Genetic modification methods

Basics and application to hops

Steve Strauss, Chris Willig, Michele Wiseman, David Gent, John Henning, and Tom Shellhammer

Oregon State University

Steve.Strauss@OregonState.Edu

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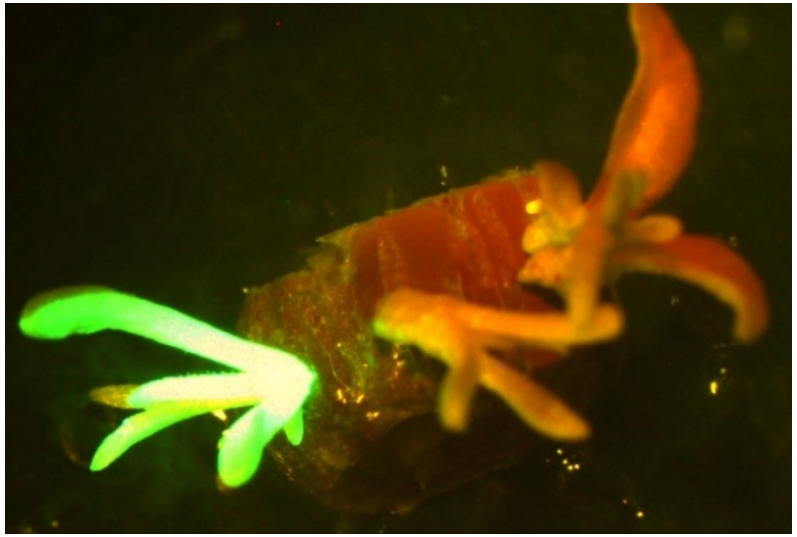
Agenda

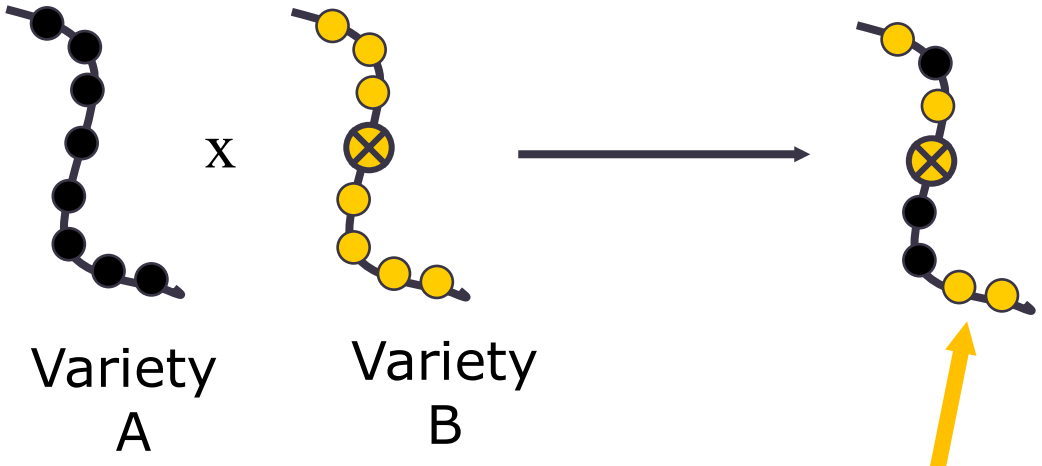
- Genetics concepts and language
  - Breeding and **biotech** (**GE**)
- Status of GE crops in USA/world
- Constraints
  - Regulations, public opinion
- Hop GE progress and potential



Genetics basics

Concept: GE vs. breeding

Traditional plant breeding



GE

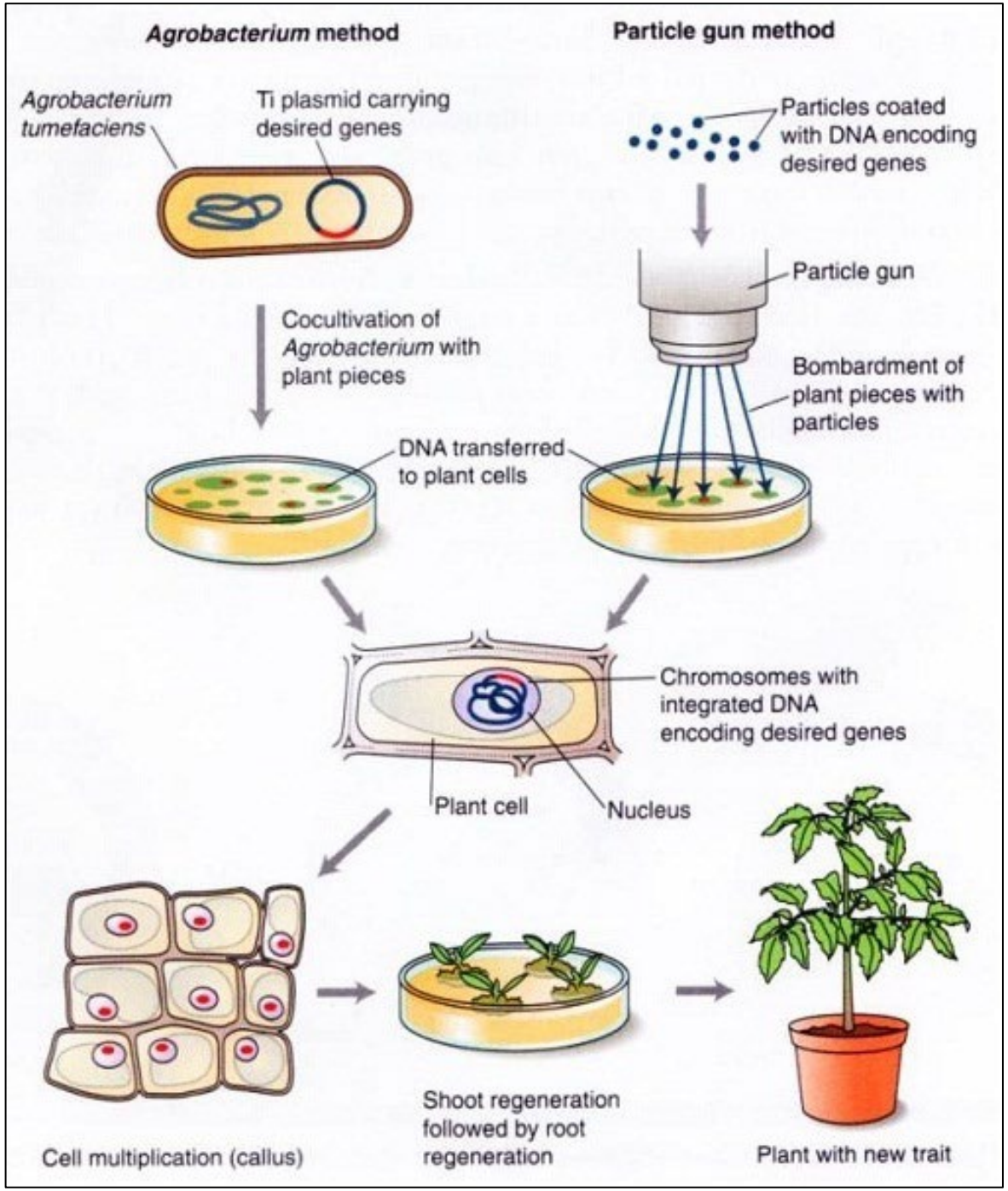
Back to breeders for integration & testing

