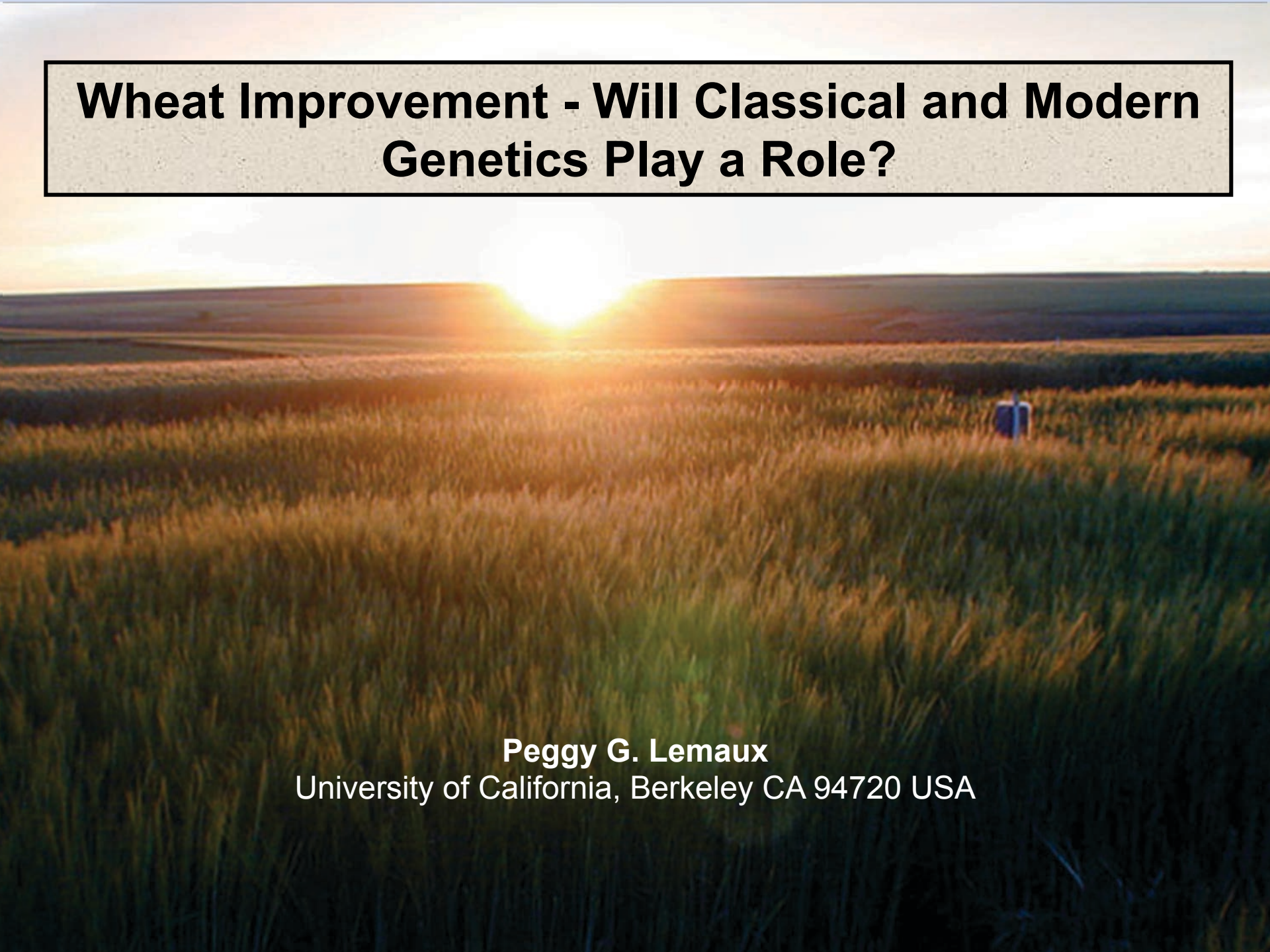
Wheat Improvement - Will Classical and Modern Genetics Play a Role?



Peggy G. Lemaux
University of California, Berkeley CA 94720 USA

1. Background on genes, genetics and genetic engineering (aka biotechnology, GMOs)

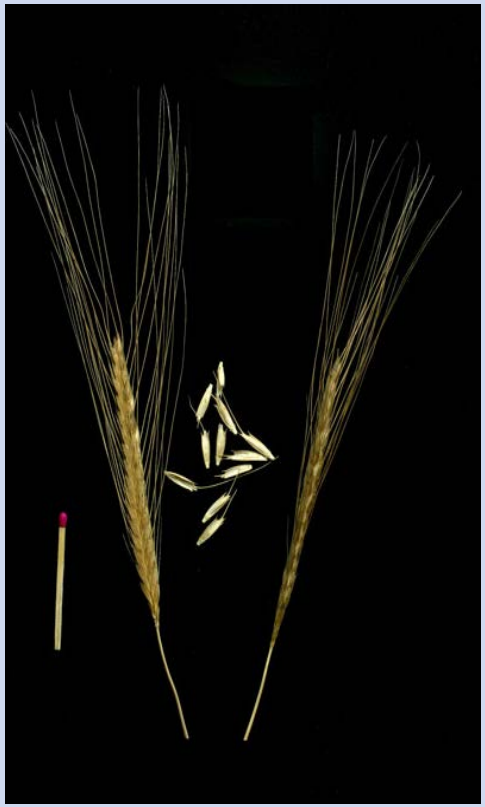
2. What engineered (GE) crops have been commercialized? What's in the pipeline?

3. What are some applications in wheat?

4. What is the regulatory structure for GE crops and foods? For wheat?

Background on genes, genetics and genetic engineering (aka biotechnology, GMOs)

How are the genes and chromosomes manipulated to create a new plant variety by classical breeding?



Triticum monococcum

Ancient variety

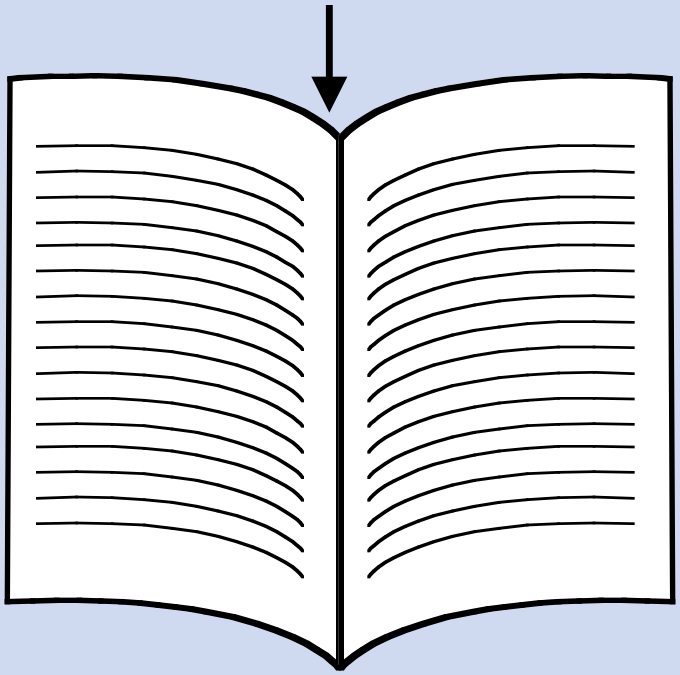
Triticum aestivum

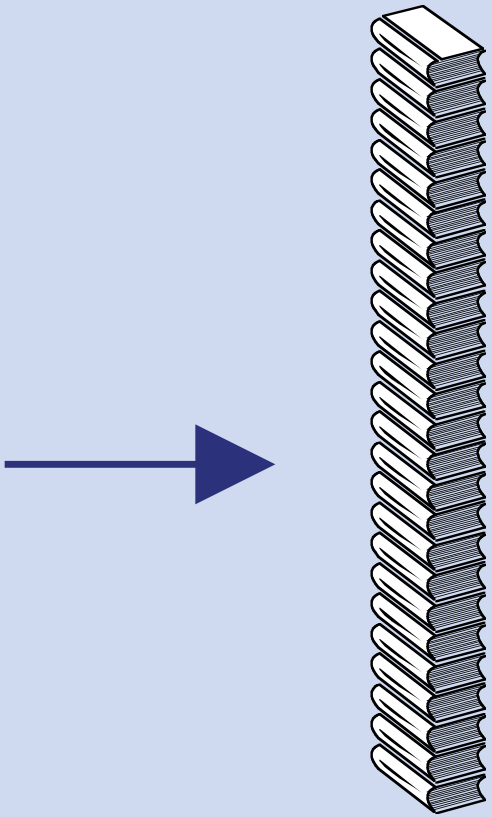
Modern bread variety

Information in the wheat genome

Chemical units represented by alphabetic letters

...CTGACCTAATGCCGTA...

