Precision Plant Breeding With CRISPR Genome Editing: Opportunities and Challenges

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Current Challenges

7.7 B →8.5 B by 2030→9.7B by 2050

Urban sprawl encroaches rapidly on farmland

Hunger

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Current Challenges

Extreme Weather/ Climatic Change

Flood

a home is surrounded by floodwaters from Tropical Storm Harvey in Spring, Texas

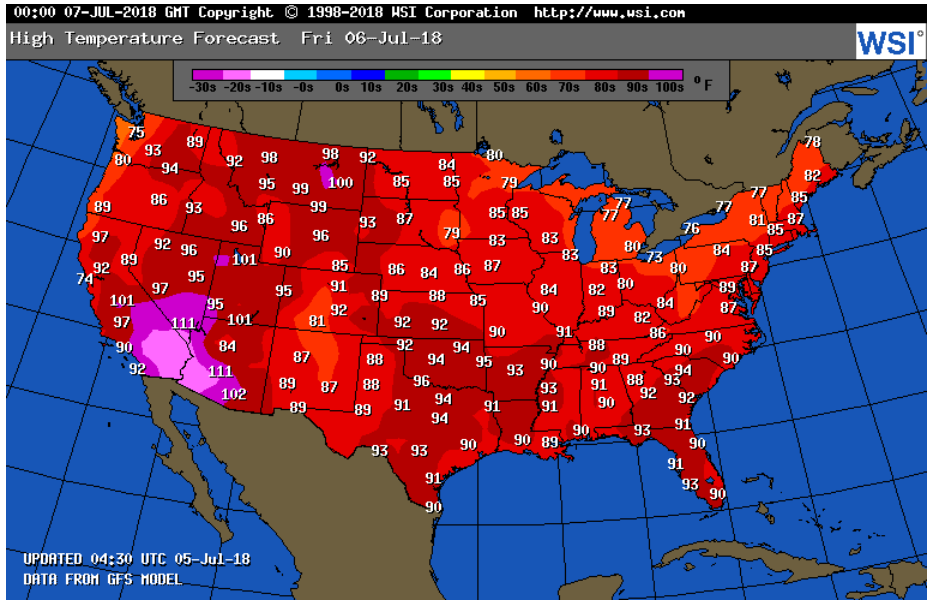
Drought

A Texas State Park police officer walked across the lake bed of O.C. Fisher Lake in San Angelo, Texas. 2012

Current Challenges

Extreme Weather/ Climatic Change

Heat



Heat Wave, July 2018

Tornado

Current Challenges

Extreme Weather/ Climatic Change

Ice covers the Lake Michigan shoreline on January 30 in Chicago 2019

Temperature increase at North Pole

Current Challenges

Extreme Weather/ Climatic Change

Hurricane

Devastated buildings damaged by the Hurricane Irma, 2017

Wild Fire

Wildfire on the hillside in Burbank, Calif. 2018

Objectives of Plant Breeding

- **Primary objective is to increase crop *yield* and improve *quality* of crop produce**

Weed Control

Integrated Pest Management

:omparison of corn with disease and Bt corn.(Photo by Biotech info center)

Insect resistant corn (top)

https://www.agronomy.org/science-news/understanding-genetic-basis-drought-tolerant-soybeans

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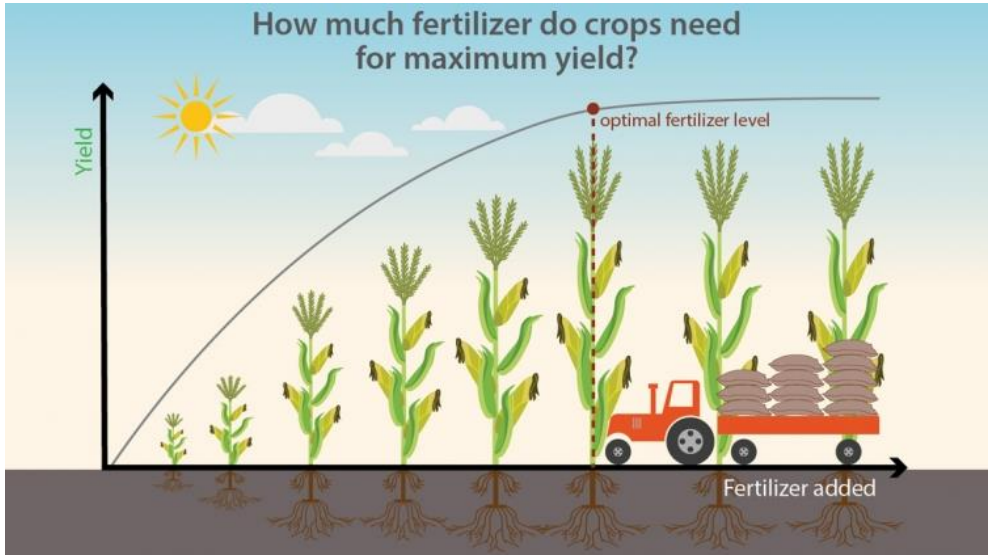
Objectives of Plant Breeding

- **Primary objective is to increase crop *yield* and improve *quality* of crop produce**

Fertilizer Use Efficiency (N, P, K)



- Solution for limiting resources (e.g. P)
- Reduce energy and cost
- Reduce environmental impact

Water Use Efficiency

Soybean plants subjected to drought stress

https://www.agronomy.org/science-news/understanding-genetic-basis-drought-tolerant-soybeans

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MILESTONES IN PLANT BREEDING

CROP DOMESTICATION

Farmers select the best wild species to develop crops

10,000 BC

Domestication of wheat

FACTS

For **10,000** years, farmers and breeders have been developing and improving crops

For **150** years, plant scientists and breeders have improved plant breeding on a scientific basis

Today, farmers feed at least **10** times more people using the same amount of land as 100 years ago

By 2050, we will need **50%** more food to feed a population of 11 billion

1865

Mendel's laws

Gregor Mendel describes the inheritance of traits from one generation to the next. His laws become the core of classical genetics

PLANT BREEDING BASED ON CROSS BREEDING

Development of improved varieties by combining good characteristics from two parents

HYBRID BREEDING

Crossing two genetically different individuals to develop better performing hybrids

More vigorous hybrid corn

1926

MUTAGENESIS

Developing new genetic diversity by exposing crop plants to chemical agents or radiation

Blast-resistant rice

1940

1953

Understanding the structure of DNA

James Watson and Francis Crick identify the double helix of DNA

GMO

Introducing foreign genes into the DNA of a plant

Insect-resistant cotton

1994

PLANT BREEDING BASED ON GENETIC INFORMATION

Development of improved varieties by working directly with the DNA

MARKER-ASSISTED SELECTION

Locating desirable traits in a plant for efficient selection and breeding

Barley resistant to yellow dwarf virus

2000

TARGETED BREEDING

Using modern tools such as genome editing for more targeted breeding

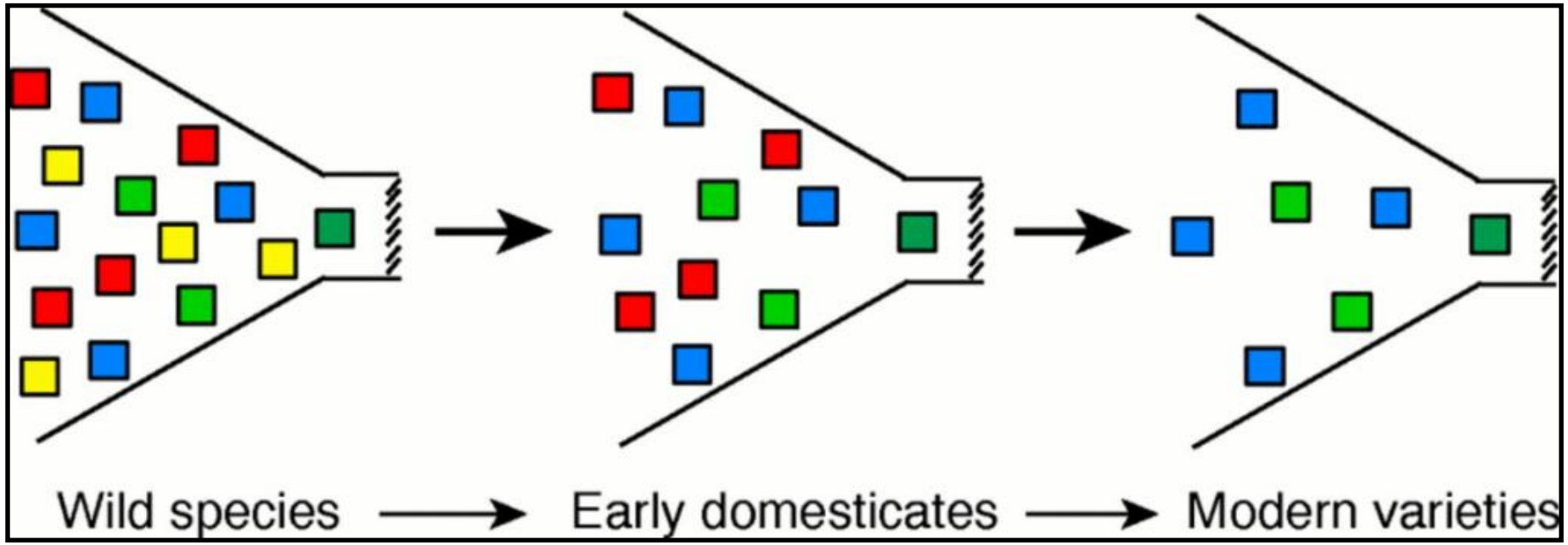
Waxy corn

now → future

2016 CRISPR/WAX Corn

Plant Domesitcation

- **Plant domestication: selection by nature power**



Desired traits: Larger grain, high yield, less bitterness

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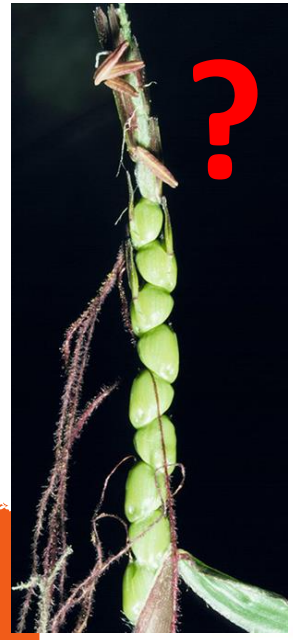
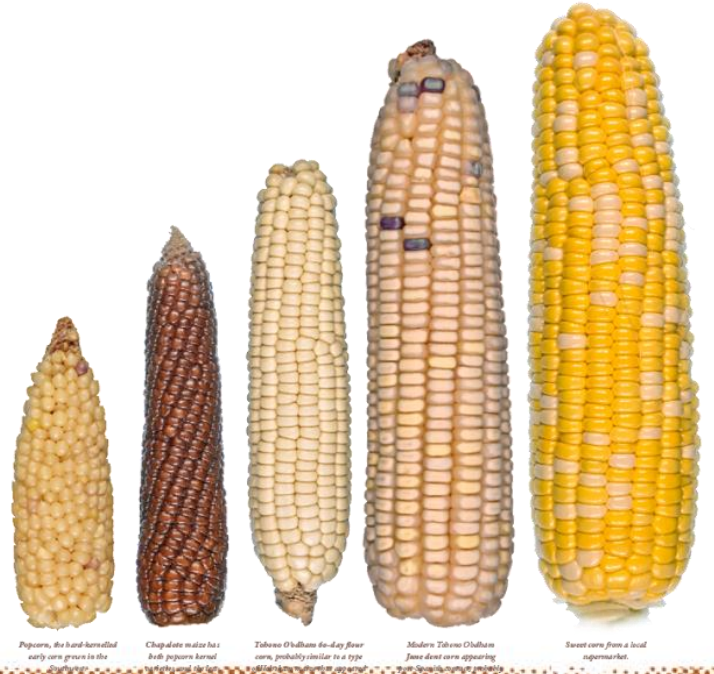
Plant Domestication







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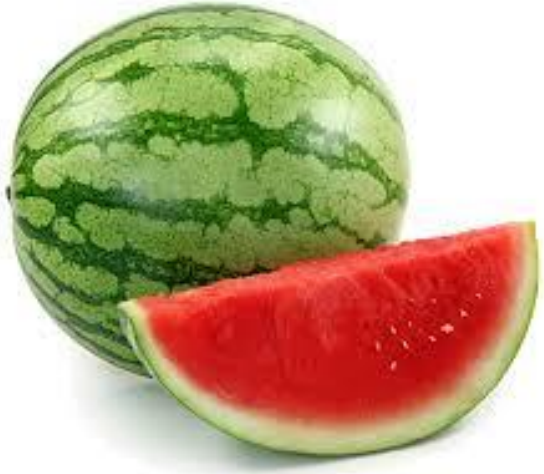
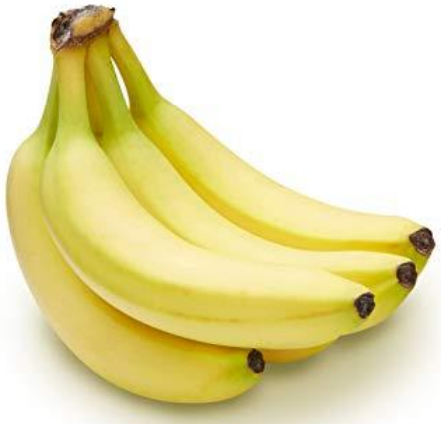
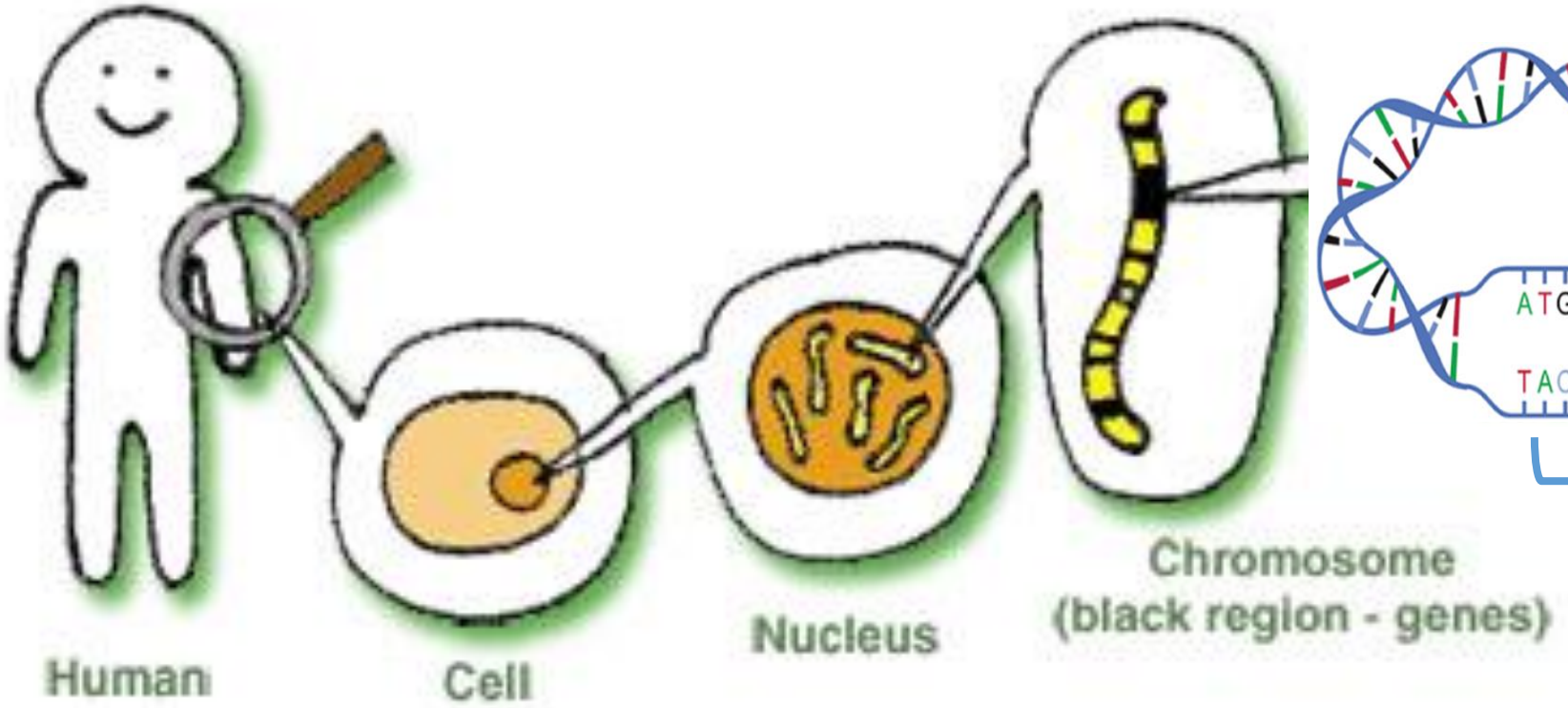
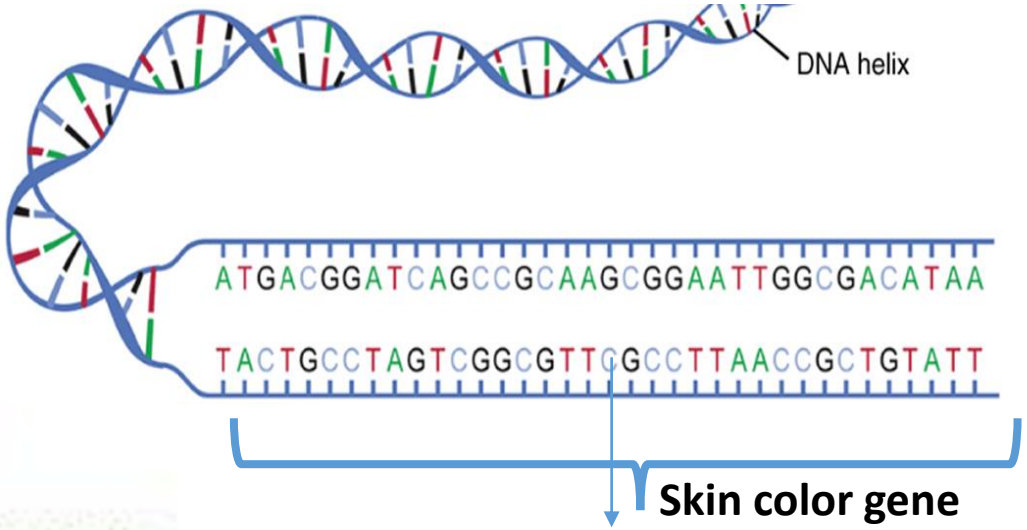