GE is defined in several ways

- GE = "Genetic modification" = **GM**, common in EU
- **= Direct** modification of DNA
  - DNA isolated, added to organisms
  - "Recombinant DNA" methods used
- Other common terms include…
  - **Genetically engineered**
  - **Biotech**
  - **Gene edited (CRISPR)**
  - **GMO**
  - **Transgenic**
  - **Cisgenic**
  - **Intragenic**
- Term meanings vary somewhat depending on context, user
  - I'll use **GE** to refer to all of these

Overview of steps to create a GE plant

- Insert genes into cells by biological agent or "gene gun"
- Find, isolate the rare modified cells
- Regenerate those cells into uniform modified plants



We use nature's biological engineer: Agrobacterium

- Bacterial plant pathogen with broad host range: Over 90 plant families susceptible
- Transfers DNA to its host to induce a gall in nature – also seen on hop
- Gall-inducing genes removed before use in biotech
- Agro DNA also a part of hop genome! (from ancient transfers)

Published: 21 September 2019

Widespread occurrence of natural genetic transformation of plants by *Agrobacterium*

Tatiana V. Matveeva & Léon Otten

Plant Molecular Biology **101**, 415–437 (2019) | Cite this article

Agro gall found on 'Crystal' hop in Oregon

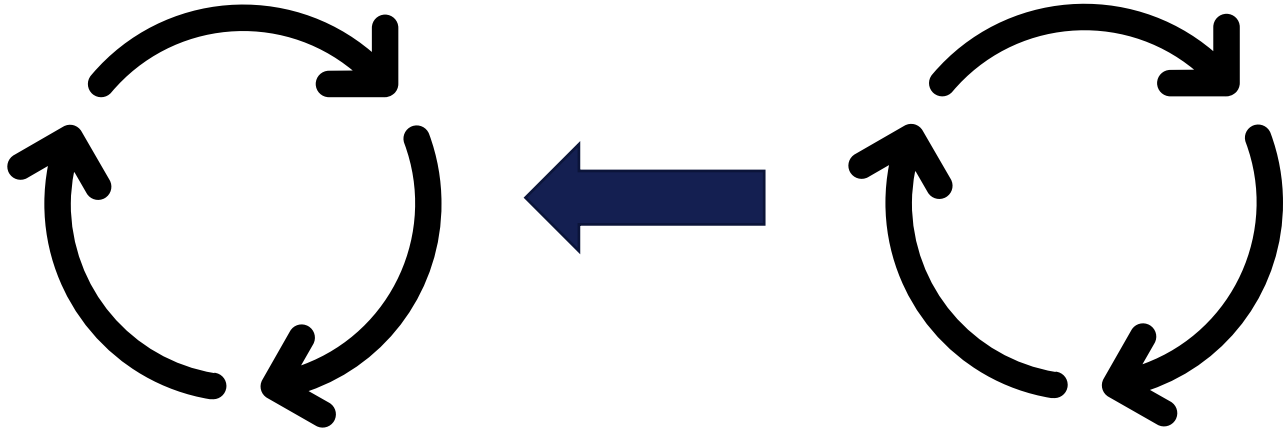
Gene editing defined

- "Stuff" you add to change **other** genes
- Highly specific, efficient modification
- CRISPR main method
- Works well everywhere!
- Routine in all crops, yeast