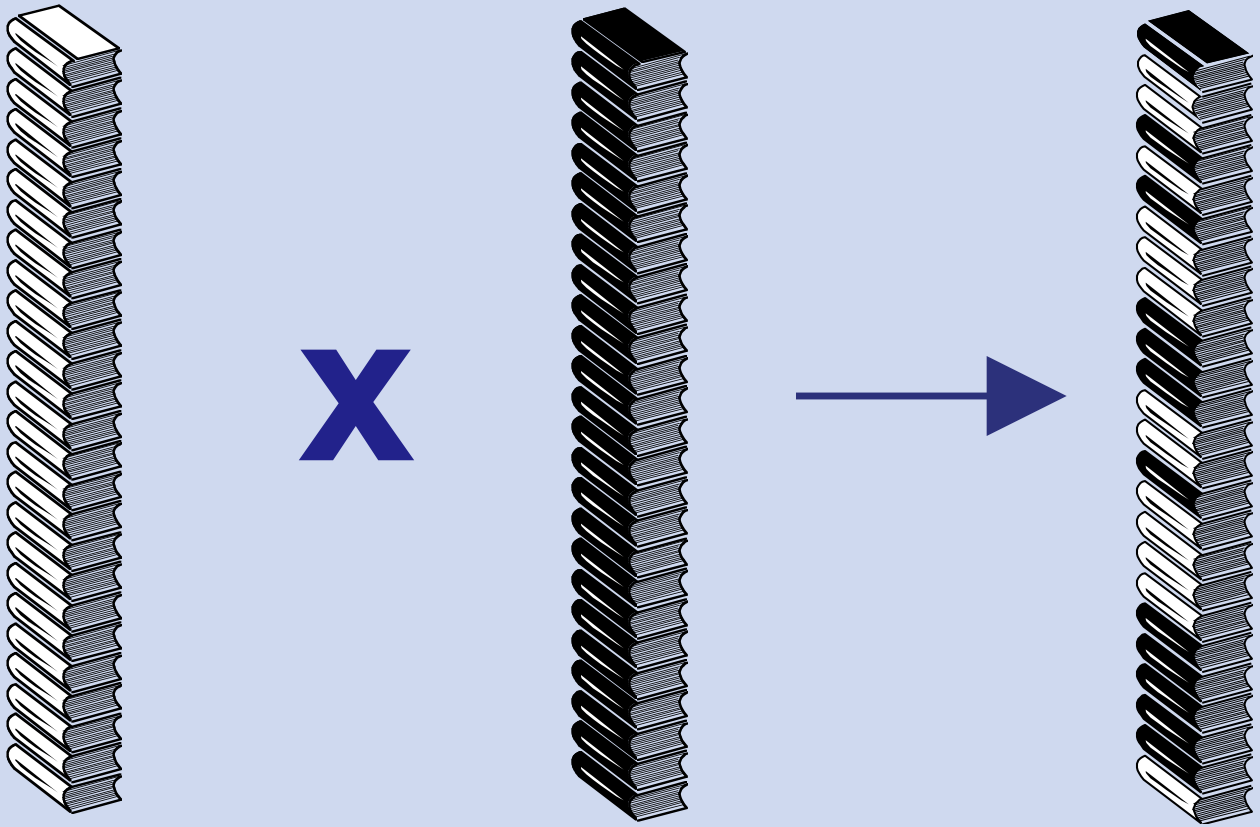




1700 books
1000 pages each

1700 books
(or 1.7 million pages)

Hybridization or crossing of wheat



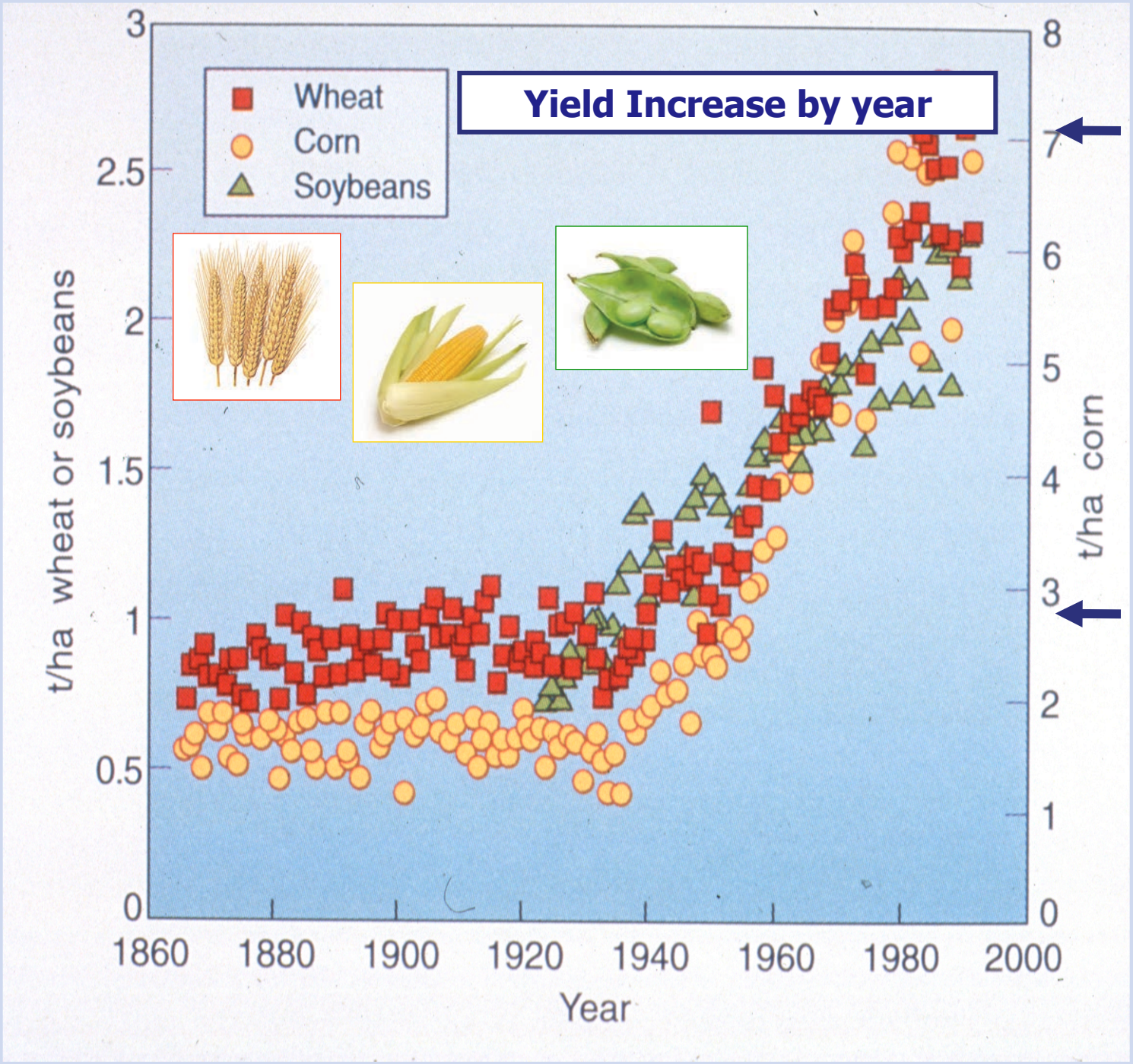
1700 books
(or 1.7 million pages)

1700 books
(or 1.7 million pages)

1700 books
(or 1.7 million pages)

Random retention of information from each parent

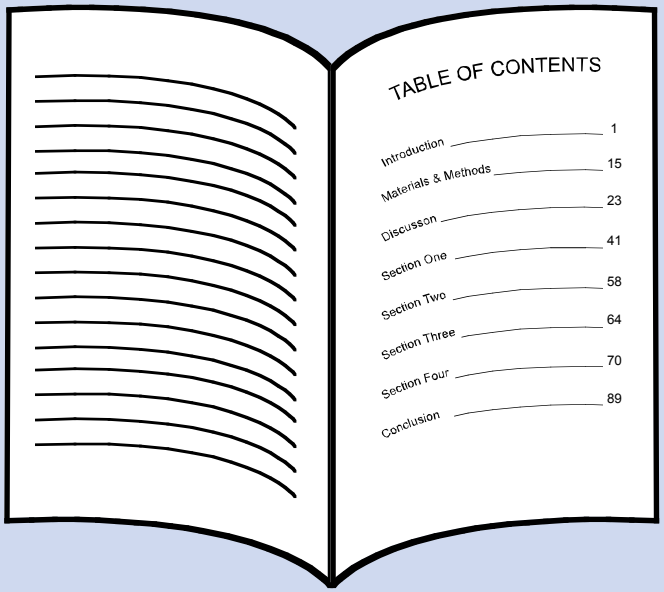
Genetic modification by hybridization is not GE or GMO


Yield Increase by year
Wheat
Corn
Soybeans
t/ha wheat or soybeans
t/ha corn
Year
0
0.5
1
1.5
2
2.5
3
0
1
2
3
4
5
6
7
8
1860
1880
1900
1920
1940
1960
1980
2000

There are new ways to do breeding…

Using a table of contents for traits to perform marker assisted selection

...CTGACCTAATGCCGTA...



Genomics

1700 books (or 1.7 million pages)

Genetic modification by marker-assisted selection is not GE or GMO

Can't We Just Do All Modification This Way?

