Gene editing as a powerful tool to advance hop research and agriculture

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Talk Roadmap

- Context of how gene editing works as a process for trait improvement
- Protocol development needed for routine gene editing
- Overview of hop biotech research at OSU
- Examples of research in hop involving gene editing—pending proposals
- Regulatory status and outlook for plant products produced using gene editing and other types of direct genetic modification

Gene editing has two very powerful applications

1) Basic research – to understand how genes are linked to traits
   - The resulting data helps inform breeders in selecting lines for crosses

2) Direct improvement of an agronomic trait for the purpose of deploying it in the field
   - For this use, many complex factors at play—economic, regulatory, social

All of our proposed projects involve modifying plants only for 1), though could also be used for 2) in the future

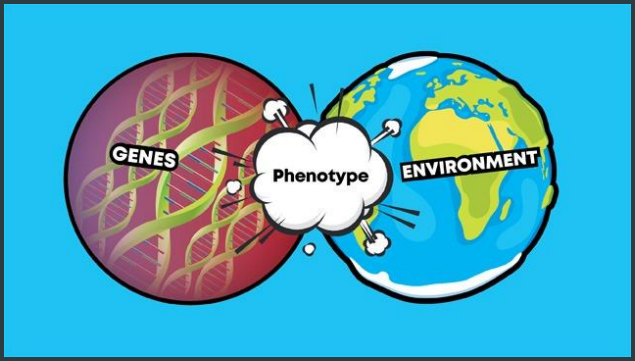
A TASC proposal to USDA with HRC aims to study techniques to explore the feasibility of 2)

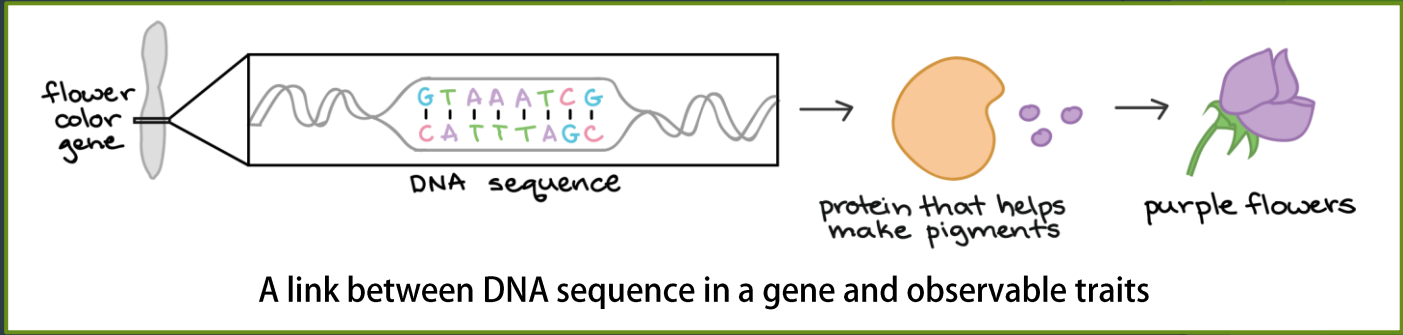
What is crop development?

- Humans have been influencing the characteristics of plants since before we started practicing agriculture
- Agriculture developed by humans selecting plants with favorable traits
- Modern plant breeding has made selection of traits systematic and has led to some huge gains in crop productivity and diversity
- We can select because of existing or amplified variation in plant populations

Genes are the source of much variation we see in plants

- We have only understood genes (DNA sequence) to be a basis for traits for the past quarter century





A link between DNA sequence in a gene and observable traits

- Most traits that we care about are influenced by a combination of genes and the environment an organism exists in

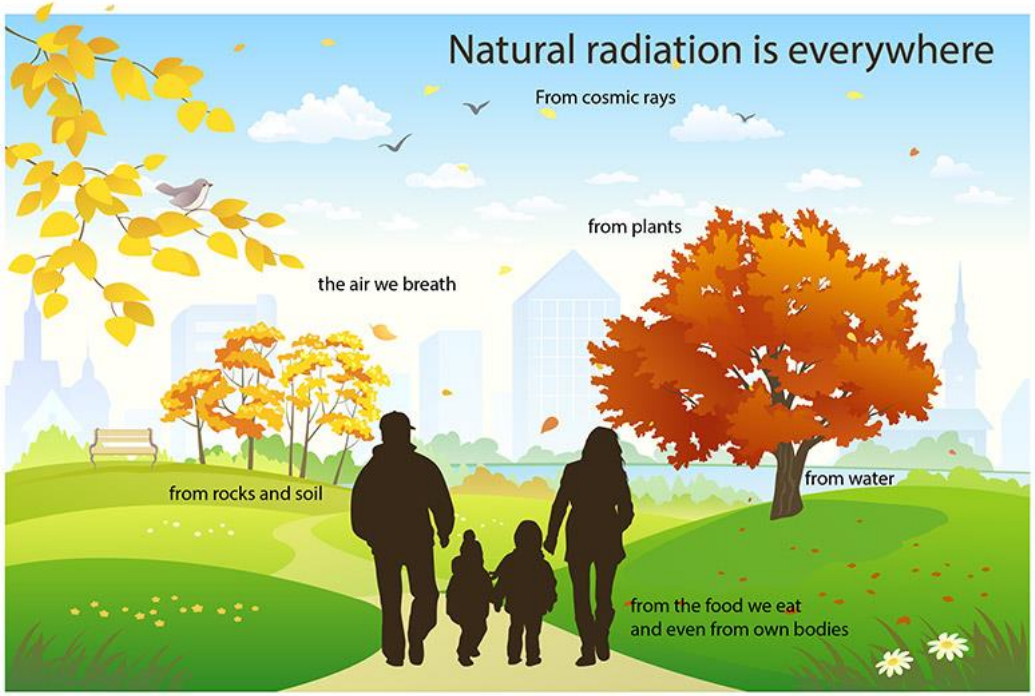
- For some specific traits, variations can be penned down to differences in DNA sequence

Sequence differences in DNA come from mutation

- The reason there are differences in DNA sequence are the continual changes caused by mutation

- Mutations aren't necessarily bad—they're natural and unavoidable

- Most types of mutations make changes at *random* DNA sites, and thus the outcome is unpredictable