Plant Domestication

Hybridization Breeding: Basic Concepts





Hybridization Breeding

Hybridization breeding Depends on genetic variation

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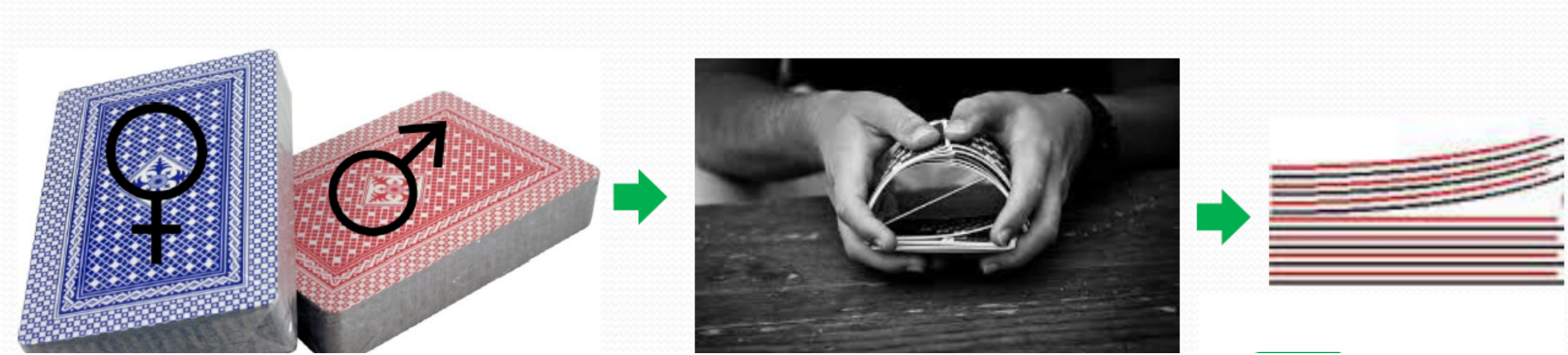
Crossing Different Parents to Create New Variations



Hybridization Breeding

+

=

The pug and beagle have been bred to produce the 'puggle', a mixed breed with both pug and beagle traits.

- Introduce desirable traits from one parent to another
- Selection based on morphological characteres
- With aritifical intervention

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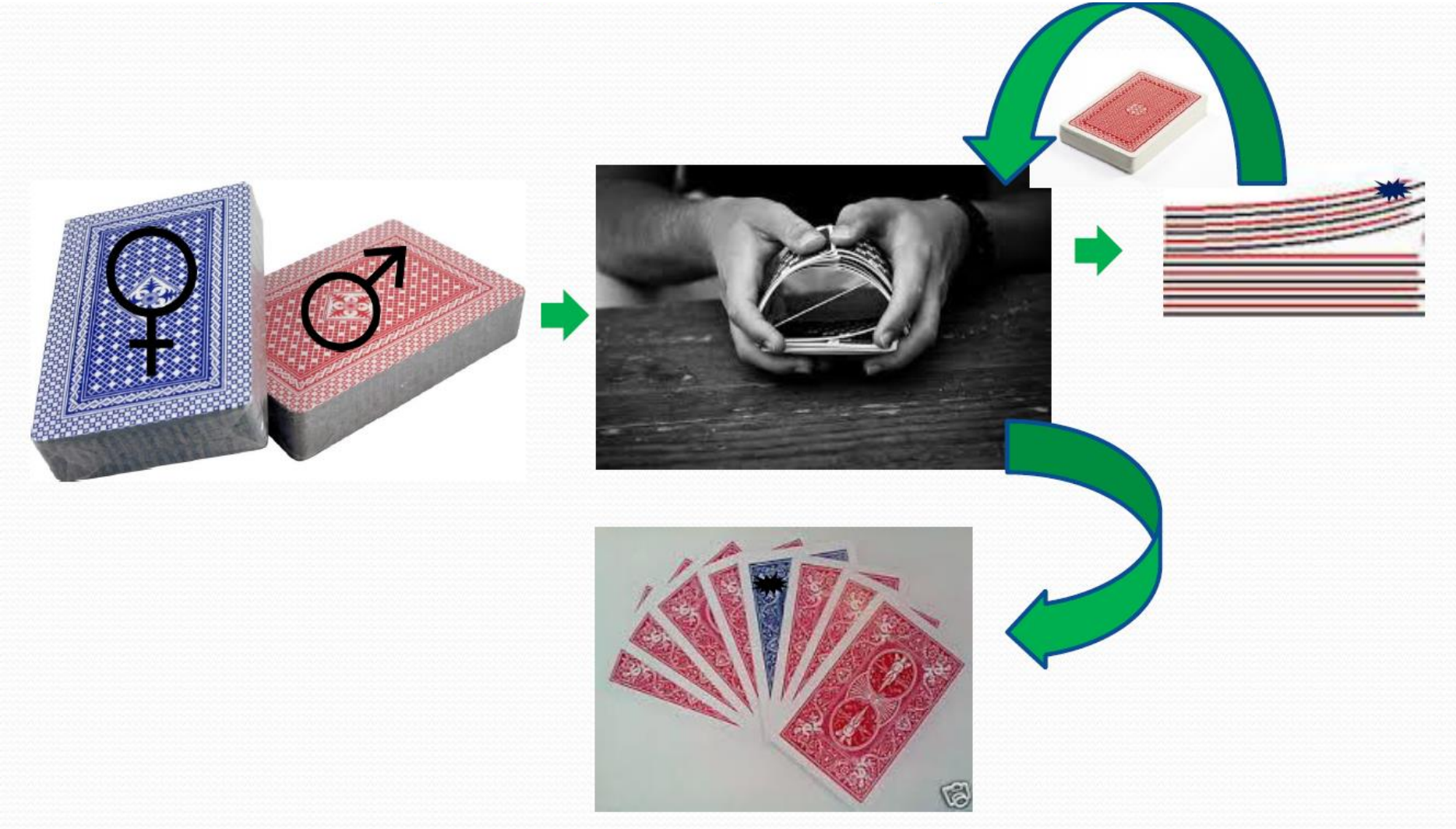
Hybridizations Breeding: Backcrossing to Clean Up Genome



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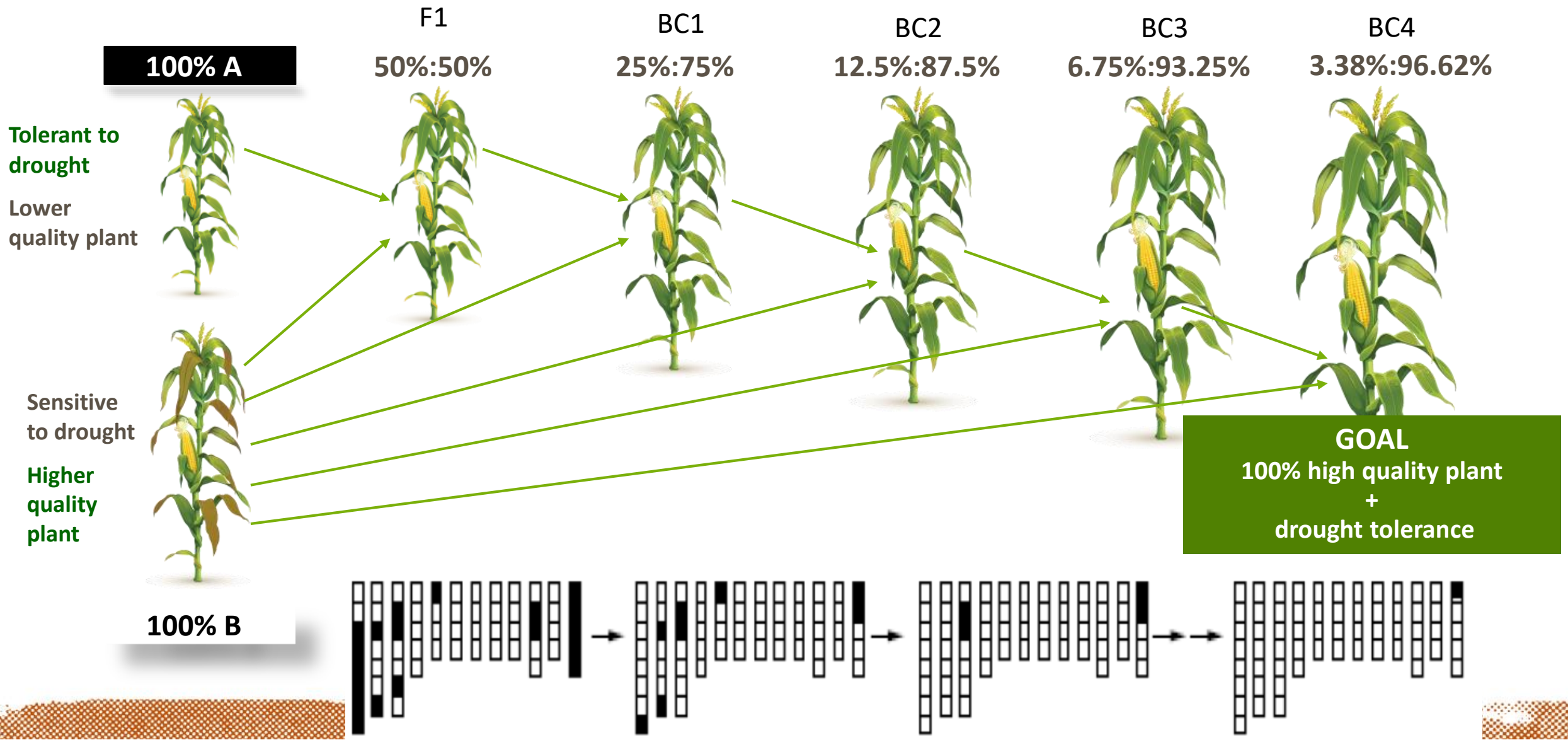
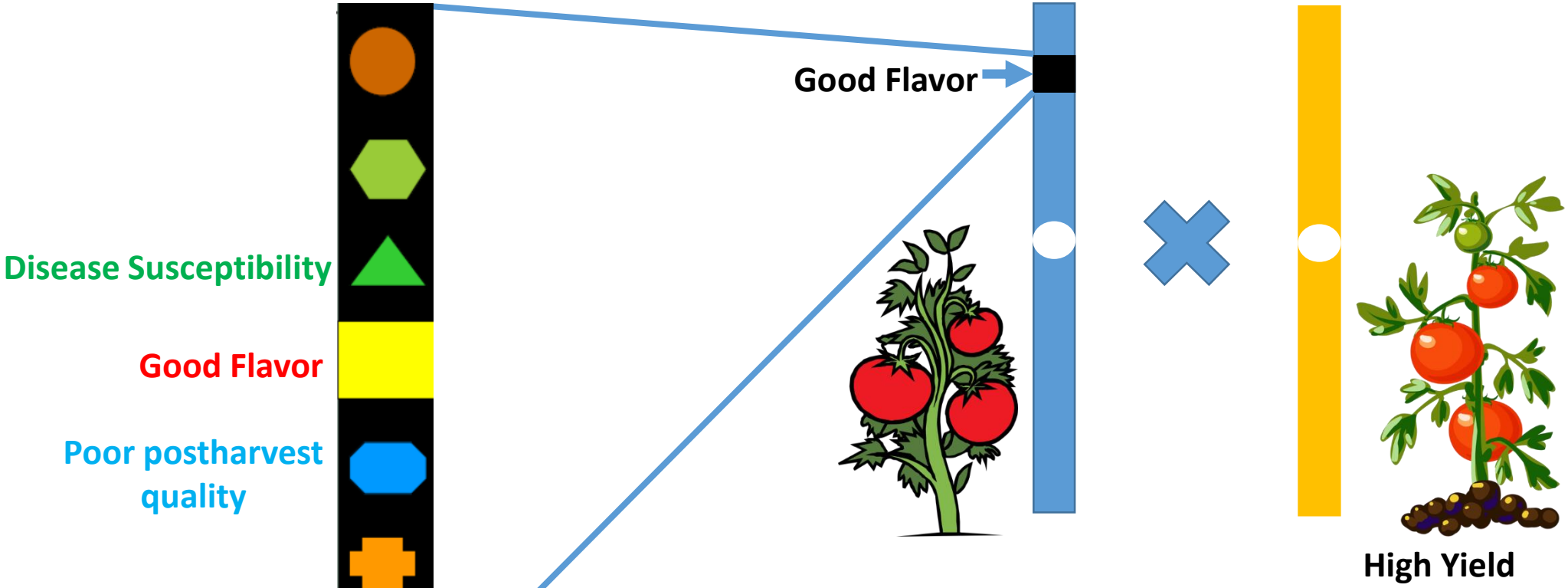


Linkage Drag: Nightmare for Traditional Plant Breeders

Never has progenies with good flavor and better postharvest quality, disease resistant tomato



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Pros and Cons of Hybridization Breeding

Pros:

- No requirement for knowing genetic and genomic background
- Not regulated by USDA, ecological, environmental friendly
- Straightforward phenotypic selection, technically easy

Cons:

- Heavily relies on genetic variation, may not exist in nature
- Time and labor consuming compatibility, embryo lethality
- Quantitative traits are hard for selection
- Linkage drag

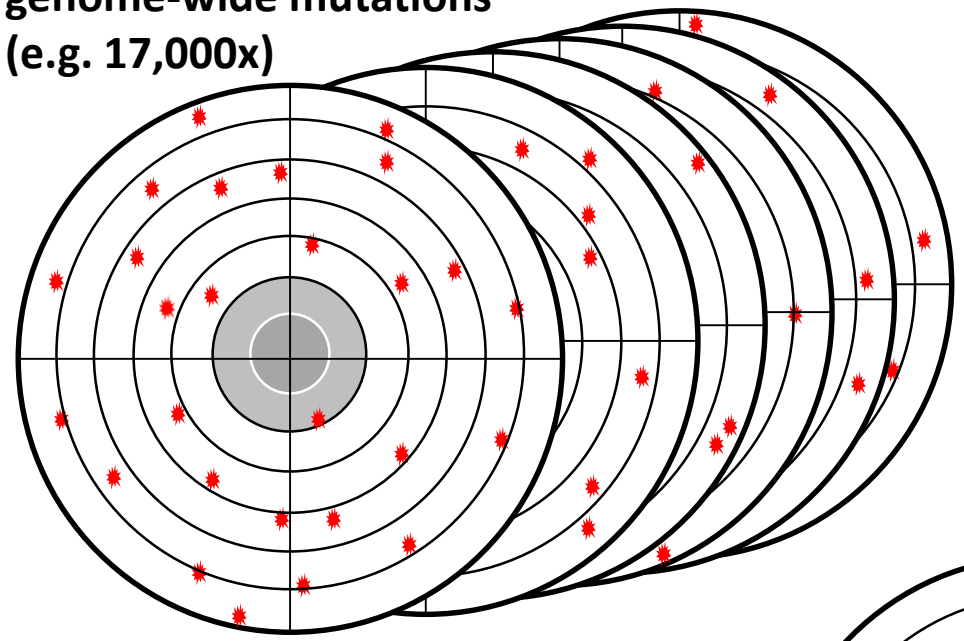
Mutation Breeding

- Spontaneous mutations: continuous source of natural genetic variation
- Traditional mutagenesis: inducing mutations by radiation or chemical mutagens