Marker-assisted selection used to protect rice against bacterial blight and blast disease

This type of modification limited to diversity in compatible relatives of crops

In wheat, emerging genetic approaches use tables of content to sort through genetic information in many wheat varieties so breeders can make more informed selections

‘Cyber breeder’ improves wheat varieties

Zhiwu Zhang sorts through field, molecular data to help breeding

By MATTHEW WEAVER
Capital Press

PULLMAN, Wash. — Zhiwu Zhang rarely actually touches wheat, but his work helps put better varieties of the popular grain into growers’ hands.

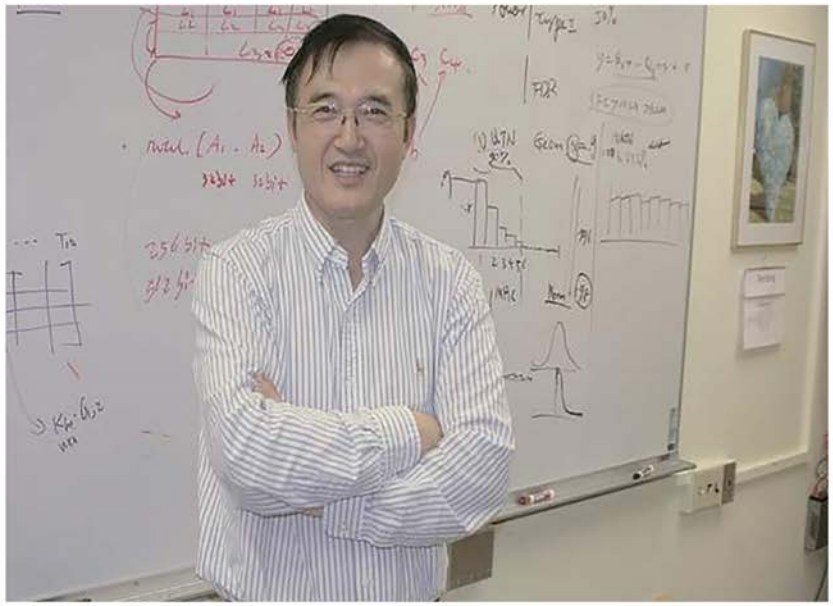
As a statistical geneticist — he calls himself a “cyber breeder” — at Washington State University in Pullman, Wash., Zhang combines information from “field” wheat breeders and researchers who identify molecular markers.

Wheat farmers are most concerned with yield, end-use quality and production cost, all traits determined by how genetics and the environment affect the farmer.”

Zhang is developing the computer programs and data

“Effectively, we are testing better material under field conditions, which should have

his master’s and Ph.D. degrees and postdoctoral training.



Matthew Weaver/Capital Press

Washington State University assistant professor Zhiwu Zhang stands in the middle of his lab Oct. 16 in Pullman, Wash. As statistical geneticist, Zhang compiles information from field breeders and molecular DNA sequencing to enable wheat breeders to improve the varieties they develop for farmers.

Western Innovator

Zhiwu Zhang

Title: Assistant professor, Washington State University; Washington Wheat Distinguished Professorship for Statistical Genetics

Age: 55

Current location: Pullman, Wash.

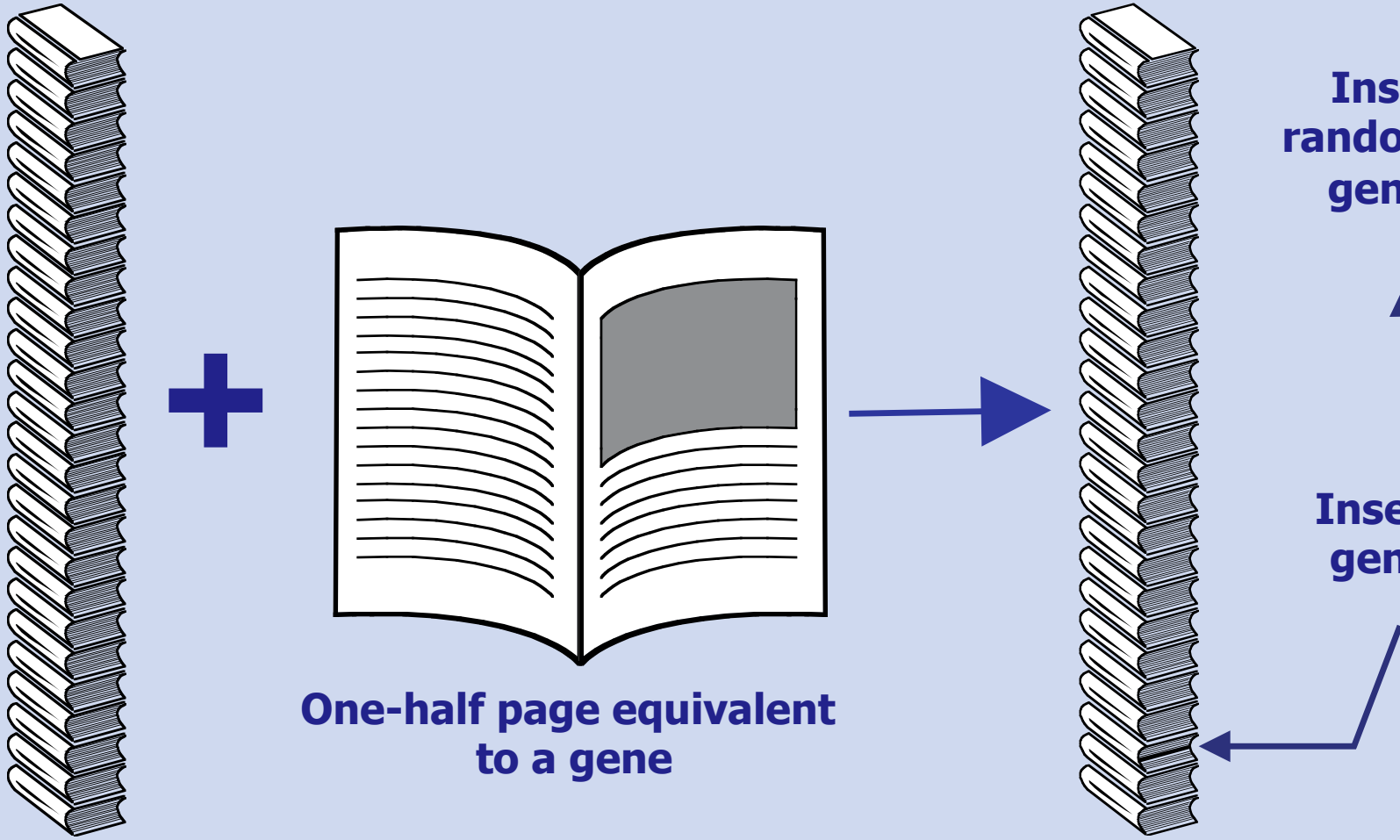
Hometown: Shulan, China

Education: Bachelor’s degree in animal science and master’s degree in animal breeding and genetics, Jilin Agriculture University, Changchun, China; Ph.D. in animal breeding and genetics, Northeast Agricultural University, Harbin, China;

SOURCE: “Cyber breeder improves wheat varieties”, Capital Press, December 28, 2015
http://www.capitalpress.com/Research/20151228/cyber-breeder-improves-wheat-varieties


But if a desired trait is from an incompatible plant or other organism, how can we create new varieties using the modern tools of genetics?
ucbiotech.org

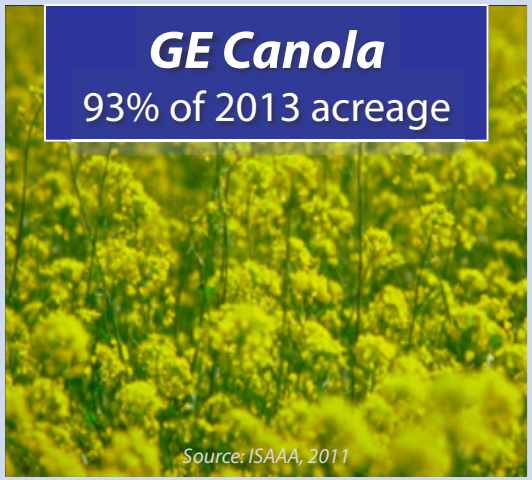
Genetic Engineering Methods

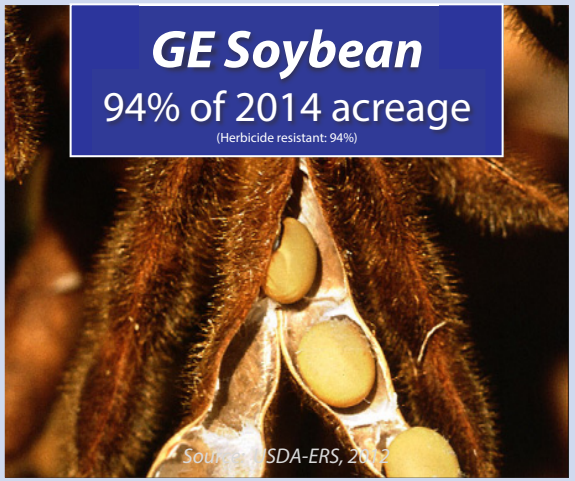


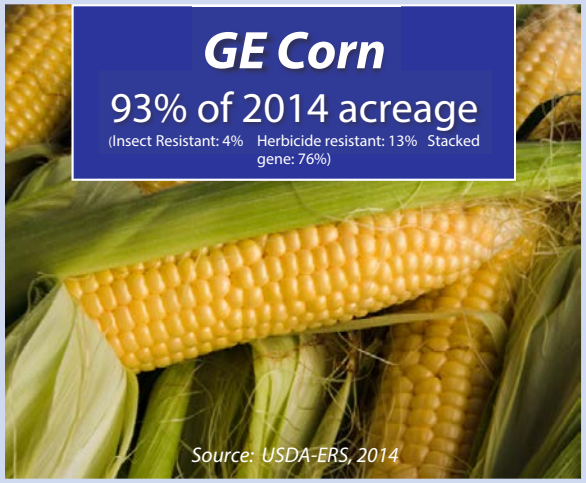
Genetic modification by genetic engineering <u>is</u> GE or GMO

