

Research Update: Characterization of MLO Edited Events

Hop Research Council / American Hop Convention
August 5-6, 2026
Bonnors Ferry, ID

Presented by: Veerendra Sharma
Oregon State University



Agenda for the presentation

- Technical details of transformation and gene editing process
- Update on Characterization of MLO edited events
- Improvements for hop transformation
- Plans for the coming year

CRISPR editing of *MLO* target genes and phenotyping

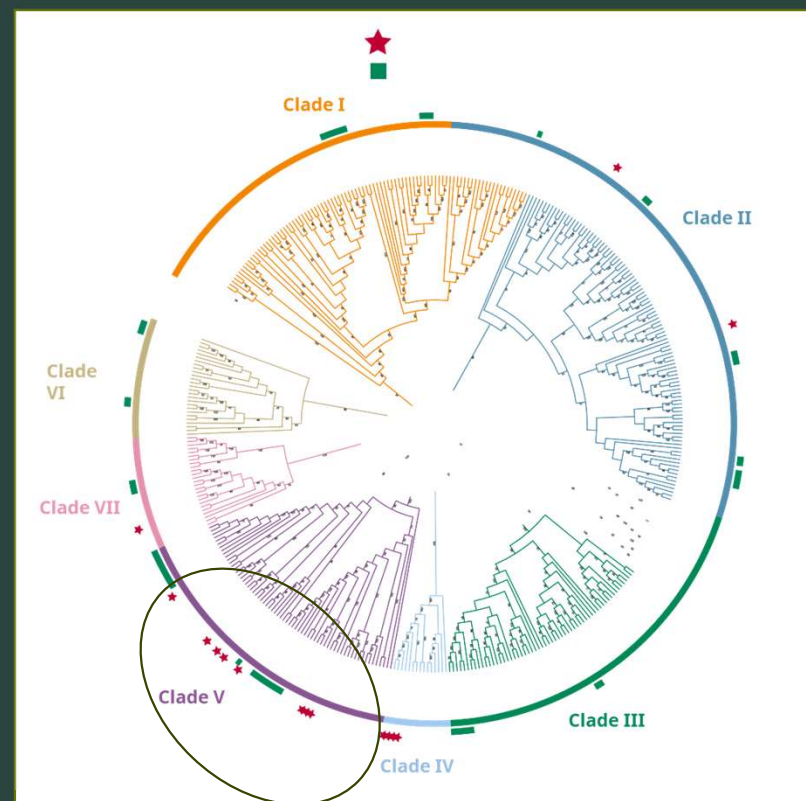
Identifying Hop *MLO* candidate susceptibility genes



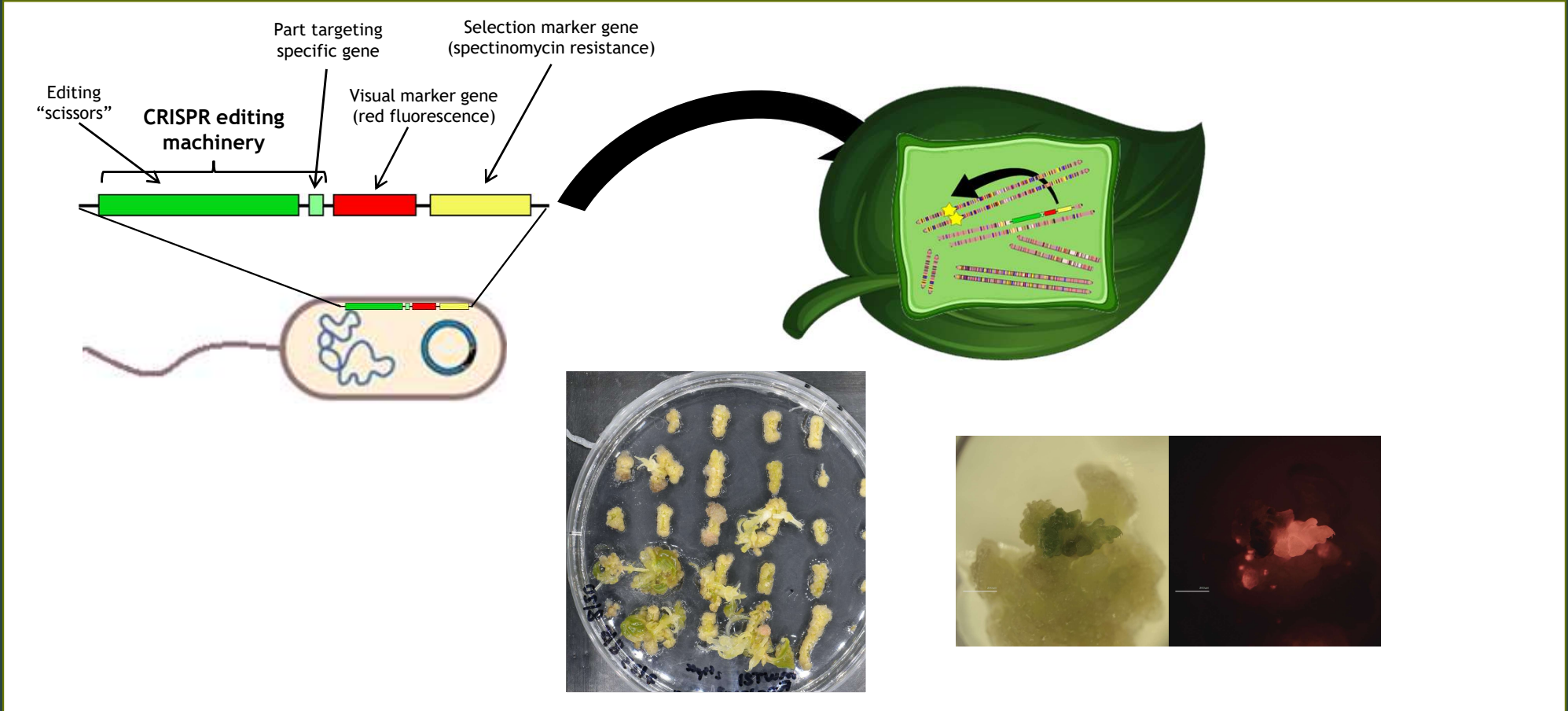
Michele Wiseman

- *MLOs* are a large and complex gene family across the plant kingdom.
- Michele 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).