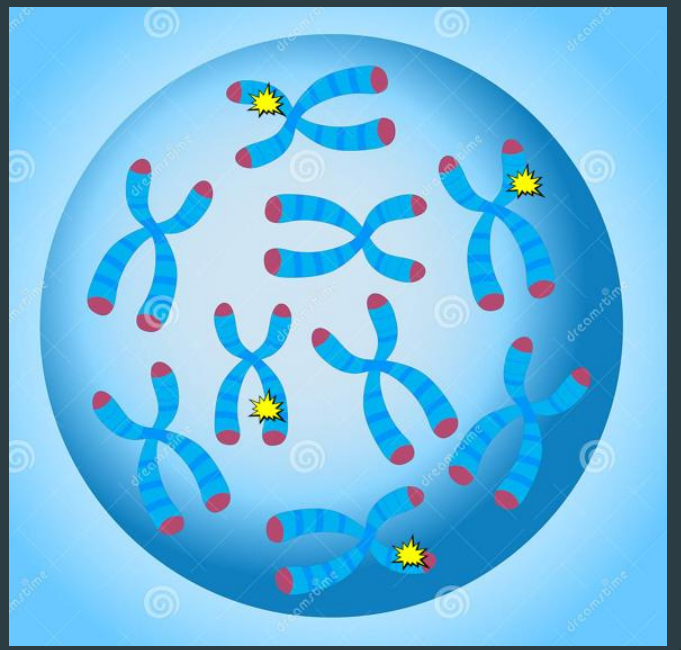
Intentionally increasing mutation rates can result in new variation

- Breeders will sometimes try to create new trait variation by exposing plant material to ionizing radiation or mutagenic chemicals

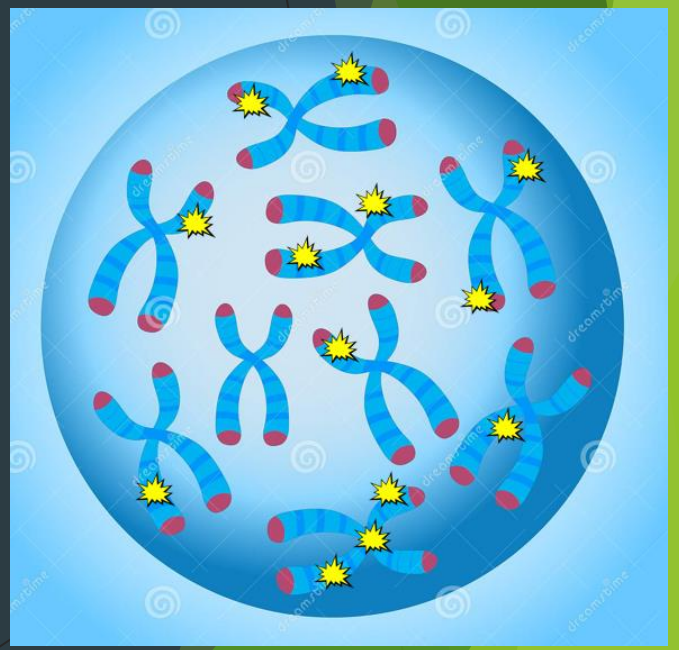
- This increases the rate of mutation, though the positions where they occur are still random

- That means beneficial and harmful mutations are both increased

= mutation site



Normal mutation rate



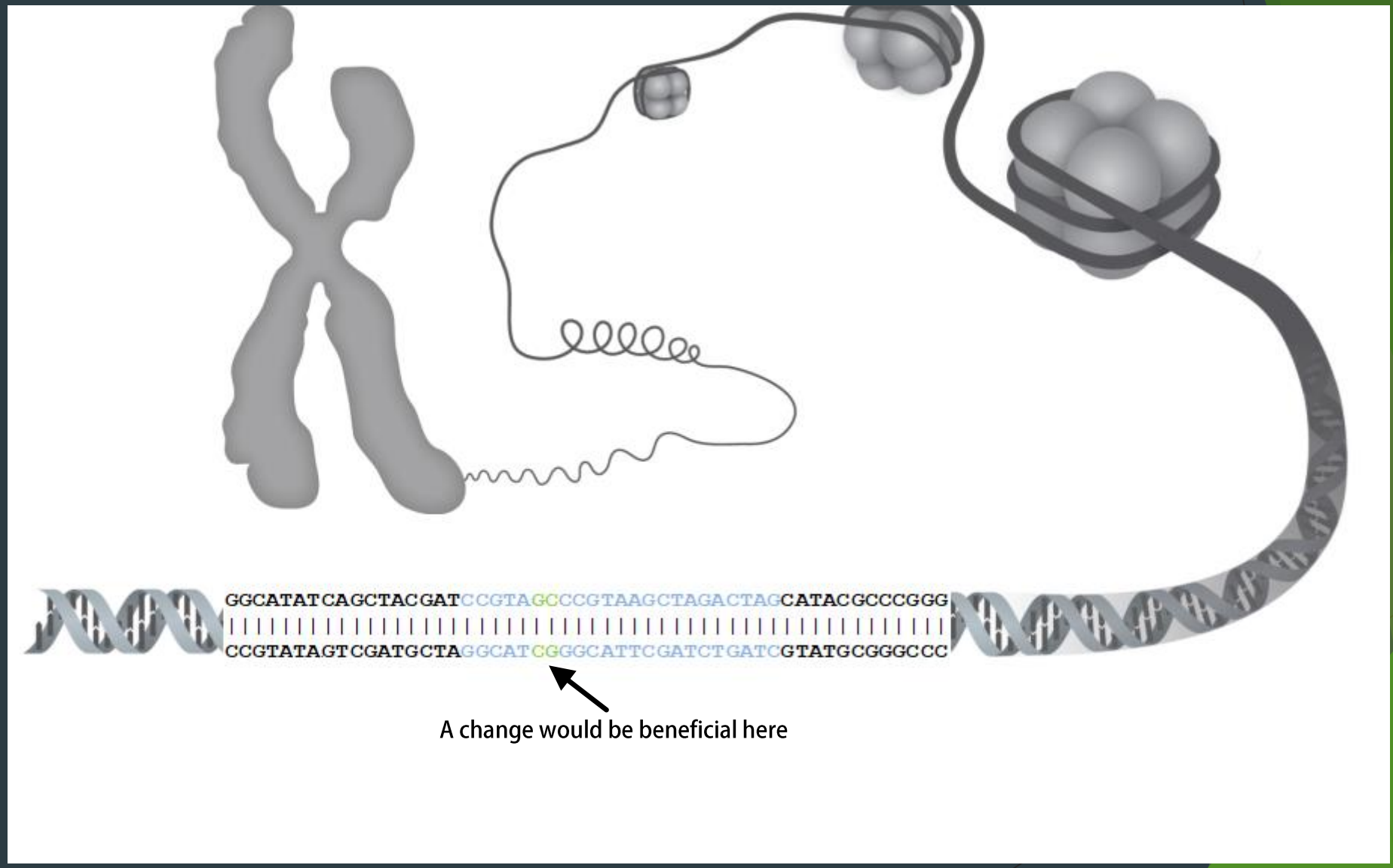
Mutation rate due to excessive ionizing radiation or chemical mutagen

