Plant Breeding Tools

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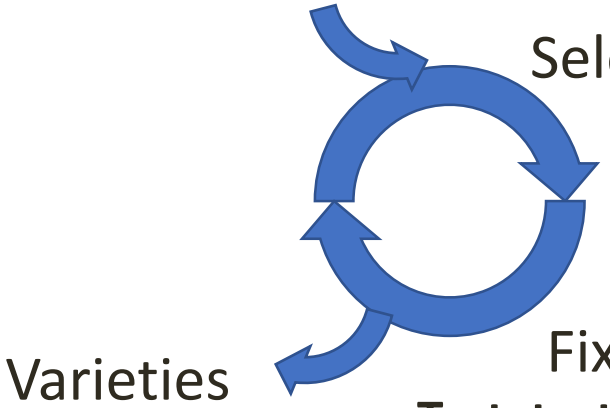
Plant Breeding

a product-oriented discipline of sciences rooted in breeding, quantitative genetics and statistics for crop improvement that encompasses an increasing number of support technologies to sustain society

Components:

Generate diversity-controlled crosses



Test, test, test!

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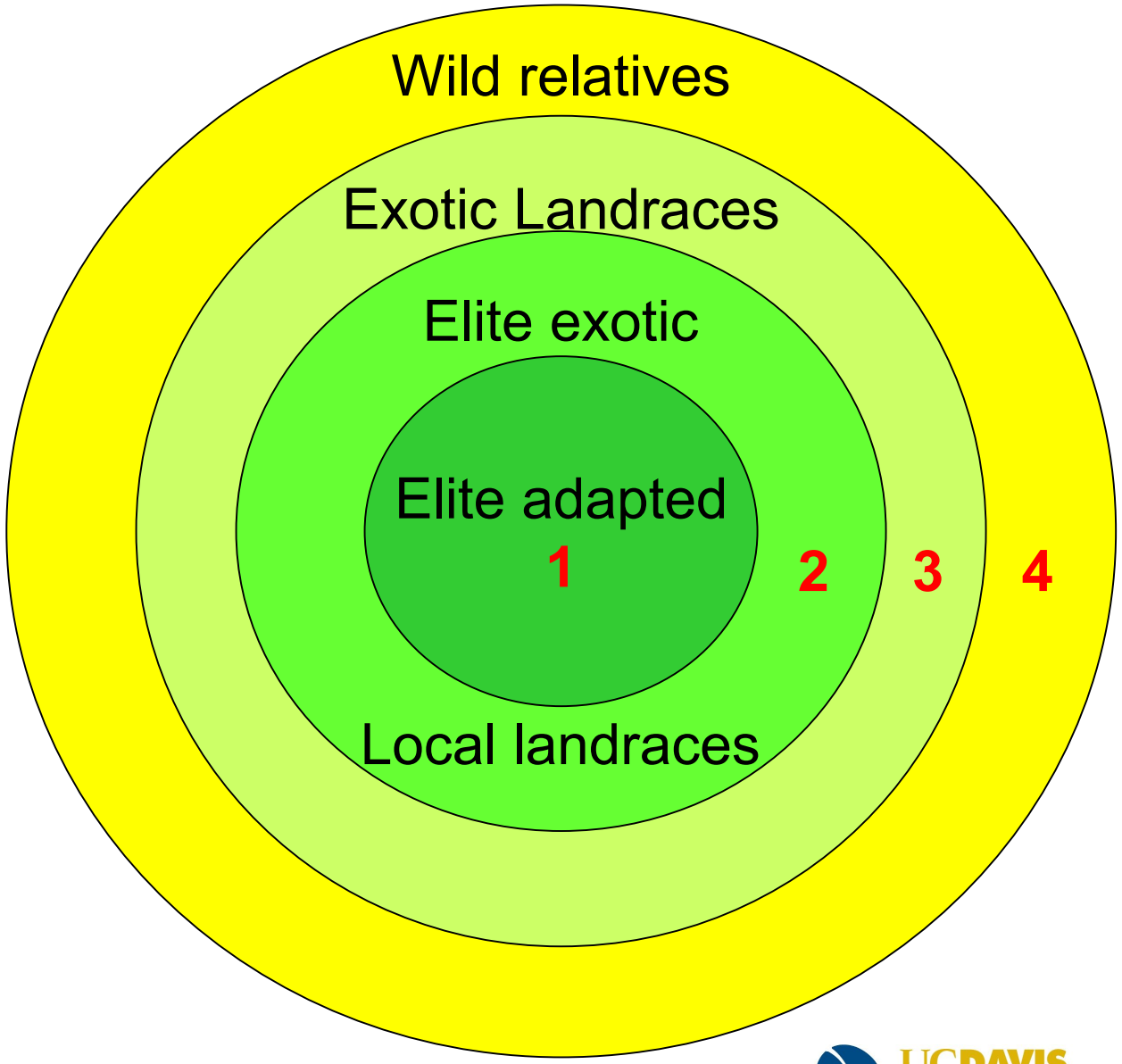
Gene pools

- Most breeding crosses are within group 1
- Group 2 has had limited use for introgressions of some diversity and/or special traits
- Group 3 and 4 almost exclusively for special traits (e.g. disease resistance), <u>found neither in 1 nor 2</u>.



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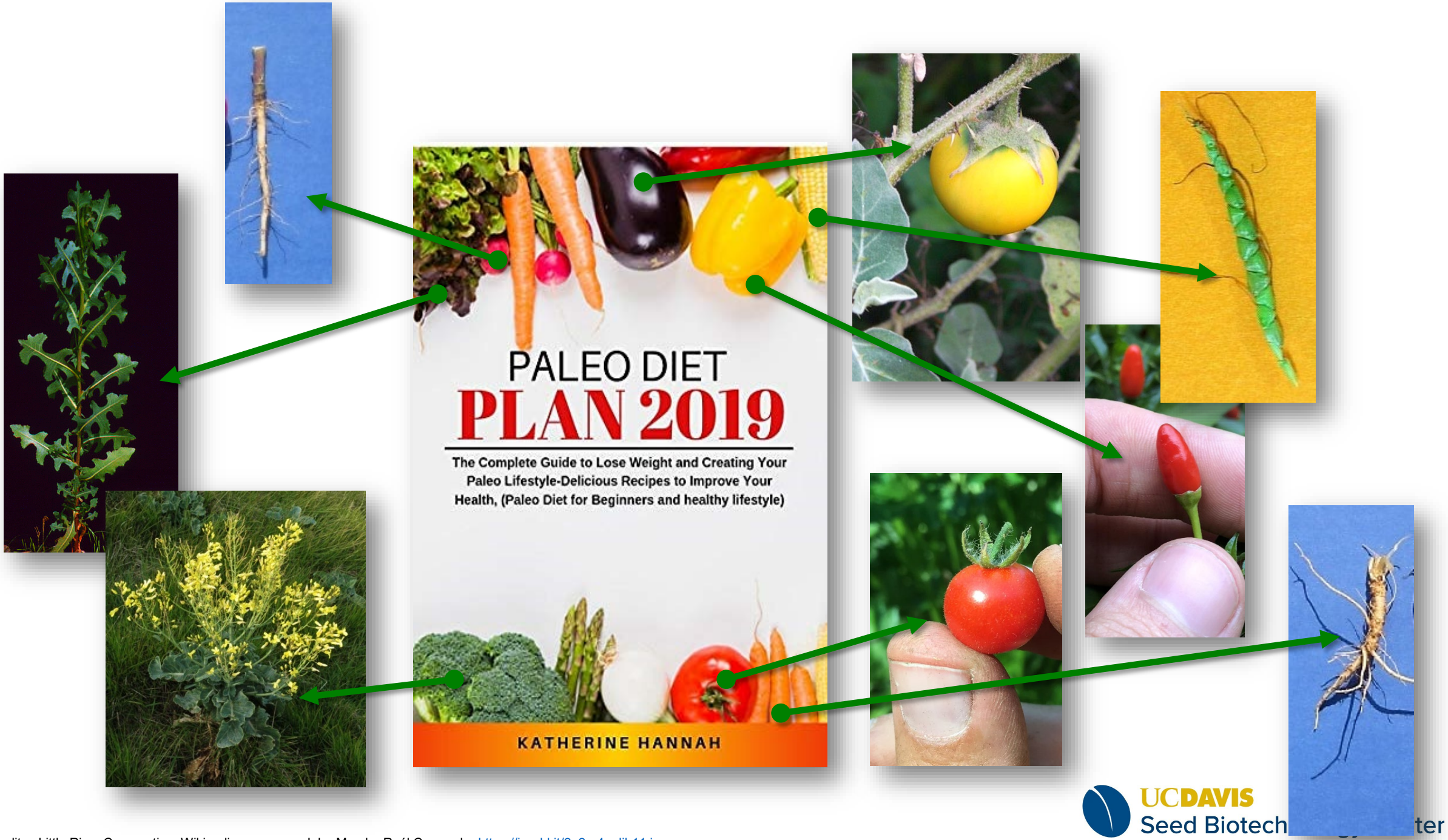