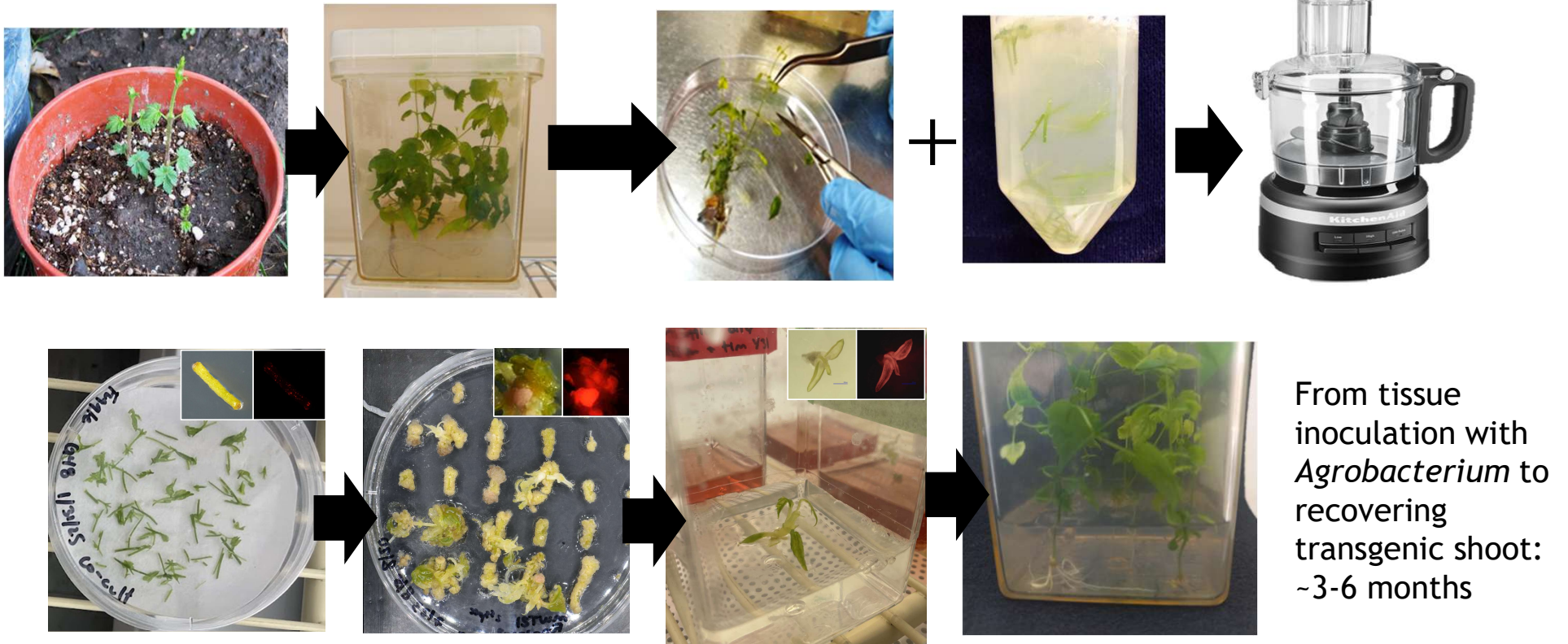
Name	p-value	Motif Locations
VvMLO3_Vitis	0.00e+0	
VvMLO4_Vitis	0.00e+0	
HUMLU_CAS0050448.t1.p1	0.00e+0	
HUMLU_CAS0068957.t1.p1	0.00e+0	
AtMLO2_Arabidopsis	0.00e+0	
AtMLO6_Arabidopsis	0.00e+0	
AtMLO12_Arabidopsis	0.00e+0	



Gene editing process- the inserted genetic material (transgene)