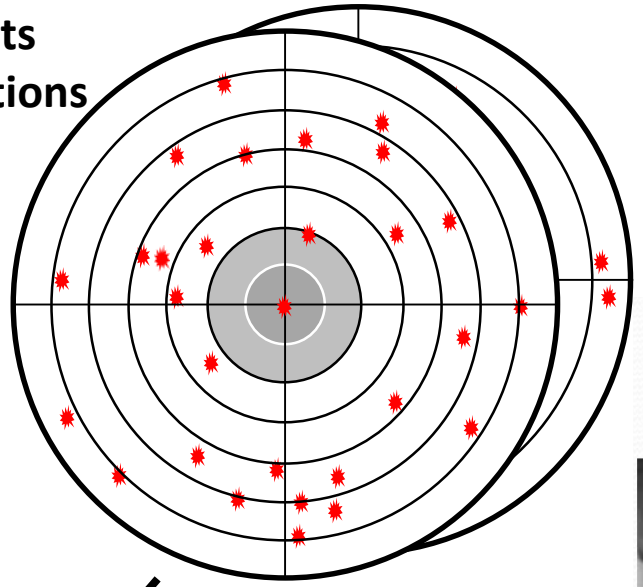
  -Large population of mutagenized plants needed (5.000-10.000)

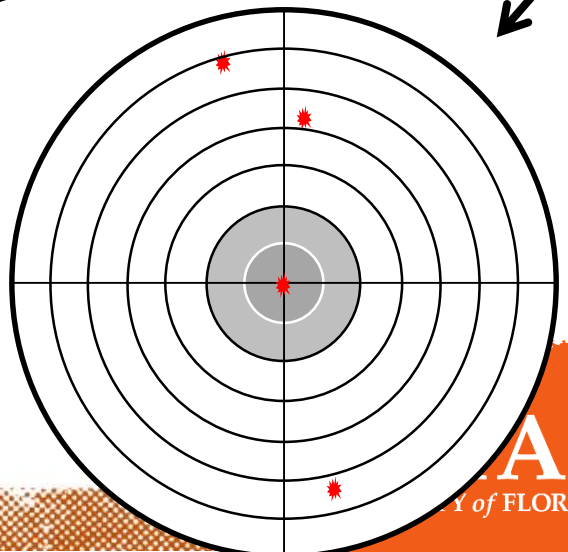
Traditional Mutagenesis

Population of plants with genome-wide mutations (e.g. 17,000x)

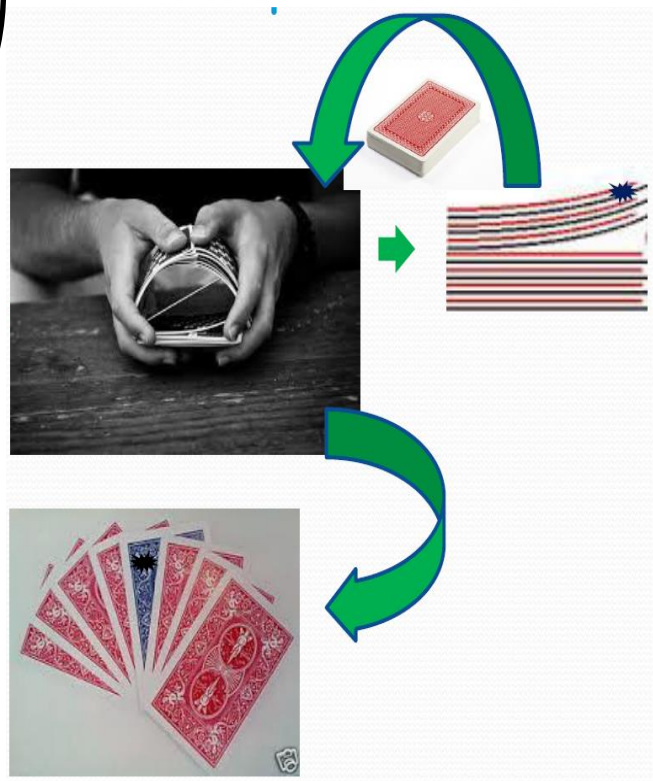


Select plants with mutations in targeted gene





Further crossing to remove undesirable mutations and to obtain optimal varieties



Examples of Mutation Breeding

Bad mutations

Bad mutations

Seedless orange

Salt Tolerant Rice

Diverse colorful flowers

Diverse colorful flowers

Foliage with different leaf pattern

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Pros and Cons of Mutation Breeding

Pros:

- Induction of desirable mutant that is not present in natural plant materials
- Not regulated by USDA, ecologically, environmentally friendly
- Straightforward phenotypic selection, technically easy

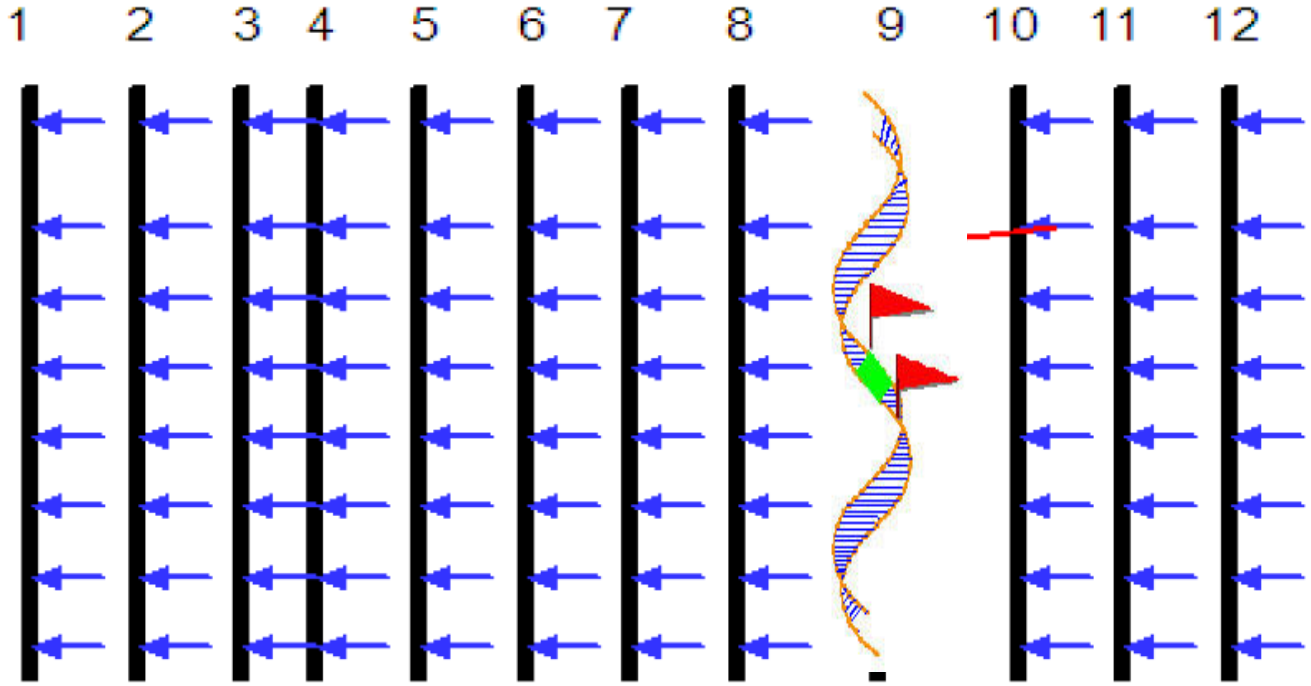
Cons:

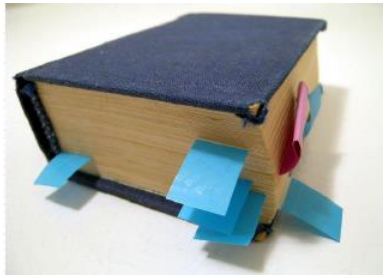
- generally random and unpredictable
- “good” mutations come with “bad” mutations
- Need large mutant pool to identify “good” one
- Costly and Slow

Molecular Marker Assisted Selection Breeding

- Marker assisted selection refers to the use of DNA markers that are tightly-linked to target loci

- Assumption: DNA markers can reliably predict phenotype





Pros and Cons of MAS Breeding

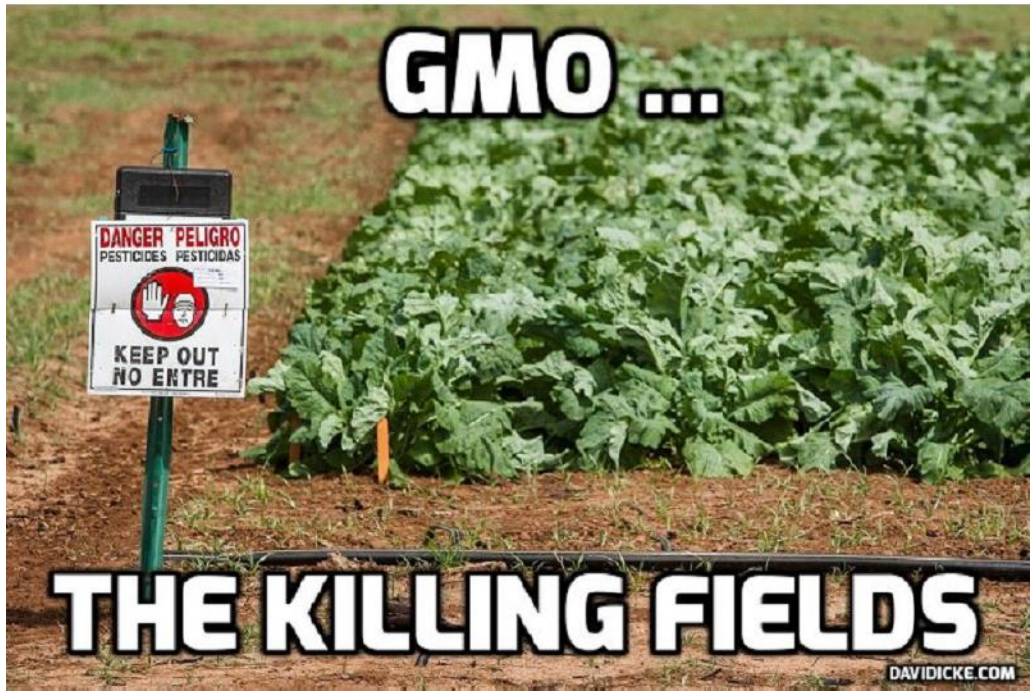
Pros:

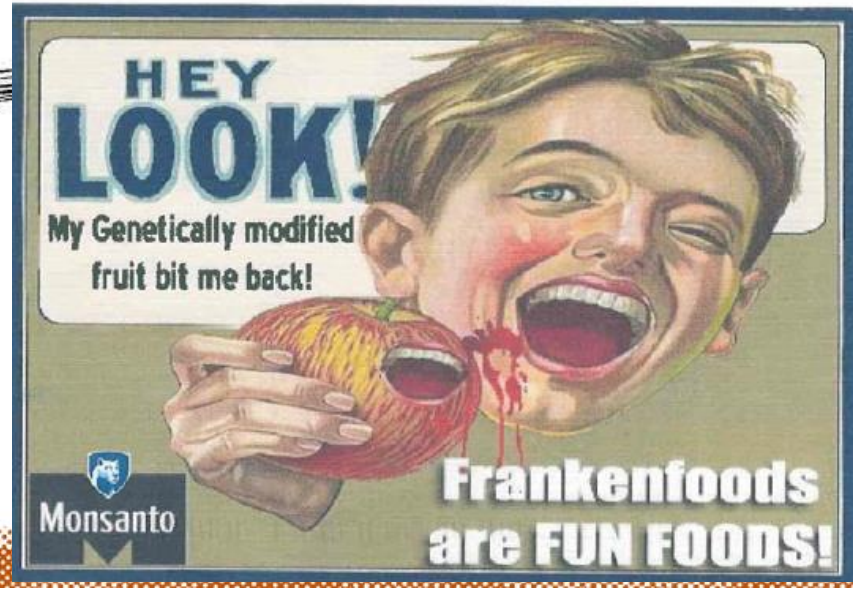
- Similar to traditional breeding, not regulated by USDA
- Accelerating breeding process
- Easier for stacking multiple traits within the same cultivar

Cons:

- Must know genomic and genetic background
- Very costly
- False markers

Genetic Engineering





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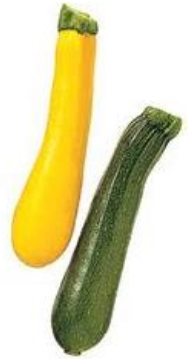
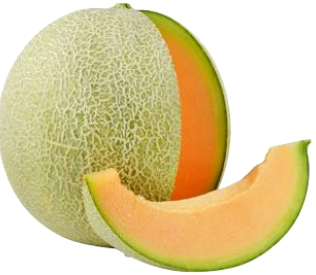
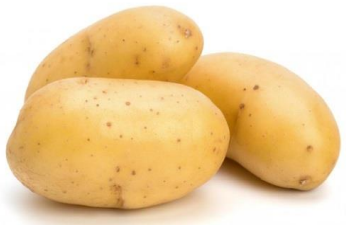
Are these crops GMOs?







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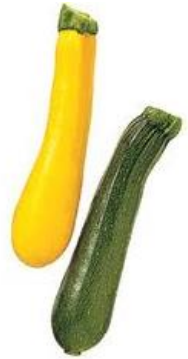

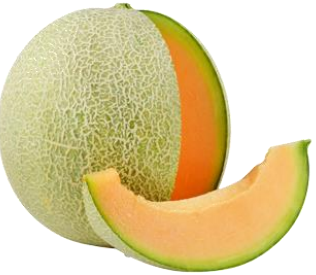

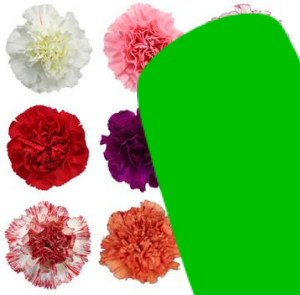

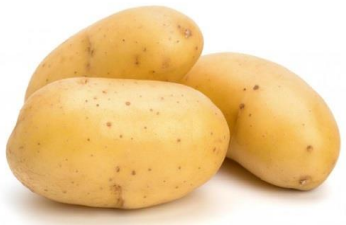

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How many approved GMOs in U.S.?

203 approved Varieties for 21 crop species

What Traits Were Modified?