Gene editing allows control over where mutations happen

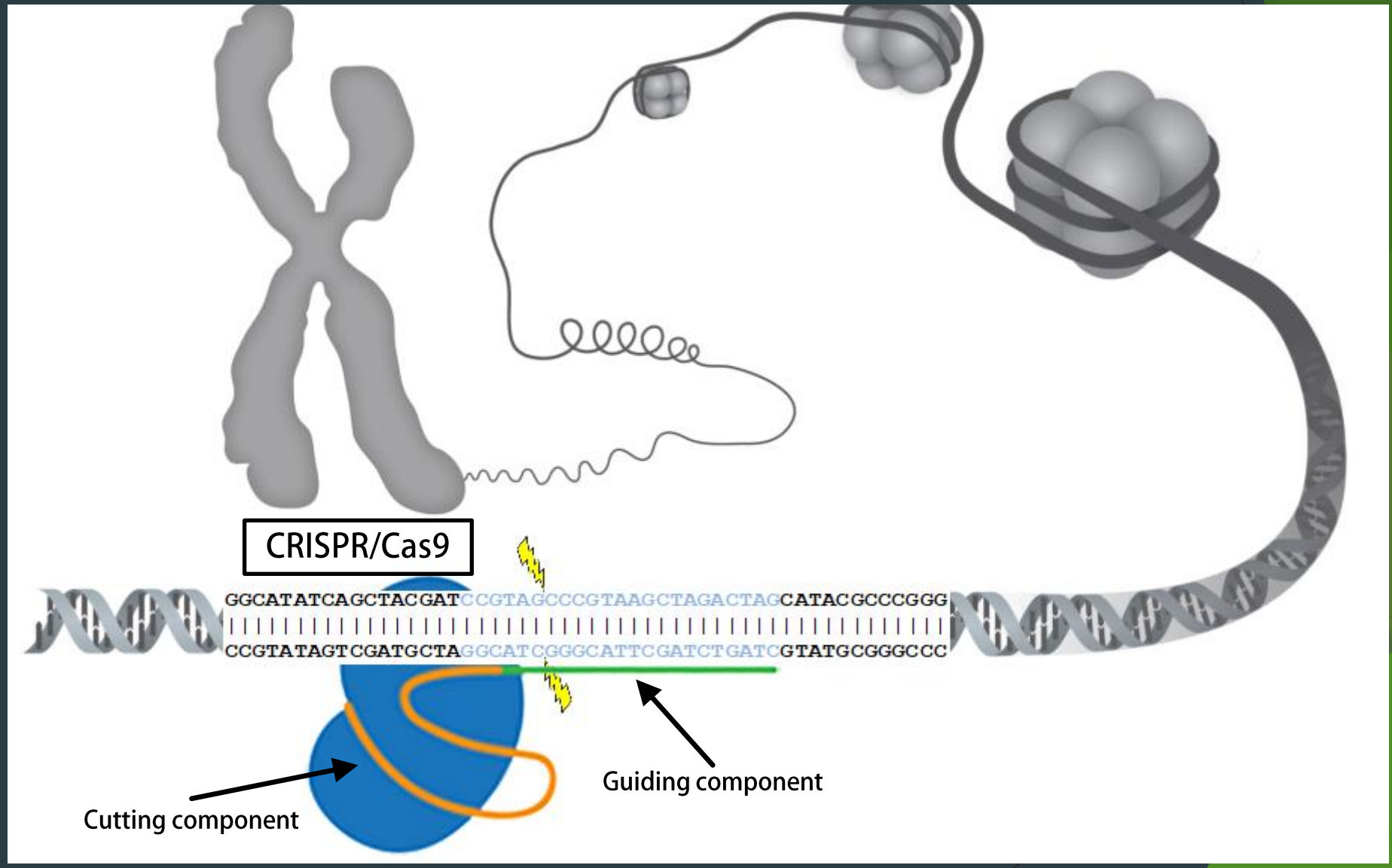
- Gene editing is such a major innovation in crop improvement because it allows us to precisely choose where mutations occur

- Avoiding randomness allows much smaller numbers of plants to be used to make a beneficial mutation