Relationship of breeding and biotech

Breeding populations

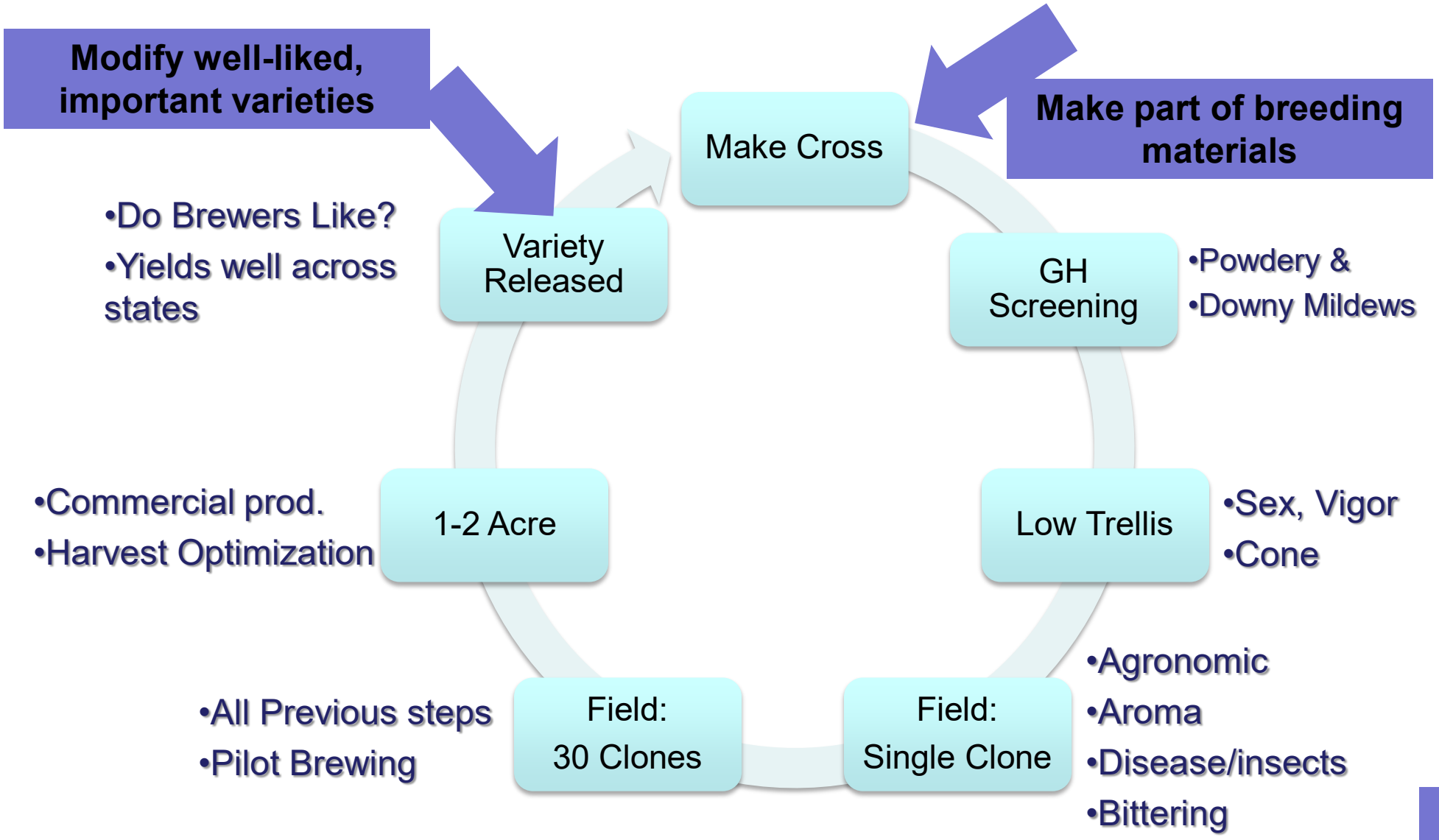
Biotech innovations



Polygenic:
Thousands of genes, growth rate and adaptation, many traits assessed

Oligogenic:
Small numbers of genes, specific modifications and one or few novel traits

Life cycle of hop variety development (12-15 Yr)



GE traits?

GE crop status

First generation herbicide and insect resistant crops were rapidly adopted by farmers, both in the developed and developing world

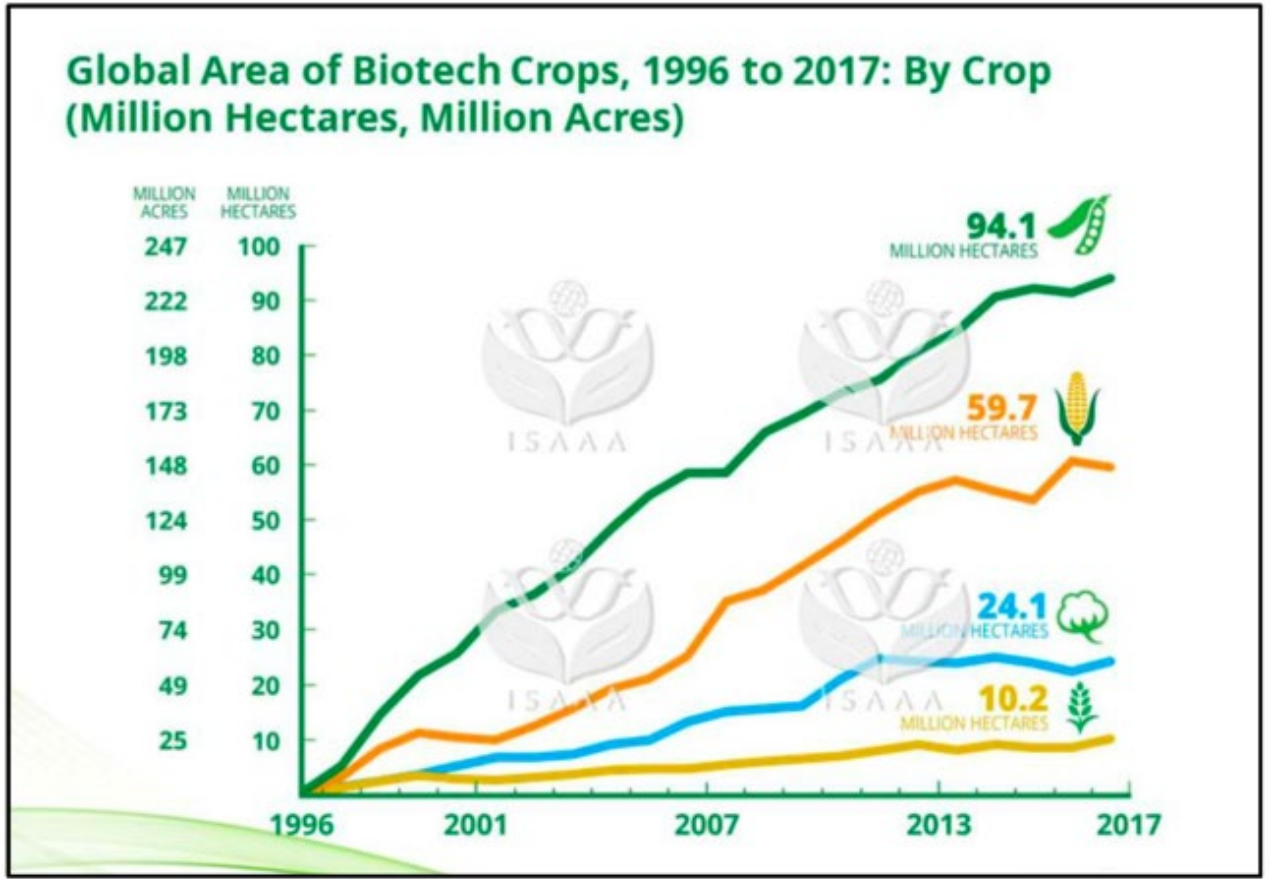


Figure 4. Global area (in million ha) of the most important GM crops in the period 1996-2017 (ISAAA, 2017)

Hop-like example: Virus-resistant GM papaya

Saved the Hawaiian industry in the mid-1990s

Courtesy of Denis Gonsalves, USDA and Cornell University

GMO, virus-resistant trees

But uptake variable

Many countries, and crop types, where GE uptake very limited or zero

European Union: Gene edit = GMO, almost no field use

Many countries reluctant to use if major EU trading partners

Cannot be organically certified

The debate is messy, multidimensional

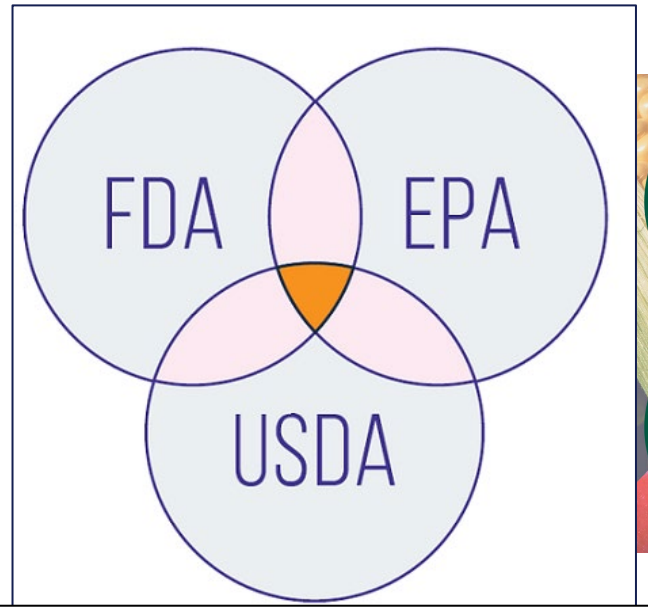
Regulatory system inertia !