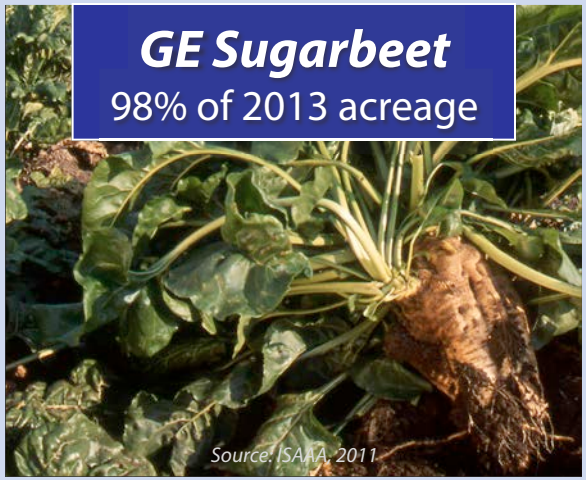

What engineered (GE, GMO) crops have been commercialized?

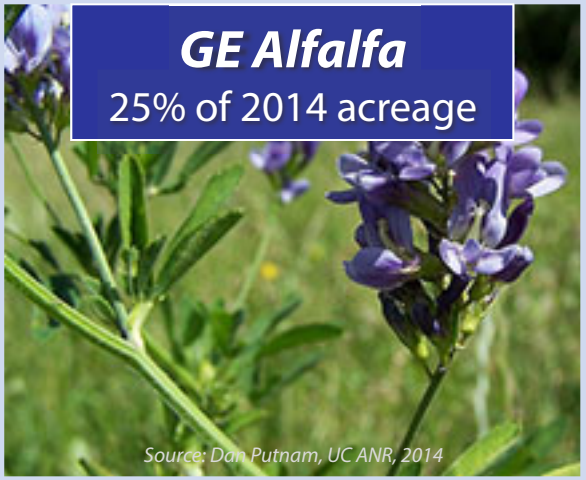
Number of different commercially available, large acreage GE (GMO) crops is limited











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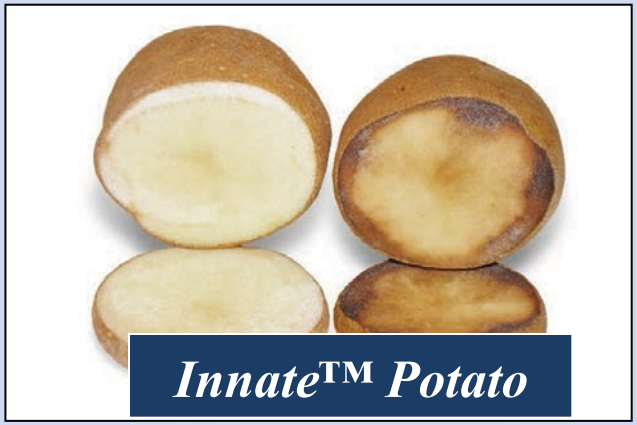
Only a few whole, GE foods are in the commercial U.S market

Two more have been approved



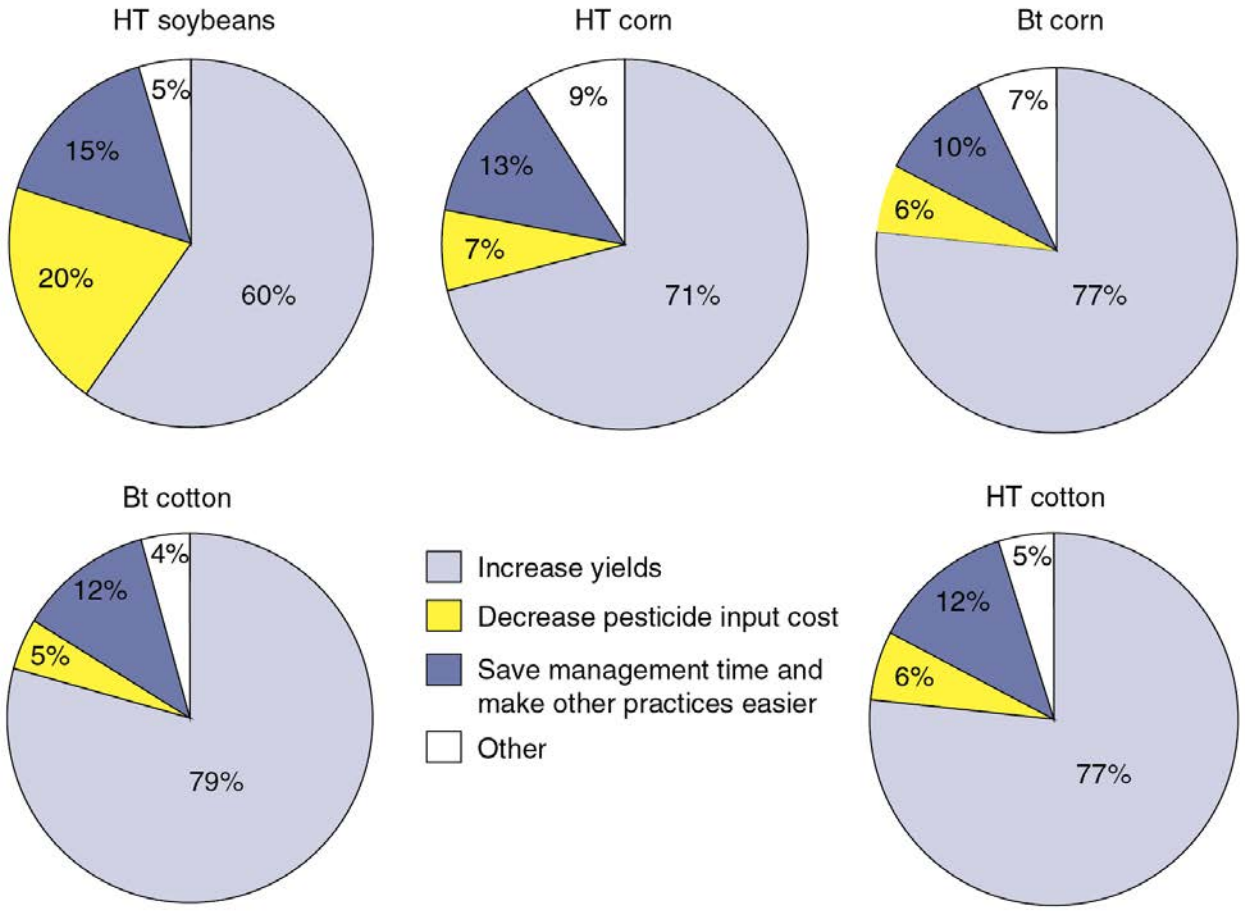
Number of different traits available in large acreage, GE crops is also limited

Bt Crops - engineered for insect resistance using gene from naturally occurring bacterium

Herbicide-tolerant - engineered with genes to tolerate herbicide application

There are also stacked Bt plus HT varieties

Why do growers adopt GE crops?