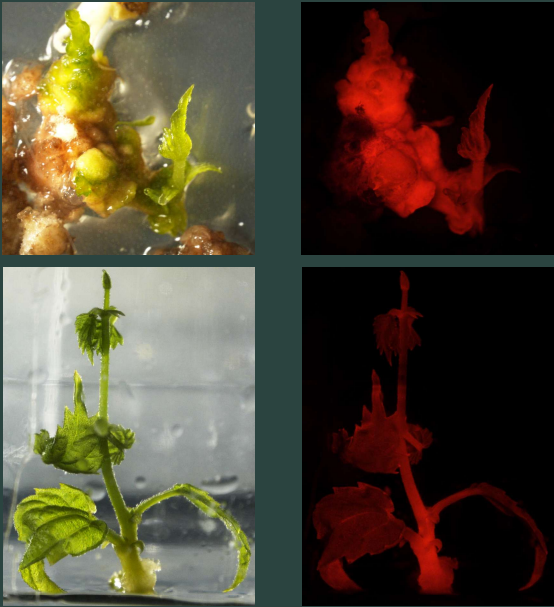


General pipeline for hop transformation

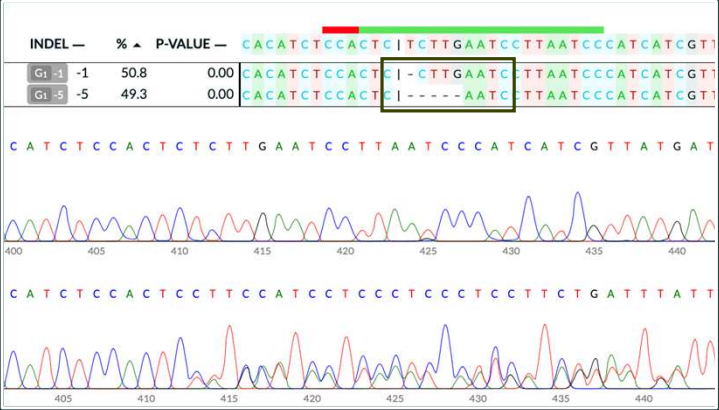


We successfully generated MLO edited lines for the first time

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



Michele Wiseman



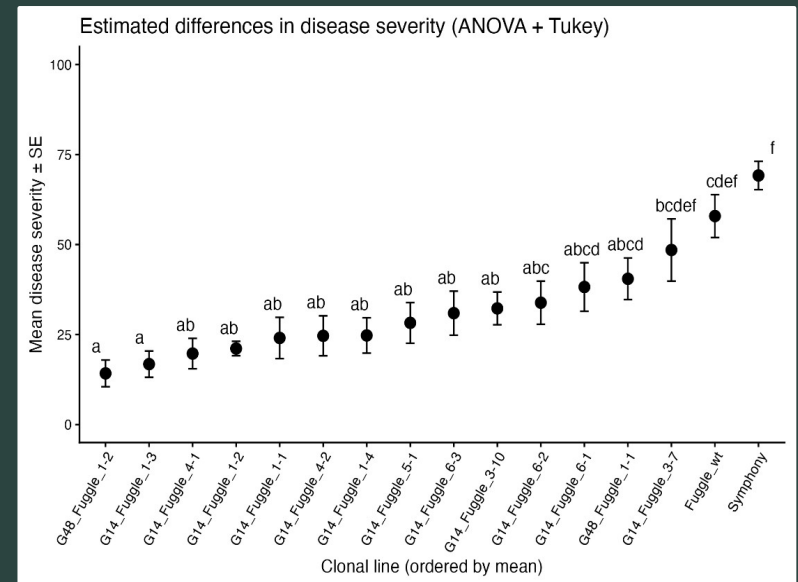
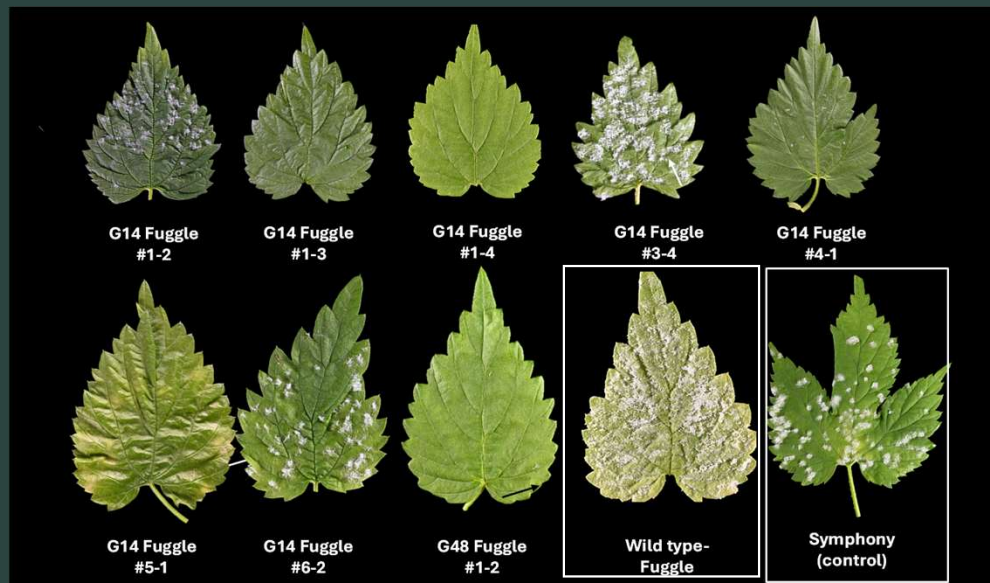
Cultivar	Guide & Event	HUMLU_CAS48594.t1.p1	HUMLU_CAS68597.t1.p1	HUMLU_CAS005044.8.t1.p1 HUMLU_CAS005035.7.t1.p1
Fuggie	G14-1	Biallelic heterozygous (+1/-4)	Biallelic homozygous (+1)	Wild type
Fuggie	G14-2	Monoallelic (+1/Wild type)	Monoallelic (-1/Wild type)	Wild type
Fuggie	G14-3	Biallelic heterozygous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