Photo credits: Little River Cooperative; Wikipedia commons; John Meade, Raúl Coronado; https://i.redd.it/2n2rp4xrdib11.jpg

Germplasm

Germplasm from Mexico
300+ accessions

- Collected with collaborators and UC Mexus grant in 2006 and 2007
  - Novel germplasm from locations where geminiviruses are endemic
  - Jose Luna-Ruiz, Aguascalientes, Mexico

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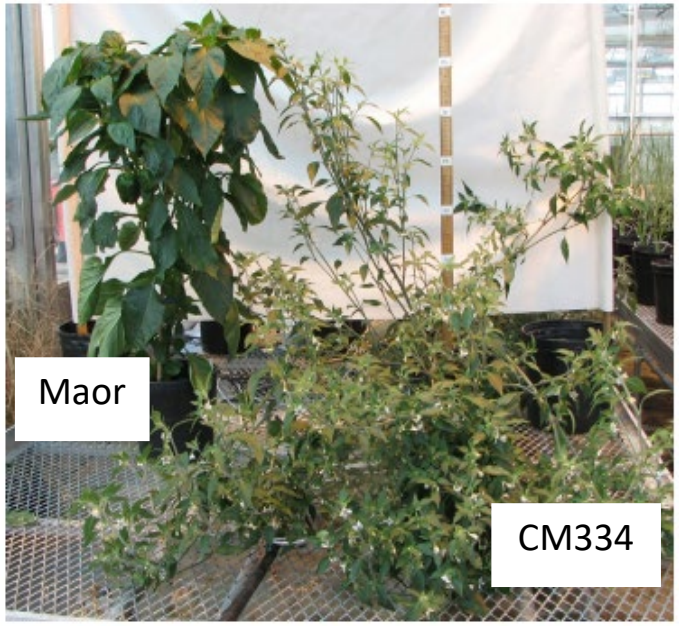
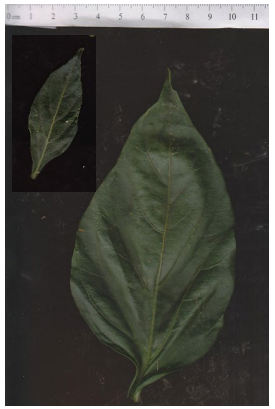
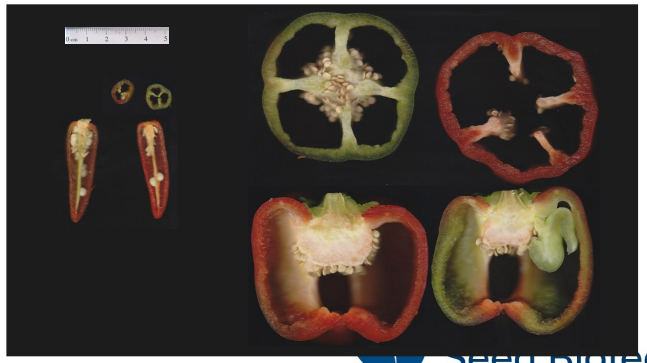
Wild germplasm is a great source of novel traits—but not (previously) practically accessible

- Breeding for resistant sources
- Unfavorable traits from resistant parents
- Difficulty in breeding programs







Breeders toolbox

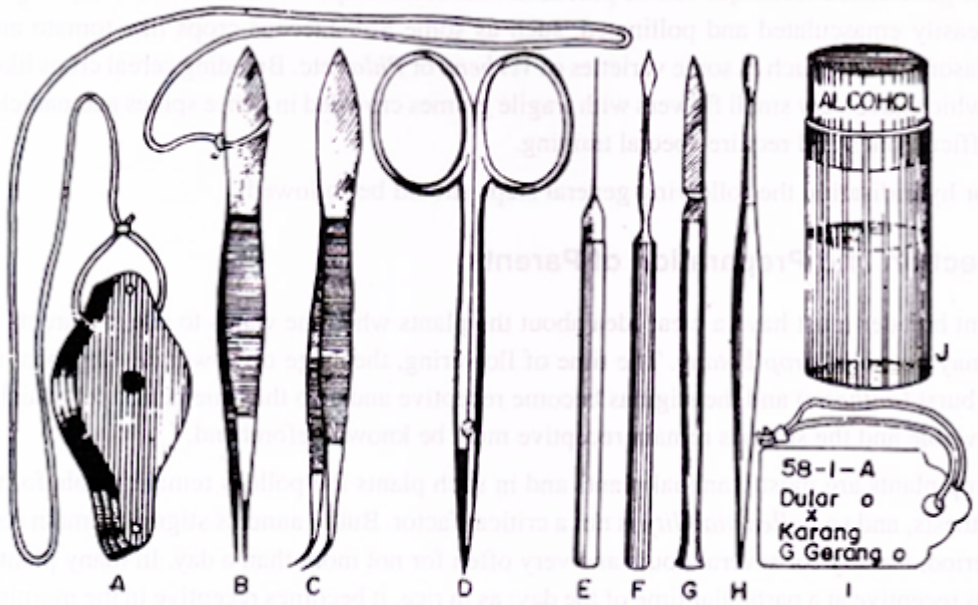
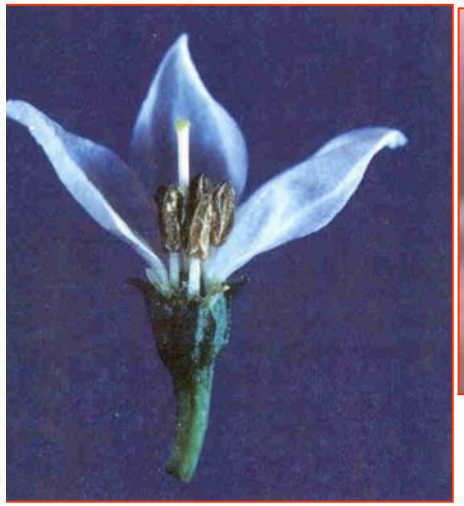
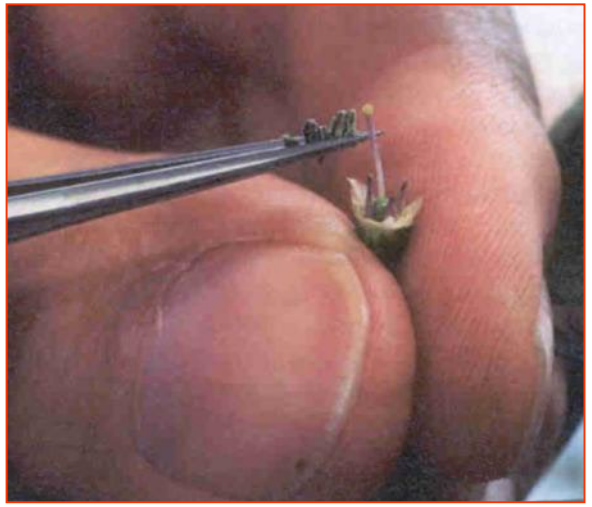


Fig. 4.1 : Plant breeding kit : (A) Magnifying glass, (B & C) Forceps, (D) Scissors, (E & F) Needles, (G) Scalpel, (H) Brush, (I) Level, (J) Alcohol tube



Emasculation (prior to anther dehiscence)

Pollination



Berke TG (1999) *J of New Seeds* 1: 49-67.

