Update: Developing gene editing technology for American hop cultivars

Hop Research Council / American Hop Convention
January 21-24, 2025
Coronado, CA

Presented by: Chris Willig

Oregon State University

Hop biotech research at OSU

- There has been active research on hop biotechnology at OSU since 2021
- Our research group consists of members of the Strauss, Gent, and Henning labs
- Initially supported by a small 2-year grant from USDA-NIFA to explore gene editing methods in hops

Strauss Lab

Chris Willig
Postdoctoral Researcher

Steve Strauss
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Undergraduate Technician

Gent Lab

Michele Wiseman
PhD candidate

Dave Gent
PI

Carly Cooperider
Undergraduate Technician

Consulting

Greg Goralogia
Postdoctoral Researcher
(Strauss Lab)

Cathleen Ma
Senior Research Technician
(Strauss Lab)

Henning Lab

John Henning
PI

USDA NIFA

Continued funding through USDA TASC program

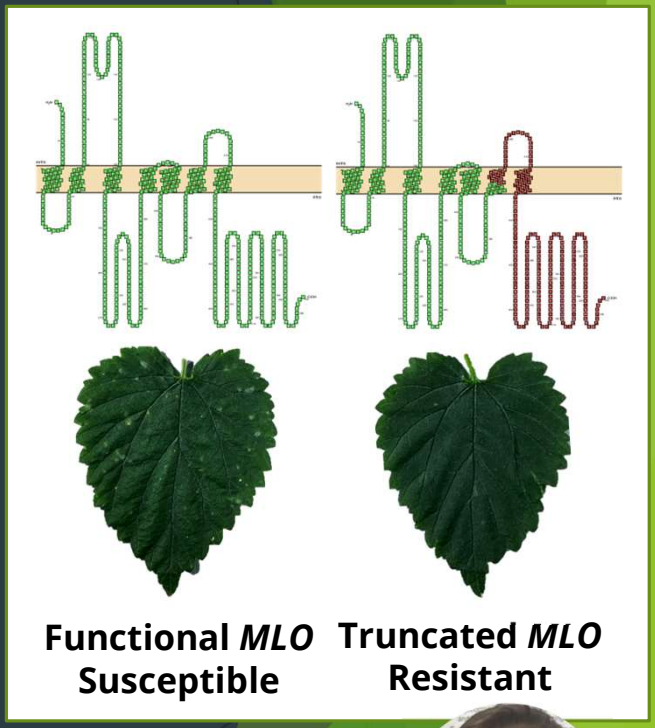
- In conjunction with HRC, we submitted a proposal to the USDA-FAS Technical Assistance for Specialty Crops (TASC) program—which was ultimately successful
- Total award amount will be >$2,000,000 over 5 years
- Two basic aims of this project:
  - Develop fundamental methods to support hop genetic research and production
  - Investigate a strategy for a long-term solution to overcome trade barriers due to powdery mildew fungicide MRLs

Rationale for the TASC project

- MRLs set by foreign markets for fungicides used to control hop powdery mildew (HPM) are a potential barrier for US hop exports
- Increasingly restrictive MRLs could limit variety of fungicide chemistries used to control HPM, making the disease more likely to develop tolerances to these
- Durable genetics-based resistance to powdery mildew could reduce dependence on fungicides to control
- Some markets with strict MRL standards are open to gene-edited products—others *moving in* that direction
  - This project aims to lay groundwork ahead of anticipated changes in global regulatory environment

Rationale for the TASC project

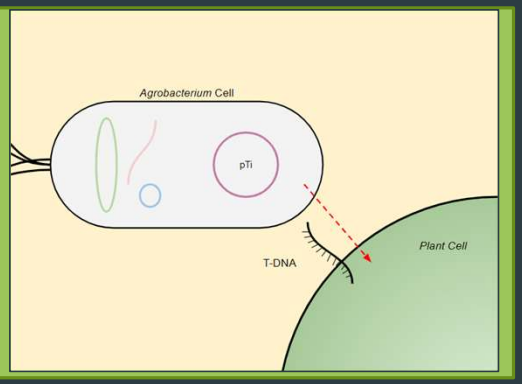
- We will explore a potential source of durable genetic resistance to HPM—members of the *Mildew Locus O* (*MLO*) gene family
- Variants of particular *MLO* genes can provide mildew resistance in several crop species
  - But some variants (not all) can cause yield trade-offs
- Individual *MLO* gene candidates we are targeting with gene editing were identified by Michele Wiseman
- Gene editing with CRISPR could allow us to:
  - Identify genes, and genetic markers associated with HPM susceptibility → supporting conventional hop breeding
  - Create plants with edited *MLO* variants that can be field-tested for yield viability