Often a polarizing issue

Regulations, public views

Regulatory environment for GE varies widely around world and within USA

- Three agencies in USA for crops and food for biotech
- FDA: Basic food safety
- EPA: Pest resistant, growth-modified crops
- USDA: Pests of agriculture, labeling of food
  - Exemptions for gene-edited crops
- Beer production and labeling: TTB



Public acceptance complex but growing

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Who trusts gene-edited foods? New study gauges public acceptance

Posted Jun 28, 2022 8:00 am

"Right now, there are a lot of people in the middle…."

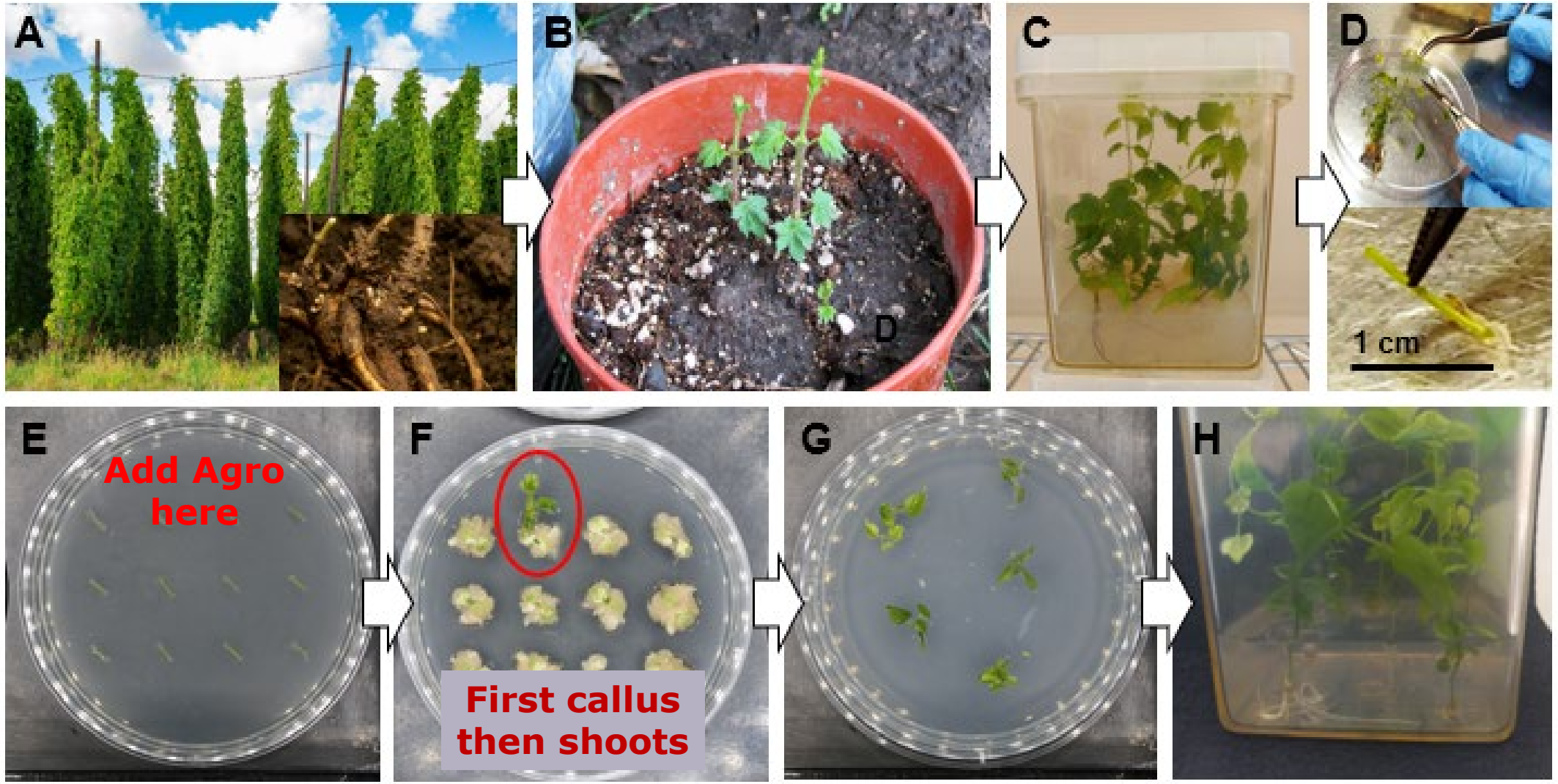
GE brewing yeast is used today, CRISPR plus

Commercial Examples	Supplier	Engineered DNA	Function
Sourvisiae	Lallemand	Fungal LDH	Produce lactic acid
Tropics	Berkeley Yeast	Bacterial carbon sulfur lyase	Release 3SH from malt, hops, grape products
Diacetyl Free	Berkeley Yeast	Bacterial ALDC	Reduce diaceyl formation
Cosmic Punch	Omega Yeast Labs	Activated yeast b-lyase	Release 3SH from malt, hops, grape products
Bananza	Omega Yeast Labs	Inactivated yeast ferulic decarboxylase enzyme	Eliminate 4-VG production

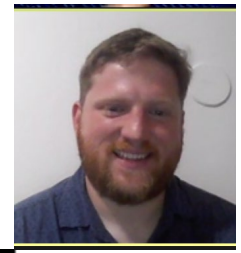
Slide courtesy of Laura Burns, Omega Yeast