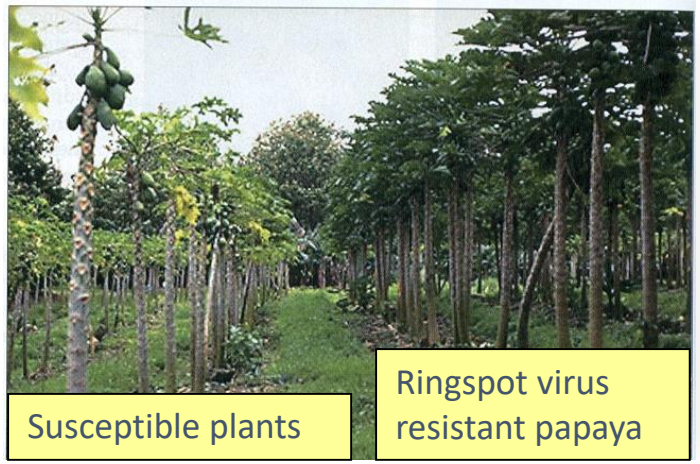
Traits	Crops
Altered lignin production	Alfalfa
Non-browning	Apple
Modified fatty acid	Canola
Herbicide Tolerance	Alfalfa, Canola, Chicory, cotton, creeping bentgrass, flax, corn, rice, soybean, sugar beet, wheat
Male sterility	Alfalfa, Chicory, corn
insect-resistance	Cotton, corn, potato, rice, soybean, sugar cane, tomato
Nutrition	Canola, corn, potato, rice, soybean
Drought tolerance	Corn
Delayed ripening	Melon, tomato
disease resistance	Papaya, potato, squash
Modified flower color	rose
Yield	soybean
Nicotine reduction	tobacco

GMO Examples

Developed by Australia
Approved in Australia, Japan, European Union

Modified color (genes from petunia)
Herbicide resistance (mutated gene from tobacco)

GMO Examples



GMO Examples

EPA approves field trials of disease-resistant GMO citrus trees

Biotechnologia Sí | November 16, 2016

Stopping Citrus Greening

American chestnuts were once a dominant tree, and a major source of food, in the forests of eastern North America. ANDREW NEWHOUSE

To save iconic American chestnut, researchers plan introduction of genetically engineered tree into the wild

By Gabriel Popkin | Aug. 29, 2018 , 12:30 PM

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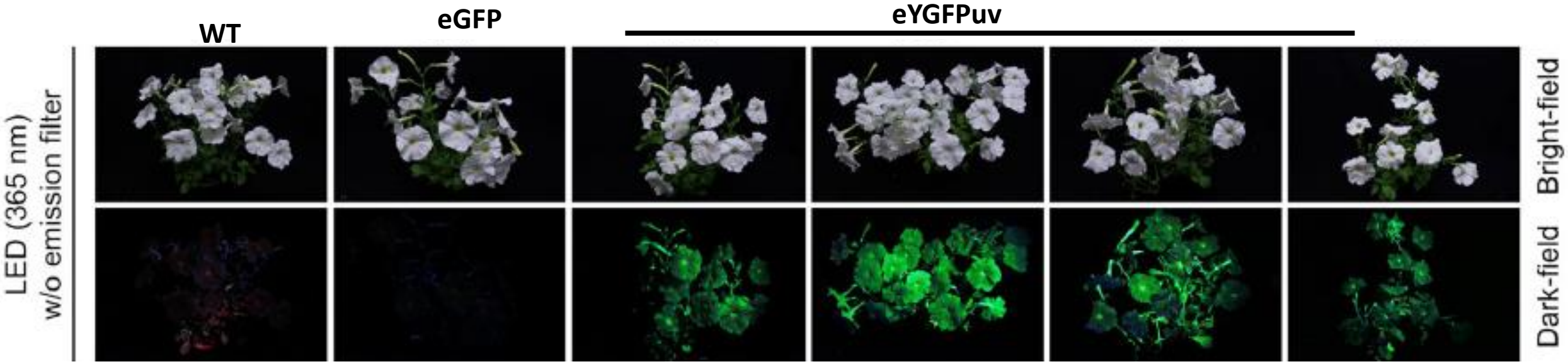
Development Of Glowing Ornamental Plants

LED(365nm) UV light



Scientific Reports (2018) 8:16556

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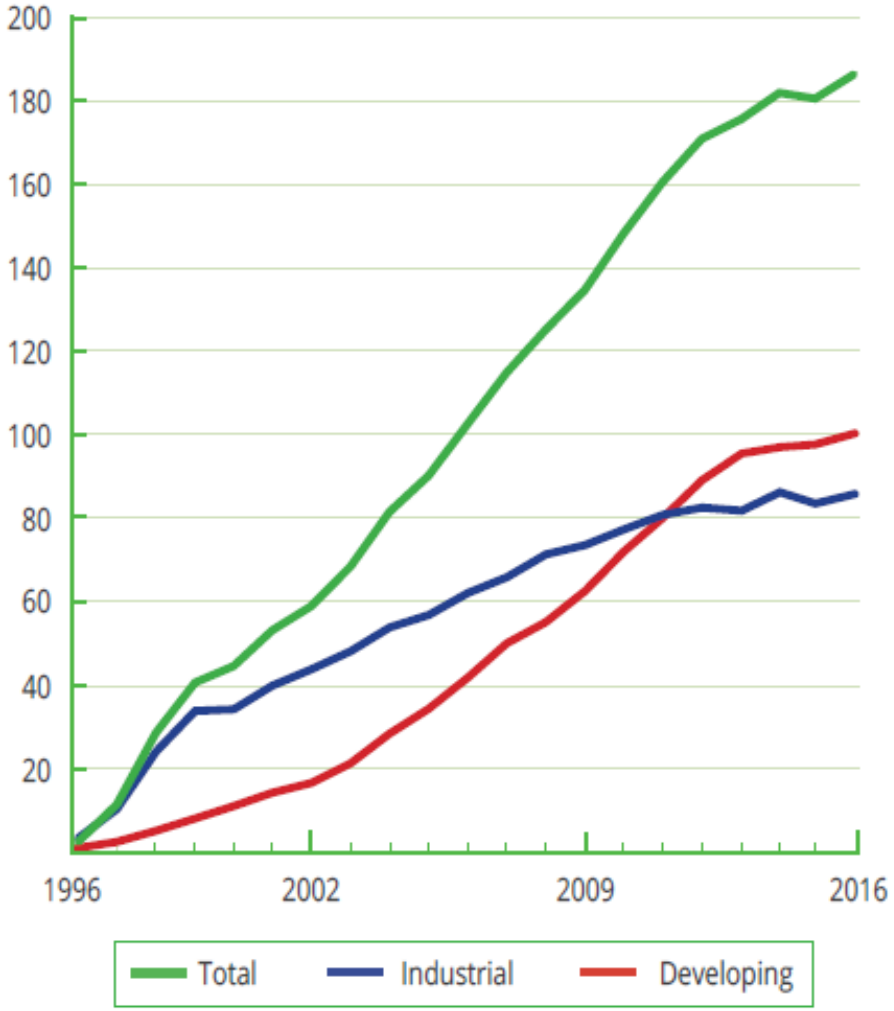
Table 1. Global Area of Biotech Crops, the First 21 Years, 1996 to 2016

Year	Hectares (million)	Acres (million)
1996	1.7	4.2
1997	11.0	27.2
1998	27.8	68.7
1999	39.9	98.6
2000	44.2	109.2
2001	52.6	130.0
2002	58.7	145.0
2003	67.7	167.3
2004	81.0	200.2
2005	90.0	222.4
2006	102.0	252.0
2007	114.3	282.4
2008	125.0	308.9
2009	134.0	331.1
2010	148.0	365.7
2011	160.0	395.4
2012	170.3	420.8
2013	175.2	432.9
2014	181.5	448.5
2015	179.7	444.0
2016	**185.1**	**457.4**
Total	**2,149.7**	**5,312.0**

Figure 1. Global Area of Biotech Crops, 1996 to 2016: Industrial and Developing Countries (Million Hectares)



Source: ISAAA, 2016

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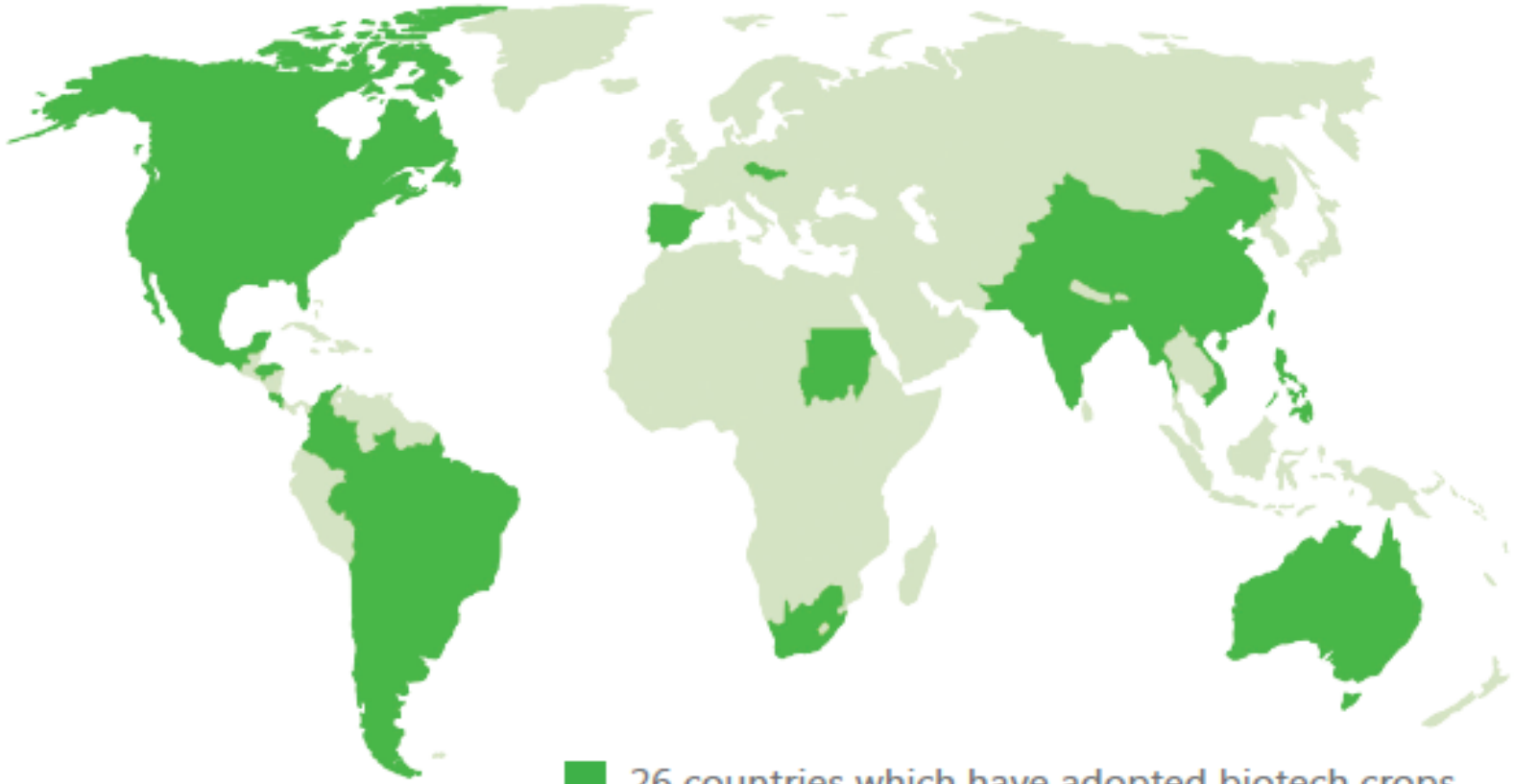


26 countries which have adopted biotech crops

3%
Increase from 2015

In 2016, global area of biotech crops was 185.1 million hectares, representing an increase of 3% from 2015, equivalent to 5.4 million hectares.

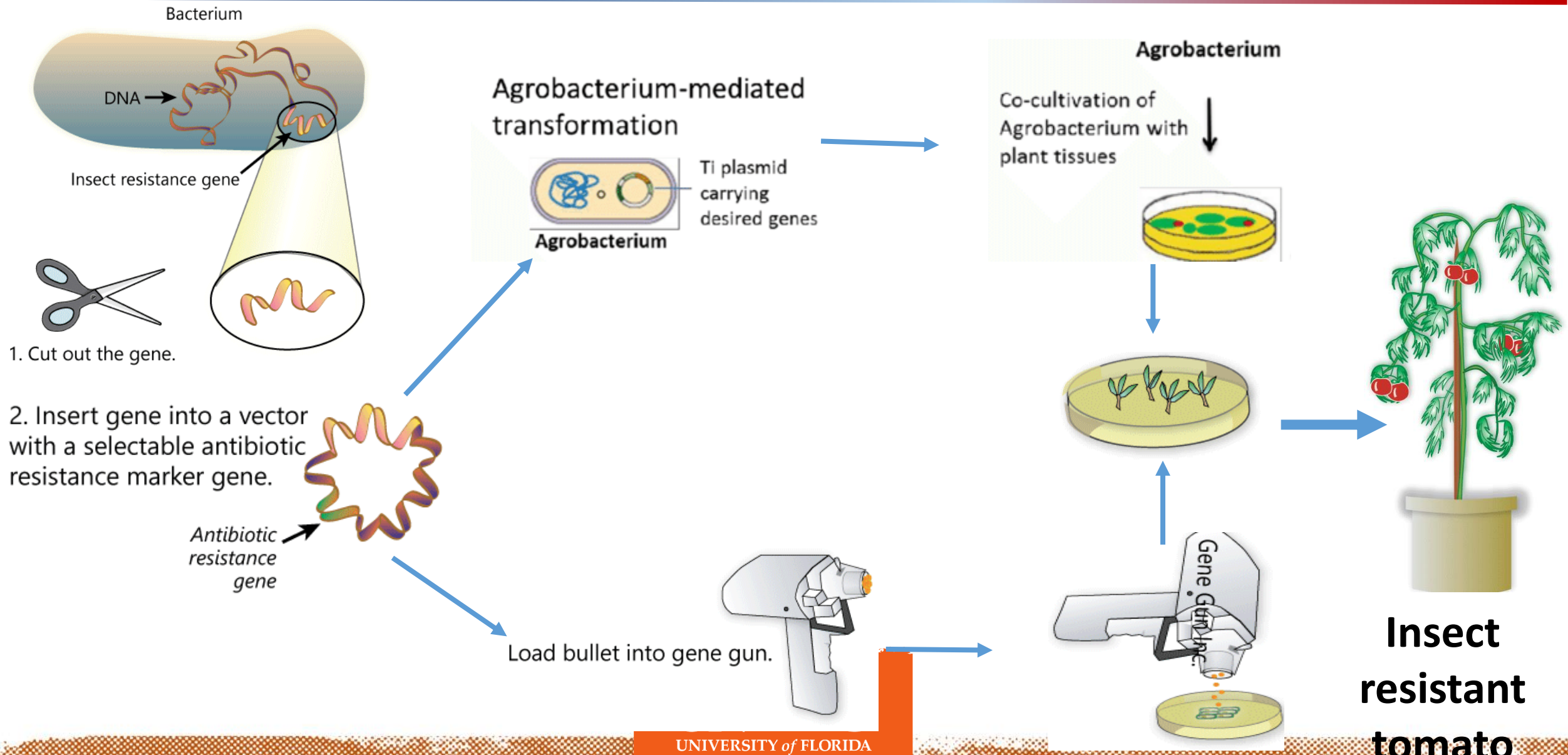
Source: ISAAA, 2016.

50,000 hectares, or more		
1.	USA	72.9 million
2.	*Brazil**	49.1 million
3.	*Argentina**	23.8 million
4.	Canada	11.6 million
5.	*India**	10.8 million
6.	*Paraguay**	3.6 million
7.	*Pakistan**	2.9 million
8.	*China**	2.8 million
9.	*South Africa**	2.7 million
10.	*Uruguay**	1.3 million
11.	*Bolivia**	1.2 million
12.	Australia	0.9 million
13.	*Philippines**	0.8 million
14.	*Myanmar**	0.3 million
15.	Spain	0.1 million
16.	*Sudan**	0.1 million
17.	*Mexico**	0.1 million
18.	*Colombia**	0.1 million

Less than 50,000 hectares	
*Vietnam**	*Bangladesh**
*Honduras**	*Costa Rica**
*Chile**	Slovakia
Portugal	Czech Republic

** Developing countries*

How Are GMOs Created?