Michele Wiseman

Process of hop modification/gene editing

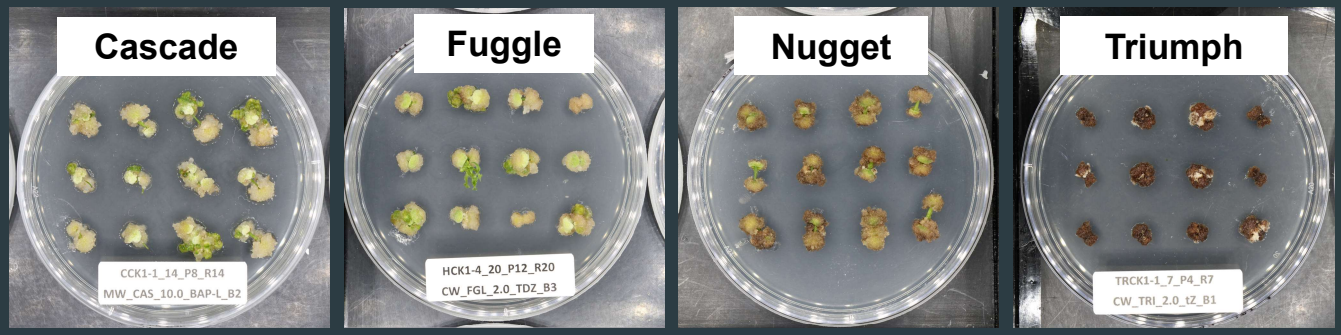
Agrobacterium as a natural genetic engineer



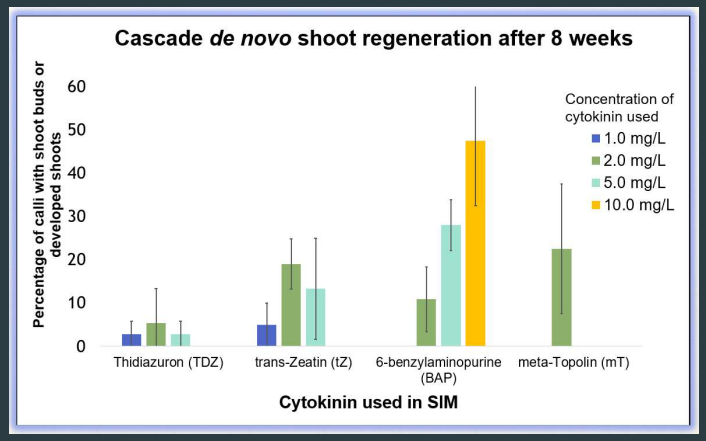
- In nature, *Agrobacterium* is a plant pathogen that causes "crown gall disease"
- *Agrobacterium* transfers a small piece of genetic material into plant cells
- Researchers have turned some *Agrobacterium* strains into a tool for crop biotechnology

Testing hop *regeneration* in tissue culture

- Screened for shoot regeneration capacity in several public hop cultivars



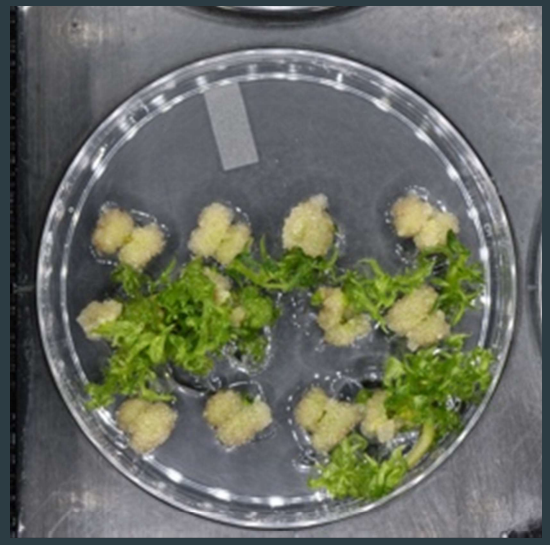
- Optimization for media hormone content in individual cultivars

