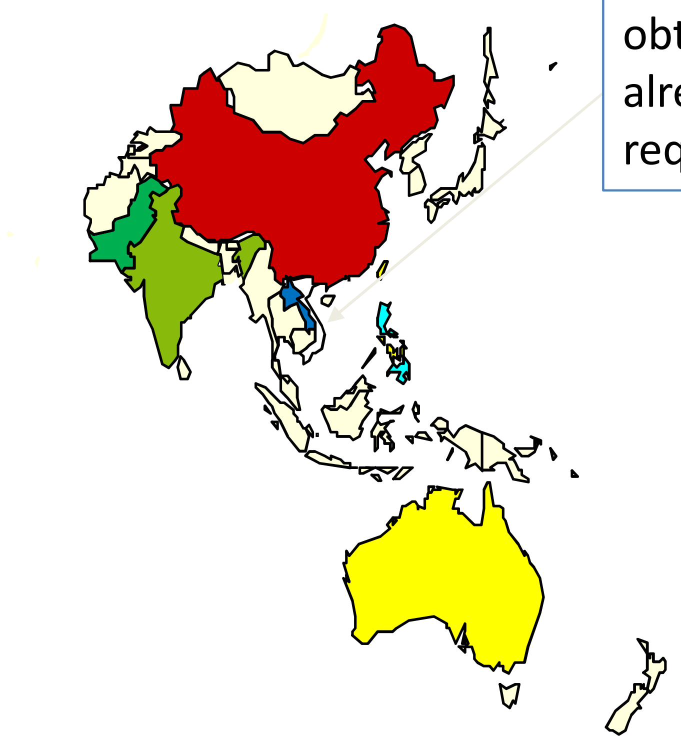

Vietnam via the WTO SPS notification system:

Single events that comprise a stacked product obtained by conventional breeding that have already been assessed for safety would not require any additional assessment in a stack

Thanks for your attention!

Honduras: Stacked trait maize MM Roca, 2011