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Innovations in Plant Breeding

- Understanding genetic principles (Mendel, Hardy and Weiberg; 1865-1910)
- Statistics and Experimental Design (Fisher; Snedecor; Pearson; 1920-30s, Melchinger 2005)
- Hybridization and Heterosis (Shull 1908, East 1936, Gardner 1963)
- **Biotechnology**: tissue culture, mutation breeding, **transgenics**, **gene editing, genome editing,** synthetic biology (1950s+)
- Speed to market technologies: doubled haploids, counter seasonal nurseries
- Genomics and bioinformatics/**machine learning** (1990s+)
- **High Throughput Phenotyping** and **Artificial Intelligence** (2010s+)
- Intellectual Property and Regulation
- A Well-Educated Workforce

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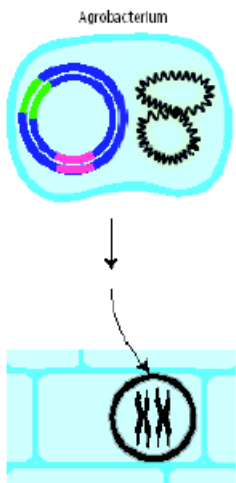
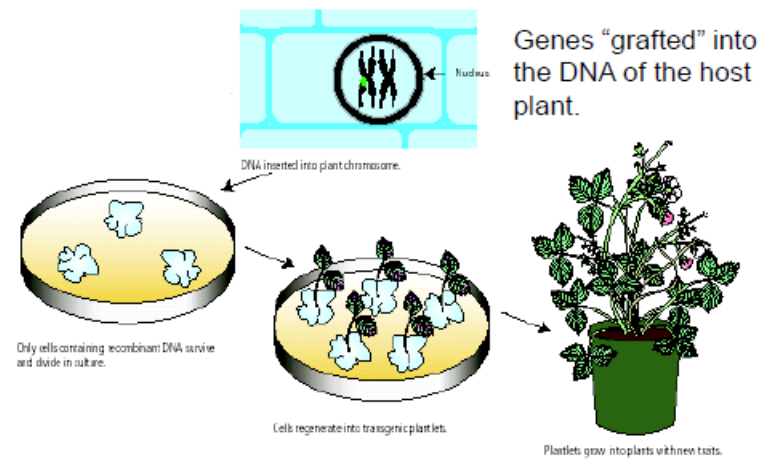
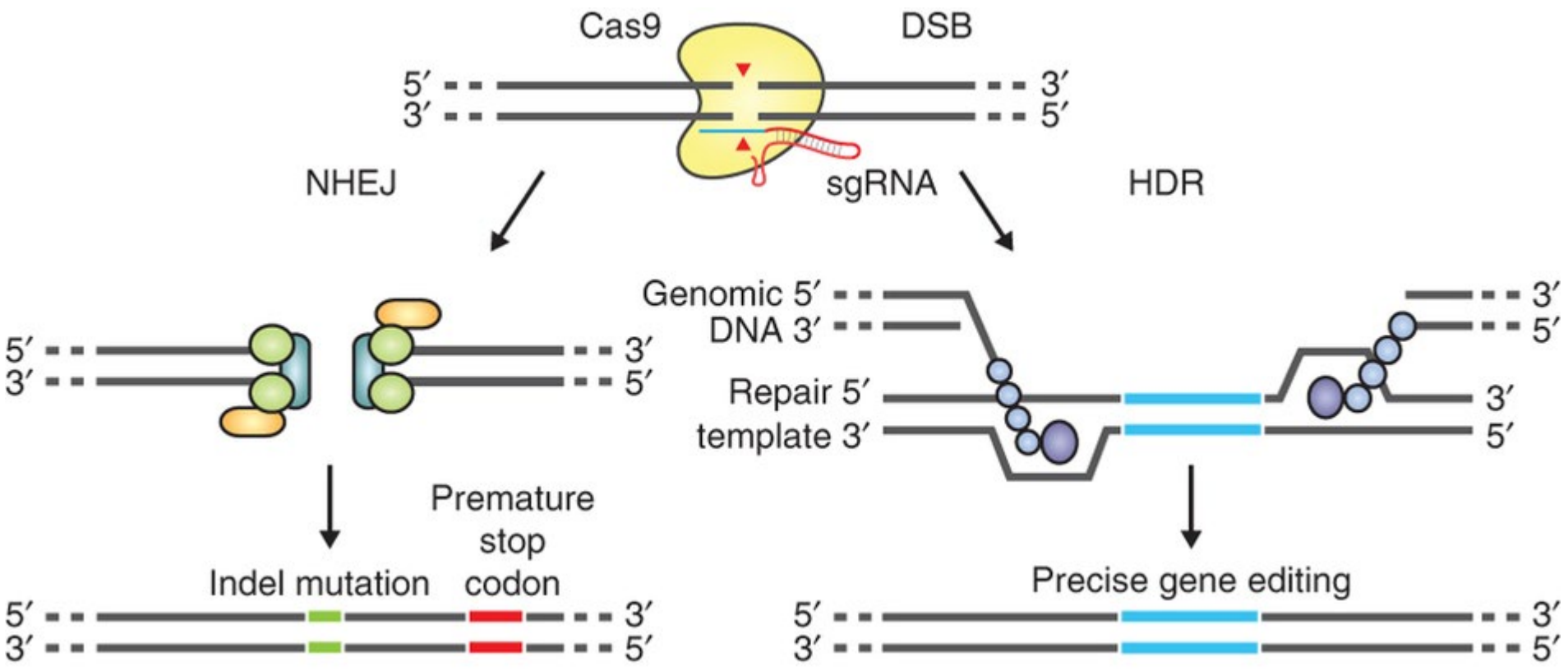
Transformation

- **Transformation** – involves transfer of genes directly into recipient cells without sexual crossing.
  - Introduces genes directly as recombinant DNA.
  - Genes can be from any source, including synthetic, and endogenous genes can be silenced (RNAi).
  - "Transgenesis" – from other species; "cisgenesis" or "intragenesis" – from same species; cisgenics may or may not be regulated in US depending on methods used.
  - Can be Agrobacterium-mediated or by direct introduction (biolistics, etc.).
  - Insertion sites essentially random and expression levels can vary.
  - Regulated if Agro is used (in US). In EU, product remains regulated even if transgene is no longer present (i.e., null segregants still regulated).





Precision Breeding-Cas9/CRISPR



DSBs induced by Cas9 (yellow) can be repaired in one of two ways. In the error-prone NHEJ pathway, the ends of a DSB are processed by endogenous DNA repair machinery and rejoined, which can result in random indel mutations at the site of junction. Indel mutations occurring within the coding region of a gene can result in frameshifts and the creation of a premature stop codon, resulting in gene knockout. Alternatively, a repair template in the form of a plasmid or ssODN can be supplied to leverage the HDR pathway, which allows high fidelity and precise editing. Single-stranded nicks to the DNA can also induce HDR.

