

Update: Developing gene editing technology for American hop cultivars

Hop Research Council / American Hop Convention
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Tucson, AZ

Presented by: Chris Willig
Oregon State University



Hop biotech research group at OSU

- ▶ Our group at OSU has been working together since 2021 on establishing biotechnology (engineering and gene editing) methods in hop
- ▶ We are funded jointly with HRC by USDA through the Technical Assistance for Specialty Crops (TASC) program
- ▶ Two 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

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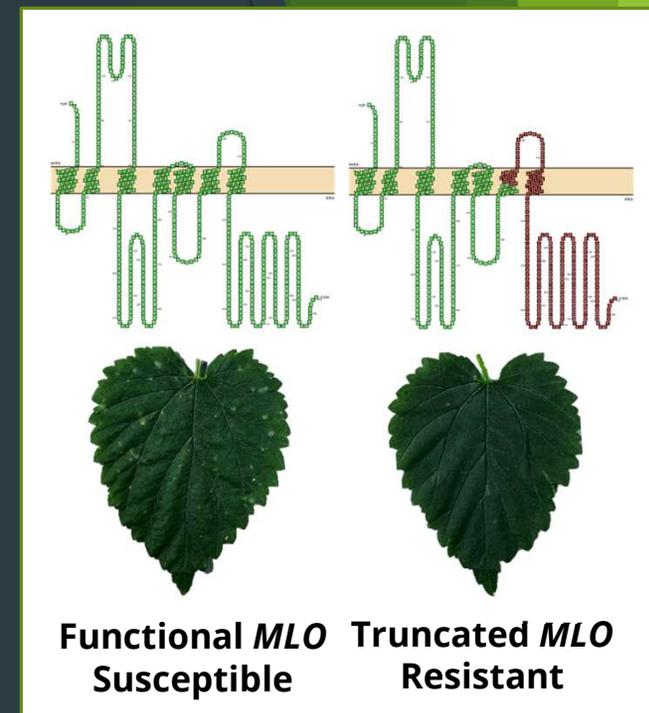
Rationale for the TASC project