Pros and Cons of Genetic Engineering

Pros:

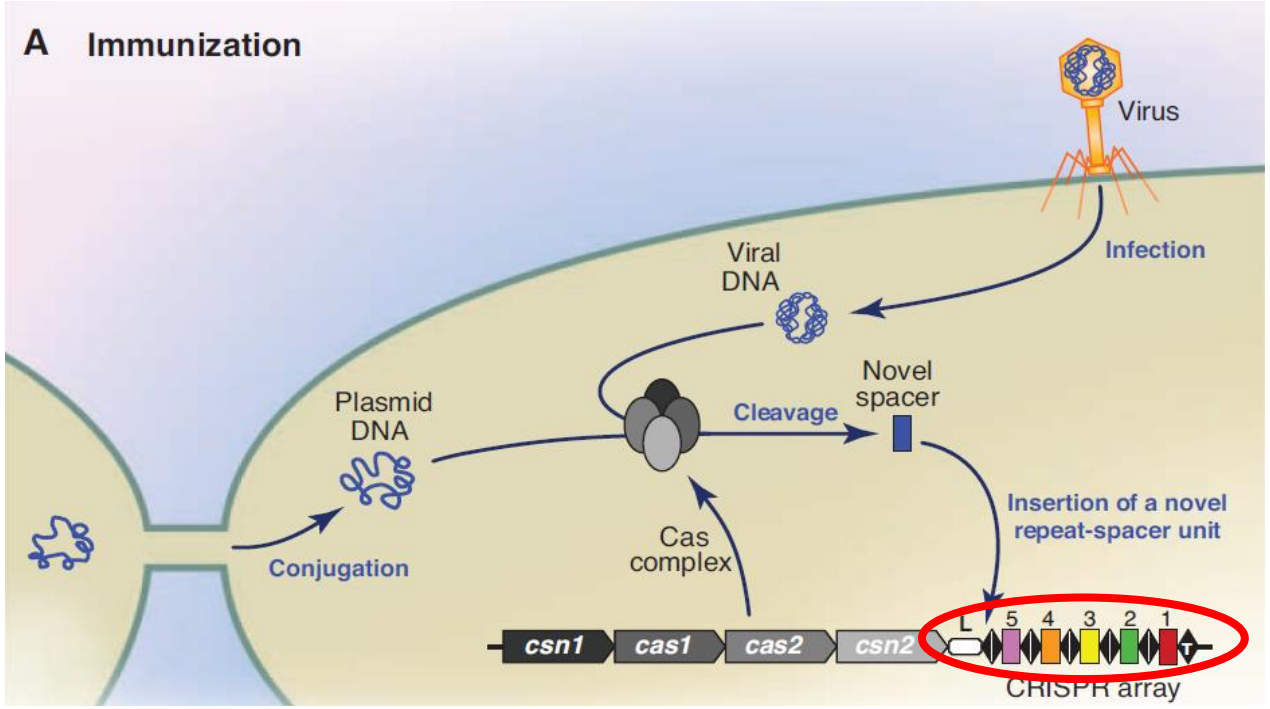
- Fast way to verify gene function
- precisely modify crop productivity and quality

Cons:

- Necessary to know gene function
- Very costly, complicated procedures
- Heavily regulated by USDA

The Latest Innovation: CRISPR/Cas9 Genome Editing

CRISPR:
Clustered Regularly Interspaced Short Palindromic Repeats



CRISPR Loci induce acquired immunity in Bacteria against the virus infection or plasmid transfer

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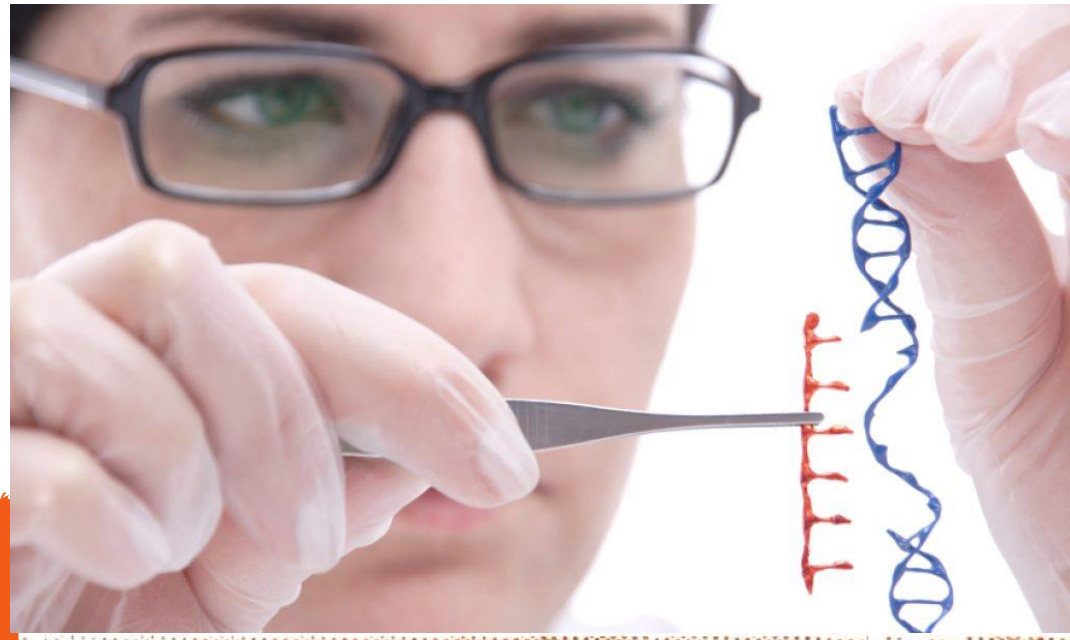
Horvath and Barrangou, 2010, Science: 167-170

Features of CRISPR/Cas9 Genome Editing

- High precision, High efficiency, Broad application
- Procedures are identical to genetic modification
- Final products are similar to traditional breeding



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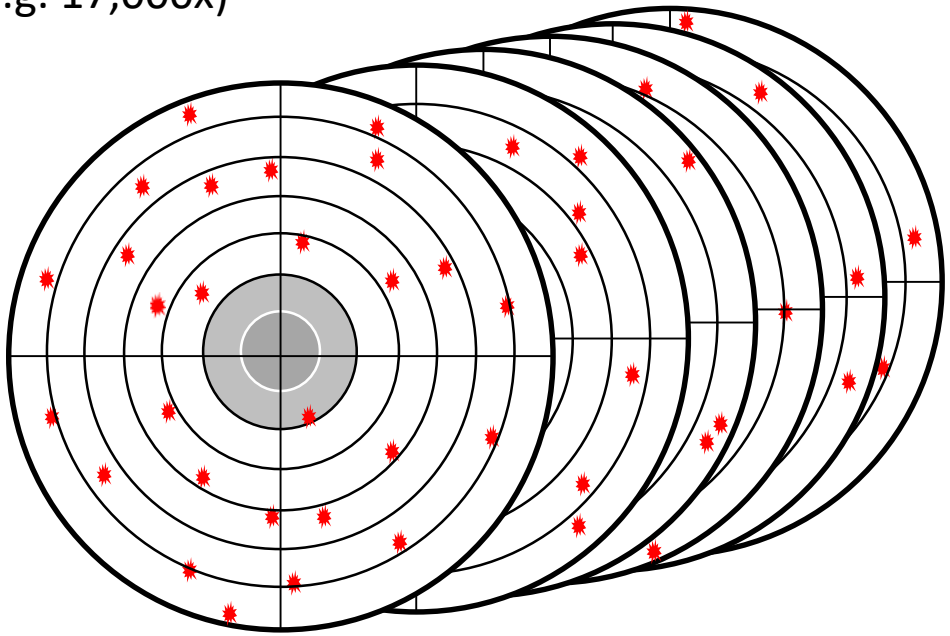
High Precision: no or less non-targeted mutations

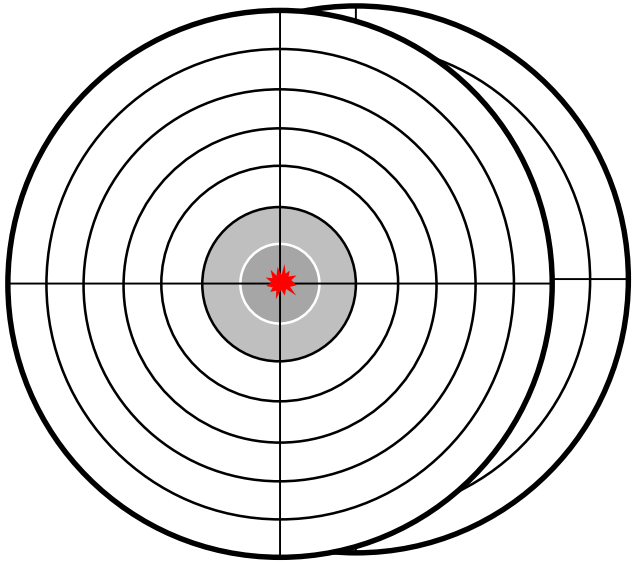
Traditional Mutagenesis Vs Targeted Mutagenesis

Population of plants with genome-wide mutations (e.g. 17,000x)



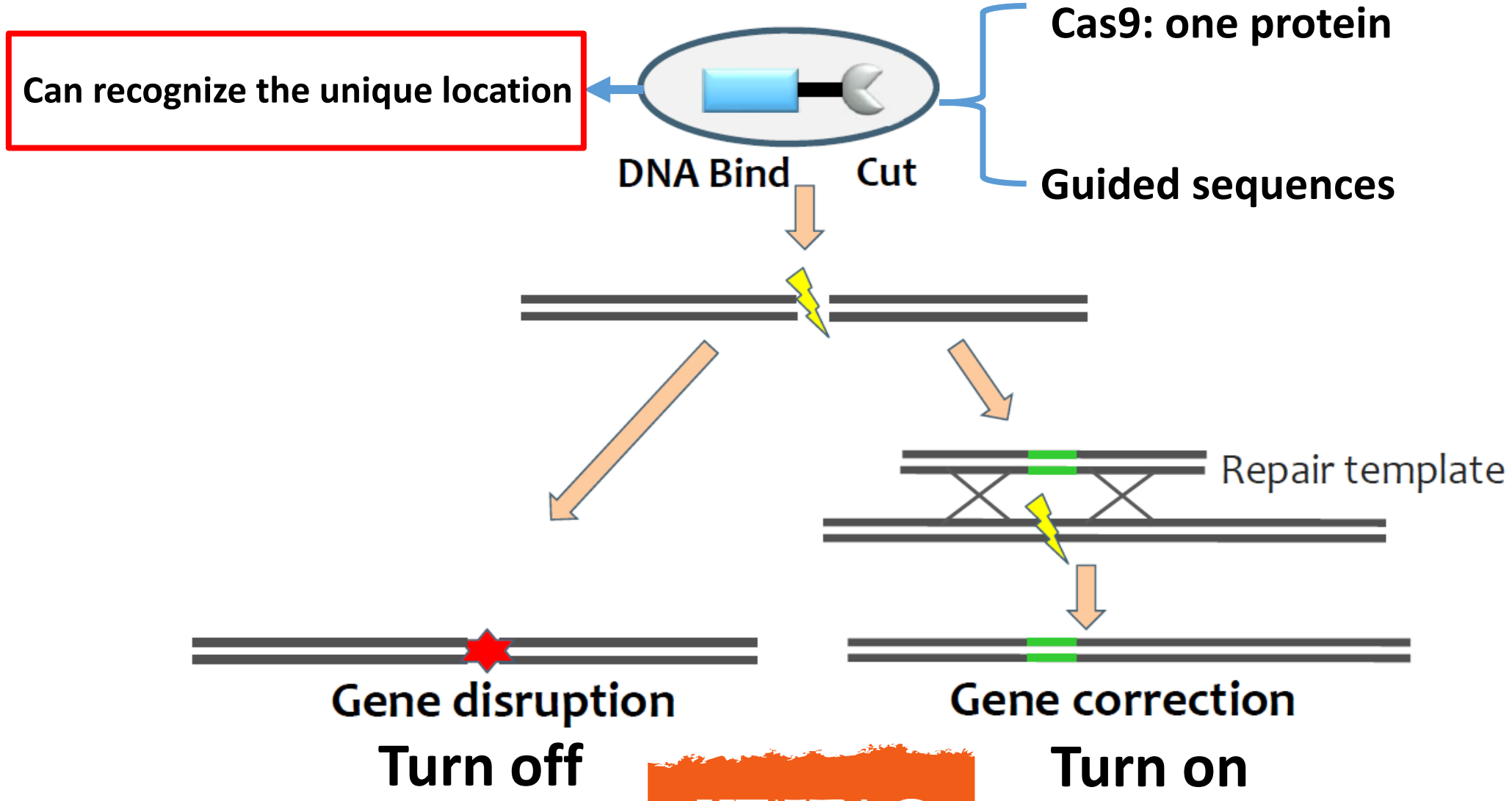
Traditional mutagenesis

A few plants with targeted mutations



Targeted mutagenesis

How CRISPR/CAS9 Can Create Precise Mutation?



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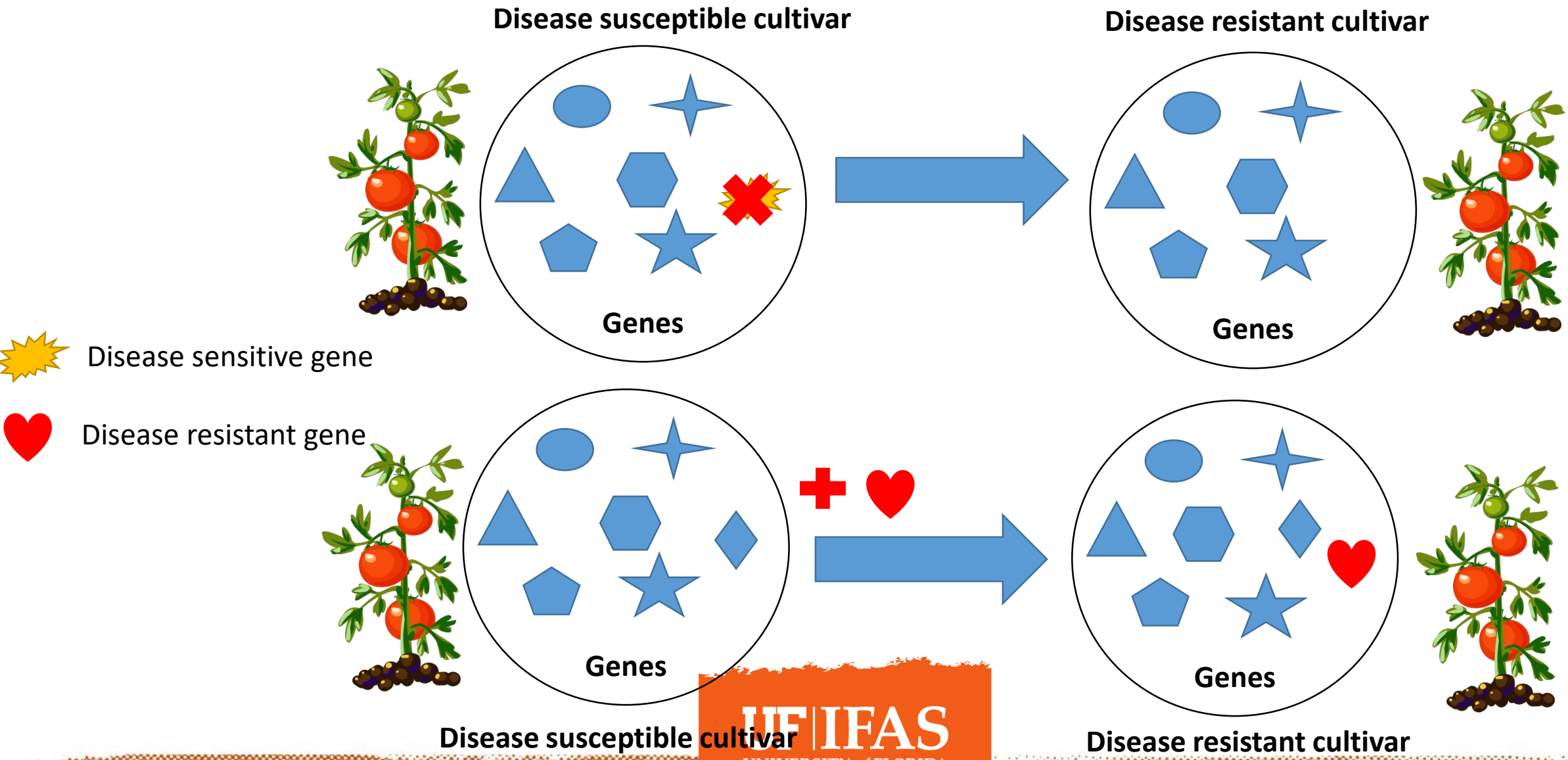
Turn Off or Turn On