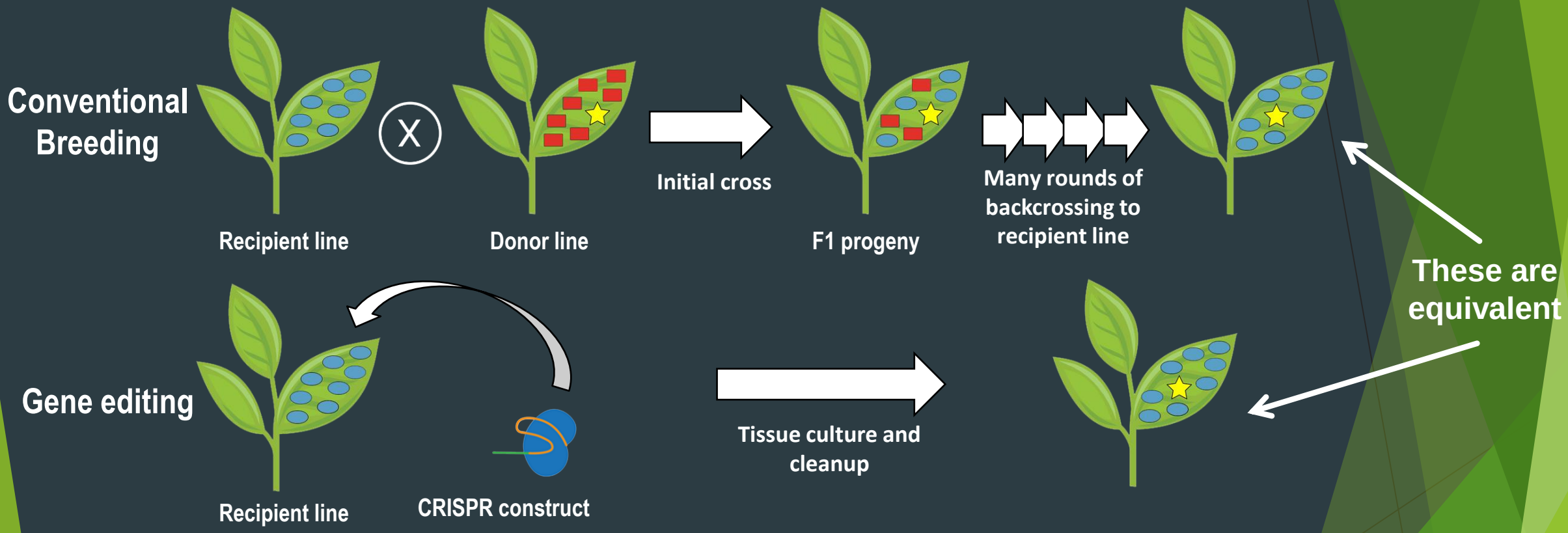
Precision mutation using gene editing



Precision mutation using gene editing



Modifying a trait by gene editing or breeding

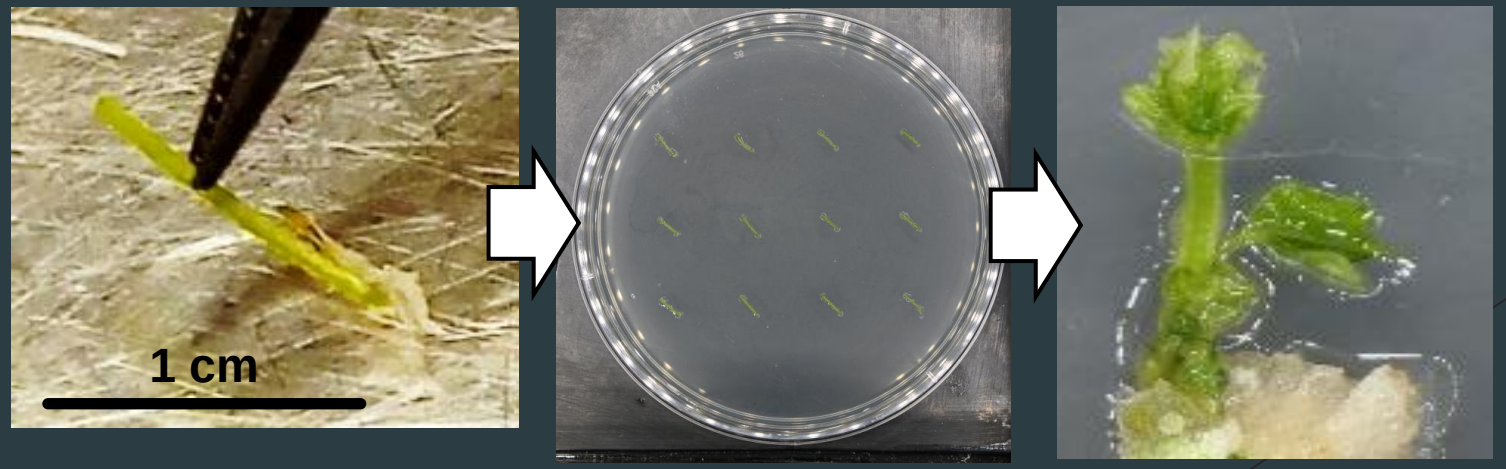


- Conventional breeding crosses and gene editing are both processes for crop development
- In this use case, the final product is the same—only the process is different

What do we need to perform gene editing?

- Repeatable methods for delivering genetic material into plant cells and then having those cells regenerate into an entire plant
  - This whole process is called plant “transformation”

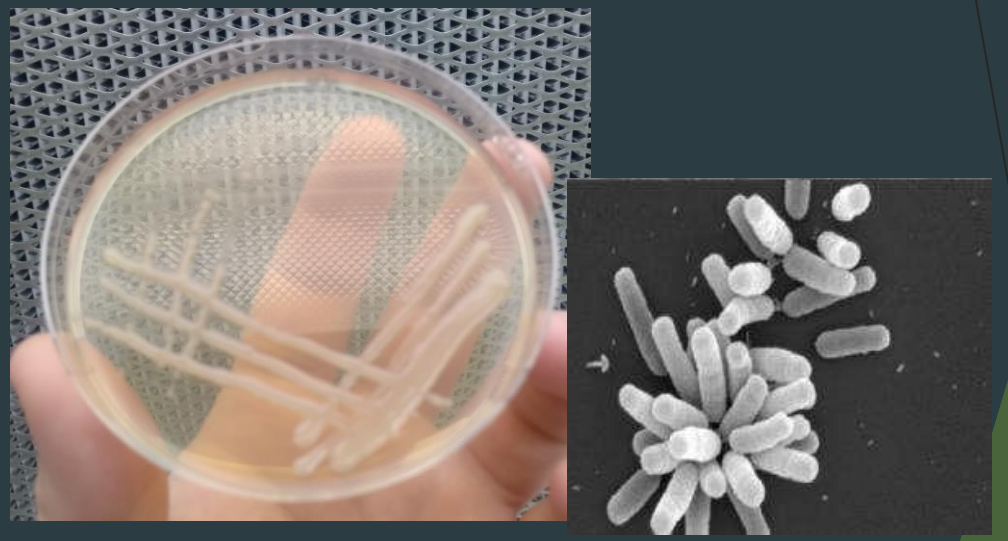
- Regeneration in tissue culture uses plant hormones to get new shoots to form from cells of a mature plant tissue, such as stem



What do we need to perform gene editing?