- ▶ 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

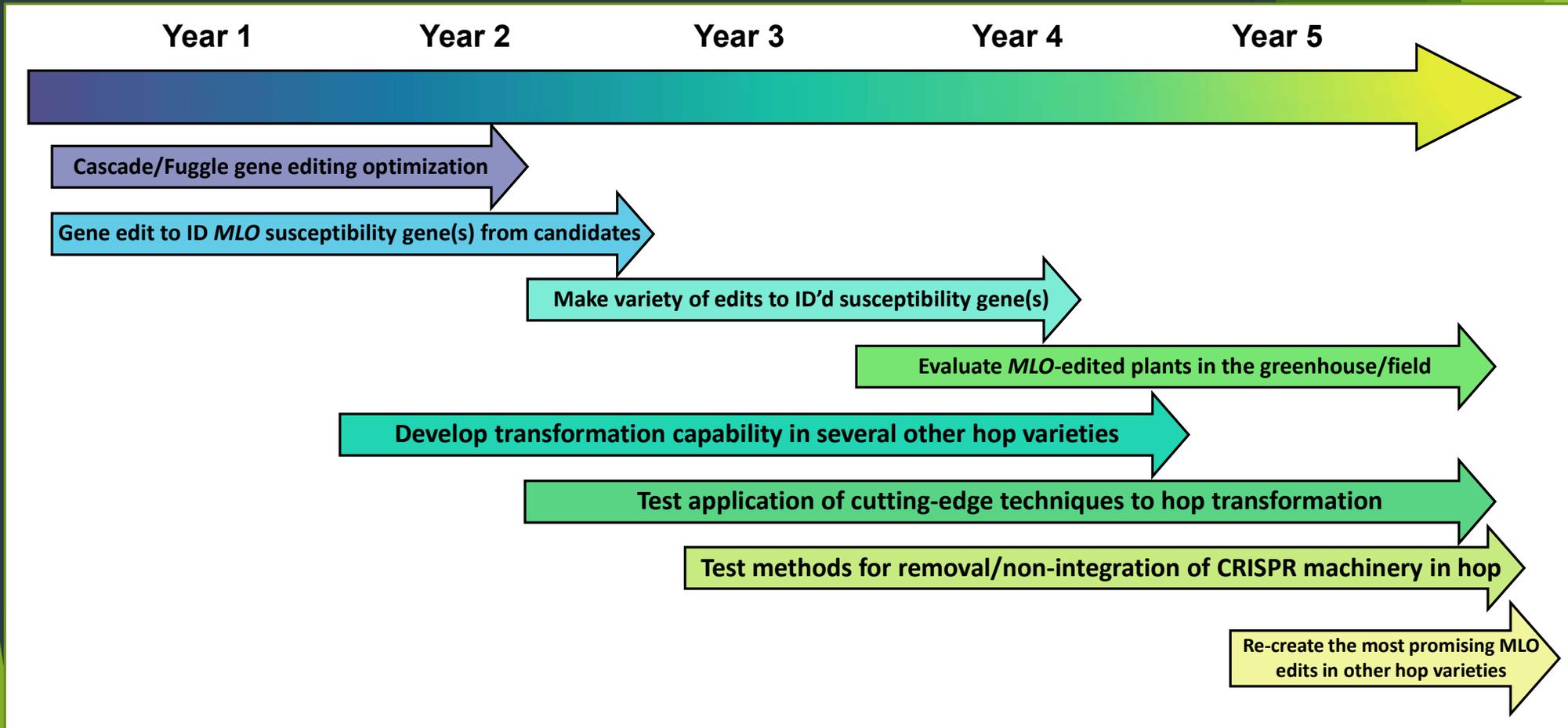


Rationale for the TASC project

- ▶ 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

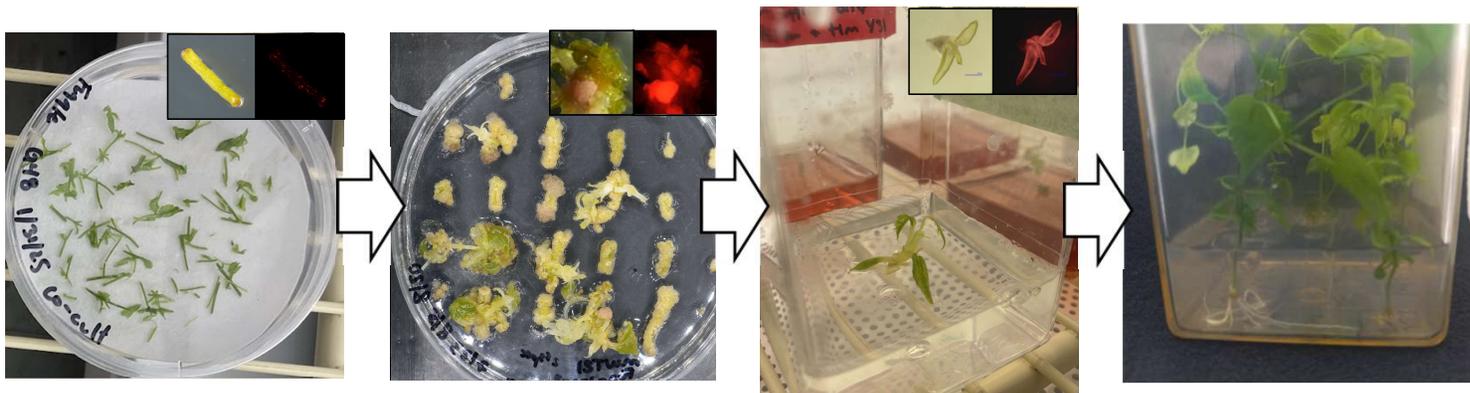


Timeline for TASC project



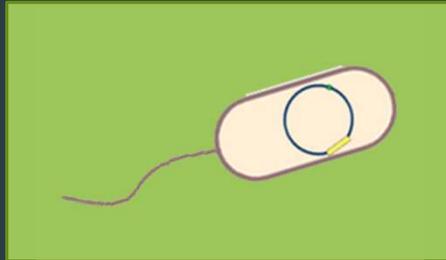
Technical Background

General pipeline for gene editing



From tissue inoculation with *Agrobacterium* to recovering transgenic shoot: ~3-4 months

Agrobacterium as a natural genetic engineer

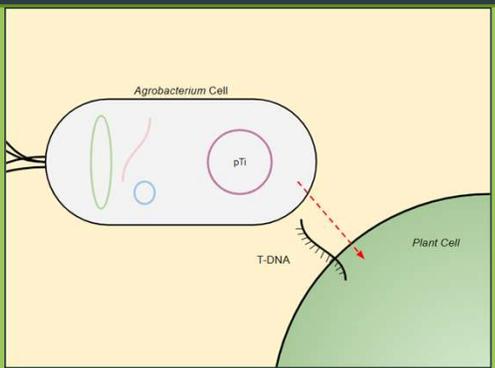


- ▶ In nature, *Agrobacterium* is a plant pathogen that causes “crown gall disease”

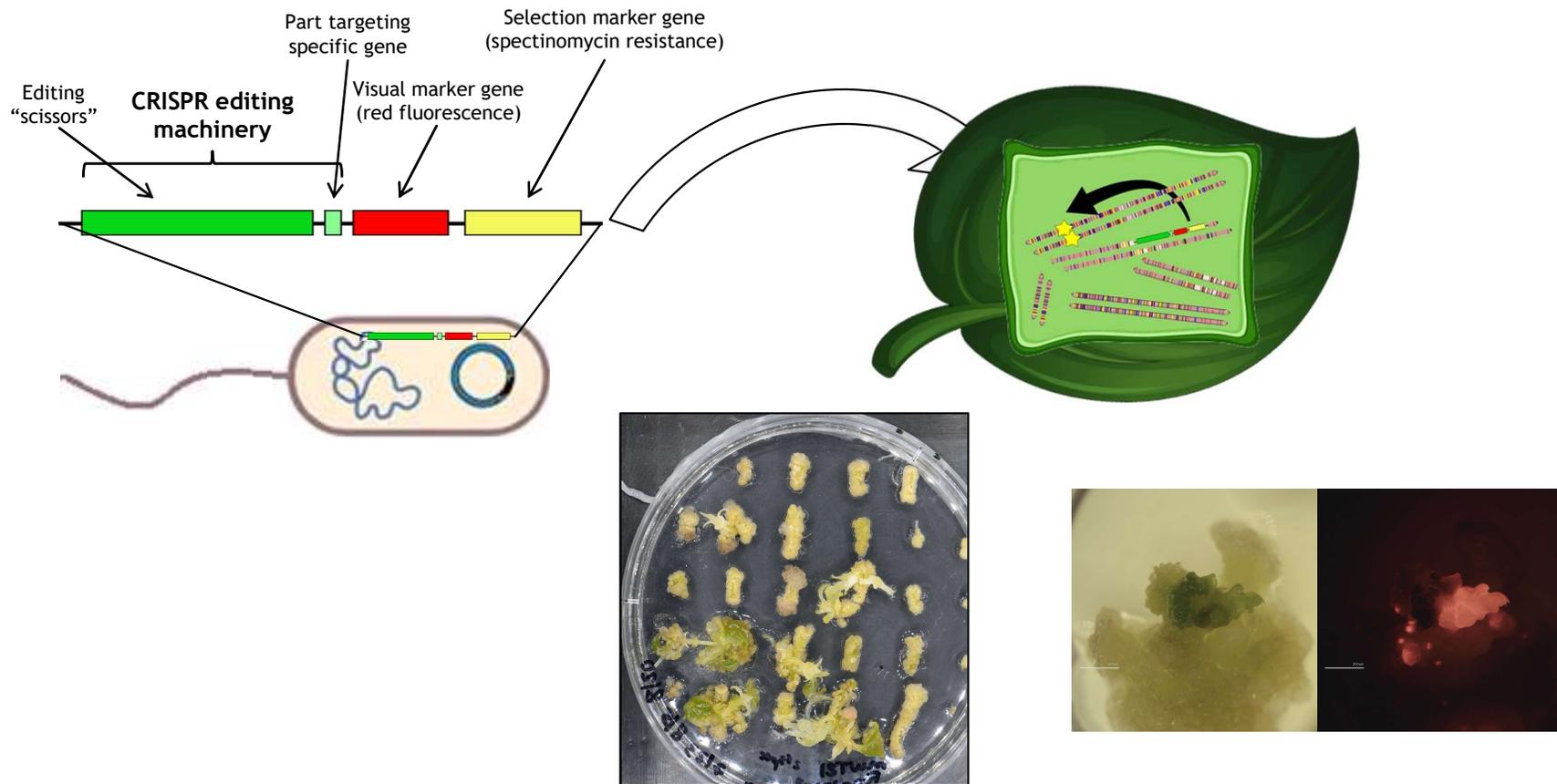


- ▶ *Agrobacterium* transfers a small piece of genetic material into plant cells

- ▶ Researchers have turned some *Agrobacterium* strains into a tool for crop biotechnology