Some edited lines show measurably reduced susceptibility



Michele Wiseman

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

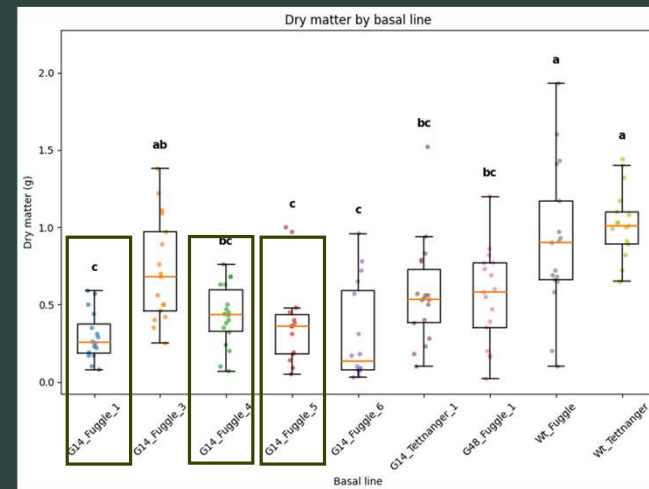


MLO edited lines with reduced susceptibility produce Lower biomass



Michele Wiseman

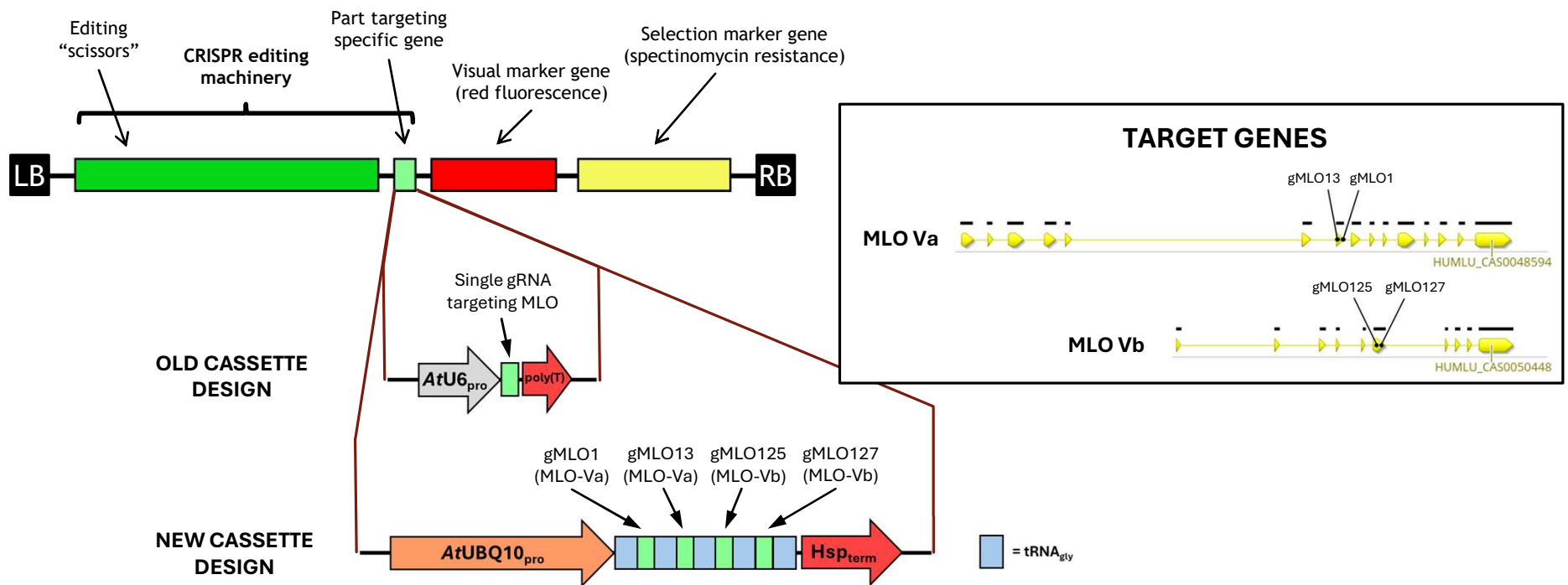
- Edited lines with higher resistance to PM produced lower biomass than wild-type controls



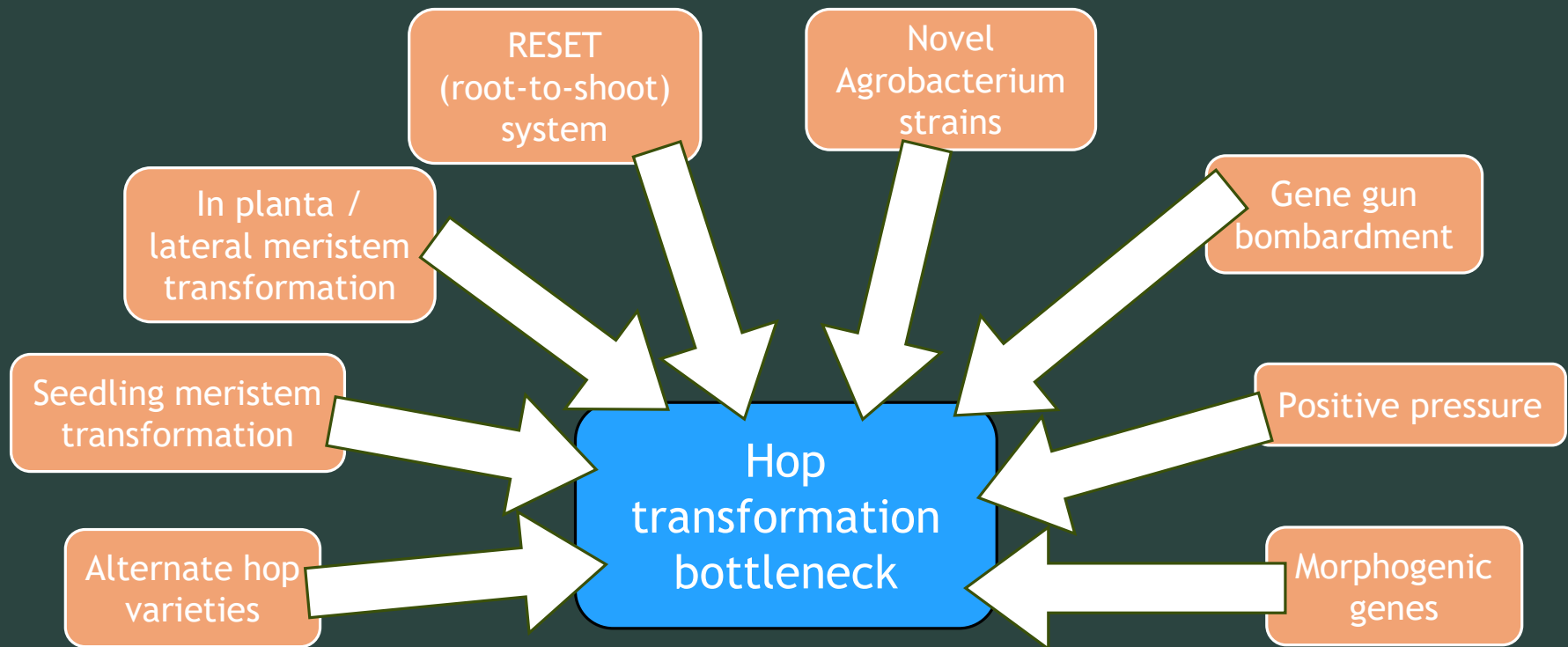
Findings on *MLO*-based susceptibility and vigor phenotyping

- 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 *MLO* genes have overlapping function
- ▶ Phenotyping of edited lines in the greenhouse suggest that *MLO* editing has undesirable effects