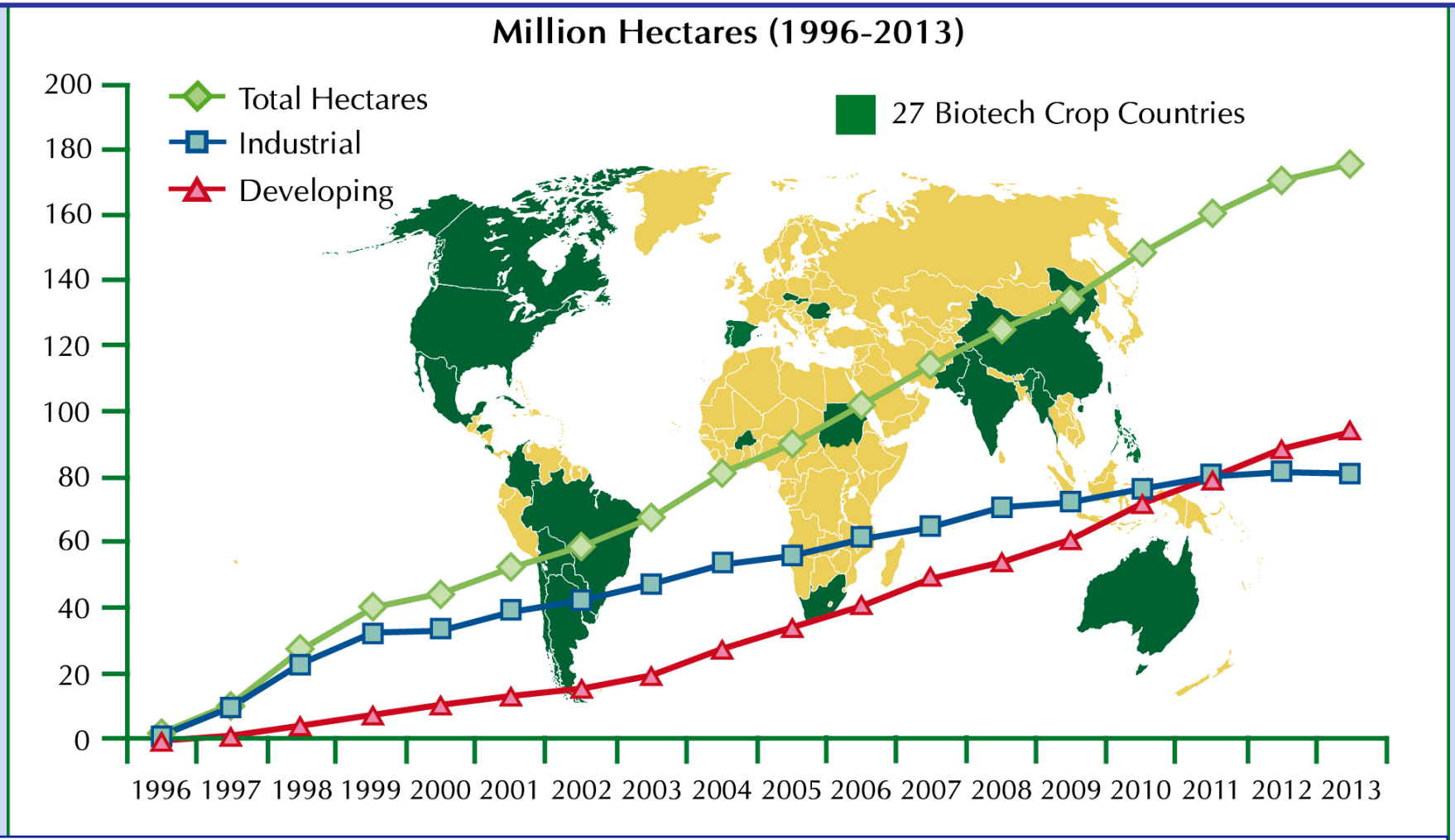
Reasons vary from crop-to-crop but predominant reason is to improve yield

SOURCE: Fernandez-Cornejo, J., Wechsler, S., Livingston, M. and Mitchell, L. 2014. Genetically Engineered Crops in the United States. USDA Economic Research Service Report No. 162. February 2014.

These types of large-acreage GE crops lead to estimates that 60-80% of processed foods in U.S. have GE ingredients

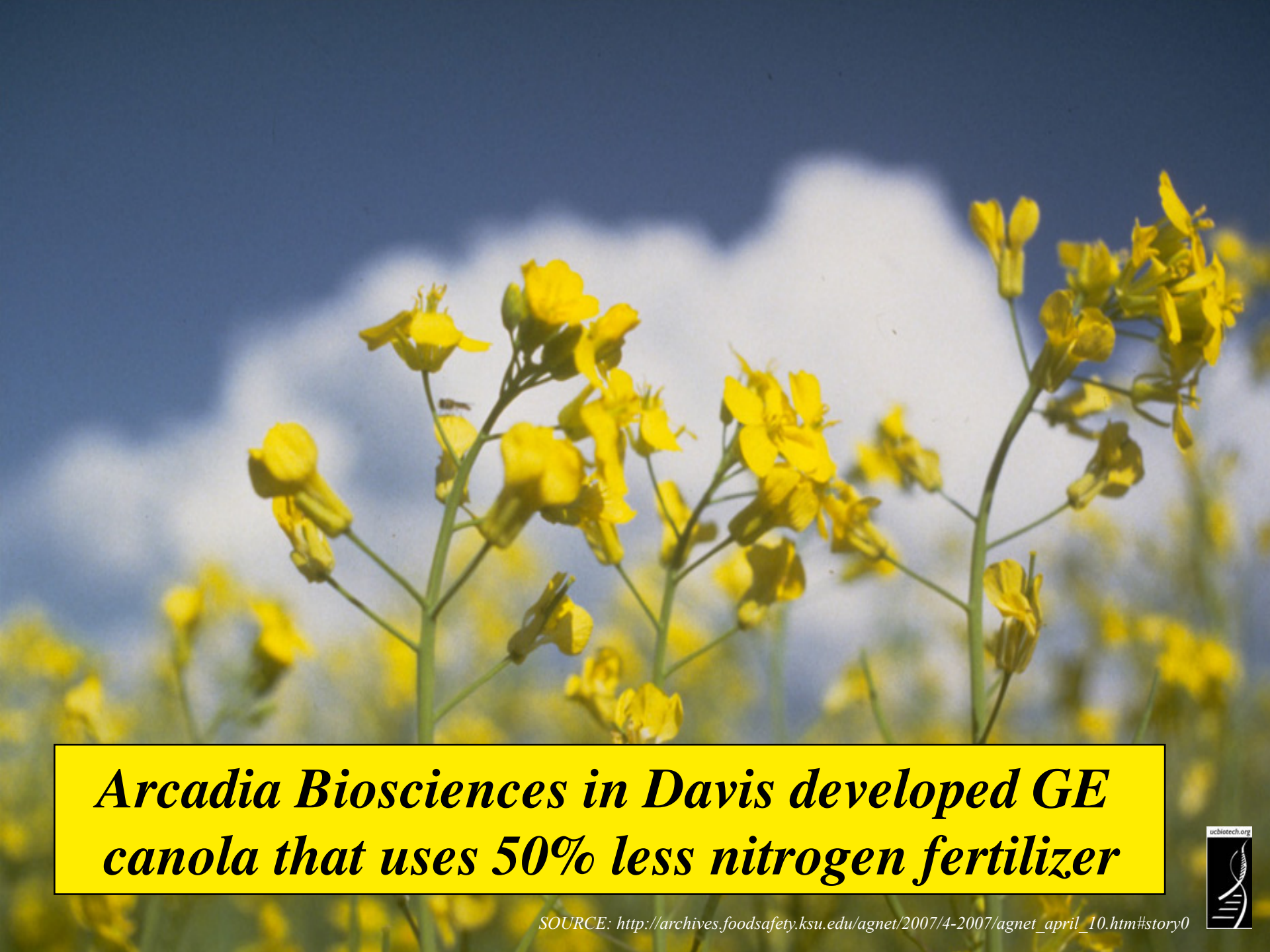
SOURCE: https://factsaboutgmos.org/disclosure-statement

Despite the same limited crop and trait types, worldwide acreage is increasing in 20 developing, 8 developed countries



2014: 18 million farmers in 28 countries
448 M acres planted: >3X size of California
>90% were small acreage farmers


What engineered (GE) crops are in the pipeline?



Arcadia Biosciences in Davis developed GE canola that uses 50% less nitrogen fertilizer

SOURCE: http://archives.foodsafety.ksu.edu/agnet/2007/4-2007/agnet_april_10.htm#story0

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Salinity and Drought Tolerance

Wild type AtNHX1
200 mM NaCl (~1/2 sea water)

Wild type IPT gene
15 days drought, 7 days re-watered

Salt-tolerance

Drought-tolerance

Hanana M. 2011. Environ Rev 19: 121-140; Anjuman A et al. 2013 Mol. Biotechnol 54: 3

UCDAVIS

2013 GE potato field study – Ireland
Desiree variety, highly susceptible to late blight, engineered with gene from wild potato variety

amiga
Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems

eagasc
Agriculture and Food Development Authority

WAGENINGEN UR
For quality of life

ucbiotech.org

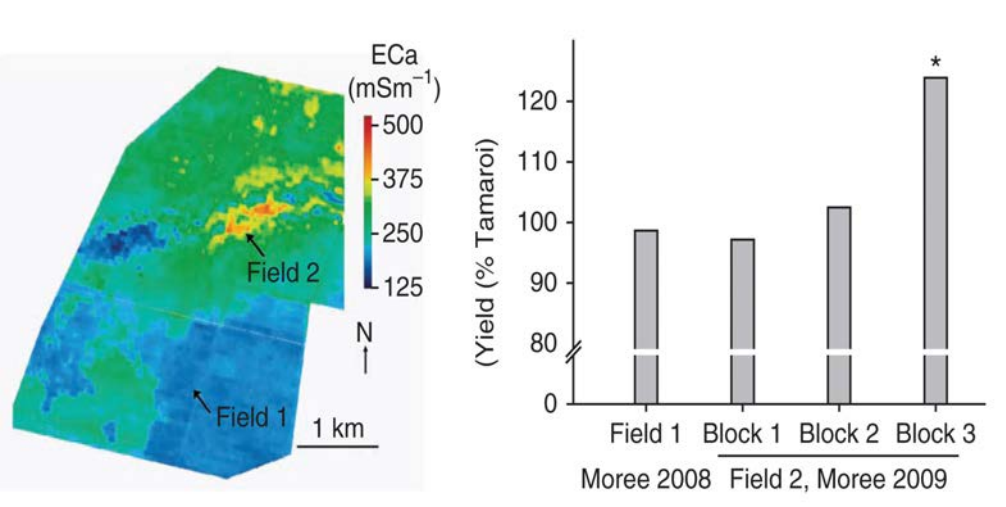
American chestnuts engineered with a wheat gene to prevent cankers from forming; replanted with $104K raised through crowd funding campaign

http://www.newscientist.com/article/dn25644-american-chestnut


What are some genetic improvement possibilities for wheat?