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Precision

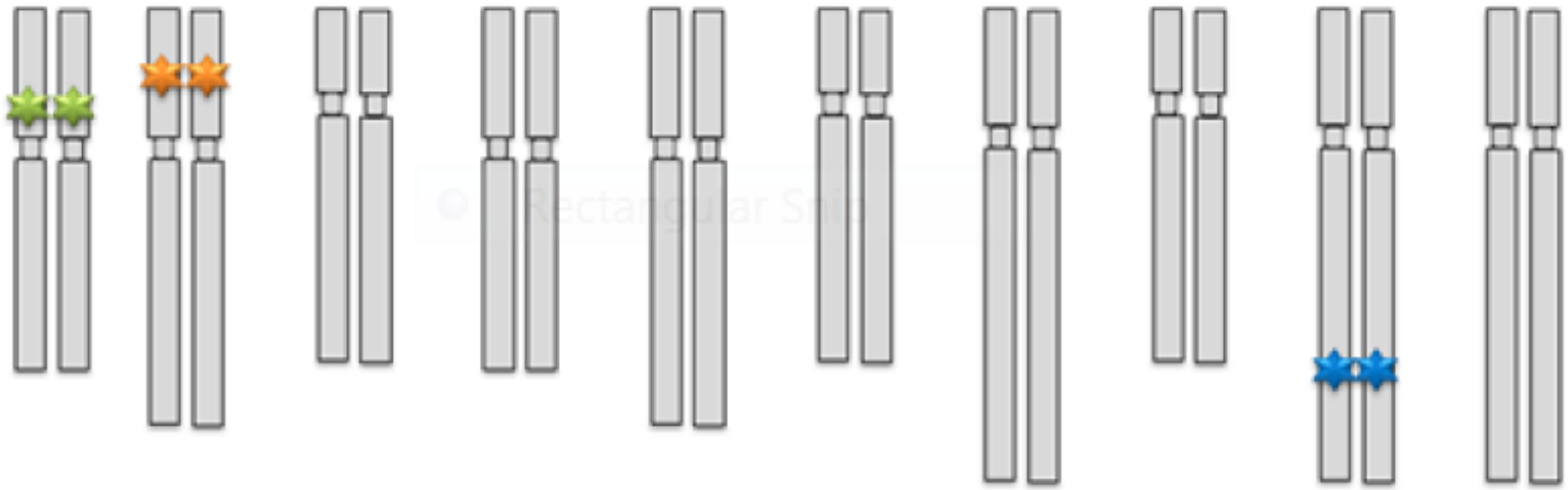
ALS2 gene editing for herbicide resistant corn

- **Ability to target ALS2, but not sister ALS1, for herbicide resistance in corn**

Herbicide-resistant

High Efficiency: Multi-Targeting

Simultaneous targeting of multiple genes



	Chrom1 *MS26*	Chrom2 Lig34	Chrom9 *MS45*
Lig34 + MS26+ MS45	Mutant	Mutant	Mutant

In rice, more than 8 targets were successfully targeted

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High Efficiency: Time

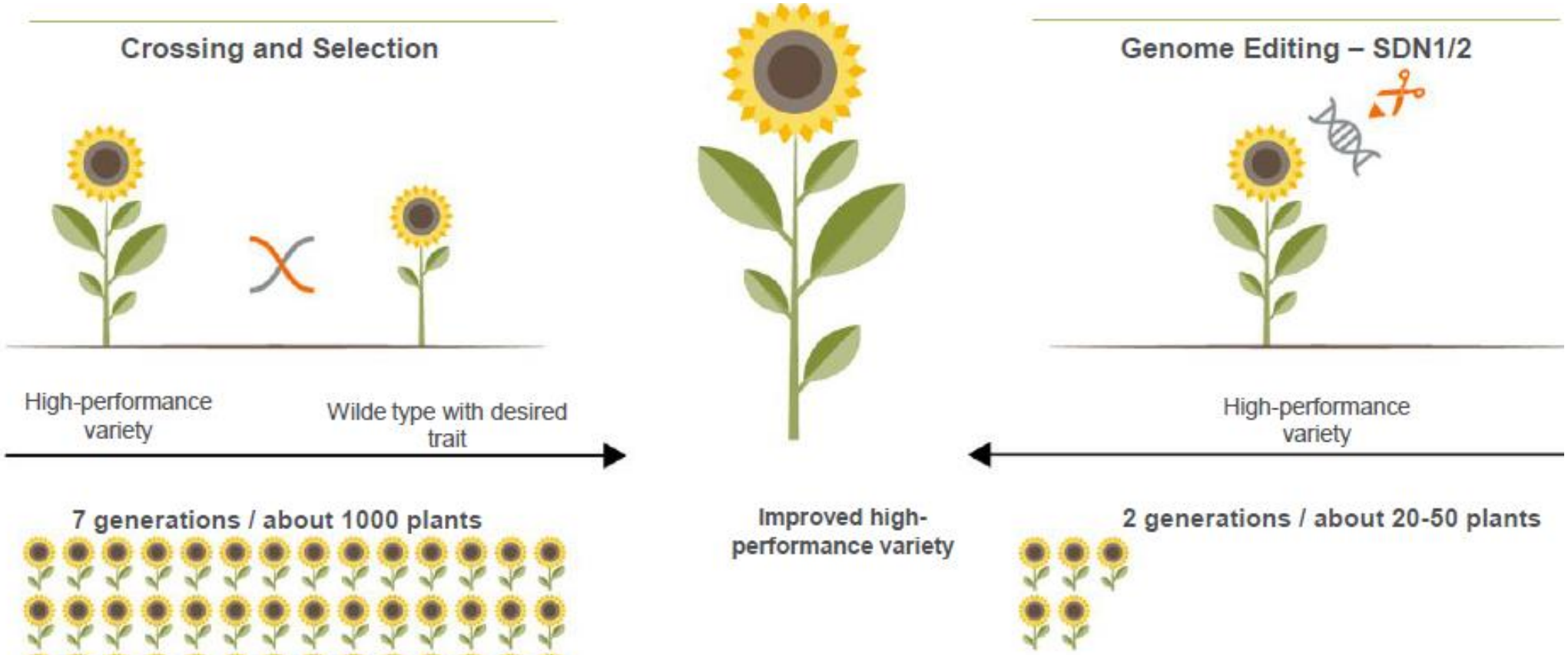
Selection and plant regeneration

2-3 months

Growth to maturity and seed set in controlled environment

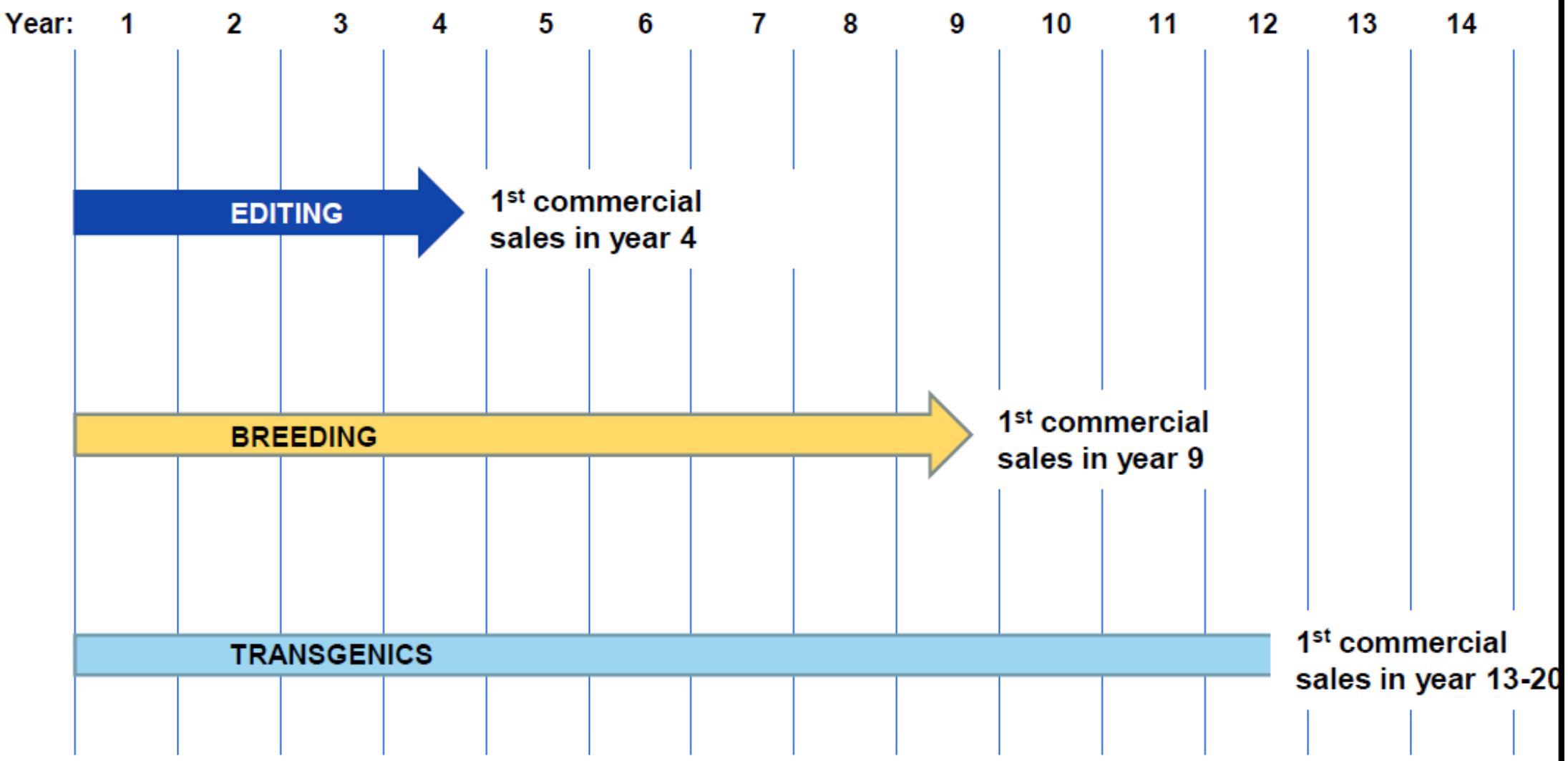
3-4 months

High Efficiency: Time



- **Significantly reduce the number of crossing cycles**
- **Significantly reduce plant number for each generation**

High Efficiency: Time

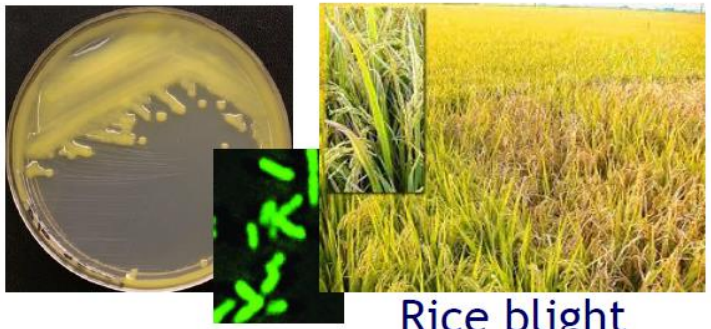


Broad applicability: Turn Off a Disease Gene

MLO1 gene, controlling powder mildew sensitivity, has been identified in wheat, barley, rice, tomato, petunia, tobacco, eggplant, cucumber, squash, melon, strawberry, lettuce, orange, and more.

Use CRISPR to knock out MLO1 gene to create powdery mildew pathogen resistant tomato

Broad applicability: Multiple Plant Species



Rice blight

Cabbage black rot

Wheat streak

Citrus canker

***Xanthomonas* infects >200 plant families**

Cassava blight

Banana wilt

Cotton blight

Figures modified from the internet

Broad applicability: Multiple Plant Species

Gene editing of OsSWEET14 to create Blight(Xanthomonas)- resistant rice

In collaboration with Dr. Deng in Gulf Coast Rec, SWEET gene has been edited for testing citrus canker disease resistance.

Similar strategy can be employed to other plant species

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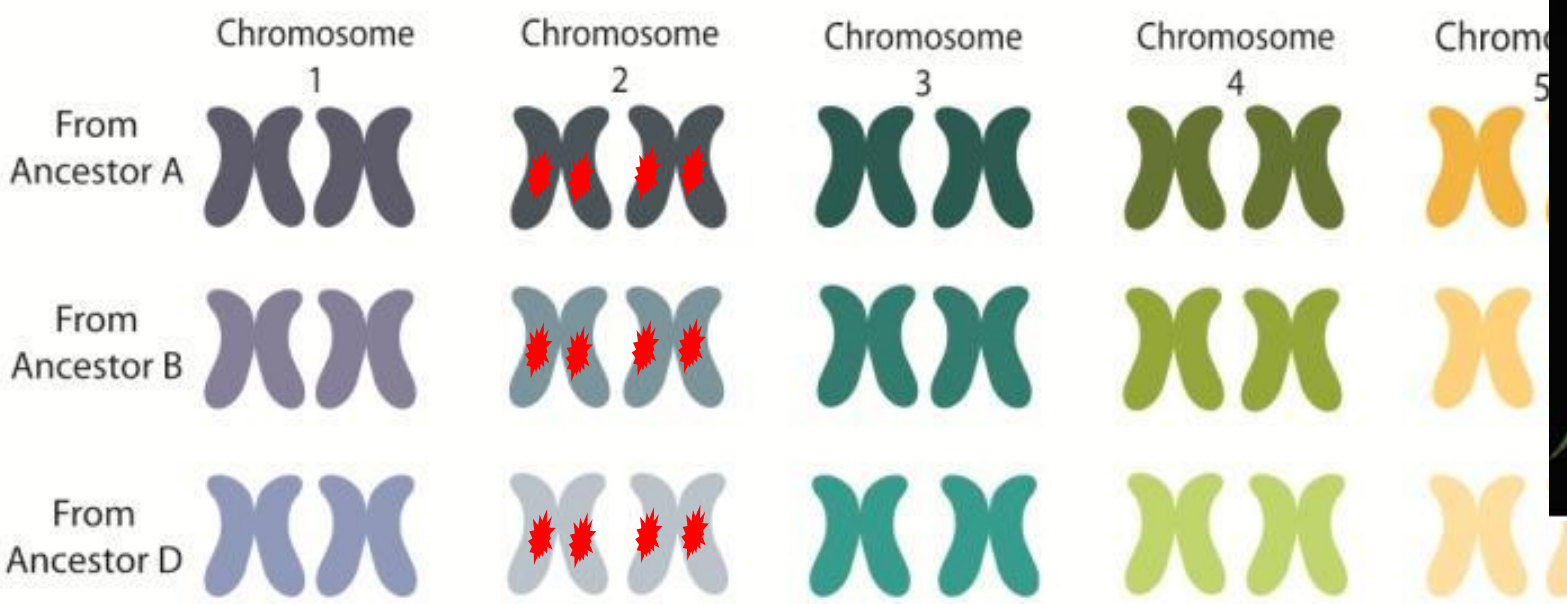
Broad applicability: polyploidy plants

Simultaneous targeting of multiple copies of same genes in polyploidy crops



WT and tadep1-aabbdd mutant (high yield)

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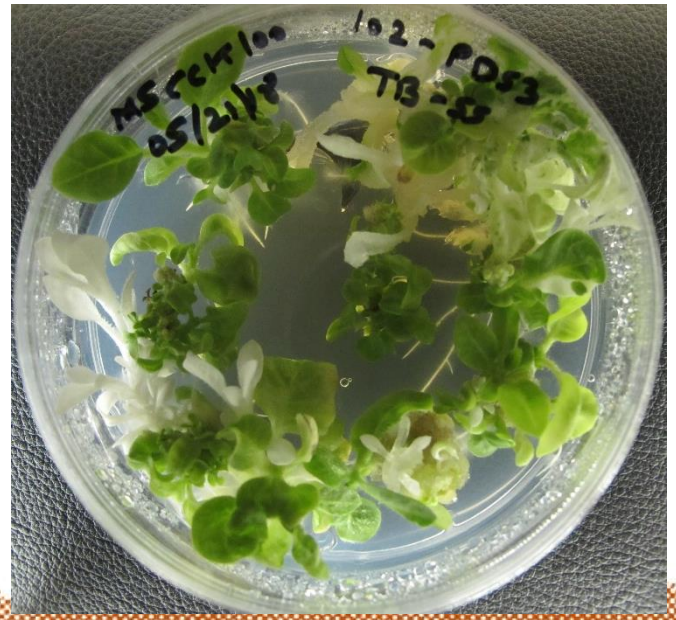
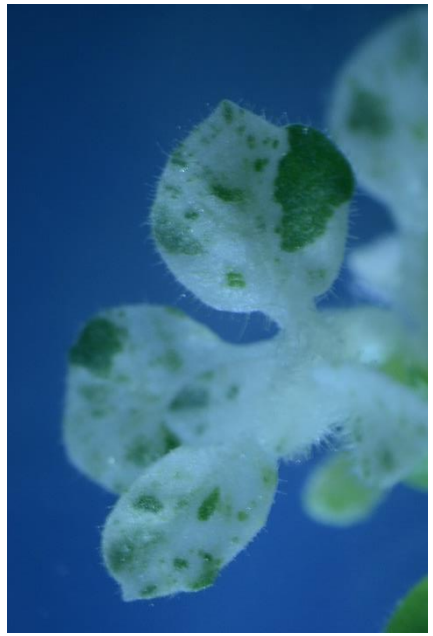
High Gene Editing Efficiency in Allotetraploid Tobacco and Octoploid Strawberry