Gene editing process- the inserted genetic material (transgene)



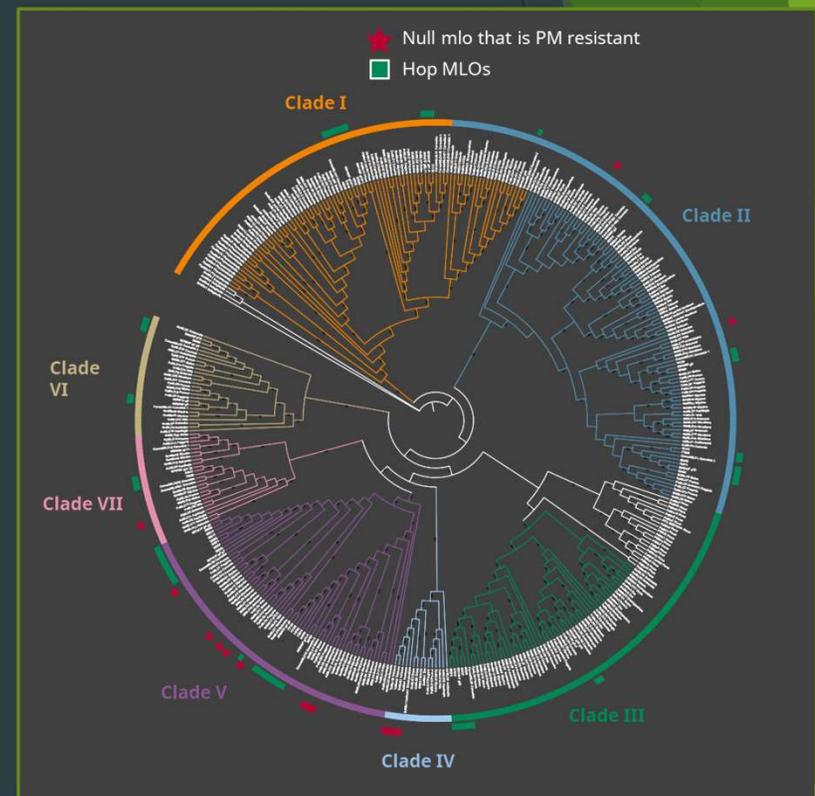
CRISPR editing of *MLO* target genes

Identifying Hop *MLO* candidate susceptibility genes



Michele Wiseman
PhD candidate

- ▶ *MLOs* are a large and complex gene family across the plant kingdom. 19-22 apparent *MLO* genes in hop.
- ▶ Michele Wiseman identified target *MLO* genes using comparative genomics and gene expression data. Top candidate genes belong to clade Vb (2 copies), then clade Va (2 copies).



Name	p-value	Motif Locations
VvMLO3_Vitis	0.00e+0	
VvMLO4_Vitis	0.00e+0	
HUMLU_CAS0050448.t1.p1	0.00e+0	
HUMLU_CAS0068957.t1.p1	0.00e+0	
AtMLO2_Arabidopsis	0.00e+0	
AtMLO6_Arabidopsis	0.00e+0	
AtMLO12_Arabidopsis	0.00e+0	

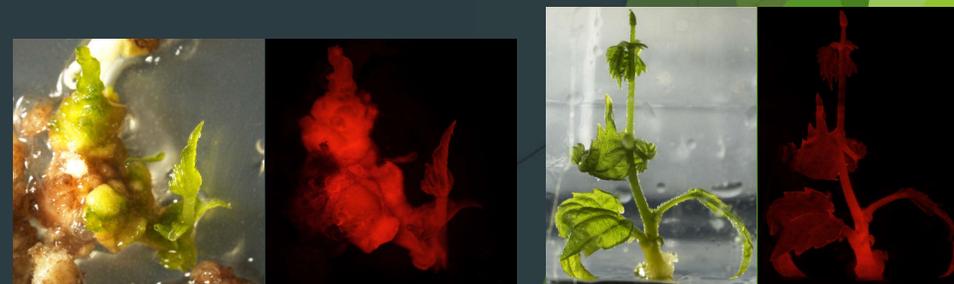
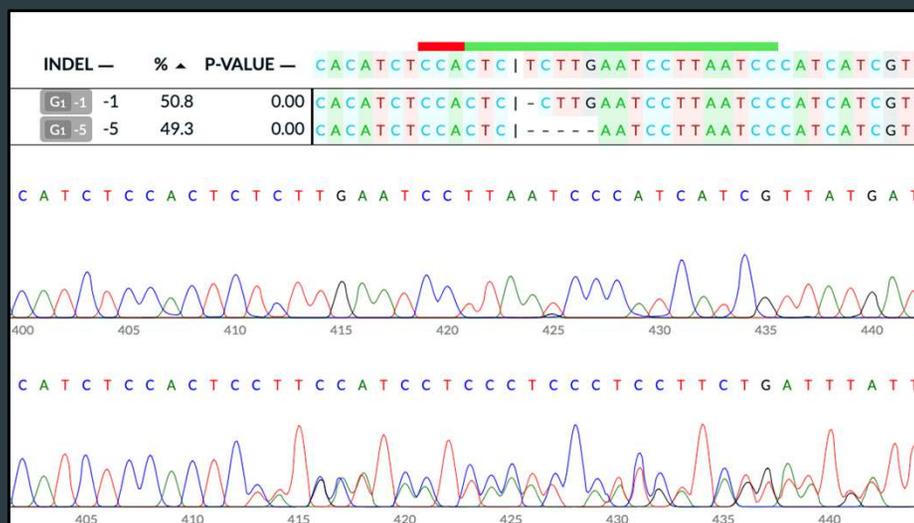
This year we generated edited hop lines for the first time!