Via classical breeding

Introduction of naturally occurring Triticum monococcum gene into commercial durum wheat increases salt tolerance and yield by 25%



SOURCE: Munns R. et al. 2012. Nature Biotechnology 30: 360-364.

Via classical breeding

Gene from Triticum monococcum protects polyploid wheat against devastating Ug99 wheat stem rust

SOURCE: Saintenac, C., Zhang, W., Salcedo, A., Rouse, M.N., Trick, H.N., Akhunov, E. and Dubcovsky, J. 2013. Identification of Wheat Gene Sr35 That Confers Resistance to Ug99 Stem Rust Race Group. Science, doi: 10.1126/science.1239022.

Capital Press
Agriculture Newspaper

More agricultural groups endorse GMO wheat

By JOHN O'CONNELL
Capital Press

Sixteen agricultural organizations in the U.S., Canada and Australia have publicly endorsed the use of biotechnology in wheat to make the cereal more competitive with other crops.

Half of the organizations involved — including the National Association of Wheat Growers, U.S. Wheat Associates and the North American Millers' Association in the U.S. — had signed a similar pledge five years ago.

Since then, the escape of unapproved genetically modified wheat into an Oregon field was discovered in the spring of 2013, prompting major trade headaches for the industry, GMO labeling initiatives have surfaced nationwide and two small Oregon counties voted in May to ban GMOs.

NAWG spokesman Will Stafford said the nine original participants worked for the better part of a year recruiting new organizations, bringing the coalition to 16 groups, and devising wording they could all support.

New U.S. groups include the American Farm Bureau Federation and the National Farmers Union.

"We're very happy to welcome the two major umbrella farm organizations on the U.S. side," Stafford said.

Other new additions include: Canadian National Millers Association, Cereals Canada, Grain Farmers of Ontario, AgForce Queensland, Victorian Farmers Federations Grains Group and Grain Producers of South Australia.

Stafford emphasized the pledge also supports science-based regulatory systems, following proper regulatory processes and maintaining customer choice.

"Hopefully, it sends a clear message to investors that our farmers are very willing to accept biotechnology and it spurs even more investment and innovation in wheat, not just in biotechnology but innovation in gen

What about engineering applications in wheat?

"16 U.S., Canadian and Australian agricultural organizations endorse use of biotech in wheat. Why? To make wheat more competitive."

SOURCE: "More agricultural groups endorse GMO wheat", Capital Press, June 10, 2014
http://www.capitalpress.com/Nation_World/Nation/20140610/more-agricultural-groups-endorse-gmo-wheat#.U7HJwo1dXog

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Wheat Community has already "experienced" Roundup Ready Wheat

Is there anything else out there?

ENERGY & ENVIRONMENT

The New York Times

Modified Wheat Is Discovered in Oregon

By ANDREW POLLACK MAY 29, 2013

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More

Unapproved genetically engineered wheat has been found growing on a farm in Oregon, federal officials said Wednesday, a development that could disrupt American exports of the grain.

The Agriculture Department said the wheat was of the type developed by Monsanto to be resistant to the herbicide Roundup, also known as glyphosate. Such wheat was field-tested in 16 states, including Oregon, from 1998 through 2005, but Monsanto dropped the project before the wheat was ever approved for commercial planting.

The department said it was not known yet whether any of the wheat got into the food supply or into grain shipments. Even if it did, officials said, it would pose no threat to health. The Food and Drug Administration reviewed the wheat and found no safety problems with it in 2004.

Monsanto GMO wheat contamination discovered in Montana

Published time: 27 Sep, 2014 02:45
Edited time: 27 Sep, 2014 09:56
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Reuters/Bogdan Cristel / Reuters

1

Monsanto's experimental genetically modified wheat has been discovered growing in the second US field in Montana, about a year after the discovery of the company's unapproved crop growing in Oregon disrupted US wheat exports.

MIT Technology Review

Chinese Researchers Stop Wheat Disease with Gene Editing

Researchers have created wheat that is resistant to a common disease, using advanced gene editing methods.

By David Talbot on July 21, 2014

Advanced genome-editing techniques have been used to create a strain of wheat resistant to a destructive fungal pathogen – called powdery mildew – that is a major bane to the world's top food source, according to scientists at one of China's leading centers for agricultural research.

Advanced genome-editing techniques used to create wheat resistant to powdery mildew by deleting genes that repress defenses against mildew

SOURCE: "Chinese Researchers Stop Wheat Disease with Gene Editing", MIT Technology Review, July 21, 2014
http://www.technologyreview.com/news/529181/chinese-researchers-stop-wheat-disease-with-gene-editing/

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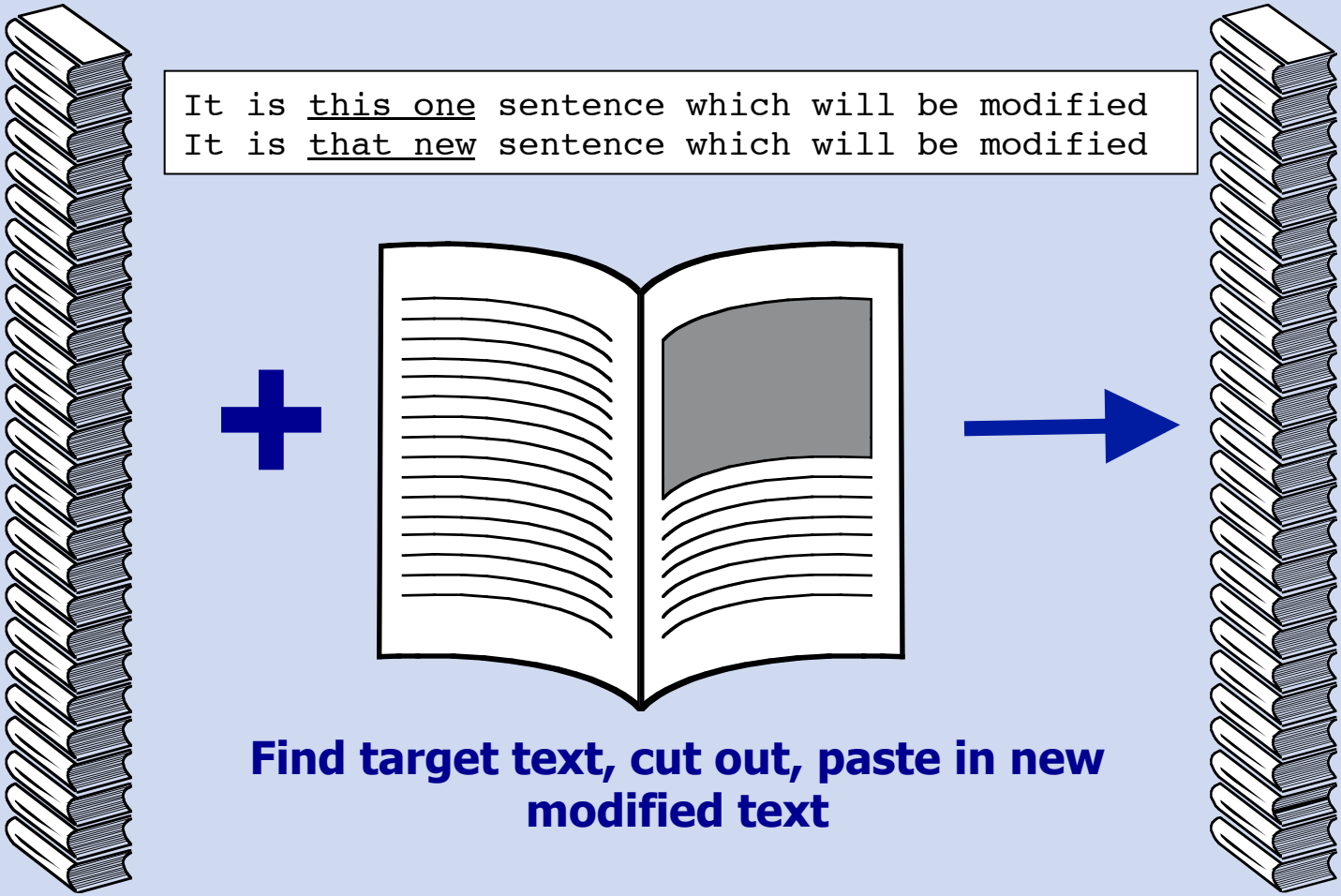
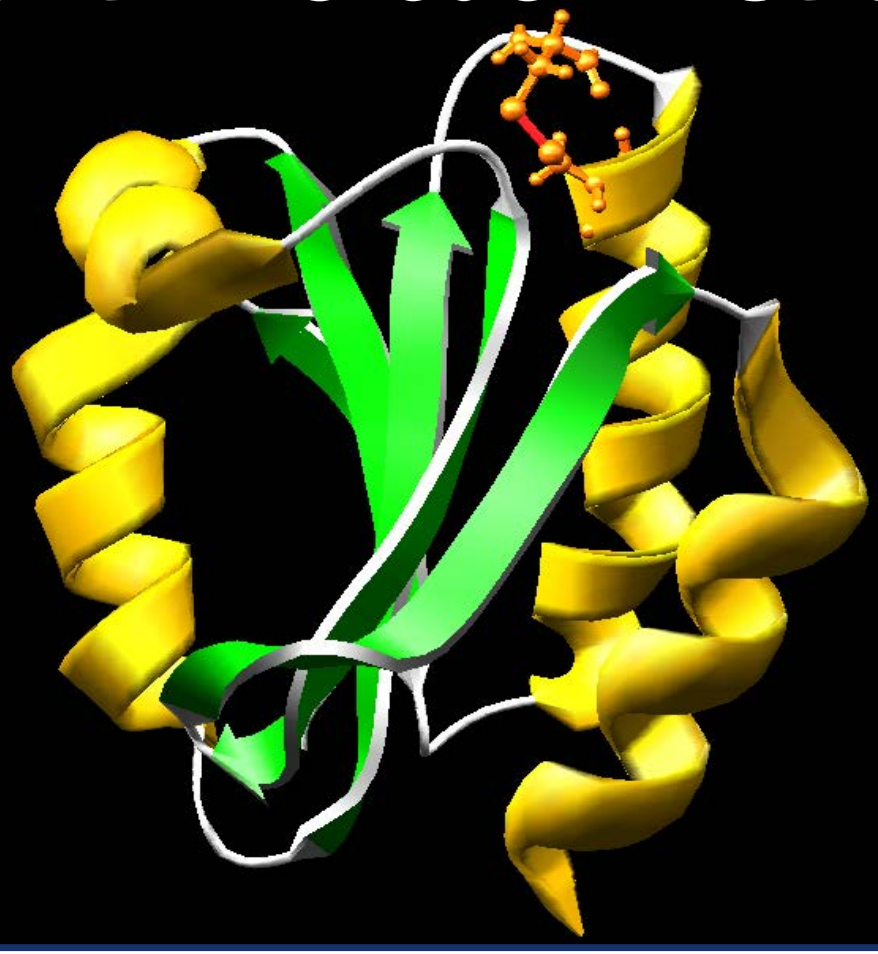
What is Genome Editing?