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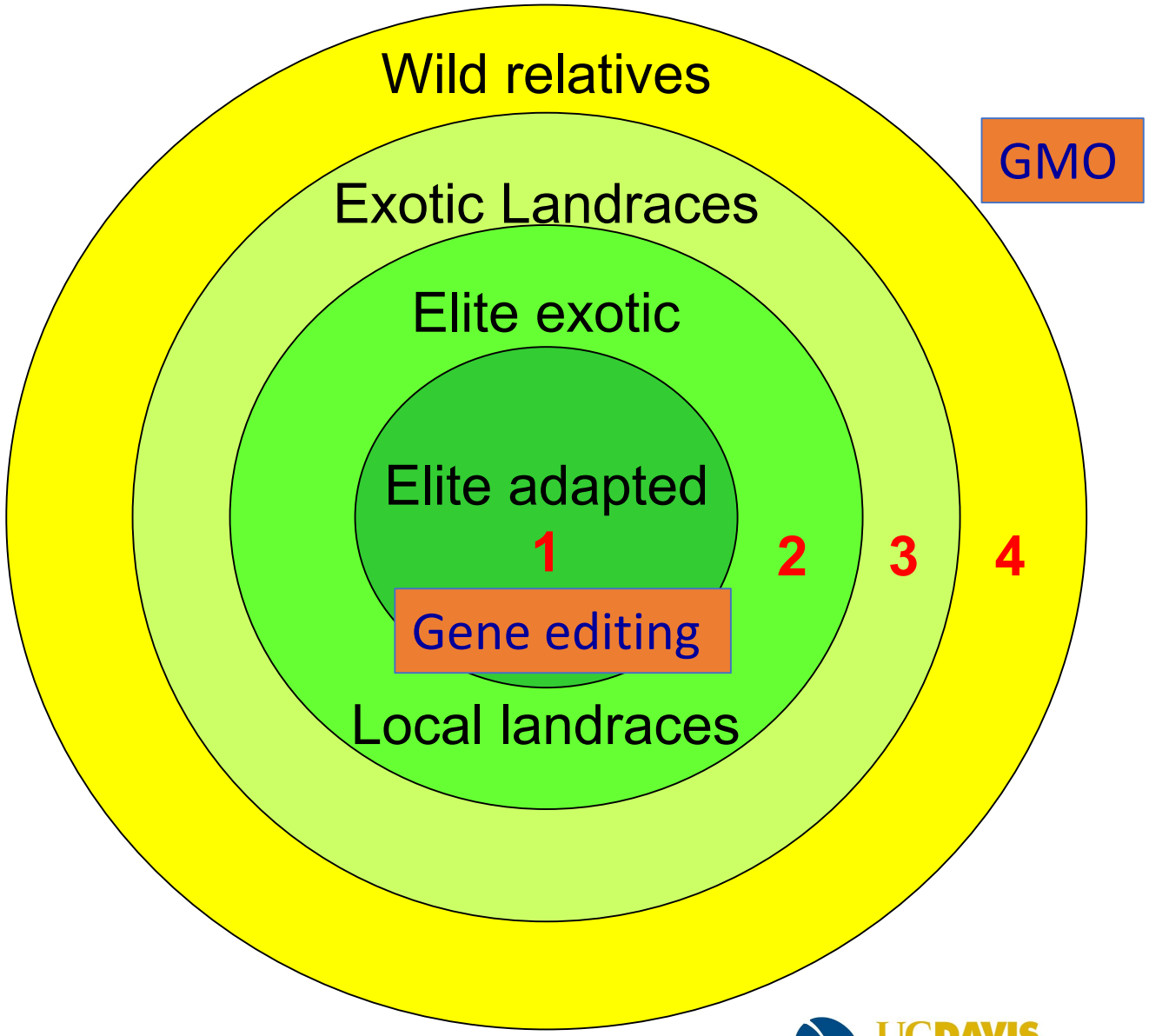

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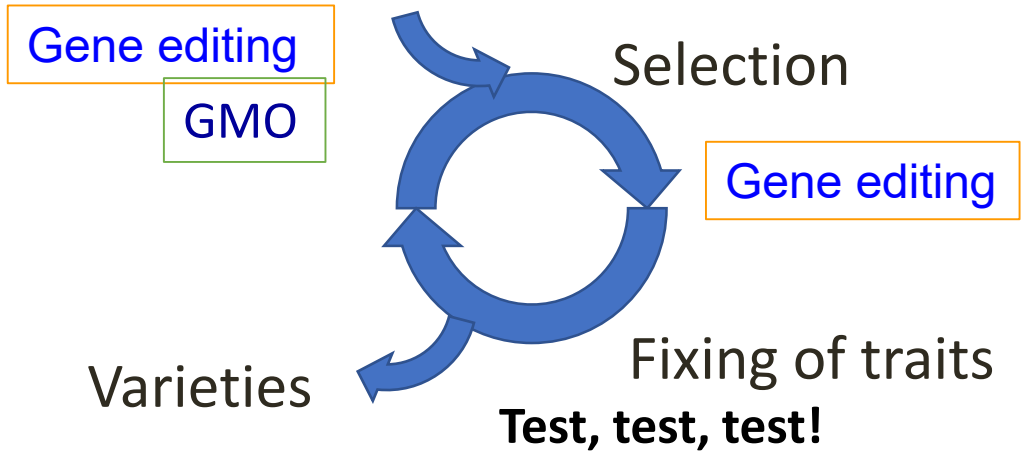

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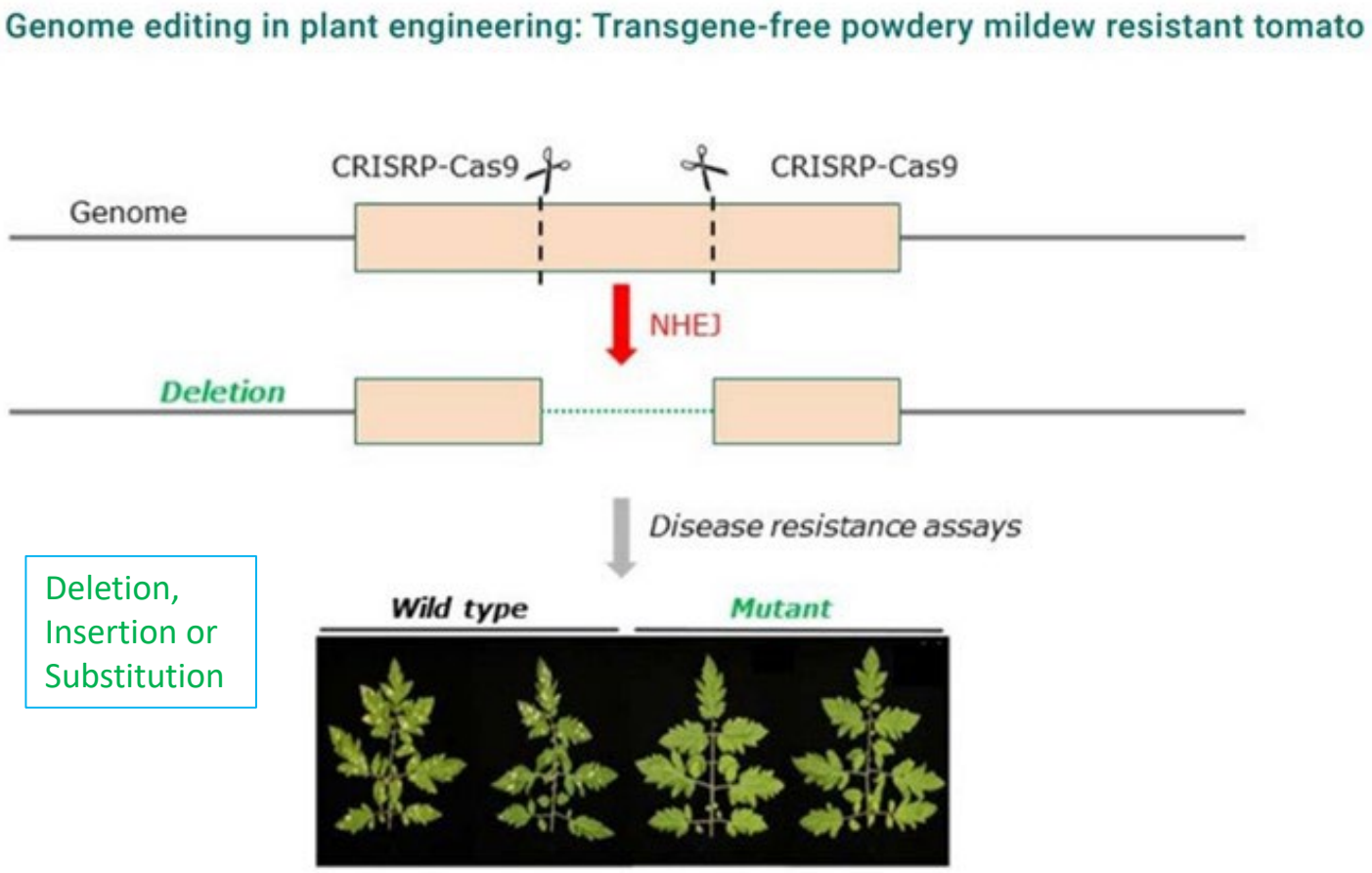
Gene Editing Advantages