- A way to get genes into plant tissue

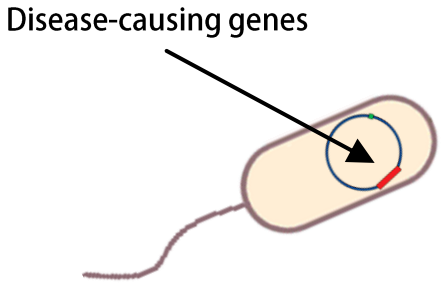
Gene gun



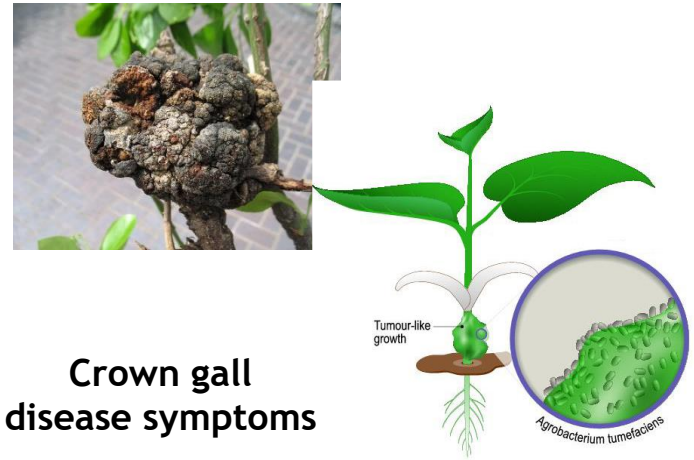
Agrobacterium tumefaciens

The most commonly-applied method for plant transformation

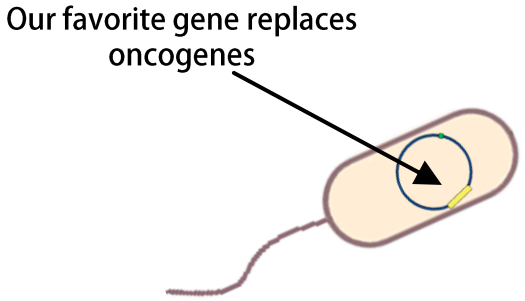
“Hacking” *Agrobacterium* for use in the lab



Wild *Agrobacterium* strain



Crown gall disease symptoms



Laboratory domesticated (“disarmed”) *Agrobacterium* strain

No disease plus intended trait modification

Hop is among plants that are “natural GMO”

- A 2019 study looked for genes originating from *Agrobacterium* in hundreds of sequenced plant genomes

Published: 21 September 2019

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

Tatiana V. Matveeva & Léon Otten

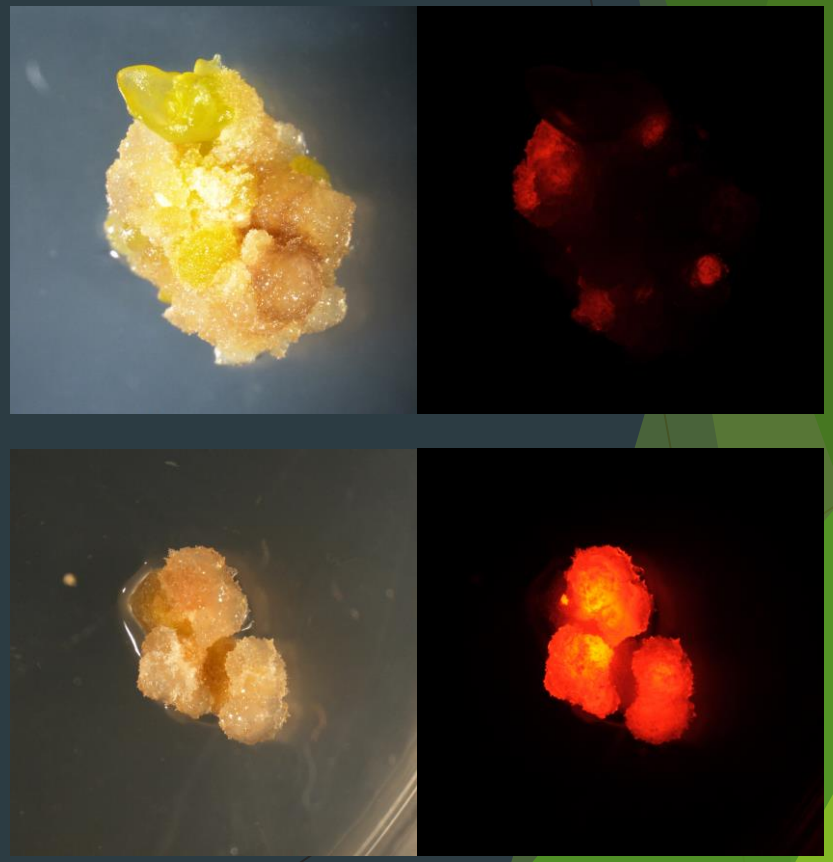
Plant Molecular Biology **101**, 415–437 (2019) | Cite this article

- Hop was among the 10% of species examined that contained one or more of the genes

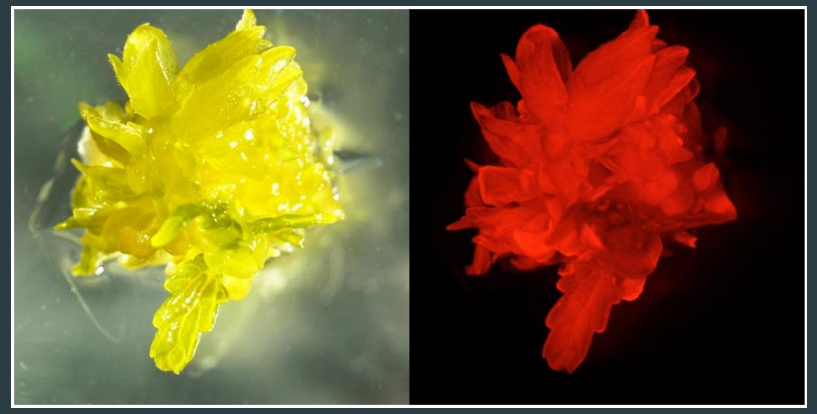
- This gene from an ancient “transformation” event by *Agrobacterium* in the wild is intact and turned on in hop