Michele Wiseman
PhD candidate

- Seven individual lines were produced and characterized for editing of *MLO* target genes

Cultivar	Guide & Event	HUMLU_CAS48594.t1.p1	HUMLU_CAS68597.t1.p1	HUMLU_CAS005044 8.t1.p1 HUMLU_CAS005035 7.t1.p1
Fuggie	G14-1	Biallelic heterozygous (+1/-4)	Biallelic homozygous (+1)	Wild type
Fuggie	G14-2	Monoallelic (+1/Wild type)	Monoallelic (-1/Wild type)	Wild type
Fuggie	G14-3	Biallelic heterozygous (+1/-2)	Monoallelic (+1/Wild type)	Wild type
Fuggie	G14-4	Biallelic homozygous (-2)	Biallelic homozygous (+1)	Wild type
Fuggie	G14-5	Biallelic heterozygous (+1/-2)	Biallelic heterozygous (-5/-8)	Wild type
Fuggie	G14-6	Biallelic heterozygous (-1/-5)	Biallelic heterozygous (+1/-9)	Wild type
Tettnanger	G14-1	Biallelic homozygous (-2)	Biallelic homozygous (+1)	Wild type
Fuggie	G48-1	Wild type	Wild type	Tetrallelic (-3)



Phenotyping - are edited lines any less susceptible to powdery mildew?



Michele Wiseman
PhD candidate

Control plant



Test plant

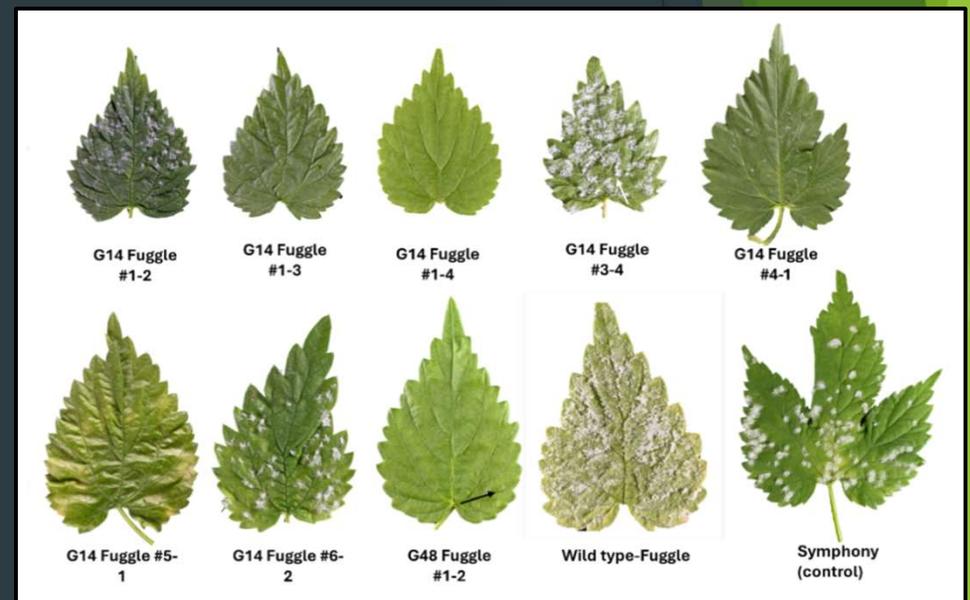
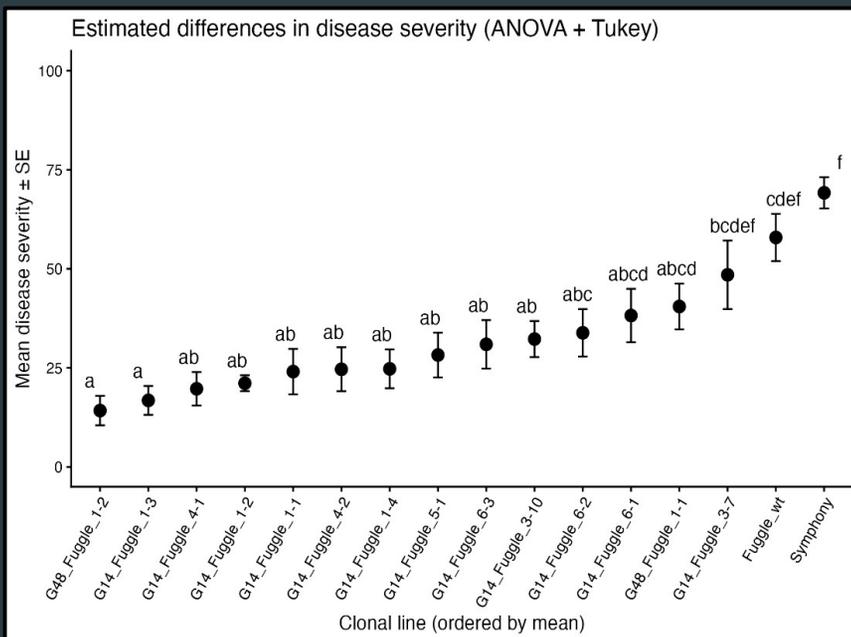


Some edited lines have measurably reduced susceptibility



Michele Wiseman
PhD candidate

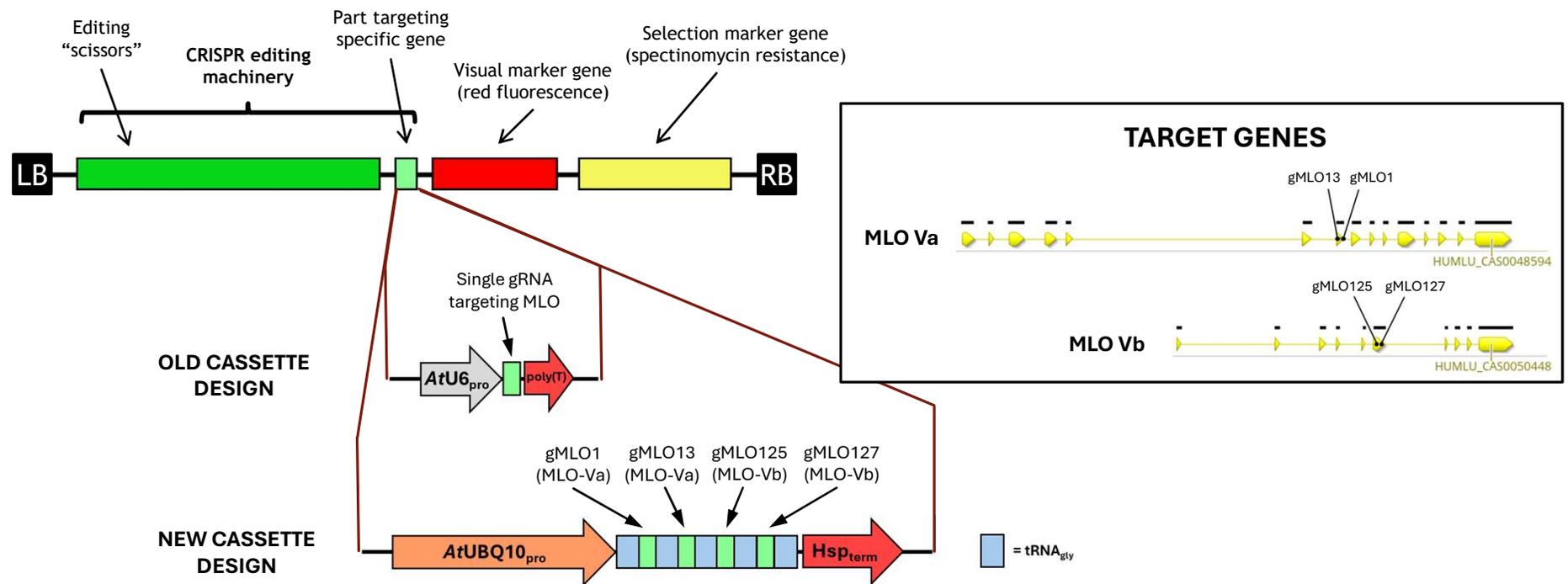
- ▶ Edited lines show a range of resistance after pathogen inoculation, with most showing lower susceptibility than wild-type controls