Allotetraploid Tobacco

Octoploid Strawberry

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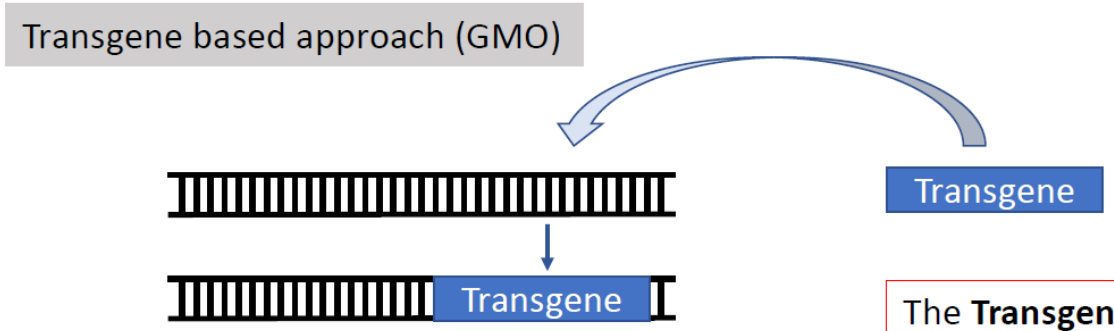
In collaboration with UF/IFAS Gulf Coast Rec

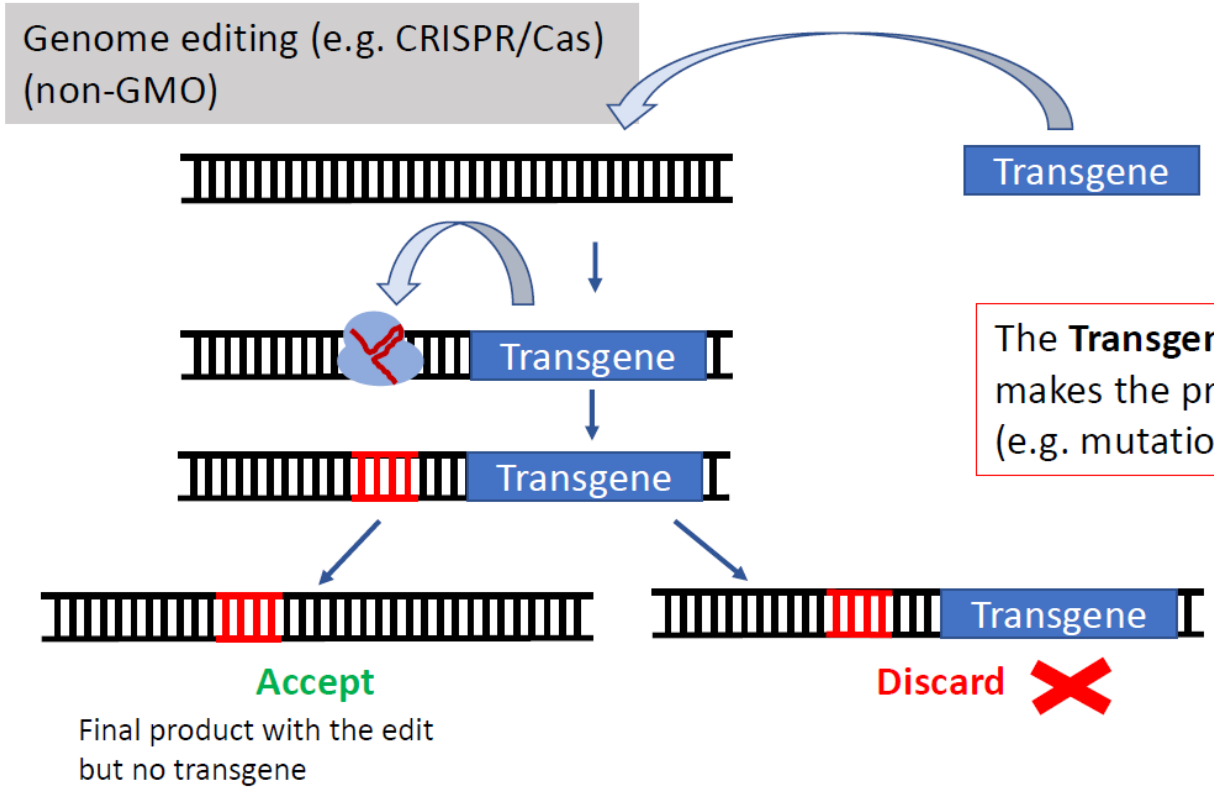
Non-GMO: NO Foreign DNA

GMO approach



The **Transgene (e.g. R-gene)** insertion makes the product

Non-GMO approach

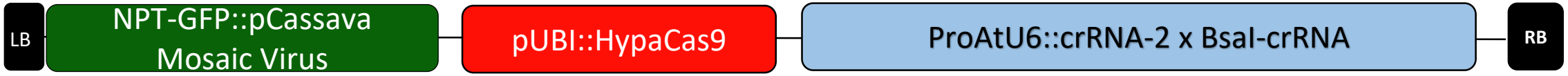


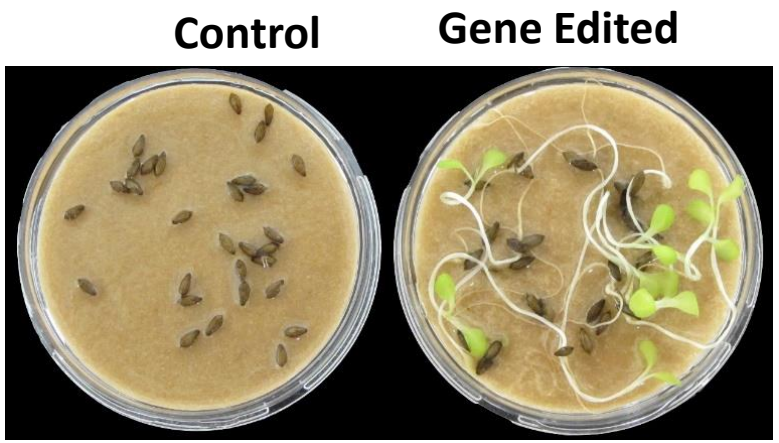
The **Transgene** delivers what makes the product (e.g. mutation in an **S-gene**)

Segregation in T1

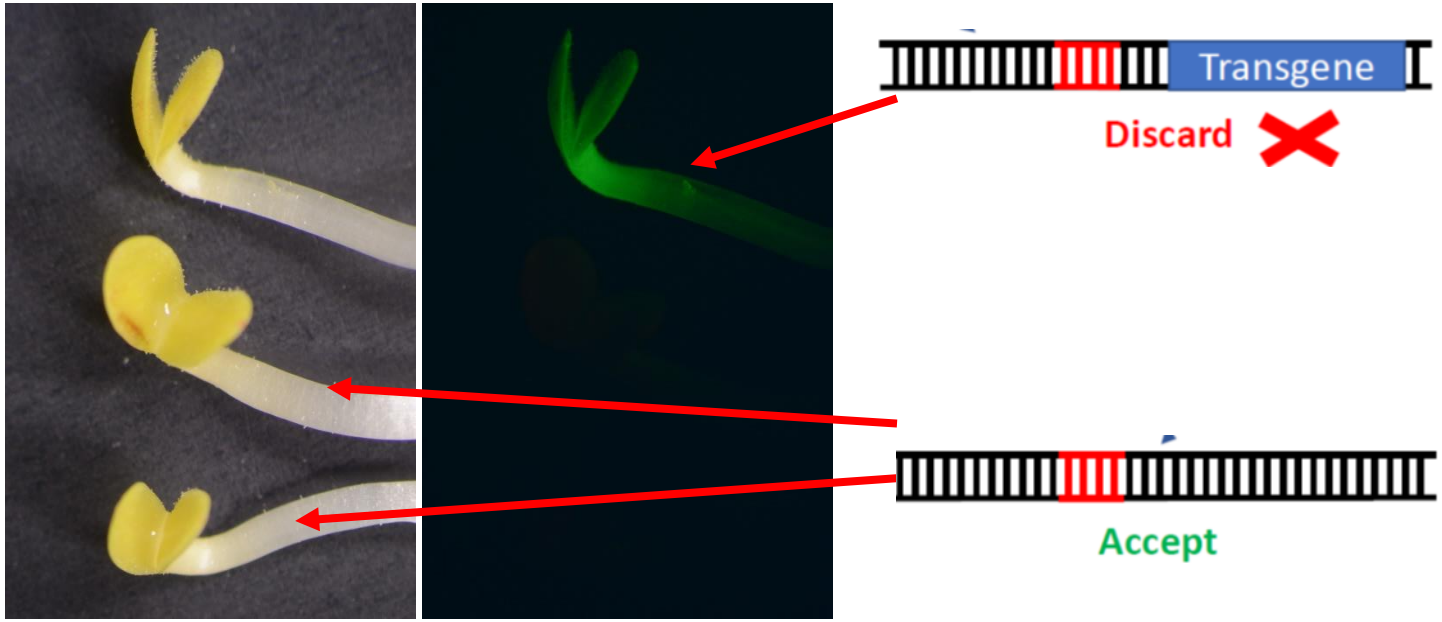
Breeding Non-GMO Lettuce with CRISPR-Genome Editing

Use your eyes to find Non-GMO plants





Lettuce Seeds are inhibited by Florida high temperature, but gene edited lettuce can germinate well



F2 Segregated Seeds

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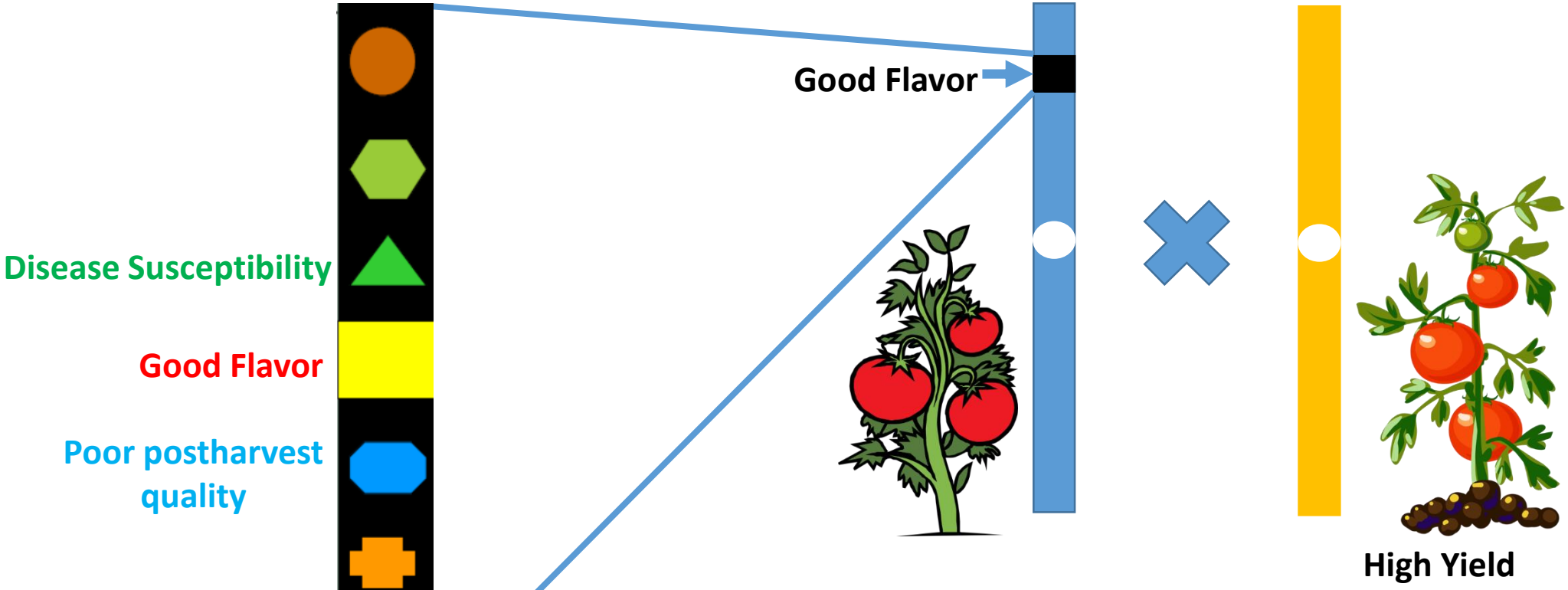
Complimentary To Traditional Breeding : Solution to Linkage Drag

Never has progenies with good flavor and better postharvest quality, disease resistant tomato



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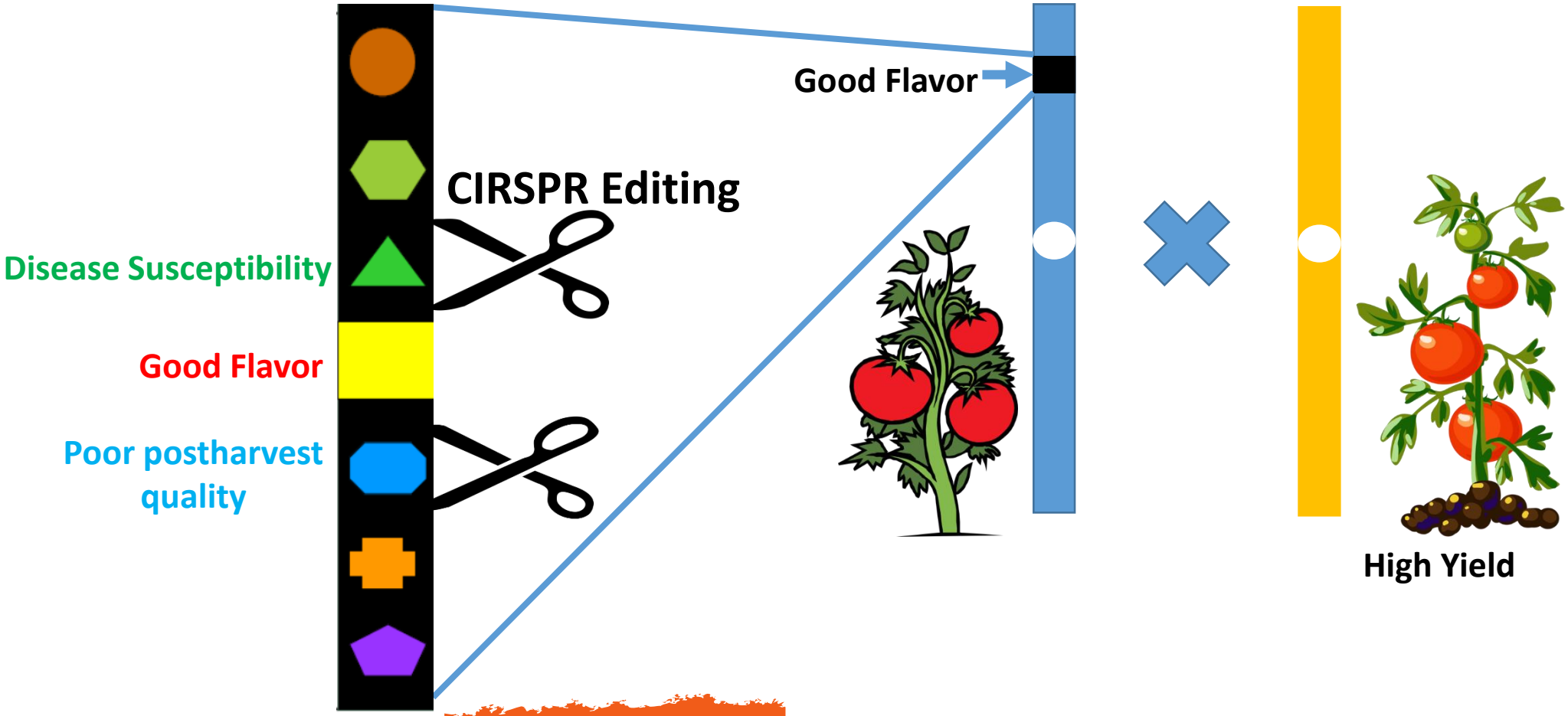
Complimentary To Traditional Breeding : Solution to Linkage Drag

Gene editing can remove linkage drag to create better plants



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How Different Is Between Conventional Breeding And CRISPR Genome Editing?