Protocol development work our lab has done

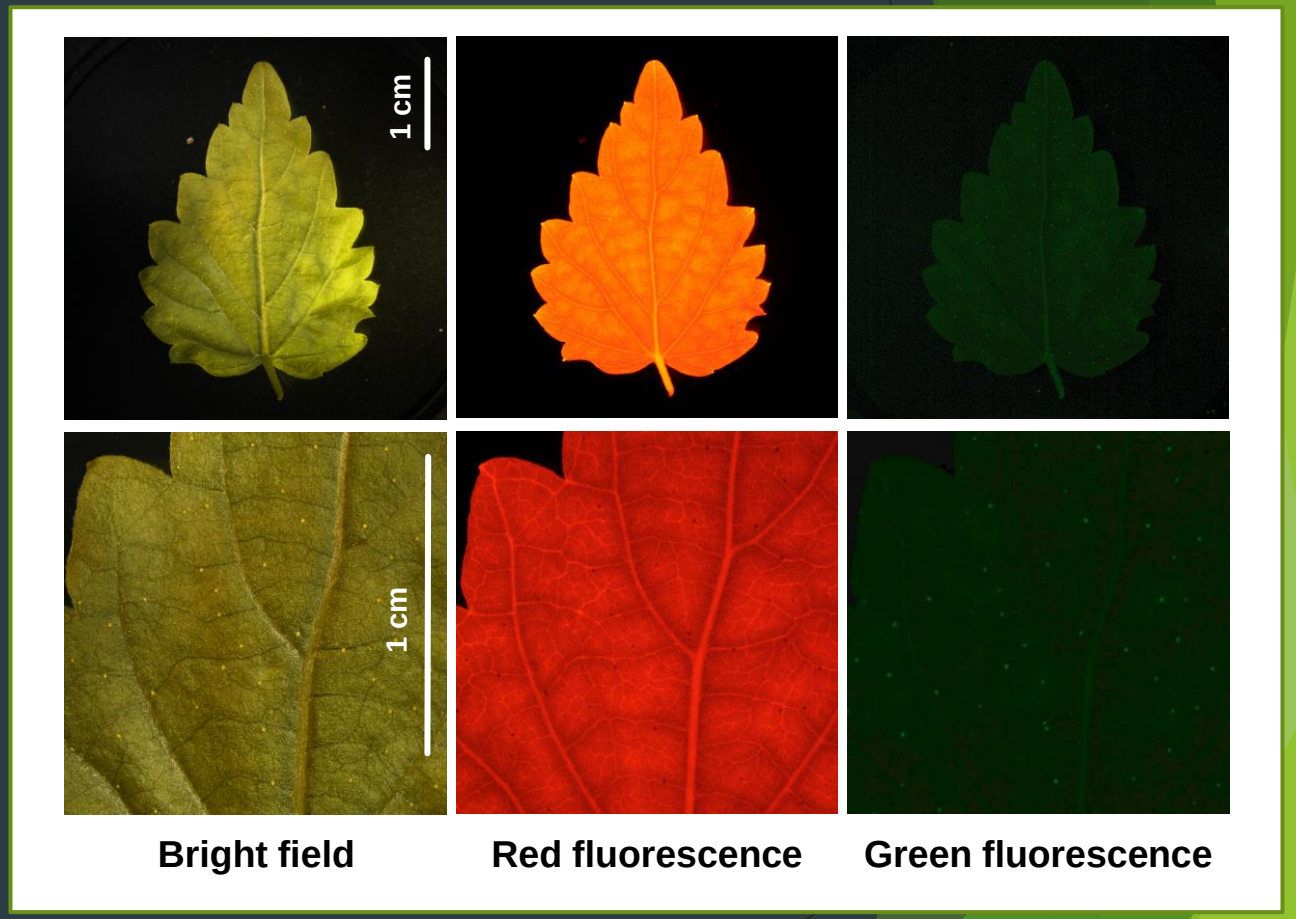
- Our research group has screened several US public cultivars for regeneration and T-DNA delivery efficiency. *Cascade was one of the top performers*



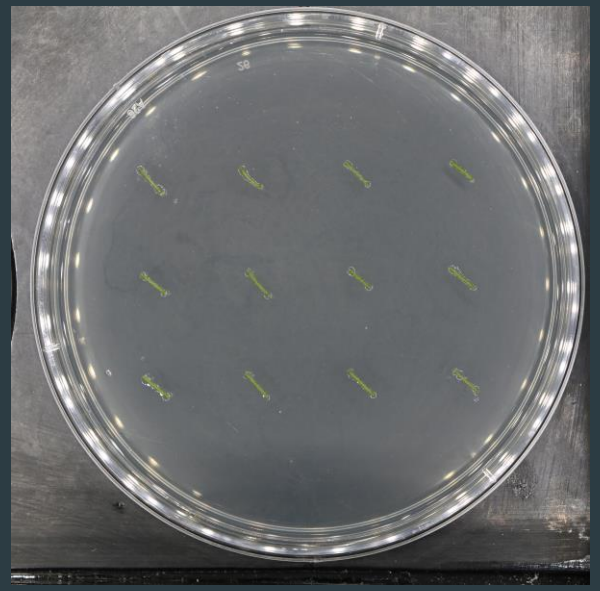
Transgenic Cascade was produced – key tool for gene editing in hand



Credit: Michele Wiseman

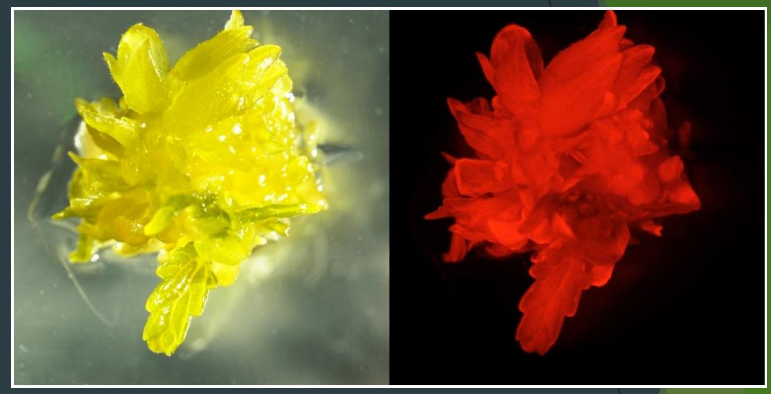


Next steps—optimizing our protocol



Rate of transgenic shoot production?