GE hops

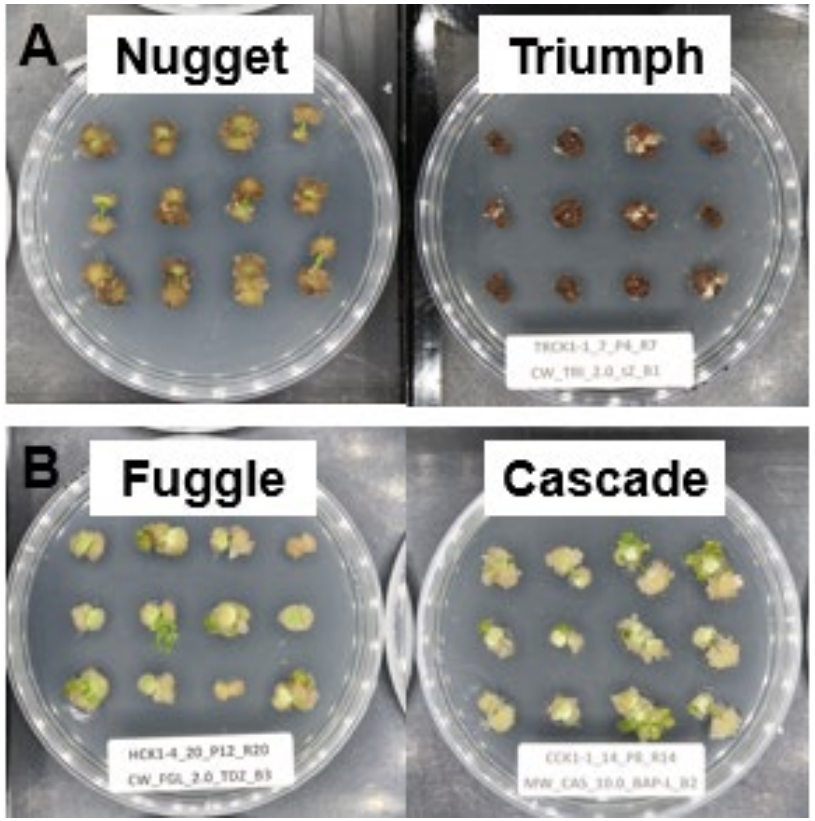
Steps for hop tissue culture & GE

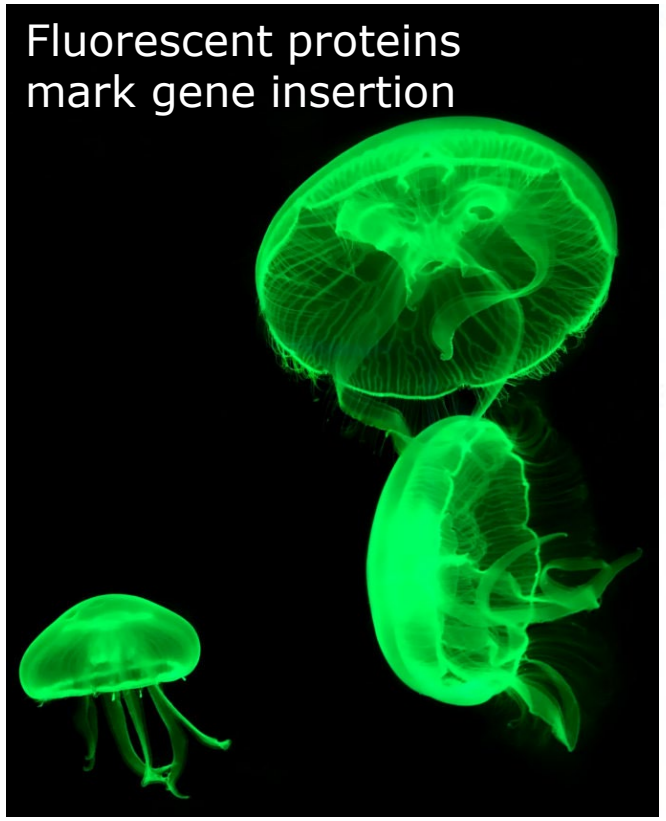


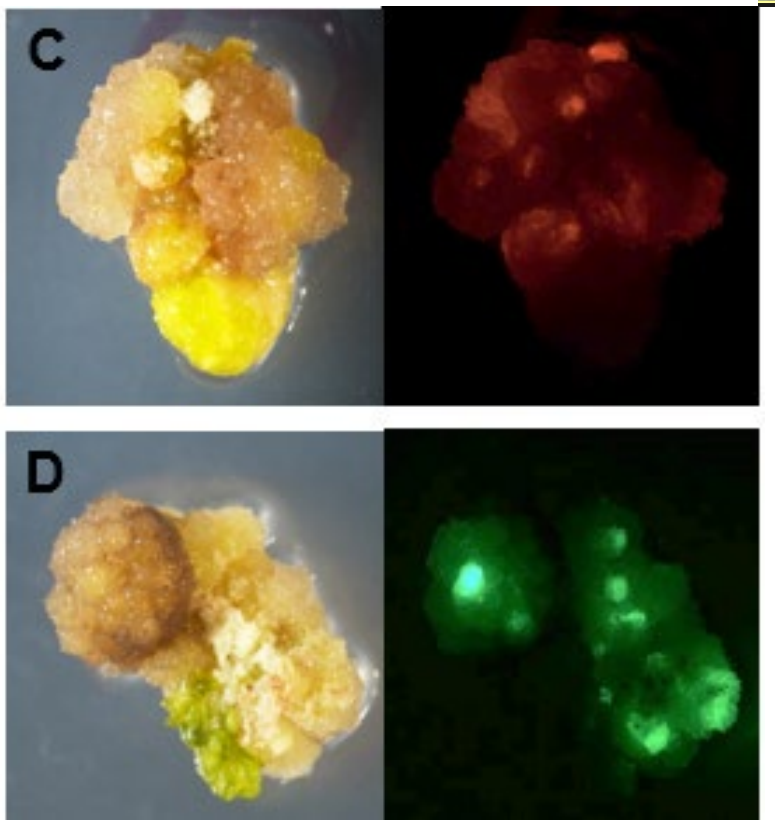
GE of hop is hard – but its been done in the EU, and is progressing on important USA varieties