	Radiation mutagenesis	Conventional Breeding	GMO	Genome Editing
genetic modification	YES, Artificial (e.g. radiation)	Yes, by nature (e.g. reshuffling of DNA fragment or recombination	Yes, Transgene	Yes, transgene
Turn gene off	yes	yes	yes	yes
Turn gene on	No(extremely rare)	yes	yes	yes
Foreign DNA	No	No	yes	No (case by case)
Distinguished from conventional breeding	no	-	yes	no
Efficiency	low	low	high	Very high
Precision	random	random	random	precise
Cost	high	high	High (mainly for dealing with regulation)	Low
Difficulties of procedures	easy	easy	complicated	complicated

Rise of Private Sector in Application of Genome Editing Techniques for Crop Improvement

Company	Location	Year Established	Selected Tools/Services	Focus Crops
Benson Hill Biosystems	St. Louis, MO	2012	CROP-OS software; gene editing using CRISPR-Cpf1 and -Cms1	Row crops edited for higher yield, stress resistance, and herbicide tolerance
Corteva (DowDuPont)	Wilmington, DE	2018	CRISPR-Cas9	Waxy corn modified for altered starch composition
Pairwise	Durham, NC	2018	CRISPR-Cas9 with base editing	Row crops such as corn and soybeans with increased productivity, disease resistance; more-convenient fruits and vegetables
Syngenta	Basel, Switzerland	2000	CRISPR-Cas9	Corn, soy, wheat, tomato, sunflower, modified to increase yield
Tropic Biosciences	Norwich, UK	2016	CRISPR and other techniques	Disease-resistant bananas, decaffeinated coffee
Yield10 Bioscience	Woburn, MA	2015	CRISPR-Cas9	Camelina engineered for higher oil content

Rise of Public Sector in Application of Genome Editing Techniques for Crop Improvement

Table 1. NIH Funding for CRISPR-Related Research, FY2011-FY2018

in dollars

Fiscal Year	Projects	Total Funding
2011	7	$5,070,129
2012	9	$7,432,520
2013	30	$12,505,507
2014	161	$85,298,742
2015	551	$267,055,410
2016	1,245	$603,205,999
2017	2,031	$947,465,783
2018	2,651	$1,155,385,840
Total	**6,685**	**$3,083,419,930**

Year	Publications
2011	87
2012	137
2013	300
2014	670
2015	1,457
2016	2,594
2017	3,738
2018	3,917
Total	**12,900**

November 20, 2018.

USDA-Regulated?

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

NEWS & OPINION MAGAZIN

Home / The Nutshell

USDA Will Not Regulate CRISPR-Edited Crops

Restrictions will remain on transgenic plants, which contain artificially inserted genes from other species.

GMO-K

The USDA says Crispr-edited foods are just as safe as ones bred the old-fashioned way

By Katherine Ellen Foley • April 2, 2018

The Packer

Newsletters

JOBS NEWS INDUSTRY HAPPENINGS PRICING + MARKETS NEWSPAPER EVENTS VIDEO PACKER 25 BLOG

USDA slams EU's decision on regulating gene-edited products

Chris Koger
July 30, 2018 06:08 PM
Print

Trending in The Packer

The USDA Just Gave the Green Light to CRISPR'd Food

Kristen V. Brown
3/30/18 2:34pm • Filed to: CRISPR

21.7K 39 6

USDA confirms it won't regulate CRISPR gene-edited plants like it does GMOs

Rich Haridy | April 3rd, 2018

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USDA-Regulated?

Not regulated as long as

- they are not plant pests or developed using plant pests.
- Noxious weed

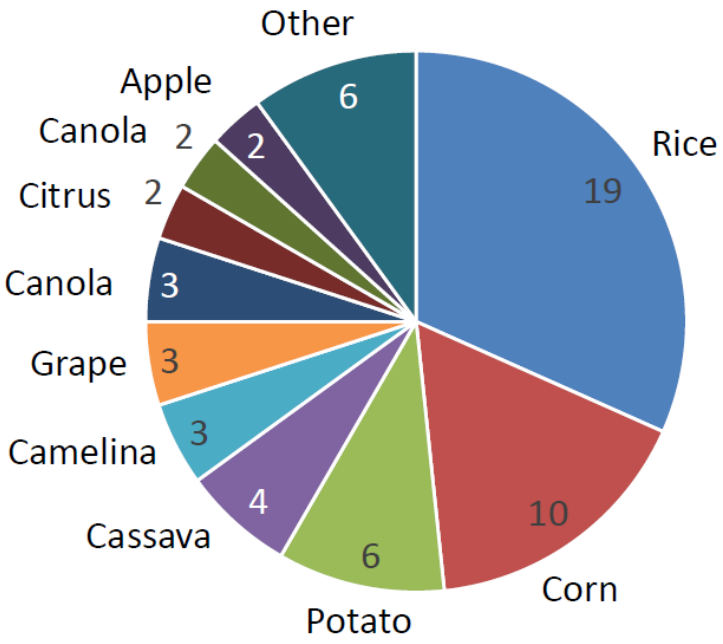
This would include plant varieties with the following changes:

- Deletions
- Single base pair substitutions
- Insertions from compatible plant relatives (foreign DNA from bacteria, insects, virus etc. will be still regulated)
- Off-springs of a genetically engineered plant that does not retain the change of its parent

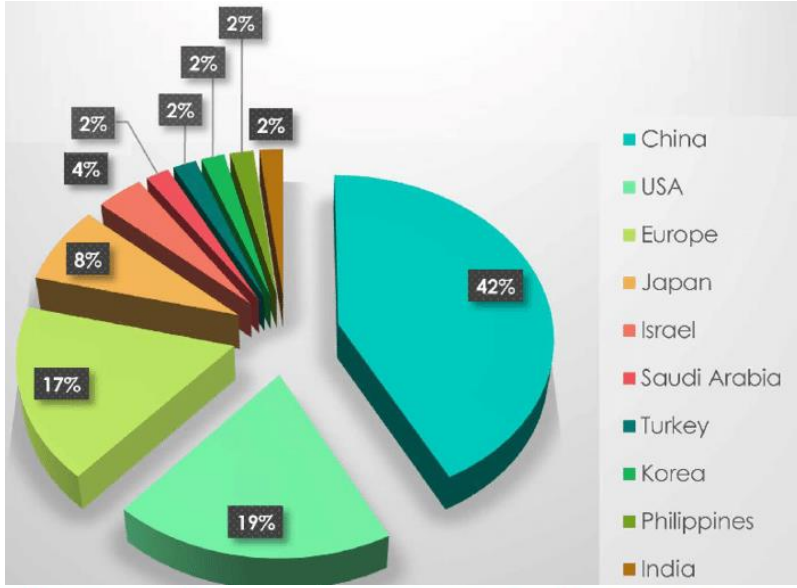
USDA Authorizations: Products Of Genome Editing

Year	Number of Permits and Notifications
2013	1
2014	21
2015	56
2016	90
2017	124
June/2018	62
Total	354

Consulted by FDA



Other
- Alfalfa
- Banana
- Eggplant
- Flax
- Pennycress
- Sorghum



Dupont/Pioneer aim to launch the first CRISPR-enabled **waxy corn** around 2020.

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Food And Drug Administration (FDA) -Regulated?

- All food is regulated, regardless of how plant varieties are bred
- No unique requirements exist for food developed with biotechnology
- All food must meet universal regulatory requirements

  -All food must be safe

  -GE food labelling? Yes

  -Genome-Edited food labeling? probably

GE Soybean with increased levels of oleic acid

New name: "High oleic soybean oil" ✗

GE soybean oil √

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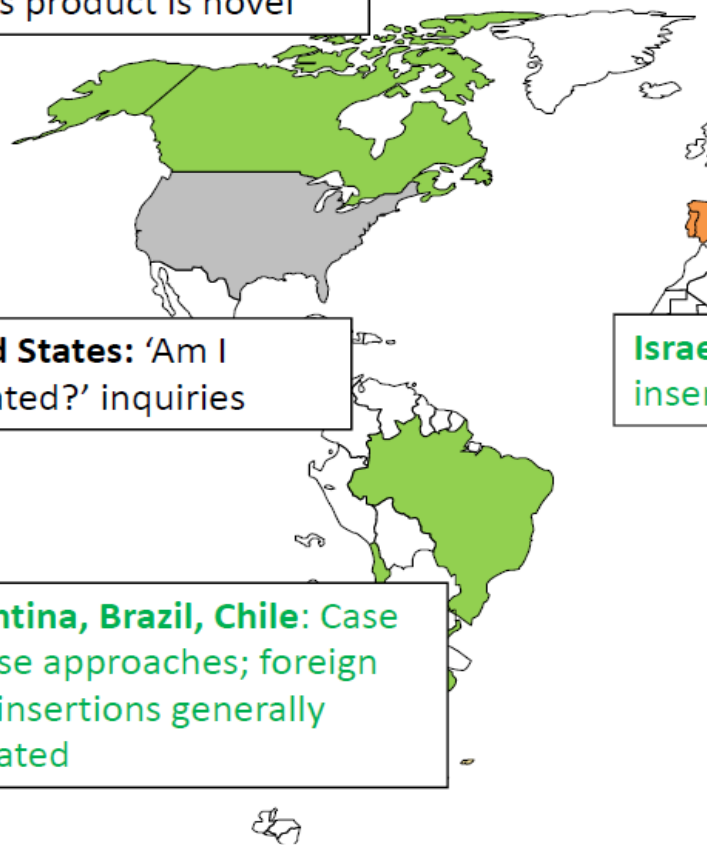
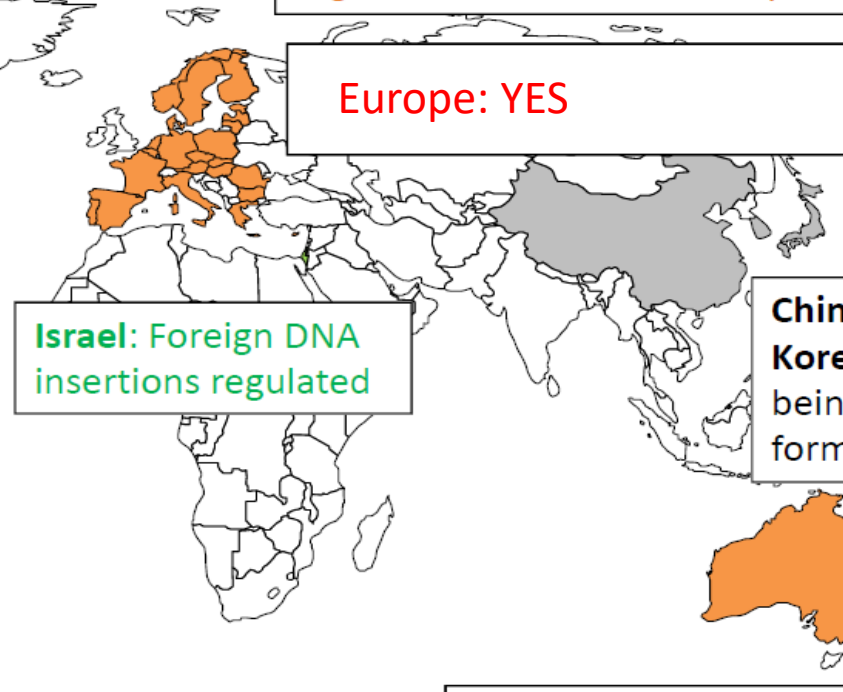
Global Regulatory Status



Canada: Not regulated unless product is novel

United States: 'Am I Regulated?' inquiries

Argentina, Brazil, Chile: Case by case approaches; foreign DNA insertions generally regulated



Norway: Proposed; foreign DNA insertion regulated; otherwise case-by-case

Europe: YES

Israel: Foreign DNA insertions regulated

China, Japan, Korea: Issue still being debated; no formal guidance

Australia: Under review; likely regulated whenever foreign DNA involved; otherwise case-by-case

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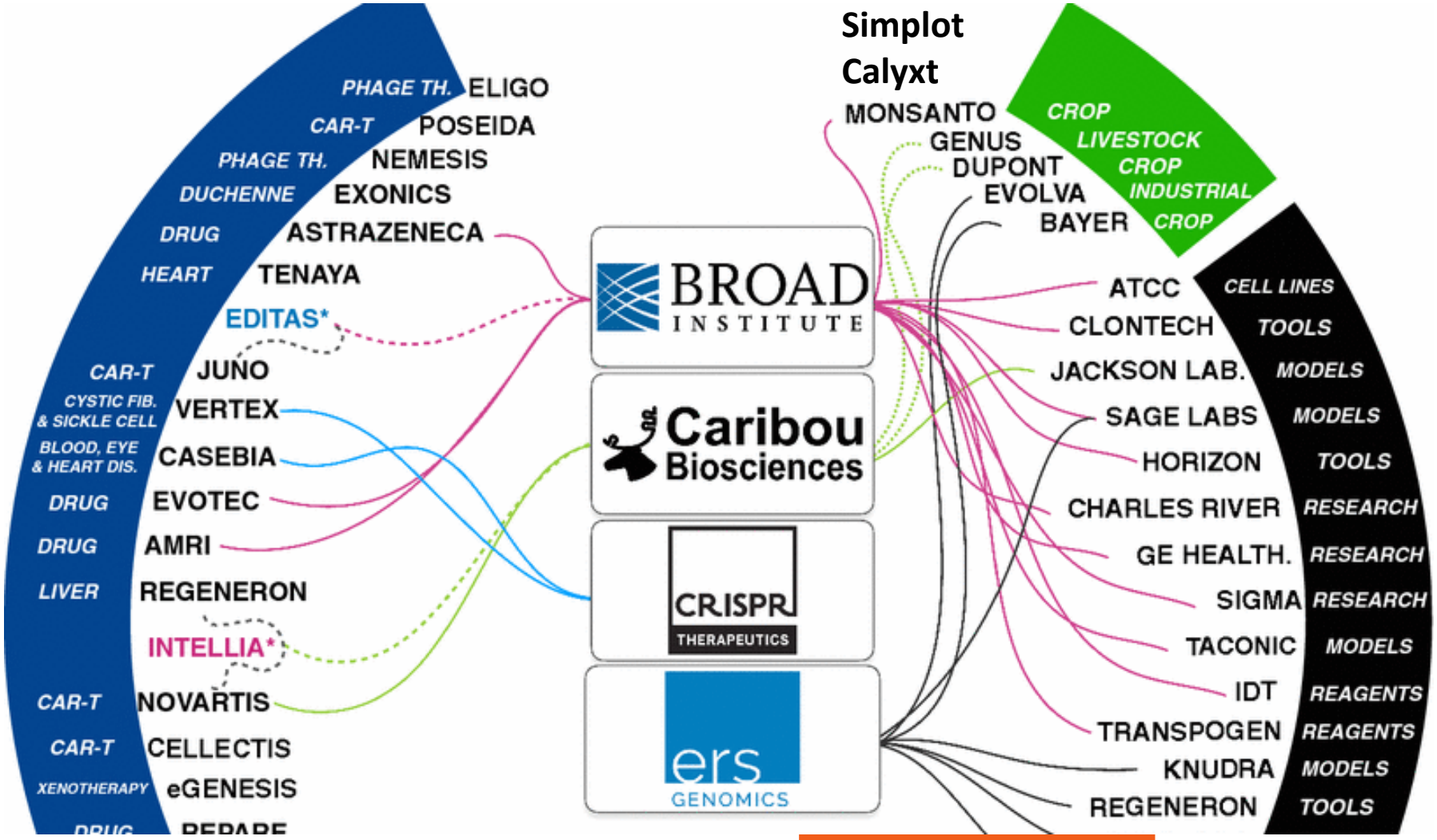
Challenges

- Availability of Genomic Sequence
- Efficient plant transformation pipe line
- DNA-free genetic transformation method for perennial plant species

IP Licensing Agreements Landscape



Free for academic researchers

Social Acceptance And Ethical Concerns

- Consumer acceptance
- Ecological concern
- Health Concern
- Ethical concern

Summary

- Genome editing requires similar procedures used for Genetic Engineering (GMOs), yet creates precise mutation in plant genomes containing non-foreign DNA
- Resulting products are indistinguishable from products of natural variability or mutagenesis, yet genome edited plants are regulated by USDA in a manner of case-by-case
- Limitation of genome editing application are plant transformation pipeline and genome availability
- Both public and private sector have significant opportunities to apply genome editing in their breeding programs

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