Estim. ~0.5%



- Transformation efficiency – how much original plant material and labor does it take to get one transgenic shoot cluster?
- In the near future, we will test whether any of a variety of tweaks to our transformation procedure can reliably boost the efficiency

Proposal submitted to the TASC program by HRC in support of further work at OSU

- We proposed to explore a strategy for a long-term solution to overcoming trade barriers due to fungicide MRLs
- MRLs limit fungicide variety used to control powdery mildew, making it more likely for the pathogen to develop tolerance to some
- Durable genetic resistance to powdery mildew could reduce or eliminate the need for fungicide application
- Some markets with strict MRL standards are open to gene-edited products, and others are moving in that direction

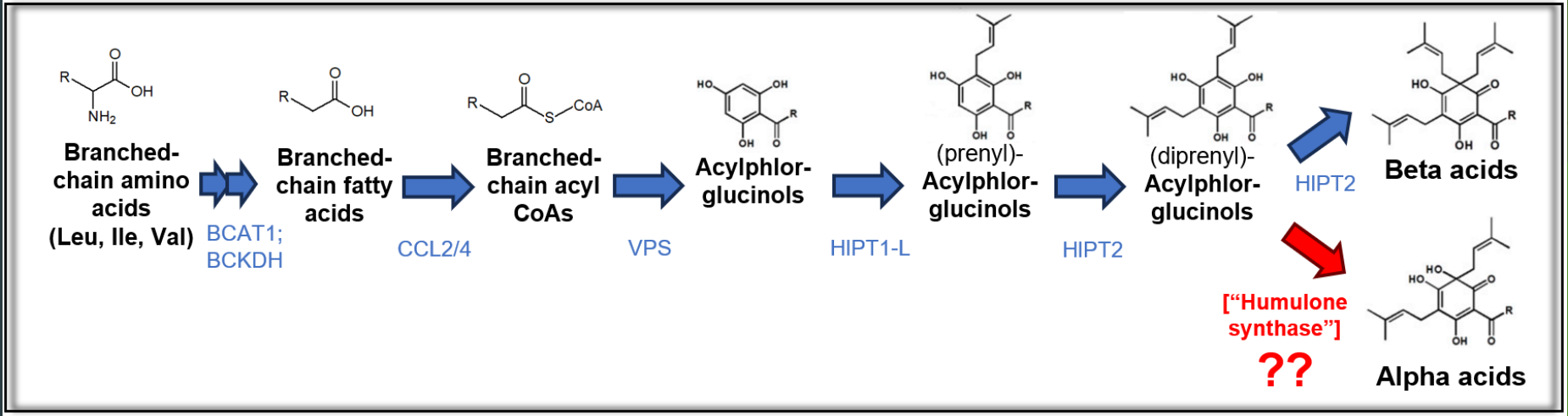
Proposal submitted to the TASC program by HRC in support of further work at OSU

- We plan to target genes of the *MLO* family to provide durable resistance—as they have for other crops
  - This is the topic of Michele Wiseman's PhD work
  - Michele has done lots of work to identify candidate *MLO* susceptibility gene sequences in hop and has transformation experiments underway to edit those genes
- In some other plant species (not all), certain *mlo* variants that provide resistance also have a yield penalty tradeoff
  - We proposed to make many *mlo* variants and evaluate in the greenhouse and field
- We will also develop transformation protocols for several additional cultivars
  - Aim is to get field-tested *mlo* resistance genes into breeding pipelines so they are used to develop new cultivars

Michele Wiseman

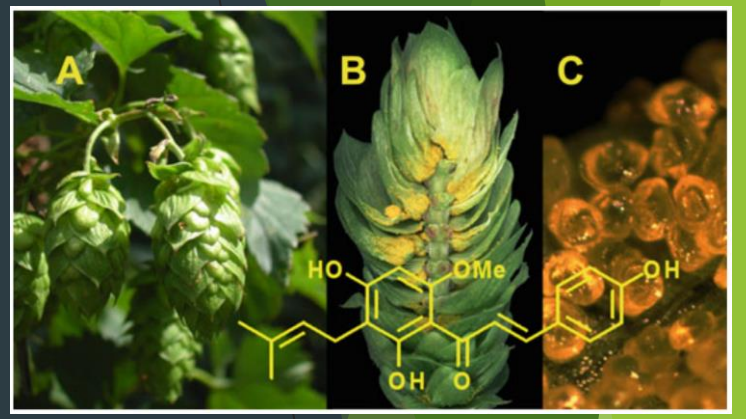
Proposal submitted to HRC

- PROPOSAL TITLE: Gene editing to modify alpha acid biosynthesis in hop
- For proposal to HRC, we will study a gene that (hopefully) plays a key role in production of alpha acids
- Successfully identifying this gene would result in breeders having a new marker to track when breeding for high alpha levels



Proposal to be submitted to USDA-NIFA "Foundational Knowledge of Plant Products"

- Xanthohumol is a compound only known to come from hop that treats metabolic syndrome and inflammatory bowel disease
- However, levels of xanthohumol in lupulin are low enough to make extraction costly
- CRISPR mutation should enable much enriched xanthohumol levels

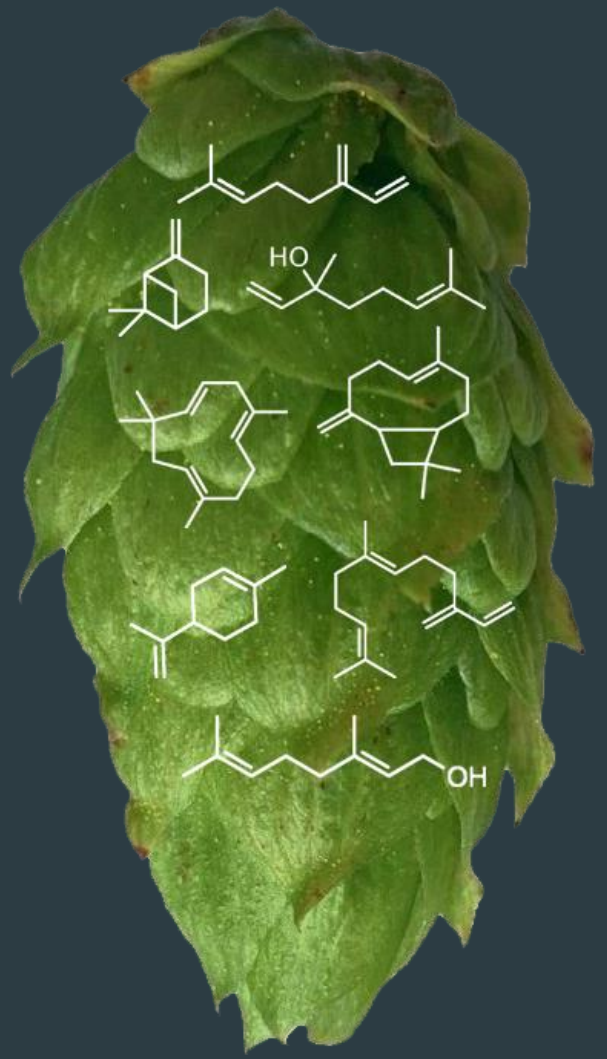


OSU study: Compound derived from hops shows promise as treatment for common liver disease