- Speed
- Cost
- Reduced risks of unintended effects
- The breeder must sort and select against negative variation in all approaches and during every generation.
- This is what a plant breeder does.



Deletion, Insertion or Substitution

© Foto: Scientific Reports ISSN 2045-2322 (online)

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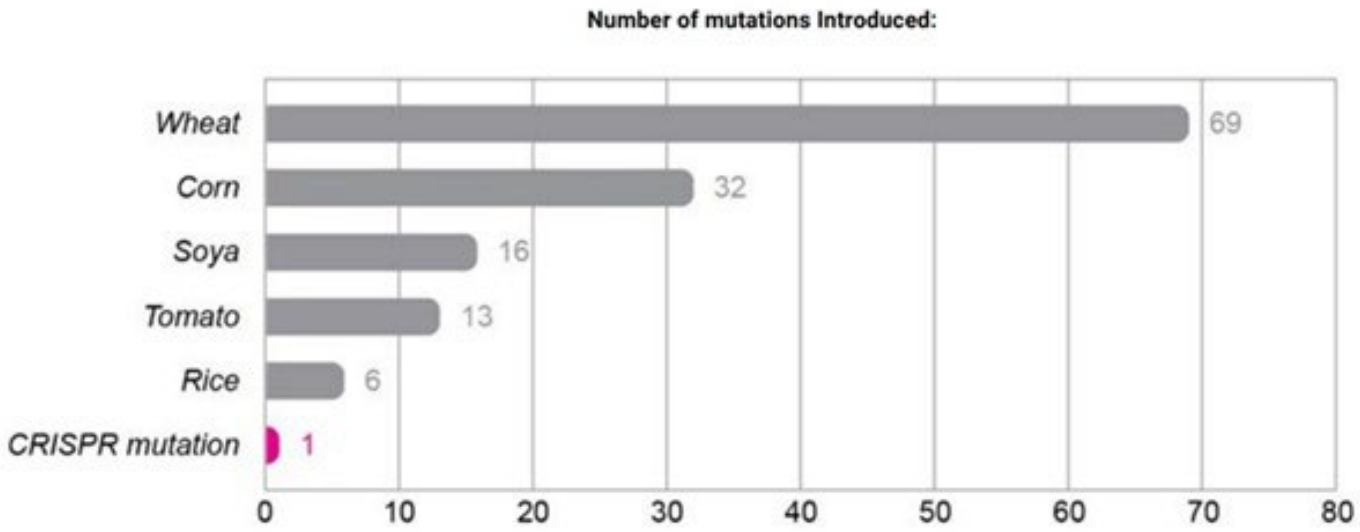
Gene Editing Enhances Precision



- Figure 1: Estimated number of natural spontaneous mutations that occur in every individual plant (grey) every generation compared to a hypothetical single base introduced using CRISPR gene editing (pink).

Discussion paper focusing on the scientific relevance of genome editing and on the ethical, legal and societal issues potentially involved - ISSUED BY THE ETHICS COUNCIL OF THE MAX PLANCK SOCIETY

Conventional Way

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Research Projects

The SBC's research activities focus on partnerships with industry collaborators that develop precompetitive information that "lifts all boats."

Melon

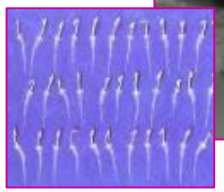
Tomato

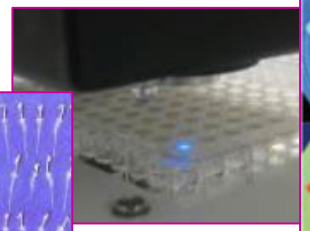
Coffee

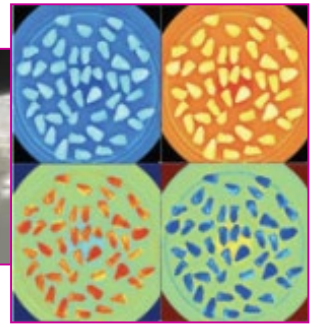
Dry Chain

Spinach

Eggplant







Seed Technology

Cotton

Pepper

Lettuce

Carrot

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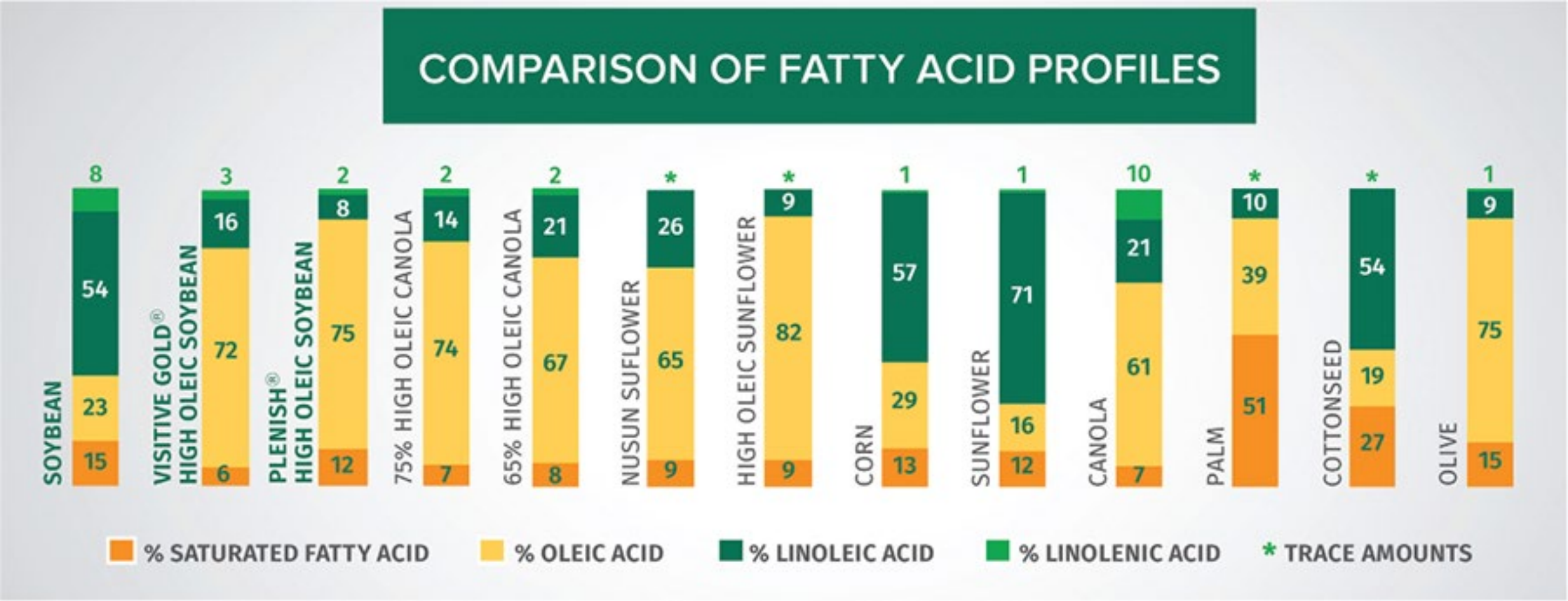