Chris Willig, postdoc, Oregon State





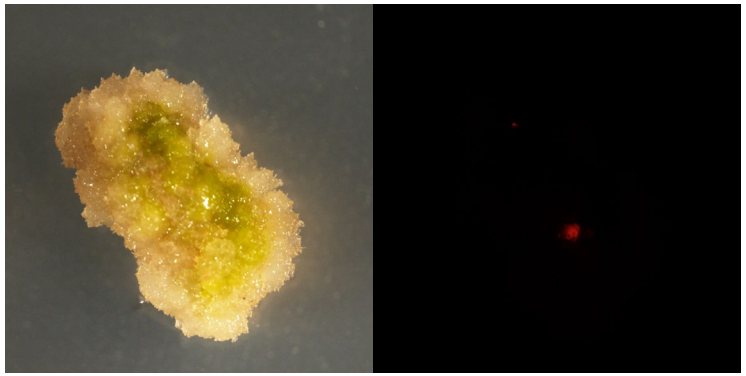


Clones vary <u>widely</u> in regeneration responses

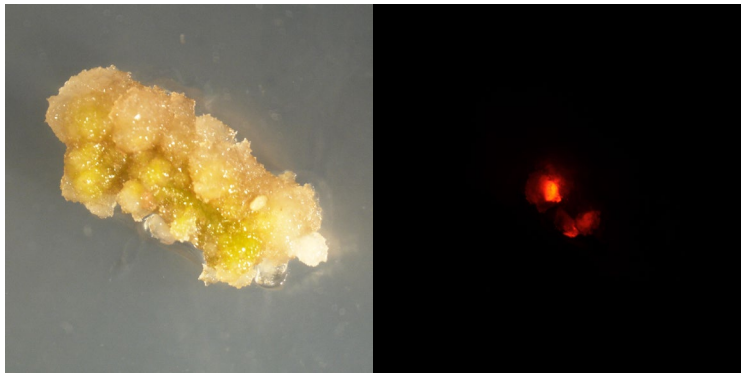
Red or green transgenic cells evident, not yet shoots