Genome editing may or may not be GE or GMO

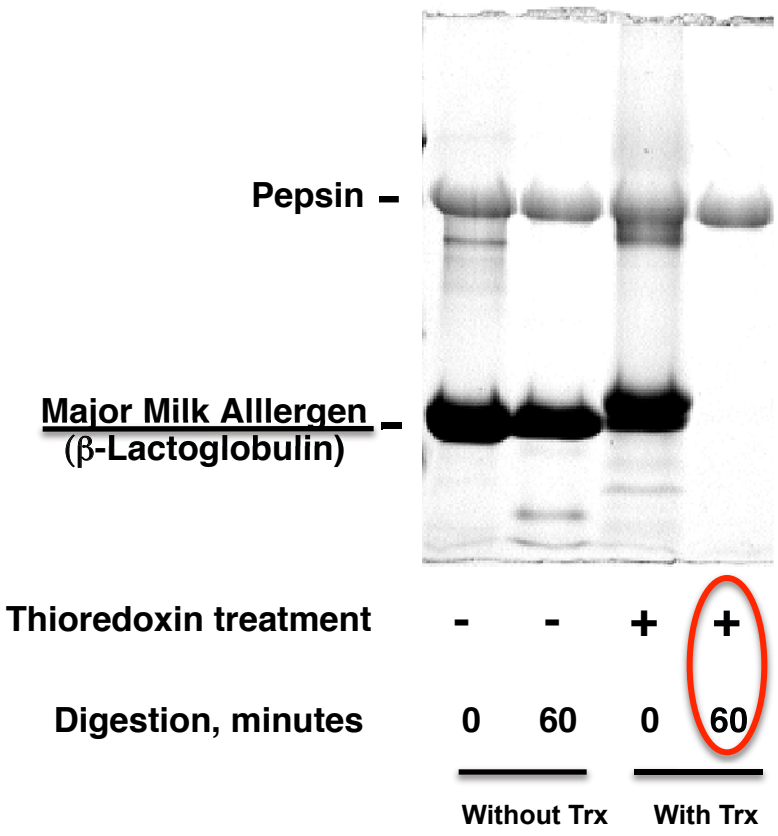

What Have We Done at UC to Engineer Wheat?

Plant Thioredoxin Structure



These approaches focused on making more or less of this naturally occurring, small protein

Treatment of Major Milk Allergen with Thioredoxin Reduced Allergenicity



Addition of Trx system in an *in vitro* digestion system leads to rapid digestion of major milk allergen.

SOURCE: del Val, G., Yee, B.C., Lozano, R.M., Buchanan, B.B., Ermel, R.E., Lee, Y.M. and Frick, O.L. (1999) Thioredoxin treatment increases digestibility and lowers allergenicity of milk. J. Aller. Clin. Immunol. ***103****, 690-697*

Another major human allergen source is wheat and wheat products

Can increasing thioredoxin in wheat grain reduce allergenicity? How do we test it?

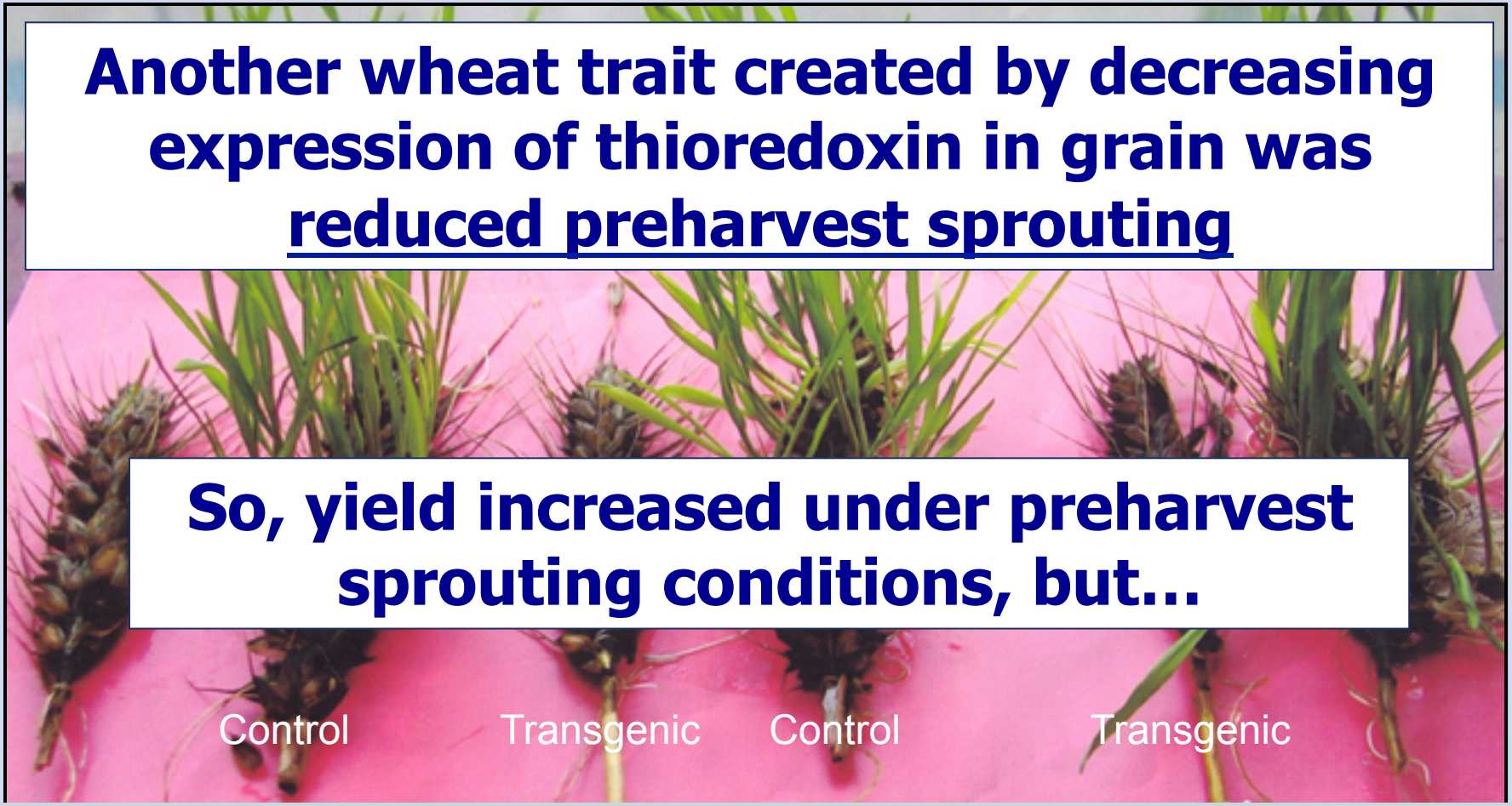
Allergenicity of Wheat Grains Making More Thioredoxin vs. Control - Tested Using Dog Model