Modifying our construct design for multiplex editing of candidate genes

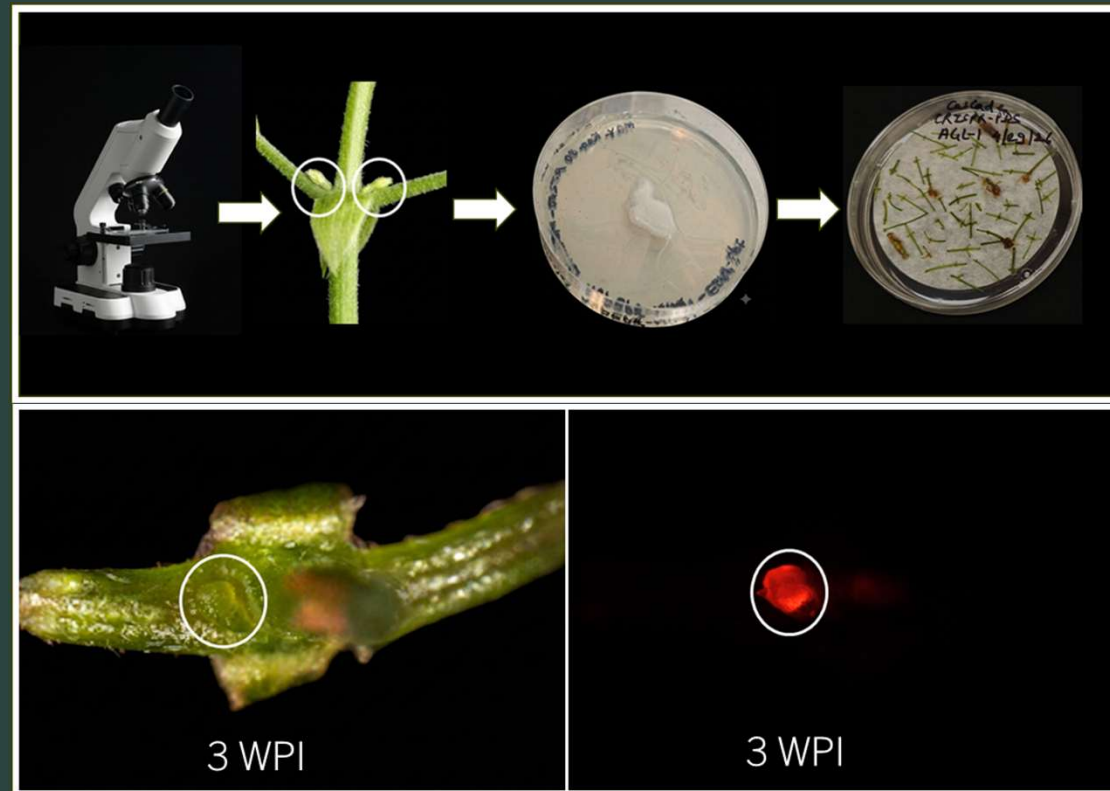


Improvement of hop transformation

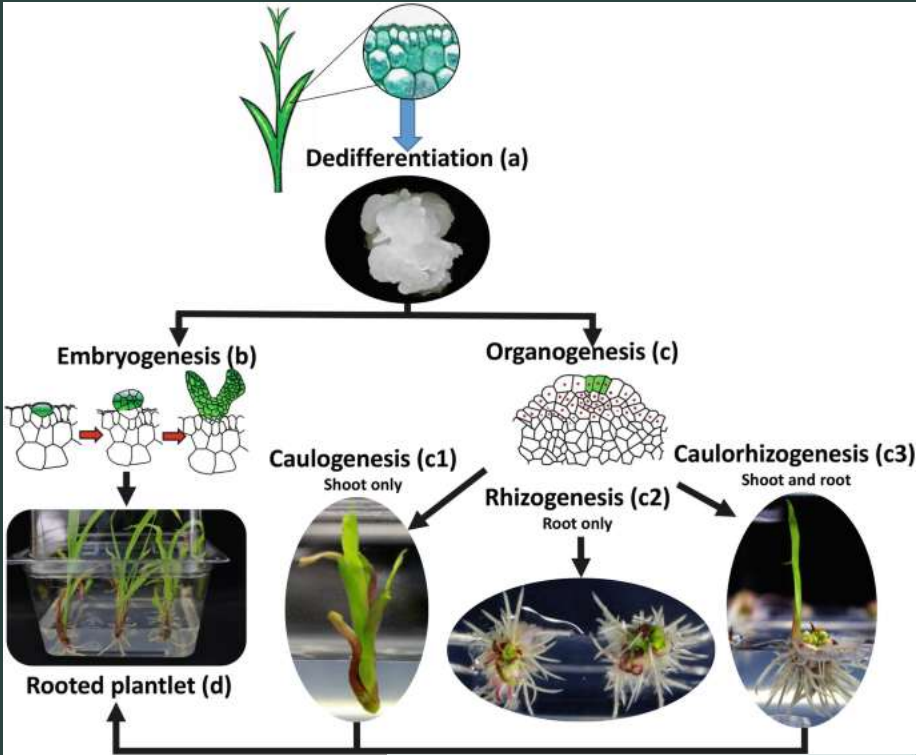


CRISPR delivery into lateral meristem can speed up shoot production

- ▶ Lateral meristem on the nodes is the source of newly emerging lateral shoot
- ▶ Delivery of CRISPR reagents into lateral meristem can speed up the recovery of transgenic lines



Addressing the *root* of the problem: Can morphogenic genes promote regeneration?



doi.org/10.1007/978-1-0716-1068-8_3

Gene(s)	Function and application	Target plant(s)
<i>AGL15</i>	Embryogenesis, transformation	<i>Arabidopsis</i> , soybean
<i>AIL</i>	Embryogenesis, organogenesis and transformation	<i>Arabidopsis</i>
<i>ANT</i>	Floral meristem, ovule development, embryogenesis	<i>Arabidopsis</i>
<i>BBM</i> , <i>BBML</i>	Embryogenesis, transformation	Brassica, maize, pearl millet, sorghum, rice, sugarcane, tobacco, sweet pepper, cacao Chinese poplar
<i>CLV</i>	SAM development and maintenance	<i>Arabidopsis</i> , maize, rice, tomato
<i>EMK</i>	Embryogenesis	<i>Arabidopsis</i>
<i>FUS3</i>	Embryo maturation	<i>Arabidopsis</i>
<i>GRF5</i> , <i>GRF4-GIF1</i>	Growth and development, regeneration, transformation	Sugar beet, soybean, canola, sunflower, maize, wheat, citrus
<i>Lec1</i> , <i>Lec2</i>	Embryogenesis	<i>Arabidopsis</i> , tobacco
<i>MYB118</i> / <i>MYB115</i>	Embryogenesis	<i>Arabidopsis</i>
<i>PKL</i>	Embryogenesis	<i>Arabidopsis</i>
<i>PLT1</i> , <i>PLT2</i> , <i>PLT3</i>	Shoot and root development, transformation	<i>Arabidopsis</i>
<i>SERF1</i>	Embryogenesis	<i>Medicago</i>
<i>SERK</i>	Embryogenesis	Carrot
<i>STM</i>	Meristem development	Tobacco, tomato, potato, grape

