Genetic Transformation and Gene Editing in Hop

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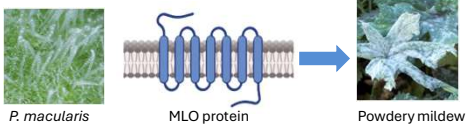
Background

Hop (*Humulus lupulus*) is a specialty crop primarily grown in the Pacific Northwest (PNW) region in the USA. USA is the second largest exporter of hop and produced worth $457M of hop in 2025. Hop production in the PNW faces substantial yield and quality losses due to powdery mildew (PM), caused by the fungus *Podosphaera macularis*.



MLO genes were identified as susceptibility factors for PM adapted species in Barley (Jørgensen 1992). Mutations, or suppression, of specific *MLO* genes confer durable, broad spectrum PM resistance in multiple plant species.

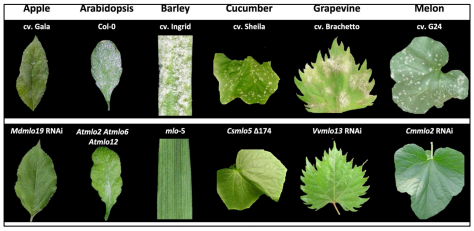


Figure 1. Examples for *mlo* resistance in various plant species. Reprinted from Kusch et al. 2017.

Modern biotech tools have the potential for hop improvement

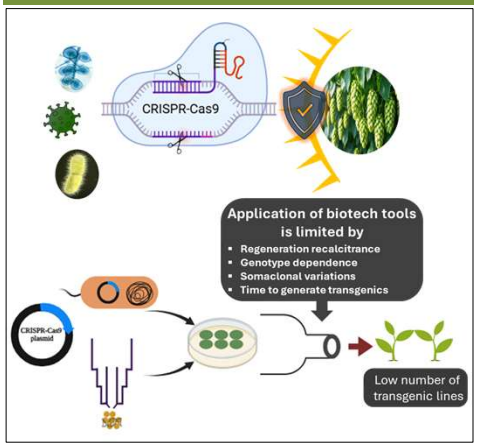


Figure 2. Potential of modern biotech tools in hop improvement and limitation in their application. Modern biotech tools such as CRISPR-Cas9 have the potential to protect hop from disease causing pathogens, but it is well known for its recalcitrance to genetic transformation and regeneration which is a limiting factor in applying biotech tools to improve hop.

Experimental design for *in vitro* transformation

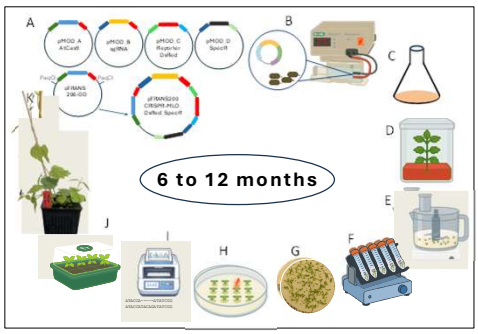


Figure 2. CRISPR-Cas construct development, transformation, and CRISPR-Cas9 editing in hop. (A) CRISPR constructs were generated via Golden Gate assembly and verified by Nanopore sequencing. **(B)** Sequence- verified constructs were transformed into *Agrobacterium* strain AGL-1. **(C-K)** Steps in hop transformation, verification of gene editing, and establishment of edited lines in the greenhouse. Detailed methodology is available in Wiseman et al. 2025.

Experimental design for lateral meristem transformation

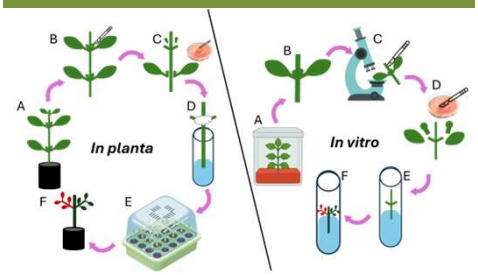


Figure 3. Hop lateral meristem transformation pipeline. (A) greenhouse/in vitro established hop plant. **(B)** A shoot with nodes **(C)** Removal of young lateral shoot bud and application of Agrobacterium paste. **(D-E)** Stem with infected node in rooting solution (*in planta*) or MS media (*in vivo*) for three days followed by transfer to rooting plugs or in multiplication medium (*in vitro*) **(F)** Potential transgenic shoot emerging from the infected node

We identified putative *MLO* S-genes in hop and designed guide RNAs for editing

Table 1. Putative *MLO* homologs in hop

Gene ID	Scaffold	Start	AA Length	UniProt Homolog
HUMLU_CAS0068957	Scaffold_49	230084079	552	MLO12_ARATH
HUMLU_CAS0050357	Scaffold_77	171879632	530*	MLO6_ARATH
HUMLU_CAS0048594	Scaffold_77	62805110	533	MLO12_ARATH
HUMLU_CAS0050448	Scaffold_77	17509059	605	MLO6_ARATH