https://www.informmagazine-digital.org/

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Laurate Canola---1995

Fatty Acid	Formula	Percent
Lauric	12:0	47.0
Myristic	14:0	3.6
Palmitic	16:0	2.6
Stearic	18:0	1.3
Oleic	18:1n-9	26.3
Linoleic	18:2n-6	14.2
Linolenic	18:3n-3	5.1

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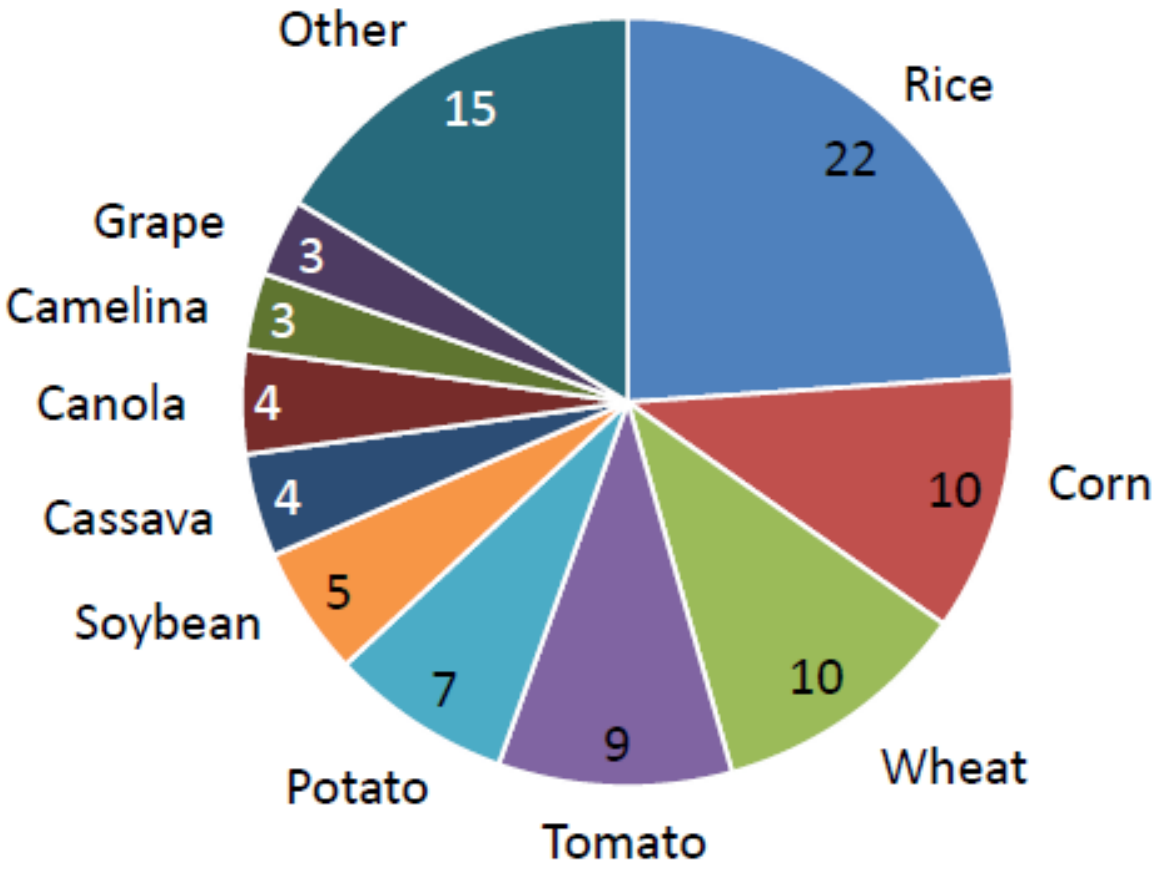
Genome editing R&D: Crops



Other
- Apple (2)
- Citrus (2)
- Cucumber (2)
- Sugarcane (2)
- Alfalfa (1)
- Banana (1)
- Eggplant (1)
- Flax (1)
- Peanut (1)
- Pennycress (1)
- Sorghum (1)

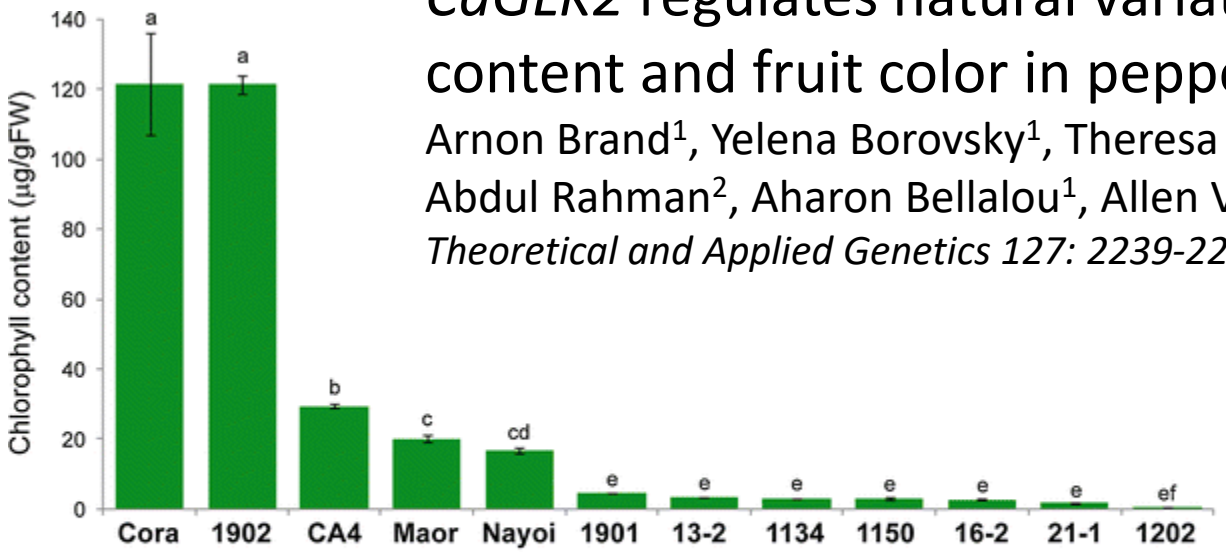
ources: Scientific literature, scientific conferences, company websites, media reports, USDA "Am I regulated?" Letters

