M-17

GENE EDITING IN HOPS: METHODS, POSSIBILITIES, AND PROGRESS

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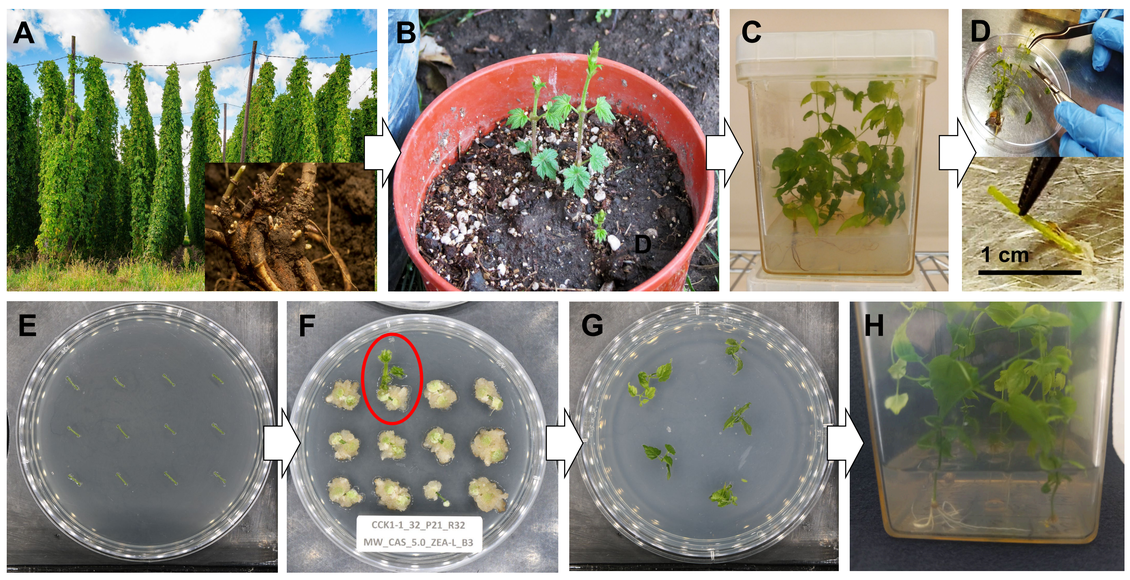
Introduction

Hops are the most expensive ingredient (by weight) used in brewing. However, hop production is threatened by diseases and climatic pressures leading to both chronic and catastrophic losses, which can have major impacts on the availability of certain varieties. Genetic engineering and gene editing may provide new methods to speed breeding and impart desirable traits in hops—while retaining the unique character of established varieties. However, these methods have hardly been explored in hop, and present significant biological and social challenges.

How does genetic engineering work?

- Genetic engineering is usually carried out via sterile **tissue culture**.
- This involves growing plant material under artificial conditions, on a petri plate or other vessel containing a nutrient mixture referred to as “media,” which is easy to modify.
- Development of a **regeneration** method in tissue culture is necessary for genetic engineering.
- By placing hop stem segments on new media containing plant hormones and other chemicals, the goal is to allow new shoots to form, which can be matured into independent whole plants.
- Finding the right conditions for regeneration takes time and varies between different hop varieties.

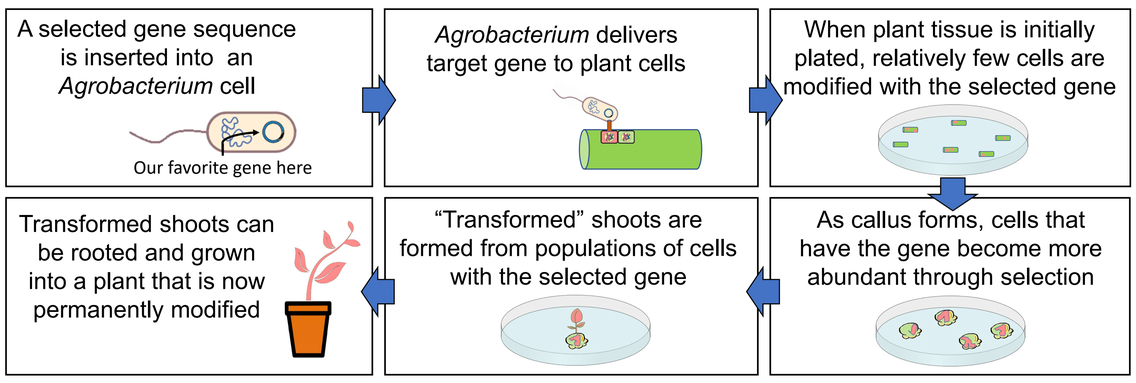
Figure 1: Steps in hop tissue culture and cellular regeneration.



Hops growing in the field (**A**) are collected by digging rhizomes (**A**-inset). Emerging shoots from potted rhizomes (**B**) are surface sterilized and propagated on tissue culture media (**C**). For regeneration, stem segments are isolated from cultured shoots and (**D**) placed on new media containing plant hormones (**E**). After 4-6 weeks callus tissue forms and under the right conditions, new shoots may emerge (**F;** shoot circled in red). These shoots can be further elongated (**G**) and then rooted (**H**) to form an independent whole plant ready for testing.