Improving Hop GE: Optimize spectinomycin to select GE cells effectively

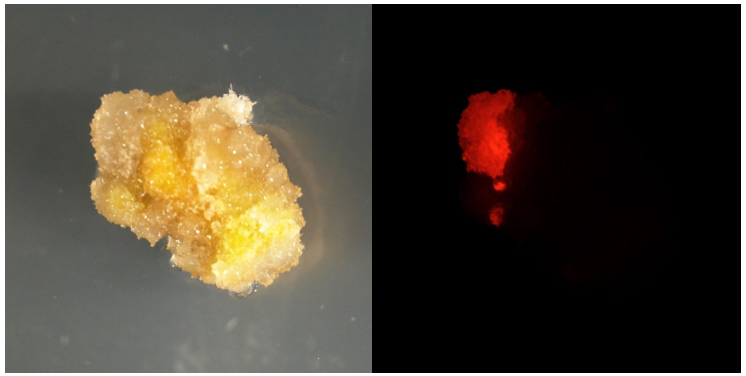
0 mg/L



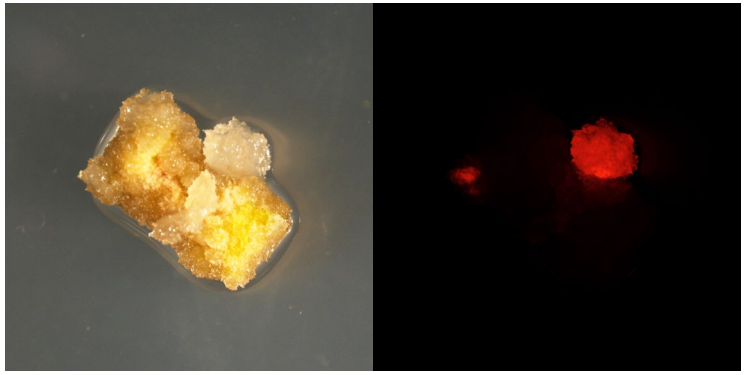
12 mg/L



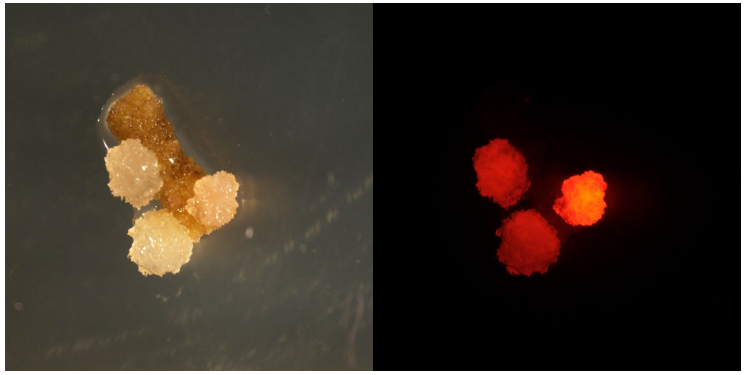
25 mg/L



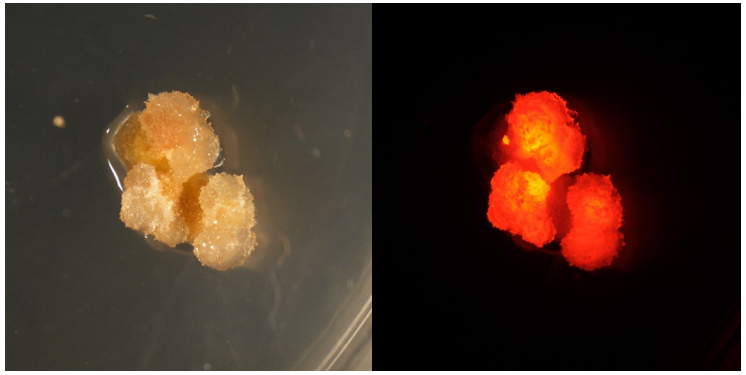
50 mg/L



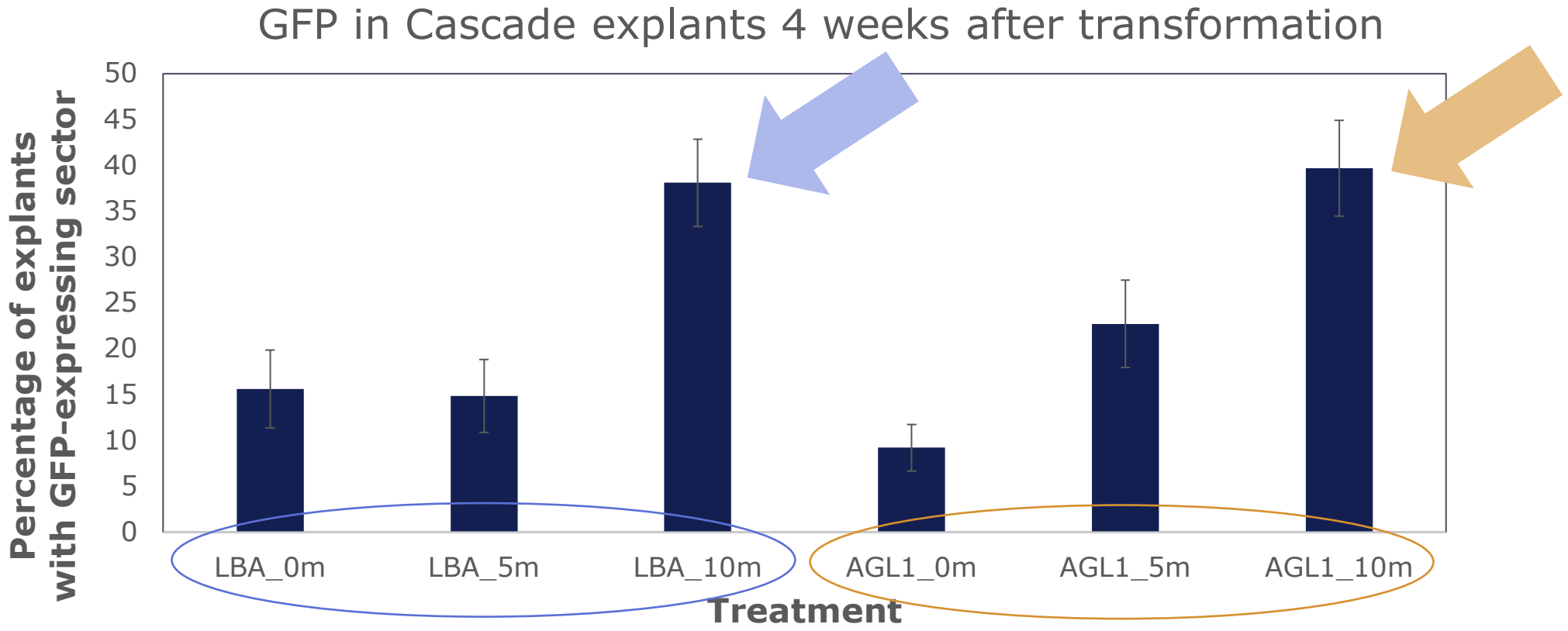
100 mg/L



200 mg/L

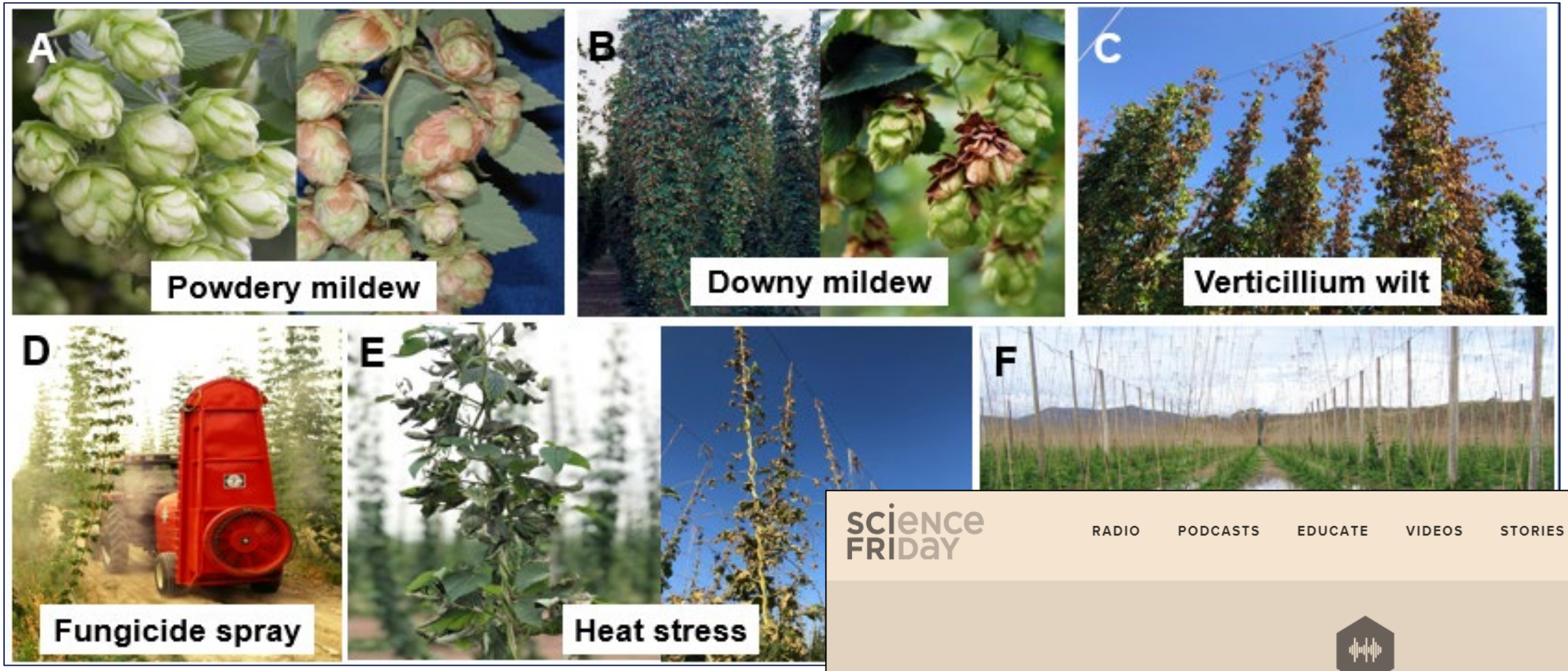


Improving Hop GE: Vacuum infiltration of Agrobacterium enhances gene transfer for two strains



Why add GE as a tool? Sustainability!