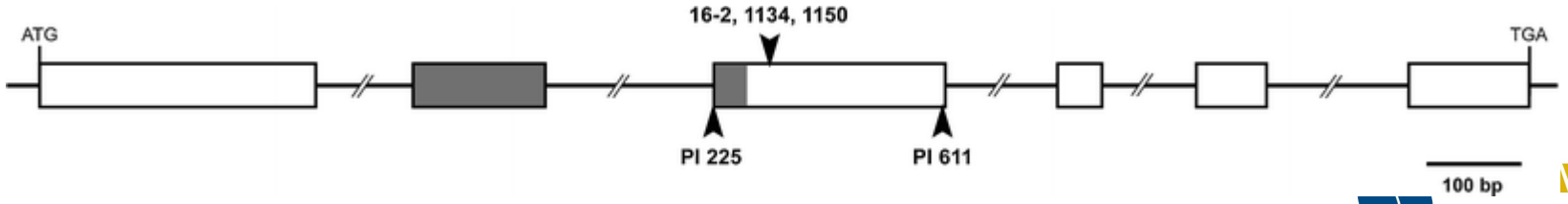
Pepper

CaGLK2 regulates natural variation of chlorophyll content and fruit color in pepper fruit

Arnon Brand[1], Yelena Borovsky[1], Theresa Hill[2], Khalis Afnan
Abdul Rahman[2], Aharon Bellalou[1], Allen Van Deynze[2] and Ilan Paran

Theoretical and Applied Genetics 127: 2239-22148. 2014.





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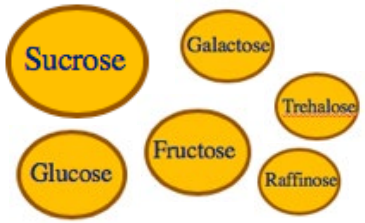
Fruit Quality and Consumer Acceptance

Color

Texture

Taste



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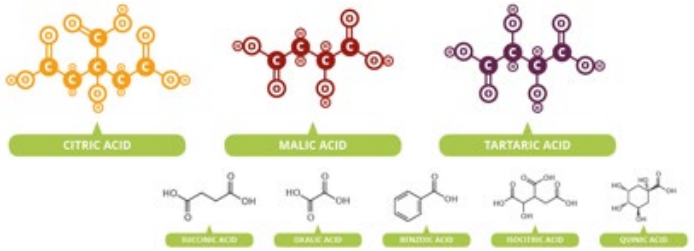
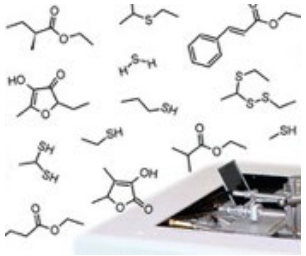


Aroma



Flavor

Vitamin C in Tomato is 1/5 to 1/10 that of Pepper

Gene editing of a single gene family can improve nutrition of US population

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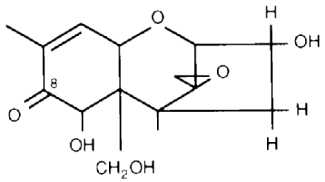
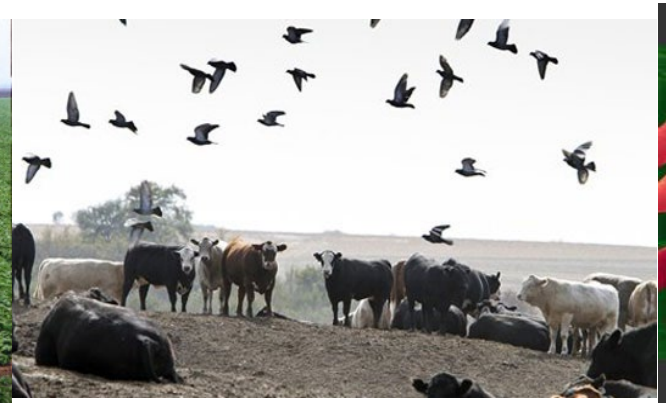
Food Safety Concerns in Crops

(Low probability, High Consequence)

- Mycotoxins
- Salmonella
- Pathogenic E. coli
- Listeria, etc.
- Heavy metals
- Nitrates,
- Allergens





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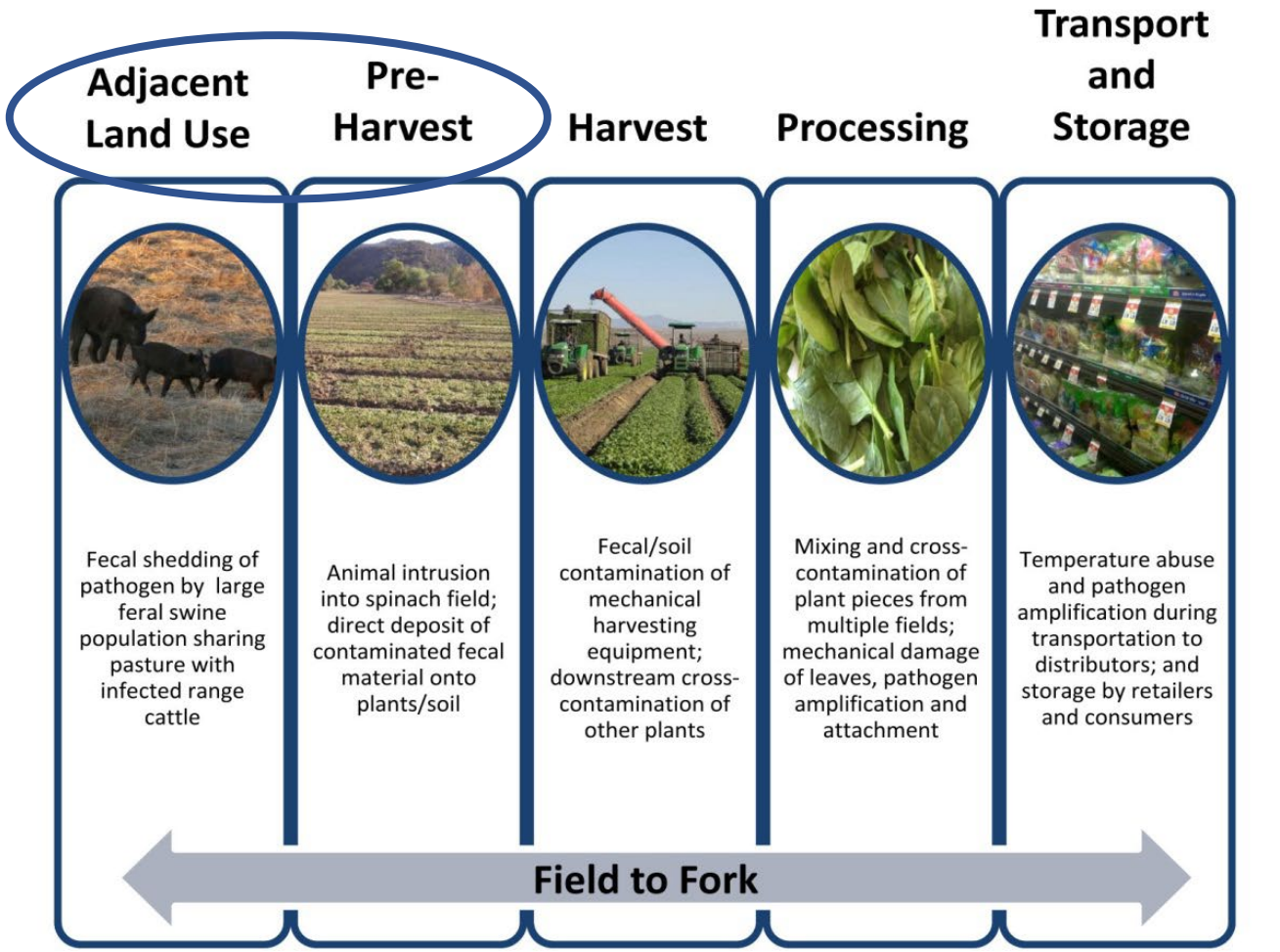
An Integrated Approach is Required