Findings on *MLO*-based susceptibility thus far in hop

- ▶ The reduction in disease severity observed in hop lines edited for clade Va *MLO* supports the conclusion that these are functional as powdery mildew susceptibility genes
- ▶ However, the small effect size suggests that there could be functional redundancy among *MLO* genes
- ▶ Thus, we have designed a new construct for simultaneous editing (multiplexing) of clade Va and Vb candidate genes in the same line, which we plan to use in experiments going forward

Modifying our construct design for multiplex editing of candidate genes



Improvement of hop transformation pipeline

Summary of parameters investigated for their effect on transformation / regeneration

Parameter	Treatments	Effect / Optimum
Cytokinin type/ concentration for SIM	BAP, zeatin, TDZ, m-Topolin, kinetin, 2iP, 4-CPPU (various concentrations)	5.0 mg/L m-Topolin
Auxin type/ concentration for SIM	IAA, NAA (each at 0.1 or 0.25 mg/L)	0.25 mg/L IAA
Carbohydrate source for SIM	Sucrose, glucose, maltose (each at 20 g/L)	Glucose ~= maltose
Selection type/ concentration in SIM	Hygromycin (1, 2.5, 5 mg/L); Kanamycin (25, 50, 100 mg/L); Spectinomycin (10, 25, 50 mg/L)	Spec at 25 mg/L
<i>Agrobacterium</i> strain type	AGL-1, GV3101(pMP90), LBA4404	AGL-1 was highest
<i>Agrobacterium</i> inoculum density	OD ₆₀₀ = 0.1, 0.3, 0.6, 1.0	OD ₆₀₀ = 0.6
Construct design	Vis. Reporter; sel. marker; promoter driving Cas	DsRed; specR; pAtUBQ10

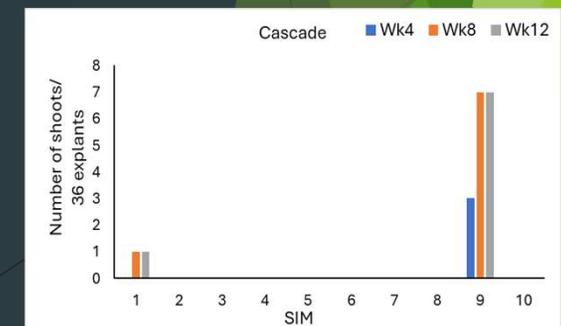
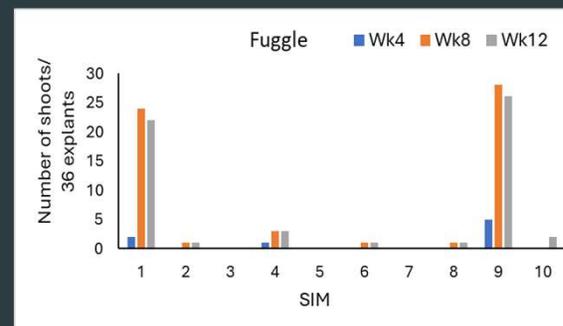
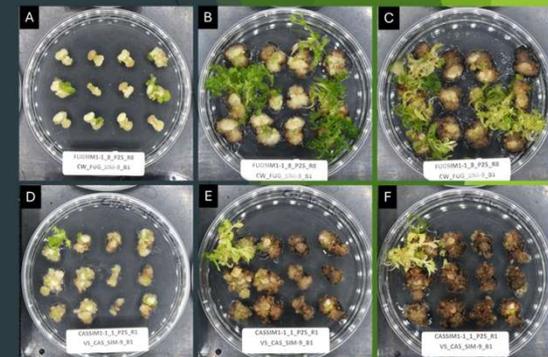
Regeneration media optimization for plant hormones (PGRs)



Dr. Veerendra Sharma
Research Scientist

- ▶ We analyzed the influence of 10 different media formulations varying in their plant hormone content on shoot regeneration
- ▶ A medium with a high concentration of m-topolin consistently gave the highest rate of regeneration in 'Cascade' and 'Fuggle'
- ▶ We will perform future regeneration tests on new cultivars using the same media panel

Medium #	Cytokinin type / concentration (mg/L)	Auxin type / concentration (mg/L)
1	BAP 2.0	IAA 0.25
2	BAP 10.0	IAA 0.25
3	BAP 10.0	NAA 0.1
4	Zeatin 1.0	IAA 0.25
5	Zeatin 1.0	NAA 0.1
6	Zeatin 2.0	IAA 0.25
7	Zeatin 2.0	NAA 0.1
8	TDZ 2.0	NAA 0.1
9	meta-Topolin 5.0	IAA 0.25
10	meta-Topolin 5.0	NAA 0.1