Effect of a Nutrition Support Formula in Adults With Inflammatory Bowel Disease: A Pilot Study

Jennifer J Ryan, ND, MS[1], Douglas A Hanes, PhD[1], Ryan D Bradley, ND, MPH[1,2], and Nikhat Contractor, PhD[3]

Terpene synthase to alter hop aroma



- Terpenes have a major contribution to aroma and flavor
- Terpene synthase genes are responsible for production and diversity of these compounds
- CRISPR will allow us to alter levels of certain terpenes and study their effect on aroma

Regulatory overview and outlook – USDA system

- USDA adopted a new "SECURE" rule in 2020
  - Shift to focus on product rather than process, and simplified requirements

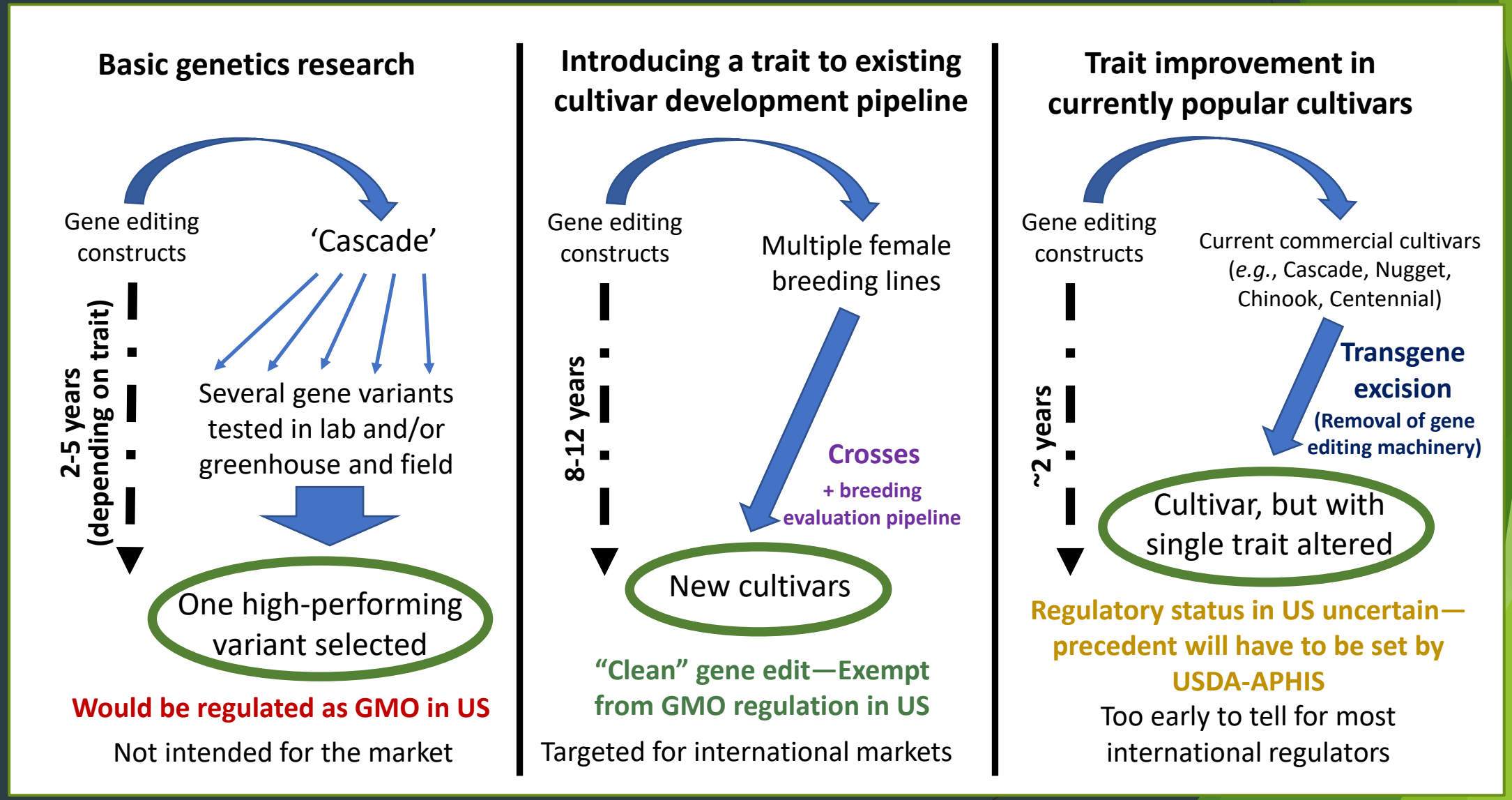
- General guideline: any plants produced using gene editing technology that *could have also been produced using breeding* are not subject to regulation
  - *i.e.*, if there is no added DNA sequence from outside the plant's breeding pool

- Everything else is evaluated on case-by-case basis through the new APHIS Regulatory Status Review (RSR) process which focuses ONLY on plant pest risk and requires one-half year for most traits

Regulatory overview and outlook – EU status

- The EU has historically taken a strict regulatory stance toward the use of biotech in agriculture
- A 2018 ruling by the European Court of Justice determined that gene editing would be regulated under the same rules established for older genetic modification techniques
- On July 5th of this year the European Commission advanced a proposal to the EU's legislative bodies that would be more product focused, similar to USDA
- The proposal includes language about prioritizing biotech applications that help the EU meet its sustainability goals laid out in the 2020 Farm to Fork strategy

Multiple paths are open for using gene editing in hop



Long-term goals and outlook

- Research priority right now is on disease resistance
- Long-term goal is to help make agriculture more sustainable and build climate resilience
- Developing editing techniques now will lay a foundation for studying/addressing difficult traits such as heat tolerance

Acknowledgements

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