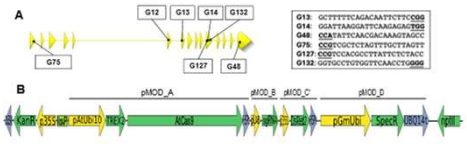


Figure 4. Intron-exon structure and identification of CRISPR targets for gene editing. (A) Schematic representation of the targeted *MLO* genomic region showing the relative positions of guide RNAs (G12 to G14 target *MLO*12 homologs, whereas G75-G132 target *MLO*6 homologs). **(B)** T-DNA region of the binary vector. *One haplotype of HUMLU_CAS0050357 appears to have a 5' truncated variant in Cascade (345 AA)

We achieved successful hop transformation and gene editing

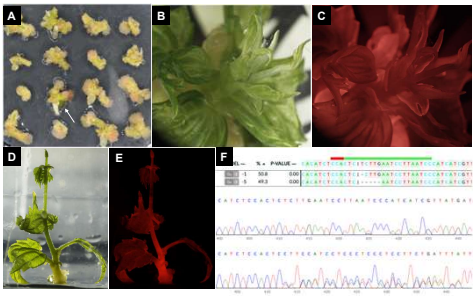


Figure 5. Hop transformation and validation of gene editing. (A) *Agrobacterium*-infected explants on shoot induction/selection medium. **(B)** A cluster of proliferating shoots emerging from an explant as seen under bright light and red fluorescent light **(C)**. A DsRed reporter positive shoot on rooting medium under **(D)** brightfield and **(E)** fluorescent conditions. **(F)** Sequencing of a transformed and successfully edited line showing biallelic heterozygous edits (-1/-5) at the guide RNA binding site.

Transgenic lines had diverse editing outcomes

Table 2. Edited events in transformed hop lines confirmed by Sanger and nanopore sequencing

Cultivar	Guide Event	HUMLU_CAS48594	HUMLU_CAS68957	HUMLU_CAS0050448 HUMLU_CAS0050357
Fuggle	G14-1	Biallelic heterozygous (+1/-4)	Biallelic homozygous (+1)	Wild type
Fuggle	G14-2	Monoallelic (+1/Wild type)	Monoallelic (-1/Wild type)	Wild type
Fuggle	G14-3	Biallelic heterozygous (+1/-2)	Monoallelic (+1/Wild type)	Wild type
Fuggle	G14-4	Biallelic homozygous (-2)	Biallelic homozygous (+1)	Wild type
Fuggle	G14-5	Biallelic heterozygous (+1/-2)	Biallelic heterozygous (-5/-8)	Wild type
Fuggle	G14-6	Biallelic heterozygous (-1/-5)	Biallelic heterozygous (+1/-9)	Wild type
Tettnanger	G14-1	Biallelic homozygous (-2)	Biallelic homozygous (+1)	Wild type
Fuggle	G48-1	Wild type	Wild type	Tetrallelic (-3)

Targeting lateral meristems accelerated transgenic shoot production *in vitro*

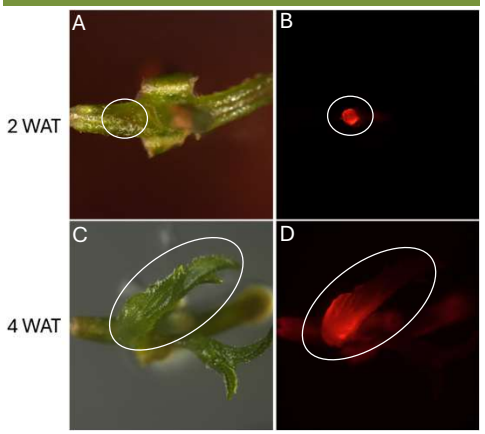


Figure 6. Lateral meristem transformation *in vitro*. (A-B) Bright field and fluorescent images of Agrobacterium infected nodes two weeks after transformation (WAT) showing reporter positive shoot bud circled in white. **(C-D)** Bright field and fluorescent images of elongating shoot 4 WAT. A non-transformed shoot without red fluorescence is emerging from the other side of the node.

We are starting to develop an *in-planta* transformation system targeting lateral meristems

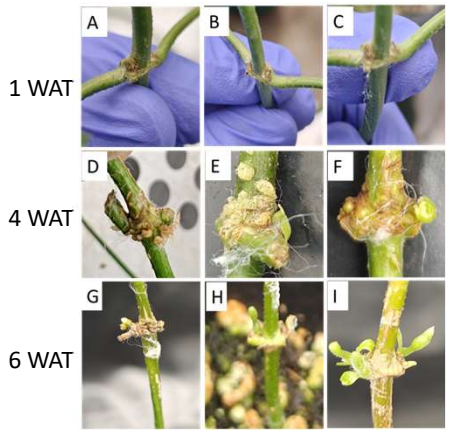


Figure 7. Hop *in-planta* transformation system under development. (A-C) Agrobacterium treated nodes one week after transformation showing the site of infection. **(D-F)** Callus formation at the site of infection 3 WAT. **(G-I)** Shoots and shoot bud formation at the site of infection. We are monitoring for transgenic shoots.

Conclusions and prospects

- We identified four putative *MLO* homologs as a potential target for powdery mildew resistance.
- We successfully developed a genetic transformation system and recovered multiple transgenic, gene-edited (KO: knockout) lines for two different *MLO* homologs.
- Targeting lateral meristem for transformation significantly reduced the time for generating transgenic shoots.
- Future efforts will be focused on improvement of hop transformation/editing rate, generation and phenotyping of additional KO lines, and editing of multiple *MLO* homologs.

Acknowledgements

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