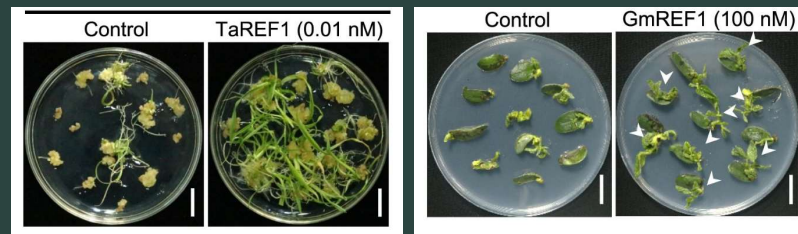
doi.org/10.1007/978-1-0716-1068-8_3

Addressing the *root* of the problem: Can morphogenic genes promote regeneration?

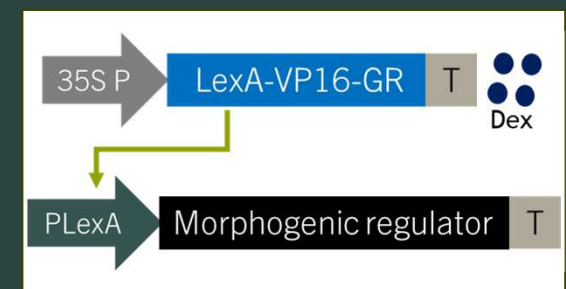
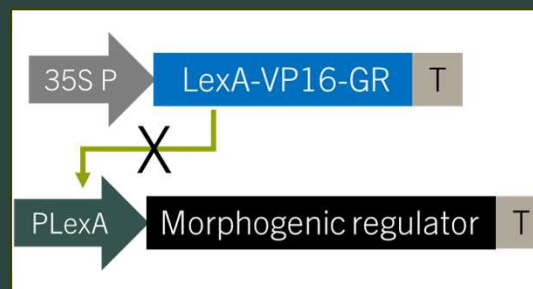


Eden Todd
Research Technician

- Morphogenic regulator genes have helped to improve transformation in other recalcitrant species
- ▶ 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



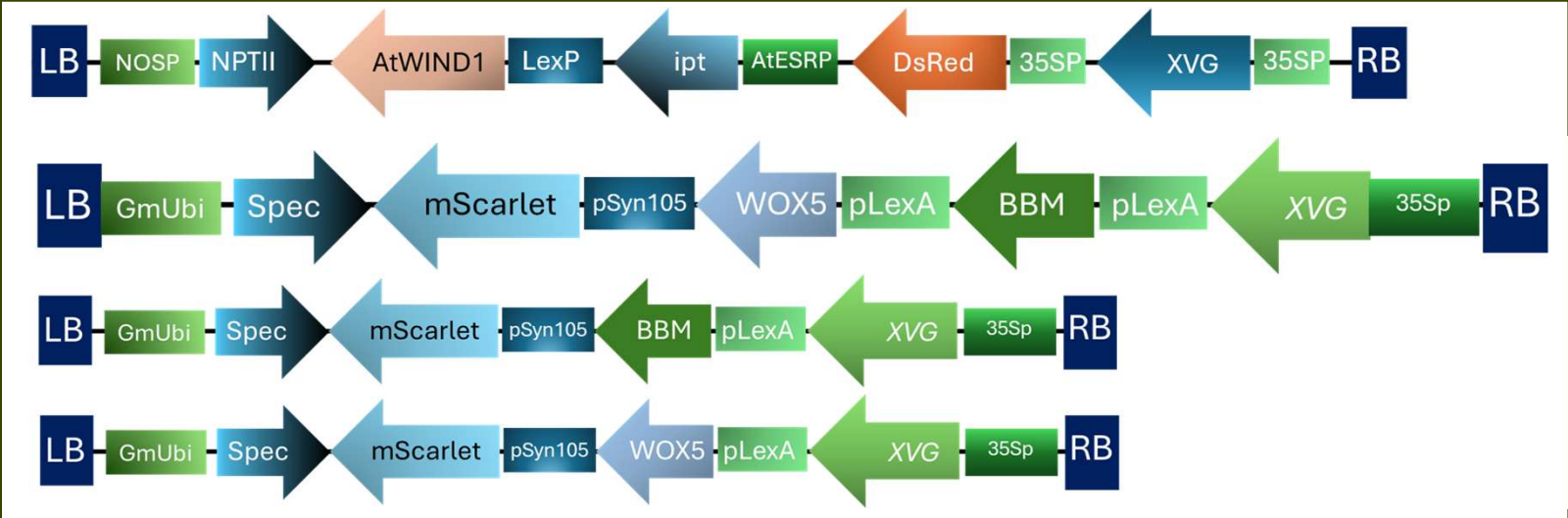
DOI: 10.1016/j.cell.2024.04.040 External Link



We built a suite of morphogenic gene construct

- We have developed several constructs of morphogenic regulators for constitutive or conditional expression
- We are testing these morphogenic genes for their effect on hop transformation and regeneration