Wheat		Wheal induction amount (ng)
Albumins/ Globulins	TRX	224
	Null	219
→ Gliadins	TRX	8,318
	Null	2,518
Glutenins	TRX	347
	Null	340

In Trx grains, gliadins, major wheat allergen, are 3 times less allergenic than control grain

SOURCE: Li, Y.-C., Ren, J.-P., Cho, M.-J., Zhou, S.-M., Kim, Y.-B., Guo, H.-X., Wong, J.H., Niu, H.-B., Kim, H.-K., Morigasaki, S., Lemaux, P.G., Frick, O.L., Yin, J., Buchanan, B.B. 2009. The Level of Expression of Thioredoxin is Linked to Fundamental Properties and Applications of Wheat Seeds. Molecular Plant 2: 430-441.

Another wheat trait created by decreasing expression of thioredoxin in grain was reduced preharvest sprouting



So, yield increased under preharvest sprouting conditions, but…

SOURCE: Li, Y.-C., Ren, J.-P., Cho, M.-J., Zhou, S.-M., Kim, Y.-B., Guo, H.-X., Wong, J.H., Niu, H.-B., Kim, H.-K., Morigasaki, S., Lemaux, P.G., Frick, O.L., Yin, J. and Buchanan, B.B. 2009. The Level of Expression of Thioredoxin is Linked to Fundamental Properties and Applications of Wheat Seeds. Molecular Plant 2: 430-441.

Grain Properties of Control, 2 Transgenic Lines from 2 Wheat Cultivars Grown in Field for 3 years

Yield

Cultivar	Line	Grain #/spike	Yield	% Yield Increase
Control		35.2± 3.0 a	7271.3± 387.2 b	-
Yumai18 weak gluten	TY18-99	37.6± 1.6 b	7304.9± 359.7 b	**8.8**
	TY18-10	39.1± 2.3 b	7394.8± 321.4 a	**9.3**
Control		31.1± 0.6 a	6684.1± 258.3 a	-
Yumai34 Strong gluten	TY34-73	33.1± 0.8 b	8245.2± 260.9 b	**11.5**
	TY34-75	33.5± 0.9 b	8415.3± 299.2 b	**13.8**

✓ **Surprisingly yield was increased 9 to 14% under normal conditions due to increased grain number.**

Grain Properties of Field-Grown Transgenic Plants from Two Wheat Cultivars over 3 years

Baking Qualities

Cultivar	Line	Stability Time	Resistance	Extensibility
Control		2.3 ± 0.28 **a**	197.5 ± 16.88 **a**	157.3 ± 7.39 **a**
Yumai18 Weak gluten	TY18-99	1.6 ± 0.12 **b**	206.0 ± 18.20 **a**	151.8 ± 4.07 **a**
	TY18-100	1.5 ± 0.13 **b**	200.7 ± 23.41 **a**	151.5 ± 3.78 **a**
Control		8.7 ± 1.85 **a**	266.8 ± 19.35 **a**	178.5 ± 2.26 **a**
Yumai34 Strong gluten	TY34-73	7.8 ± 0.47 **a**	279.8 ± 24.68 **a**	179.5 ± 5.72 **a**
	TY34-75	7.5 ± 0.65 **a**	286.0 ± 12.78 **c**	177.0 ± 5.29 **a**

What about baking quality? Remained same in strong gluten variety

But none have made it to market. They're in a cabinet in the basement of my building

What would have to happen for them to go into the market place?

U.S. Regulatory Agencies

USDA

- Field testing
  - -Notifications
- Determination of non-regulated status

FDA

- Food
- Feed safety

EPA

- Pesticidal
  - -tolerance exemption
  - -registrations
- Herbicide registration

Undergo Federal Regulatory Scrutiny

Plant pest?

Danger to people?

Risk to environment?

USDA variety release requirements for conventional varieties require data on:

- agronomic performance
- proximate analysis
- antinutritive factors

Paperwork required for registration of conventional flax variety

A. McHughen

USDA variety release requirements for engineered varieties require that data plus…

Paperwork required for registration of GE flax variety

A. McHughen

- Molecular characterization of inserted DNA,
- Southern and restriction analyses
- PCR for several fragments,
- Various enzyme assays (ALS, NOS, NPT-II)
- Copy number of inserts
- Size of each fragment,
- Source of each fragment
- Utility of each fragment
- How fragments were recombined
- How construct was delivered into flax
- Biological activity of inserted DNA (genes)
- Quantitative analyses of novel proteins (western analyses)
- Temporal activity of inserted genes
- Spatial activity of inserted genes
- Complete amino acid analysis
- Detailed amino acid analysis for valine, leucine and isoleucine
- Toxicity
- Allergenicity
- Biological analysis:
- Pathogenicity to other organisms
- Dormancy,
- Outcrossing
- Potential for horizontal gene transfer
- Seed production
- Flowering time,
- Flower morphology
- Analysis of relatives
- Stability of inserted genes over seed generations
- Survivability in natural environment
- Survivability in agricultural environment in presence of herbicide
- Survivability in agricultural environment in absence of herbicide
- Interaction with other organisms- alterations to traditional relationships
- Interactions with other organisms- novel species
- Changes to persistence or invasiveness
- Any selective advantage to the GMO
- Any selective advantage to sexually compatible species
- Plan for containment and eradication in the event of escape

This isn't cheap. Industry estimates costs are $10-$20M for each event! Well beyond resources of academics and small companies.

And…USDA APHIS created new regulations for GE wheat after discovering GE varieties in Oregon and Montana - where they shouldn't have been

Can be field tested **under permit process (**as with trees and perennial grasses) **rather than the notification process**. This will **require, for example, more monitoring and reporting**. Key risk mitigation factors (7 CFR part 340.3) include:

1. **Plants and plant parts must be contained or devitalized** when not in use.
2. Field trial conducted so **regulated article will not persist in environment** and **no offspring** can be produced that could persist.
3. **Upon termination of field test, no viable material shall remain**, which is likely to volunteer later or volunteers are managed to prevent persistence in environment

Regulatory system for biotech products has remained same since 1986, with minor revisions in 1992 resulting in:

- **New products emerging with no rules to govern them;**
- **No clear commercialization path for older products;**
- **New products being created to avoid regulation**

USDA clears GMO tall fescue

68

Mateusz Perkowski
Capital Press

Published:
December 30, 2014 9:21AM

Last changed:
December 30, 2014 9:28AM

A new GMO variety of tall fescue turfgrass that's resistant to glyphosate herbicides has been cleared for cultivation by USDA.

The USDA has cleared the way for cultivation of genetically modified tall fescue without conducting an environmental review of the new crop.

The Scotts Miracle-Gro company developed the glyphosate-resistant turfgrass variety with genes from other plants through a process known as "biolistics," in which a "gene gun" essentially shoots DNA-coated metal particles into the plant cell.

Because the method does not involve the use of a plant pest for gene transfer, the USDA has no authority to regulate the tall fescue, according to a document recently released by the agency.

Like GE turfgrass: created to side-step regulation by not using a plant pest or plant pest parts

Like longer storage life potatoes created with genome editing tools that USDA APHIS will not regulate

ucbiotech.org

These examples have resulted in calls for revamping regulatory oversight

Genetically engineered crops that fly under the US regulatory radar

To the Editor:

Recently, the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) has categorized as outside the scope of its regulations several genetically

cisgenesis/intragenesis and site-directed nucleases, may be a deliberate strategy for smaller entities to navigate the US GE crop regulatory framework. The fact that the US Coordinated Framework is on the one hand

First steps in this direction taken on July 2, 2015 by a White House Initiative to require USDA, FDA and EPA to modernize biotech regulation.

their products has been on the increase. Many of these inquiries originate from public institutions or small biotech companies, suggesting that the use of technologies, such as null segregants, novel delivery systems,

build a system that is based on science, with enough flexibility to evolve with accumulating scientific knowledge and technologies and, importantly, that allows the participation of small companies and public sector institutions.

Camacho et al. 2014. Nature Biotech. 32:1087-1091

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