- **Transformation** is the process of delivering genes into cells for the purpose of altering traits.
- In hop we use a natural genetic engineer, called *Agrobacterium*, to deliver genes into stem tissue.
- Different hop cultivars vary in their susceptibility to *Agrobacterium*, and thus must be screened.
- So called “**marker genes**” assist transformation by promoting survival of modified cells in tissue culture (selectable marker) or producing a fluorescent signal in modified cells (visual marker).

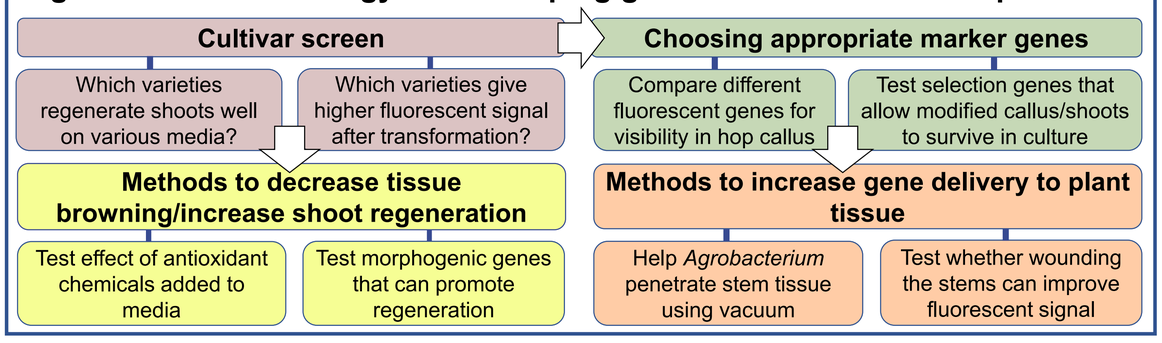
Figure 2: Schematic of plant transformation process



Key terms

- **Genetic engineering**: The use of modern technology to alter plant traits by adding to or changing its DNA
- **Tissue culture**: Growing whole plants or parts under artificial laboratory conditions such as on a Petri plate
- **Regeneration**: Causing a plant part—a stem for example—to develop into a whole individual, independent from the parent plant it was taken from
- **Transformation**: Delivery of a gene (or other DNA) into plant cells from outside, often via *Agrobacterium*
- **Marker gene**: A gene causing a trait (visual or selectable) that allows us to easily determine which cells in a plant tissue have been successfully transformed
- **Gene editing**: A method of genetic modification that allows trait alterations by making changes to an organism’s native DNA rather than adding new gene sequences

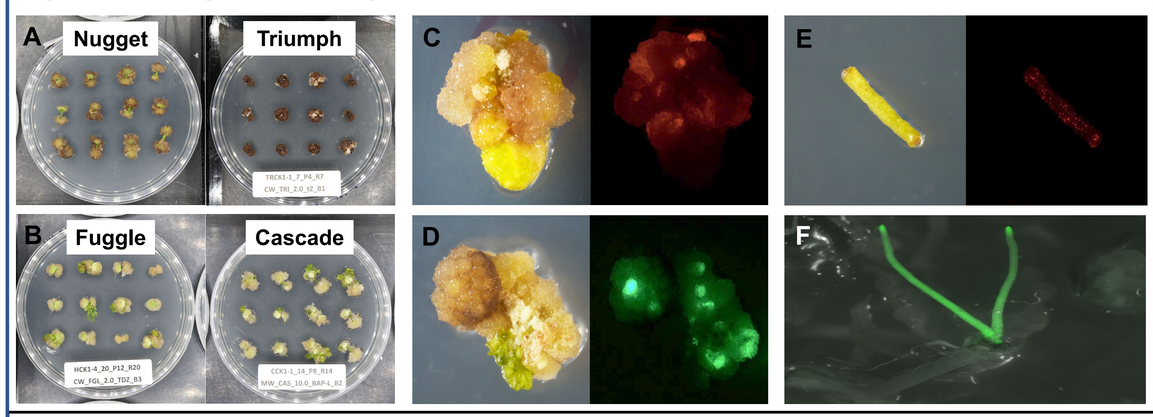
Figure 3: Current strategy for developing genetic modification in hop



What is the status of our research?

- We are evaluating regeneration and transformation in a number of cultivars important to hop culture in the USA, including Cascade, Nugget, Fuggle, Triumph, and Centennial.
- For regeneration testing, we see wide variation among varieties with responses ranging from good shoot formation under multiple hormone treatments to widespread tissue browning and death.
- Fuggle and Cascade have performed best in our hands, achieving rates of shoot regeneration from stem segments up to 40-50%, albeit under different ideal conditions.
- Transformation is challenging in hop. Though we can produce transformed callus tissue, we have not yet recovered transformed shoots.
- Work going forward will focus on testing many factors to optimize the regeneration / transformation process in Cascade and Fuggle (see Figure 3 for strategy).