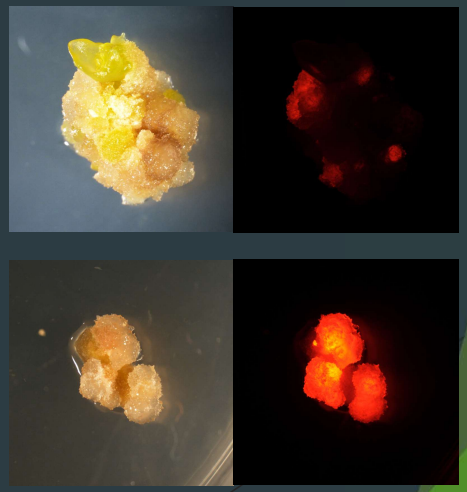


Testing hop *transformation* in tissue culture

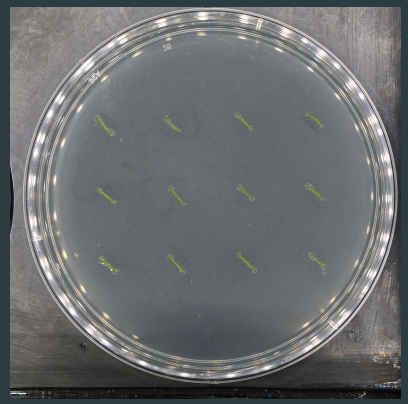
- Marker genes (fluorescent red) are used to tell where and how much genetic material is making it into plant tissue

- For the most part, genes are not getting into the same cells that end up regenerating