Stresses on hop are growing – biological and climatic



Serious damage to Citra in W
with 2021 heat dome ev

Dought tolerant wheat approved in Argentina

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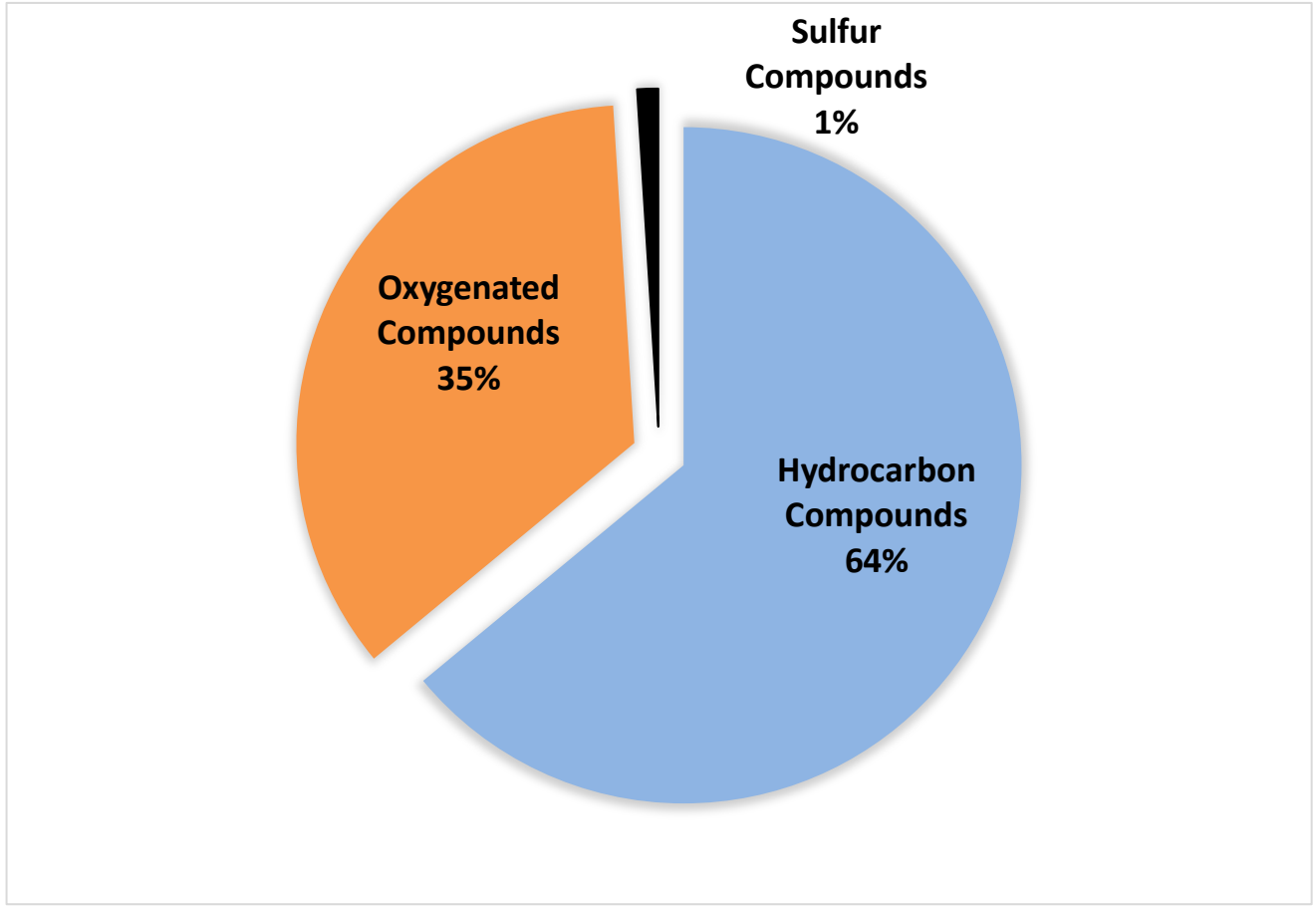
News in Brief | Published: 10 June 2021

Argentina first to market with drought-resistant GM wheat

GE appears capable of helping to improve complex traits like drought tolerance in commercial crops

Flavor modification another reason to consider GE approaches

Three major classes of compounds make up hop oil

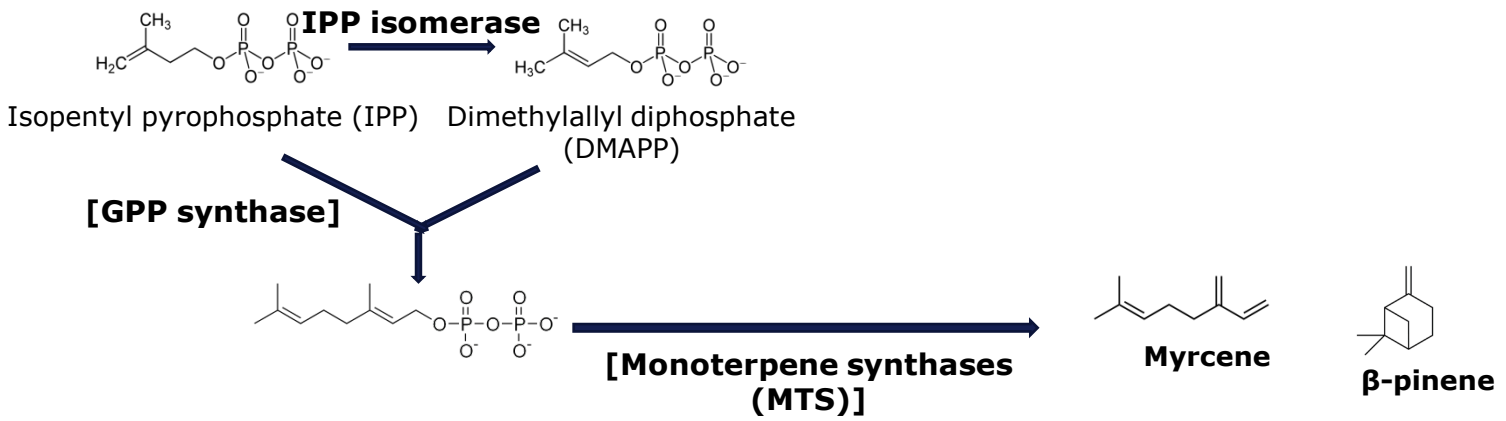


- More than 1,000 compounds in total
- Most show extensive variation among varieties
- Growing scientific understanding of biosynthesis

Monoterpene oils are critical to flavor variation

- Linalool – lavender/”Froot loops”
- β-pinene – pine/minty
- Geraniol – rose/floral
- Myrcene – citrus/metallic flavor, 10-70% of total oil

Hop essential oil pathways being mapped to key genes



The Plant Genome | OPEN ACCESS

ORIGINAL RESEARCH | Open Access

A draft phased assembly of the diploid Cascade hop (*Humulus lupulus*) genome

Lillian K. Padgitt-Cobb, Sarah B. Kingan, Jackson Wells, Justin Elser, Brent Kronmiller, Daniel Moore, Gregory Concepcion, Paul Peluso, David Rank, Pankaj Jaiswal, John Henning, David A. Hendrix

Summary of target traits for hop breeding and GE

- Tolerance to disease, heat, and drought stress – key concerns in a climate change world
- Altered bittering and aroma qualities—to produce distinctively flavored beers

- Plant height—dwarf hops are easier to harvest and require less costly infrastructure
- Flowering time—expanding capacity for hops outside of major production regions
- Storage stability—preserving flavor for longer periods

Take-home messages

- GE can add specific traits to crops using asexual methods
- GE crops used on massive scale globally, but uptake highly variable
- Regulatory barriers appear to be receding and consumer acceptance growing in USA
- GE of hop is hard, but promising given research
- Many options for GE to help improve hop traits
  - ***Need research to explore***

Acknowledgements

- We thank the USDA-NIFA, through AFRI grant #2021-67013-34739, for support of postdoc Chris Willig on gene editing in hop

- Also my thanks to a great team doing hop gene editing research, and that helped put this talk together


Chris Willig


Michele Wiseman


John Henning


David Gent


Tom Shelhammer


Cathleen Ma