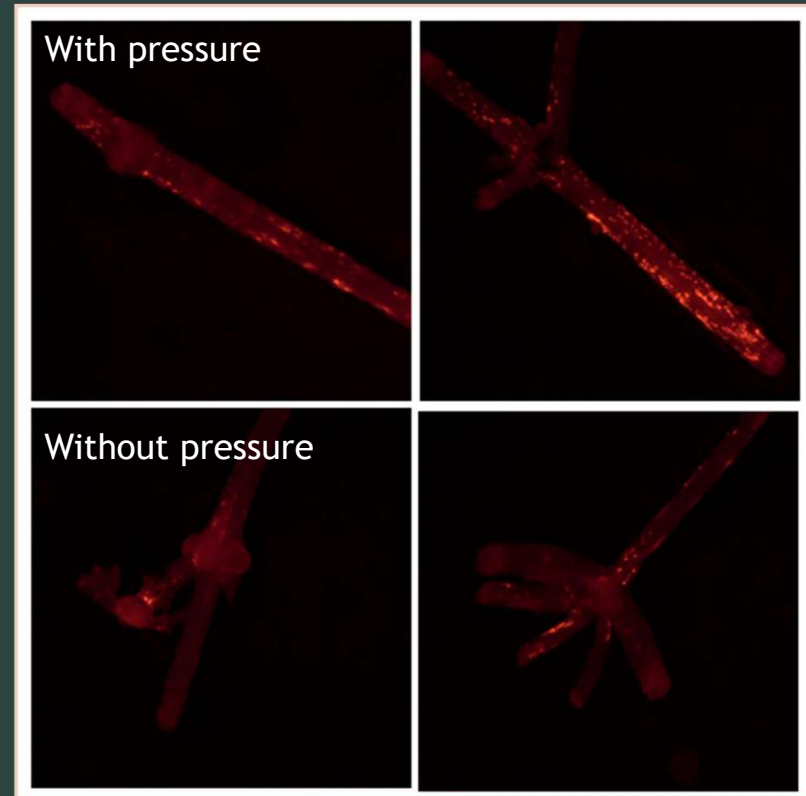
Morphogenic regulators	Expression system
AtPEP6	Inducible/ constitutive
AtWIND1	Inducible
GRF-GIF	Constitutive
PLT5	Constitutive
BBM	Inducible/ constitutive
WOX5	Inducible/ constitutive
IPT	Inducible/ constitutive



Improving agrobacterium penetration using positive pressure?



- Agrobacterium penetration is a critical factor cells for T-DNA delivery.
- ▶ We used a pressure chamber to improve Agrobacterium penetration in hop explants.
- ▶ We will test different ranges of pressure and time duration to further improve T-DNA delivery

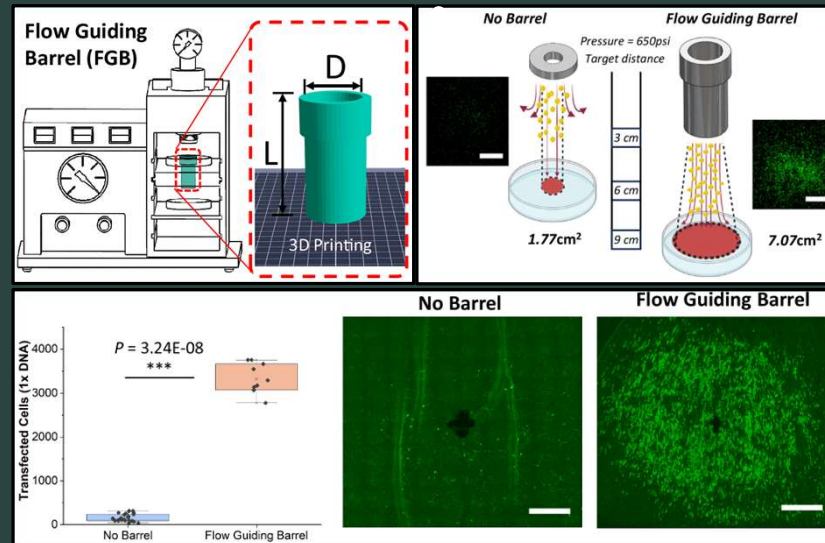


Gene gun an alternative to deliver CRISPR reagents in hops?

- Gene gun has been successfully used to deliver transgenes in various species including hops
- A recent modification called flow guided barrel (FGB) when attached to gene gun can significantly improve transgene delivery
- We are in the process of procuring FGB for hop transformation



Gene gun in Strauss lab



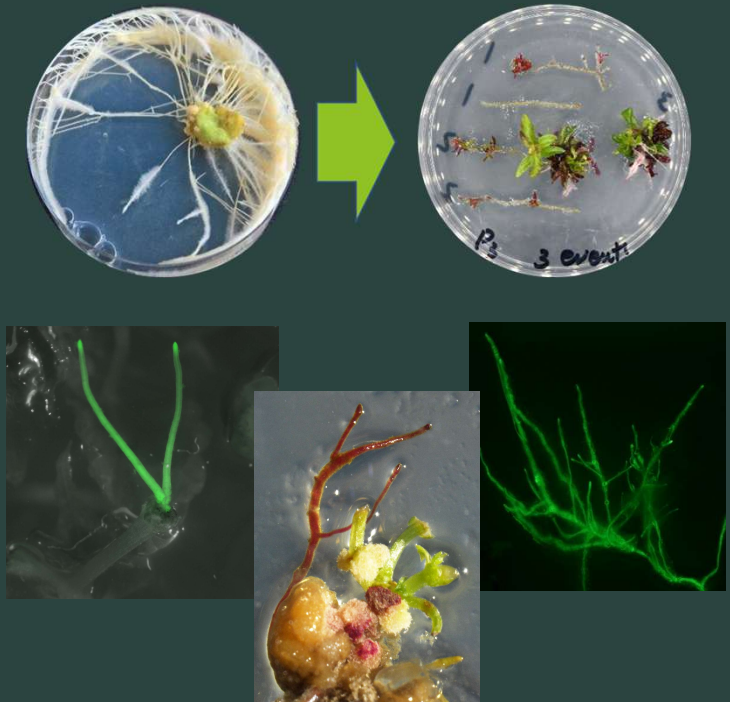
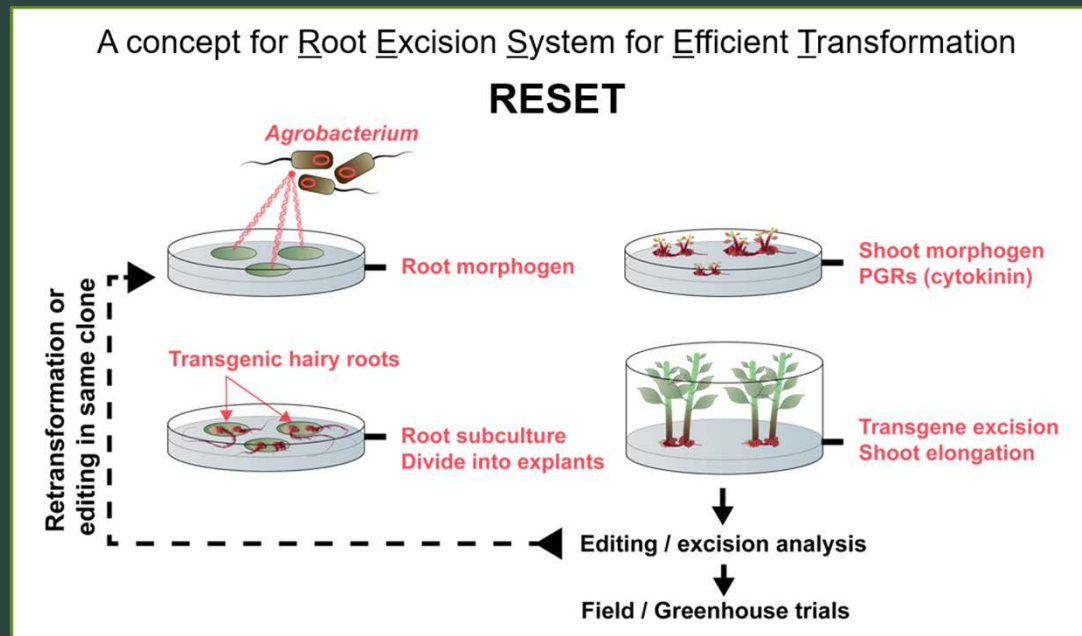
DOI: 10.1038/s41467-025-60761-x