-1 log CFU/g contaminated field product

1 log CFU increase per day due to temperature abuse (optimal conditions)

1.56-28.8 cells/ 85 g serving
4,112 estimated illnesses

Danyluk and Schaffner, 2011; Jay-Russell , CAB Reviews 2013

The Seed industry is Highly Regulated

Seed Trade Organizations

International Seed Federation
Seed is Life

Coordinate safe and fair movement and trade of seed based on Federal Seed Act (FSA)

asta | american seed trade association

- International Seed Federation
- American Seed Trade Association: 700 members
- California Seed Association - 120 members

California Seed Association

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Seed Certifying agencies

ISTA
Seed Quality Assurance

AOSCA

- International Seed Testing Association
  - to develop, standardise and validate methods for sampling and testing of seed quality, using the best scientific knowledge available.
- Association of Seed Certifying Agencies:
  - To promote and facilitate the movement of seed or plant products in local, national, and international markets
- California Crop Improvement Association

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International Seed Regulations

Convention on Biological Diversity

- Convention on biodiversity: Access and benefit sharing of genetic resources
- Cartagena Protocol of Biosafety: Safe movement of Living Modified Organisms = Genetic Modified organisms- 172 member countries

Food and Agriculture Organization of the United Nations

- International Treaty on Plant Genetic Resources: USA signed in 2018. Alternative to CPB.

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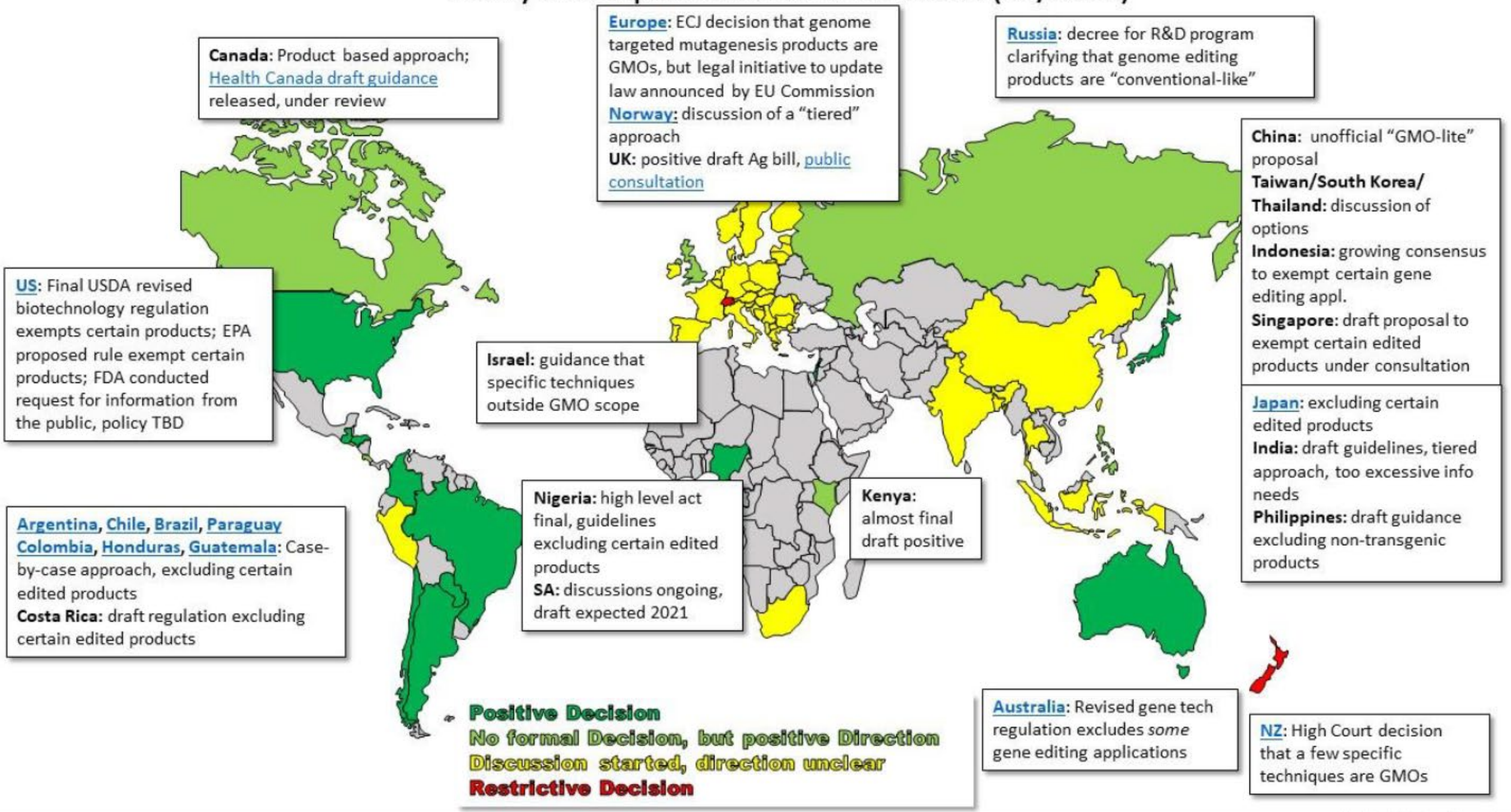
Three Agencies Regulate Biotech Crops in the US

The US Dept. of Agriculture determines whether the *crop is safe to grow* based on authority to regulate *plant pests*. For example, is it a threat to become a weed; what are its growth and flowering characteristics? Were any plant pests used in its development (e.g., *Agrobacterium*)?

The Food and Drug Administration determines whether the crop is *safe to eat*. Is it substantially equivalent to other crops with respect to composition, nutrition, allergenicity, digestibility, etc.?

The Environmental Protection Agency regulates crops that have pesticidal properties. Are they *safe for humans, for non-target organisms, and for the environment?*

Policy developments around the world (09/2021)



US Plant Breeders Rights

- Plant Patents (U.S.) – asexually propagated plants (trees, grape vines); tuber-propagated excluded. **Right to breed**
- PVP (U.S.) – any plant, **Right to Breed, Farmer saved seed**
- Utility Patents (U.S.) – all plants; plant deposit may be necessary
- Plant Breeders' Rights – all plants, **Right to Breed**

African Plant Breeding Academy CRISPR Course

An Initiative of the African Orphan Crops Consortium

Contact:
Rita Mumm, ritamumm@illinois.edu

UCDAVIS Plant Breeding Academy

AOCC

Enabling African scientists to use CRISPR gene-editing technology for

- A quantum leap in **nutrition**, climate resiliency, and sustainability in African food systems
- An explosion of **innovation** and economic growth across the continent
- **Capacity building and mobilization for** African molecular scientists and national programs

AFRICAN PLANT BREEDING ACADEMY CRISPR COURSE

PARTNERS

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IITA Transforming African Agriculture

CGIAR

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ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

MORRISON FOERSTER

World Agroforestry

Breeding Goals (some) for the Next 20 Years

Sustainable production of food, feed and fiber: customer driven

- Increase yields to feed an increasing population
- Increase yields per acre as acres of arable land decline
- Increase drought tolerance as water becomes more scarce
- Increase disease and pest resistance as we move away from chemical controls to genetic controls.
- Improve nitrogen use efficiency
- Develop products adapted to indoor factory farms
- Enhance attributes of quality
  - Flavor
  - Nutrition
  - Uniformity

We need all of the tools!

QUESTIONS??