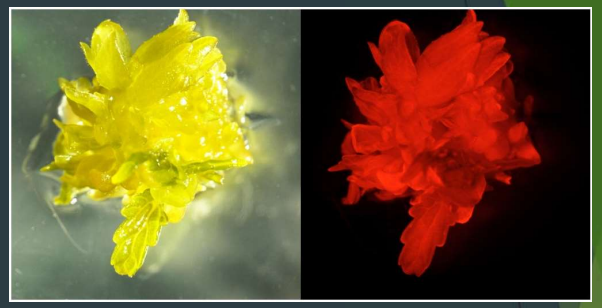


Improving low transformation efficiency



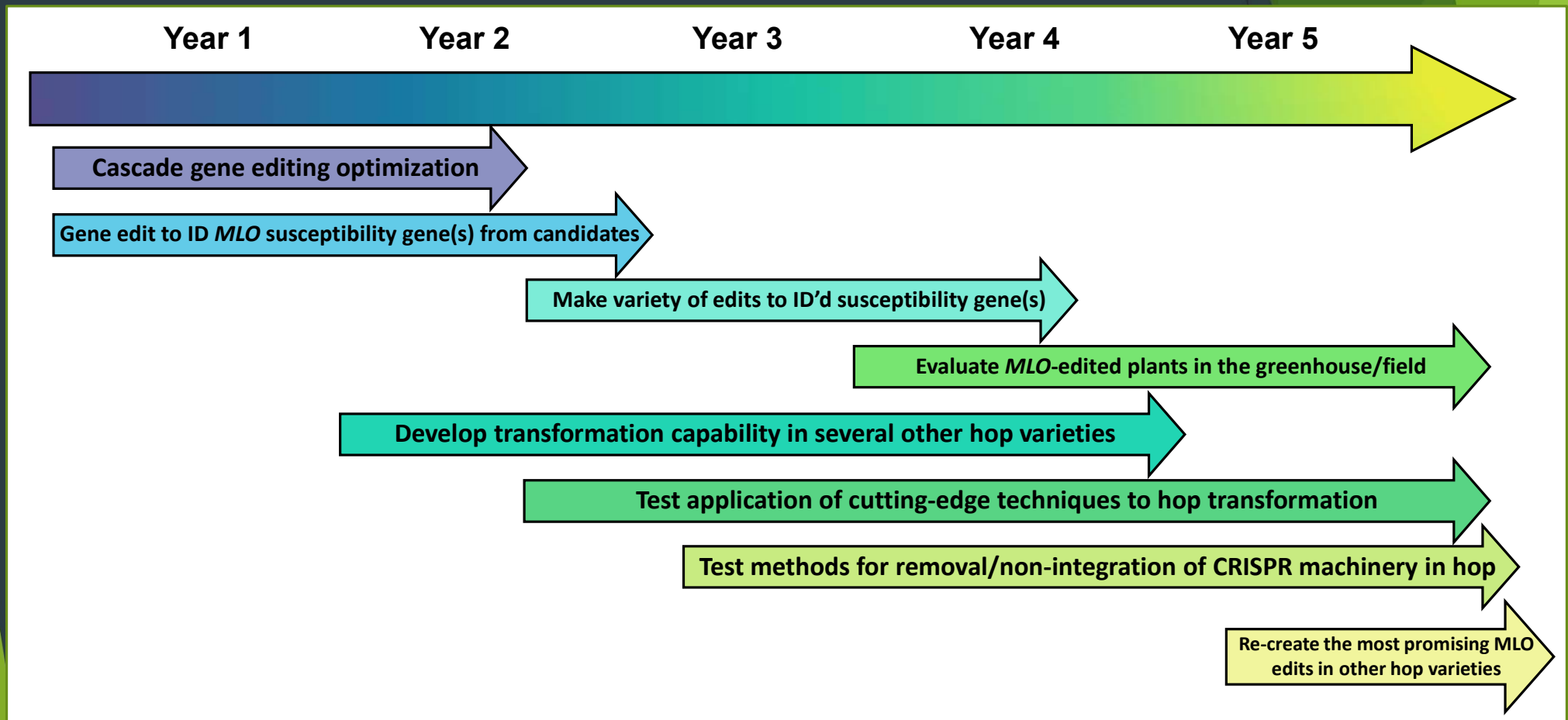
Rate of transgenic shoot production?

Estim. ~0.5%



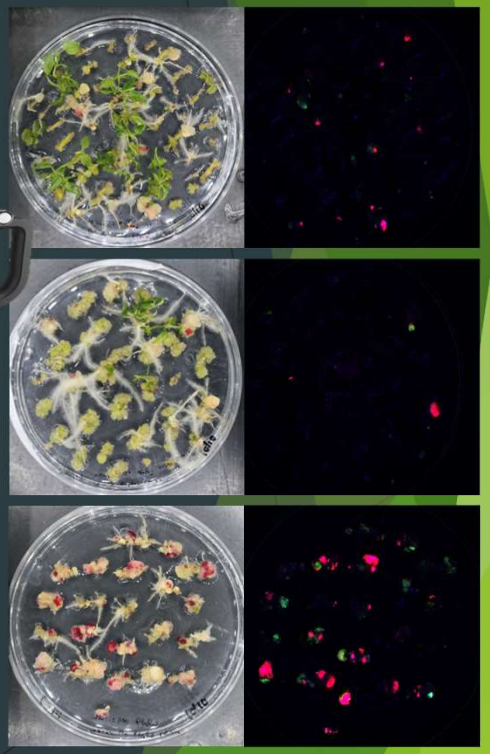
- We have been able to generate several plants with our marker gene
- Are now trying this method with gene editing for our *MLO* target genes—have not recovered any *confirmed* edited plants yet
- Tried many modifications to media recipes, incubation conditions for plants, and tweaks to *Agrobacterium* culture—only a couple of major effects seen

Timeline for TASC project



Current focus of work on TASC project

- The priority right now is to generate plants edited for *MLO* genes as quickly as possible while testing additional variations on our basic methods
- Have been exploring a method that involves mixing two *Agrobacterium* strains and putting plant tissue into a food blender
- The Strauss lab has recently hired a new research scientist who will be working full time on this project