Test clean editing 'RESET' system in hop



Dr. Greg Goralogia
Research Scientist

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

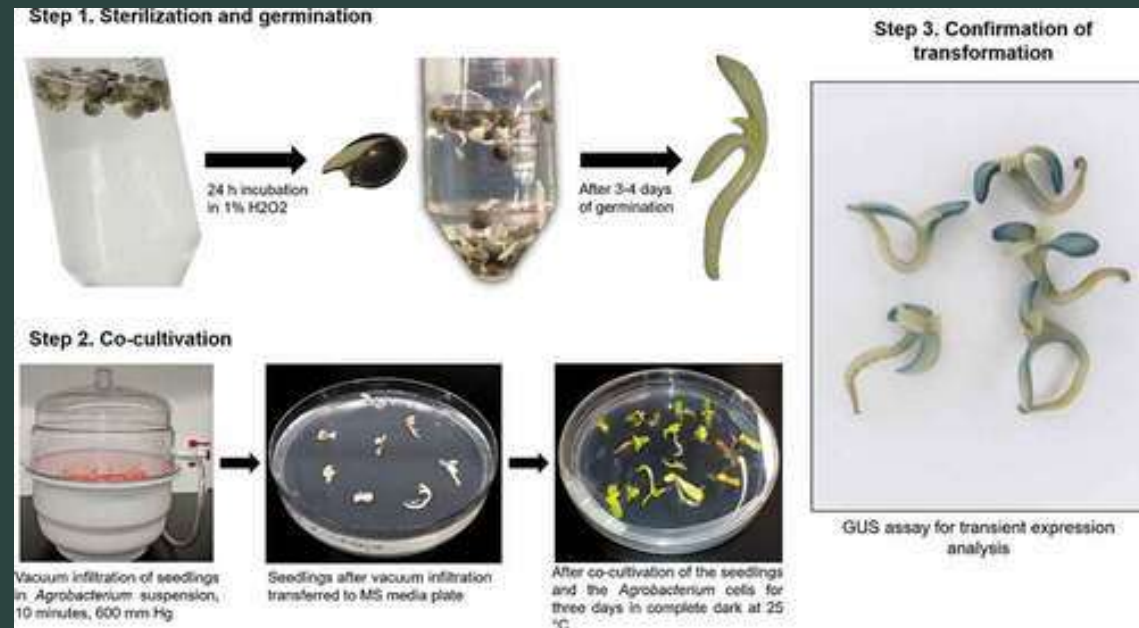


Targeting seed meristem for transformation



Kayla Altendorf

- ▶ Transformation of germinated seedling explants is a common practice in various species including cannabis, a close relative of hops
- ▶ With our collaborator Kayla Altendorf, we will test a seedling-based transformation system in hop
- ▶ We will use both *Agrobacterium* and gene gun delivery methods



DOI: 10.1080/15592324.2020.1780037

Finding regeneration and transformation amenable hop cultivars

- ▶ We plan to test more hop varieties to test their amenabilities to genetic transformation and regeneration

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

- ▶ If you know a regeneration amenable cultivar, please let us know

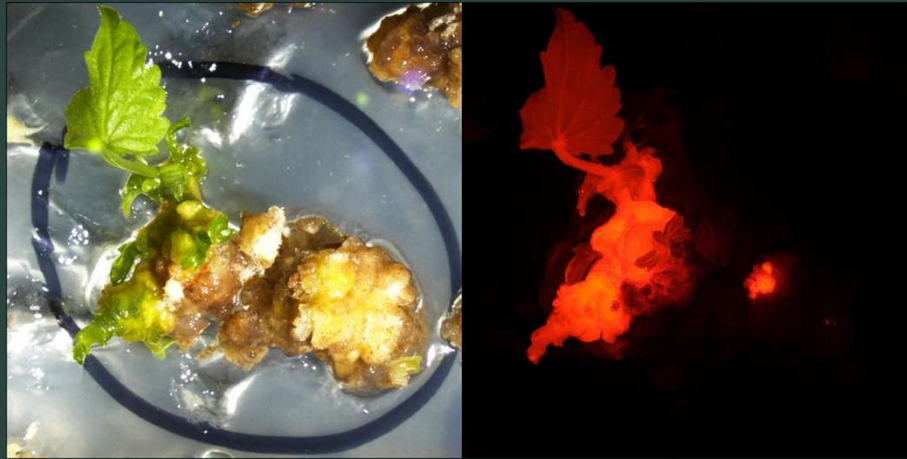
Plans for the coming year



Research agenda

- ▶ Continue producing and evaluating MLO-edited hop lines
- ▶ Continue experiments to improve transformation / regeneration / editing pipeline
- ▶ Test “clean editing” techniques in hop
- ▶ Test gene gun mediated hops transformation
- ▶ Evaluate new hop varieties we have not tested yet for their compatibility with gene editing methods
- ▶ Continue collaborating with Eva Chappell to analyze the global regulatory environment for use of gene edited hops in commerce

Thanks / Questions?



Connect with the Strauss lab

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