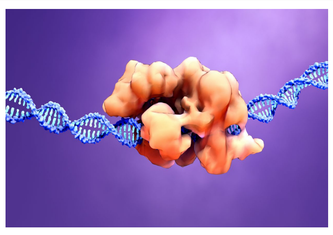
Figure 4: Progress on regeneration and transformation in hop



Four weeks after initiation on regeneration media, varieties including Nugget and Triumph were poorly regenerable and showed a browning response (**A**), while Cascade and Fuggle gave a high rate of new shoot formation under certain hormone treatments (**B**). The visual markers of gene transfer DsRed (**C**) and jellyfish green-fluorescent protein (GFP, **D**) could be detected in transformed Cascade callus tissue, but the shoot buds that formed did not have a fluorescent gene expressed. DsRed could be detected in stem as early as 10 days after transformation (**E**). Though no fluorescent shoots were detected, we were able to produce transgenic, GFP-expressing hairy roots, an alternative genetic engineering method (**F**).

What is CRISPR gene editing?

CRISPR is a genetic tool that allows us to make very precise changes to genes already present in an organism’s DNA, resulting in altered traits (this is called **gene editing**). It is often described as “molecular scissors” and can be used to create trait alterations similar to those used in conventional breeding. Once we develop a hop transformation system, *Agrobacterium* can be used to deliver the CRISPR editing components into cells. CRISPR is easily customizable to modify any hop gene, and the first successful application of gene editing in hop has recently been reported[1].



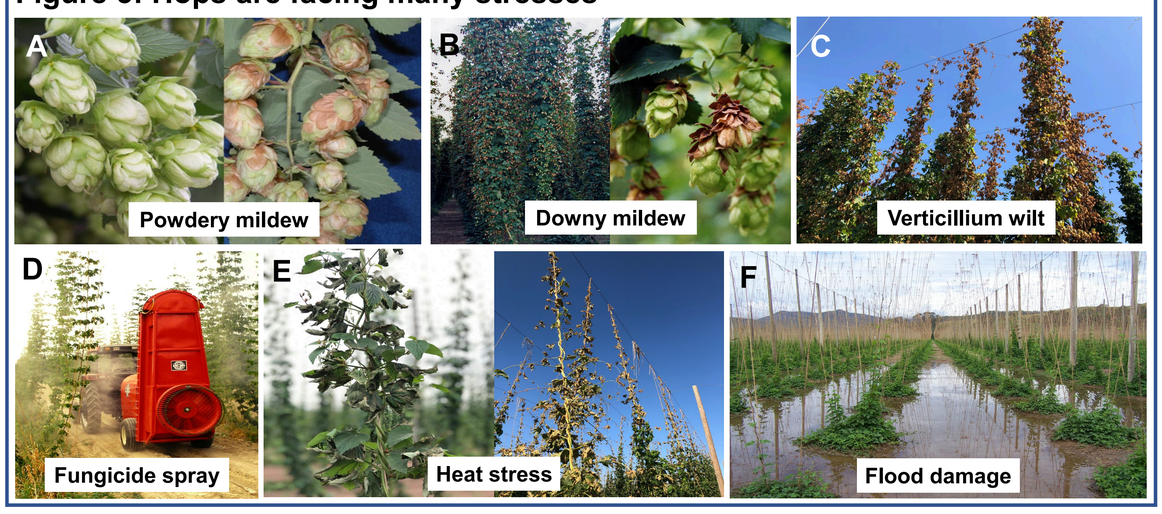
Goals for genetic modification in hop

There are many possibilities for genetic modification to produce useful traits to aid brewing, production, or resistance to the growing diseases and stresses, some of which are linked to climate change (Figure 5). One of our research aims is to use CRISPR to confirm the function of a hop gene linked to resistance to powdery mildew disease.

Many traits could potentially be altered with gene transfer or gene editing, including:

- Tolerance to disease, heat, and drought stress—key concerns in a climate change world
- Plant height—dwarf hops are easier to harvest and require less costly infrastructure
- Flowering time—expanding capacity for locally grown hops outside of major production regions
- Storage stability—potentially preserving flavor for longer periods
- Altered bittering and aroma qualities—to produce distinctively flavored beers

Figure 5: Hops are facing many stresses



Market and social considerations

Historically, brewers and consumers have been hesitant to embrace genetically engineered plants. That appears to be changing as consumers become more familiar with the technology. USDA regulations for engineered products are becoming less onerous, as the serious threats to production from climate change becomes increasingly apparent. Many also see gene editing as a more “natural” form of trait enhancement than conventional engineering. If the biological challenges can be overcome, we expect to see hop varieties with edited traits begin to enter the marketplace within the next 10 years.

References and Acknowledgements

1. Awasthi et al. 2021. *Plant Phys and Biochem*, *160:*1-7.

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