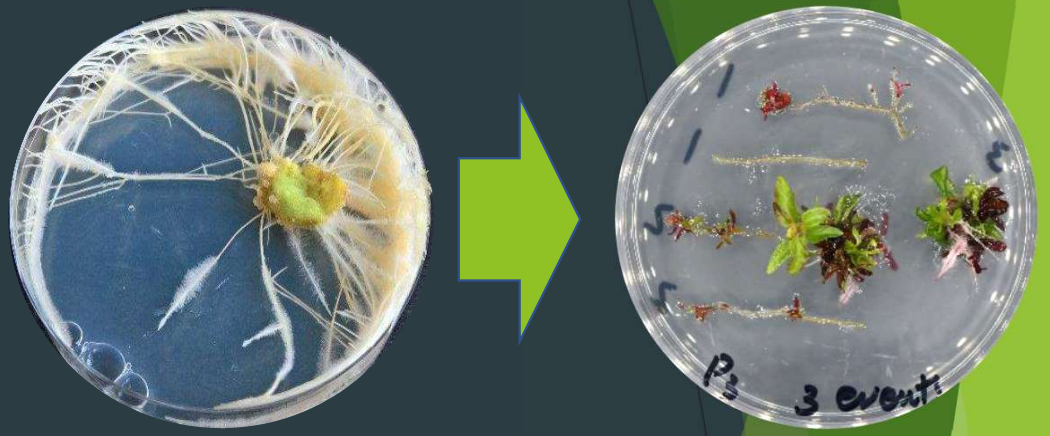
Veerendra Sharma
Research Scientist

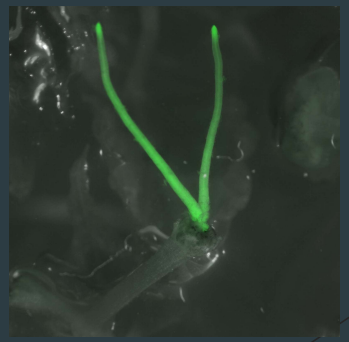
Identifying useful wild agro strains through natural interaction with hop

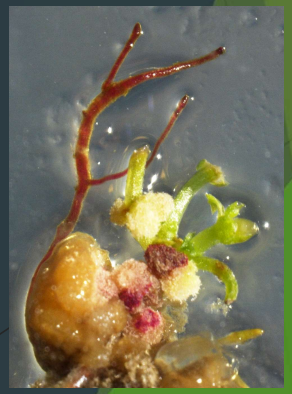
- The *Agrobacterium* strains we use as transformation tools in the lab may not be best adapted to all plant species
- Since strains have differences in host ranges, certain ones may be better adapted to transforming hop
- We isolated a new wild strain from a crown gall found on a hop plant, and recently disarmed it (made non-gall-forming)

Hairy root to shoot method

- Testing an alternative transgenic “hairy root”-to-shoot transformation approach
- This method has been reported worked in other crops recently
- We have shown that we are able to get transgenic hairy roots in 4 hop varieties