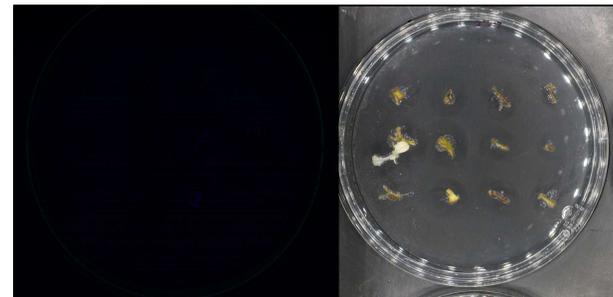
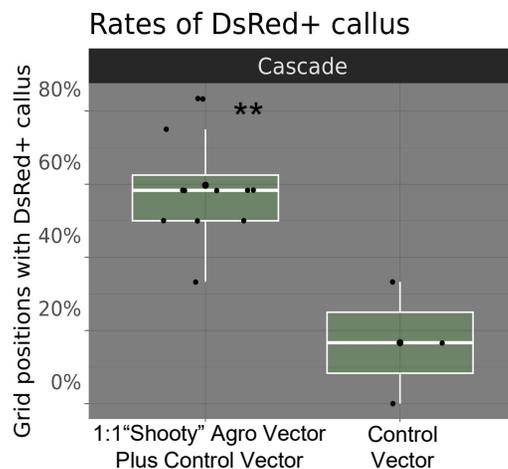


Use of “shooty genes” from *Agrobacterium* increases the rates of transgenic callus production

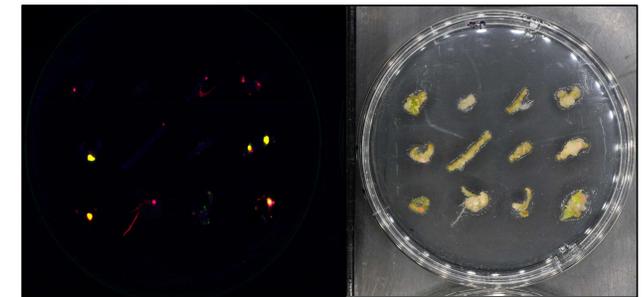


Dr. Greg Goralogia
Research Scientist

- ▶ Mixing an *Agrobacterium* strain carrying a suite of shoot-inducing genes with a strain carrying our gene editing construct resulted in more callus tissue with the visual marker



Without developmental genes, 3wk post cc



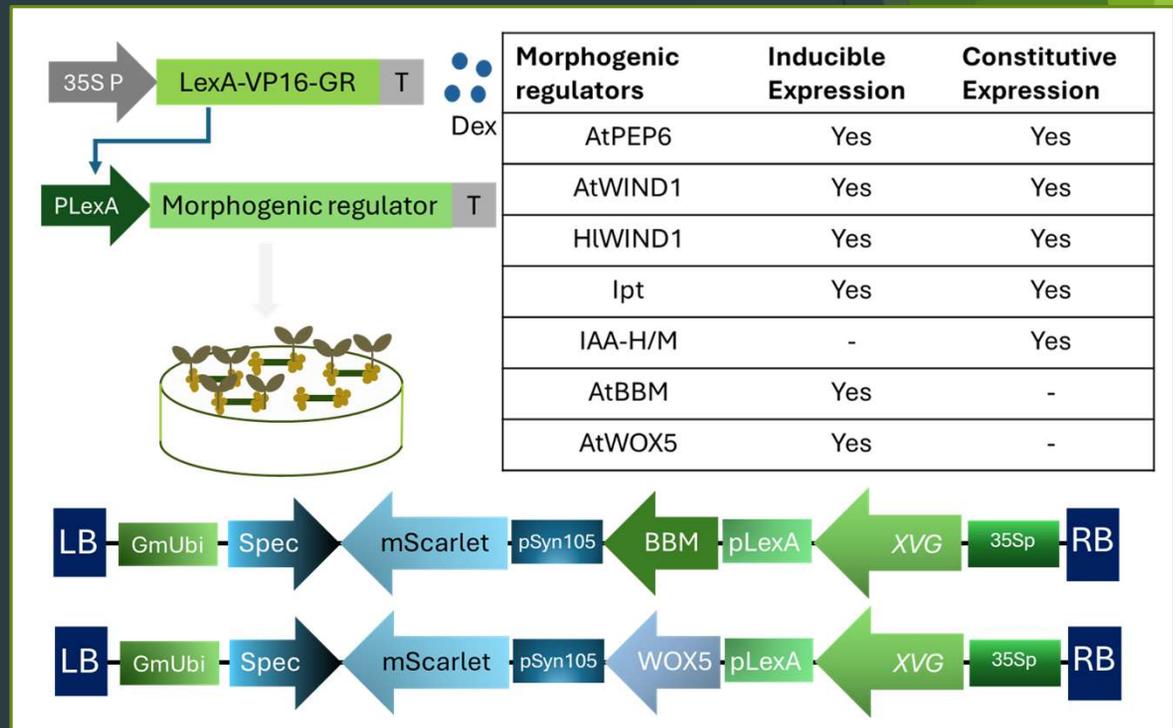
With “shooty” developmental genes, 3wk post cc

Addressing the *root* of the problem: Can we “tell” transformed cells to regenerate?



Dr. Veerendra Sharma
Research Scientist

- ▶ Morphogenic regulator genes have helped to improve transformation in some plants, but aren't a silver bullet
- ▶ One issue is that they need to be “turned on” at only a particular time and place, or else they can cause growth defects
- ▶ Using a chemical “switch” may allow us to control expression of these genes during tissue culture enough so they are helpful without causing other issues