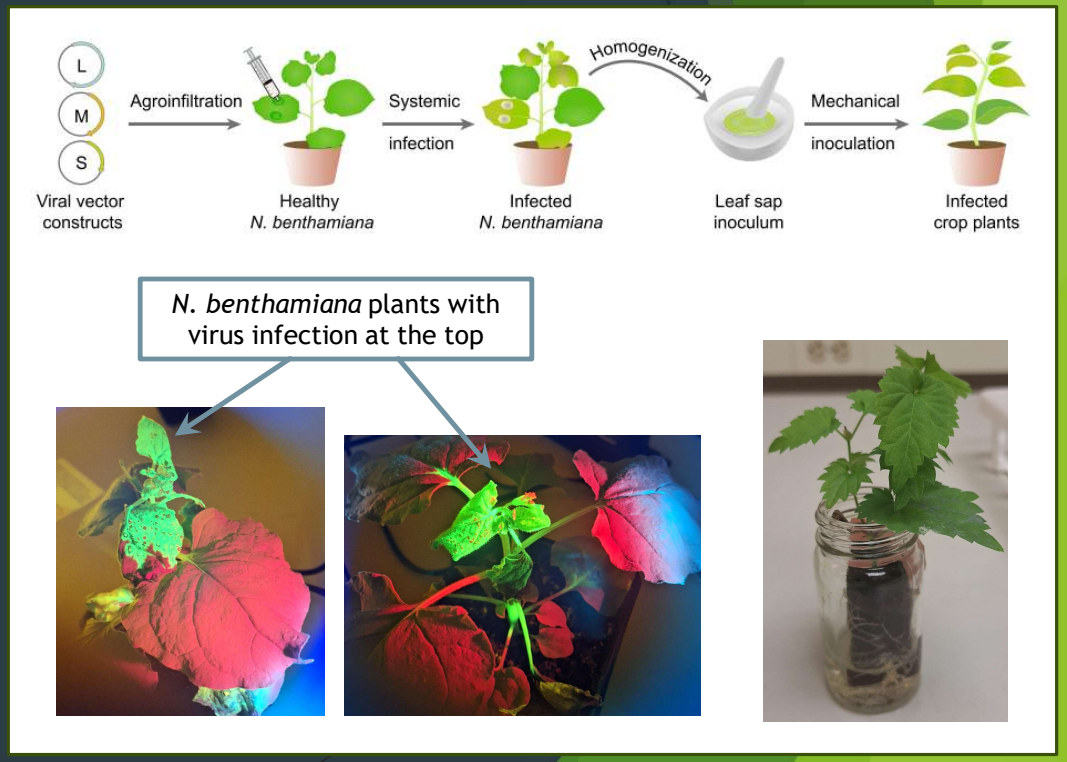






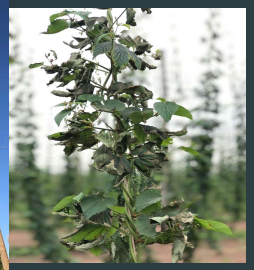
Testing viral vectors

- Deactivated virus can be used to carry gene editing machinery without leaving anything in the genome
- We are testing multiple viral vectors to see whether they will spread in hop tissue (*e.g.*, TSWV, TRV)
- If existing tools won't spread in hop, we may try to build our own vector using HpLV or AHLV



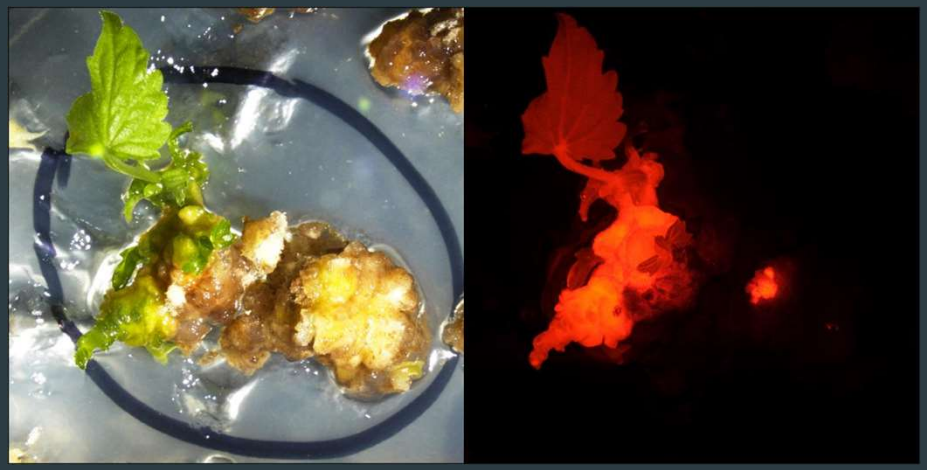
A long-term investment into future hop genetics research

- Hop agriculture is facing threats due to a changing global climate
  - Extreme temperature waves
  - Periodic drought
  - Disease and pest outbreak

- Will be addressed by accelerating genetic research and breeding hop varieties with improved traits that offer better resilience to these pressures

Thanks / Questions?



Connect with the Strauss lab

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