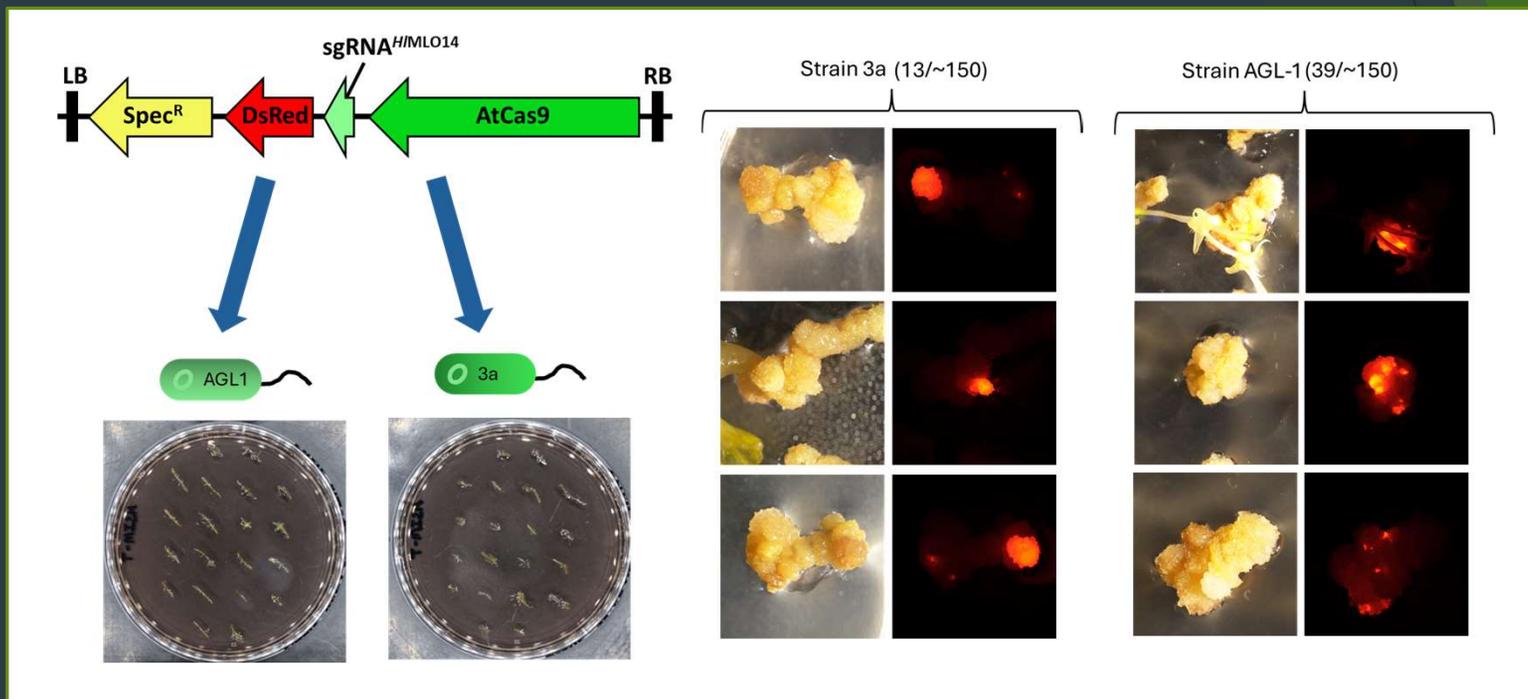
Leveraging wild *Agrobacterium* strains as new biotechnology tools in difficult crops

- ▶ 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 delivering DNA into hop (and other crops)
- ▶ We are testing wild-type agro strains isolated from natural host plants as well as a large strain collection at OSU for their potential to be developed as biotech tools



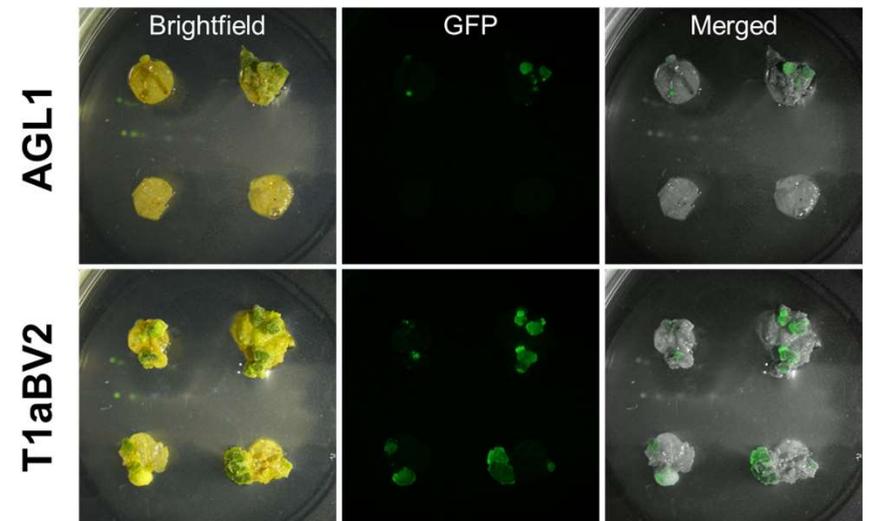
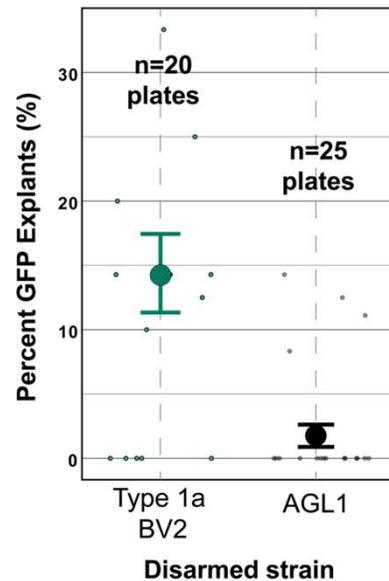
How do newly developed strains compare to our default ones usually used for gene editing in plants?

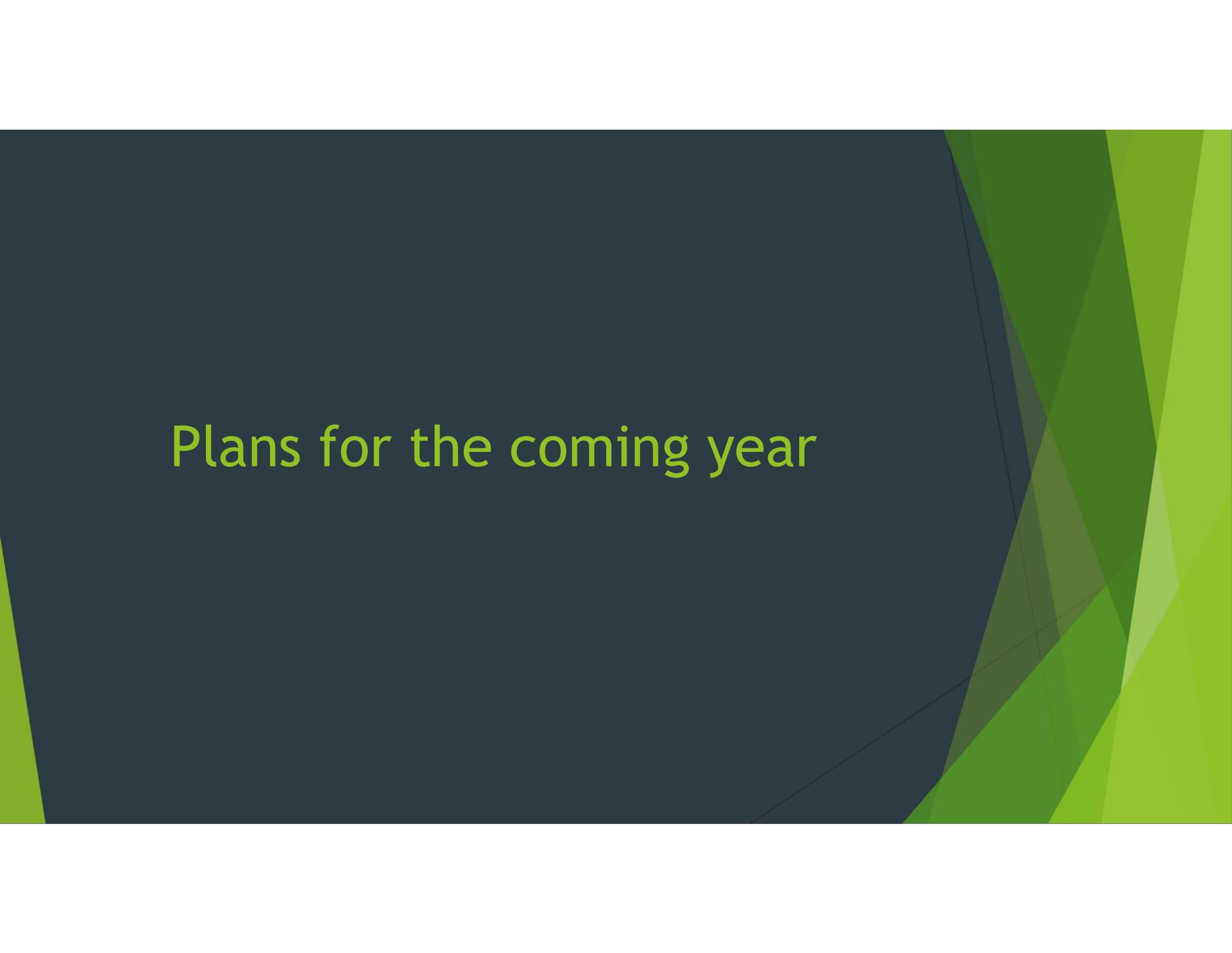
- ▶ Strain “3a”, derived from a gall found on a hop plant, seems to perform poorly compared to conventional strain



How do newly developed strains compare to our default ones usually used for gene editing in plants?

- ▶ One novel strain (originating from a gall on rose) in initial testing is showing much improved DNA delivery in multiple plant species, including hop
- ▶ However, more replication is needed to clearly demonstrate its effect



The background features a dark blue-grey area on the left and a series of overlapping, semi-transparent green and yellow-green geometric shapes on the right, creating a modern, abstract design.

Plans for the coming year

Research agenda

- ▶ Continue producing and evaluating *MLO*-edited hop lines
- ▶ Continue experiments to improve transformation / regeneration / editing pipeline
- ▶ Test “clean editing” techniques in hop
- ▶ Evaluate new hop varieties we have not tested yet for their compatibility with gene editing methods

An alternative route to transformed hops — root to shoot

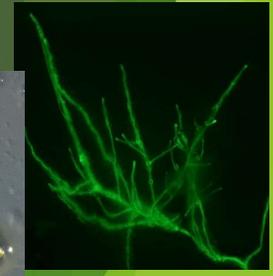
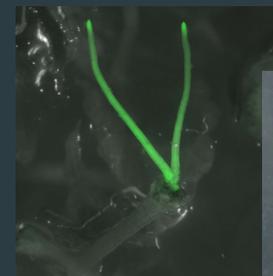
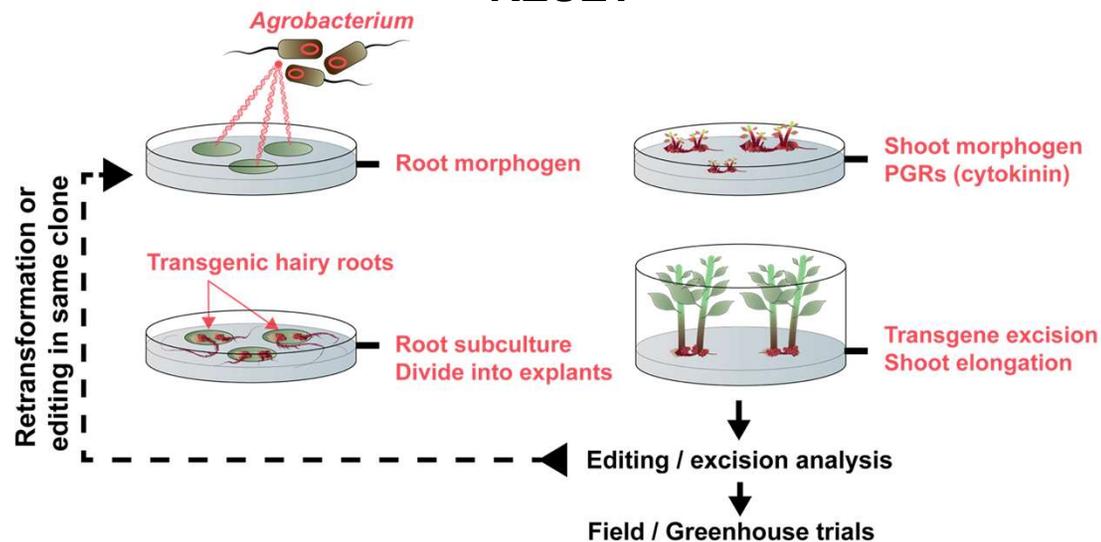
- ▶ Our lab has successfully employed this method in poplar trees, potato
- ▶ We have generated transgenic hop hairy roots in the past



Dr. Greg Goralogia
Research Scientist

A concept for Root Excision System for Efficient Transformation

RESET



Testing new hop varieties for amenability to tissue culture/gene editing techniques

- ▶ The following varieties have been recommended for evaluating:

Variety	Significance
CTZ	Major US high alpha variety, but susceptible to PM
Bullion	Breeding parent
Brewer's Gold	Breeding parent
Cashmere	Unique aroma, but susceptible to PM
Magnum	High levels of DM resistance, susceptible to PM
Mt. Rainier	High levels of DM resistance, susceptible to PM

- ▶ Others to recommend? Please let us know...

Thanks / Questions?



Connect with the Strauss lab

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