Toward Gene Editing in Hop:

Application of a Cutting-Edge Tool for Accelerating Breeding and Improving Traits

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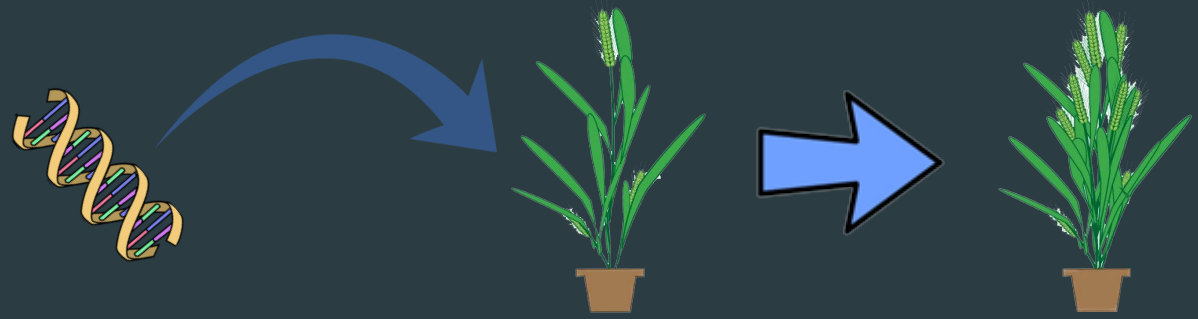
Genetic engineering vs. gene editing

- Genetic engineering / transformation – method for delivering a "package" of genetic material into a plant to alter a trait

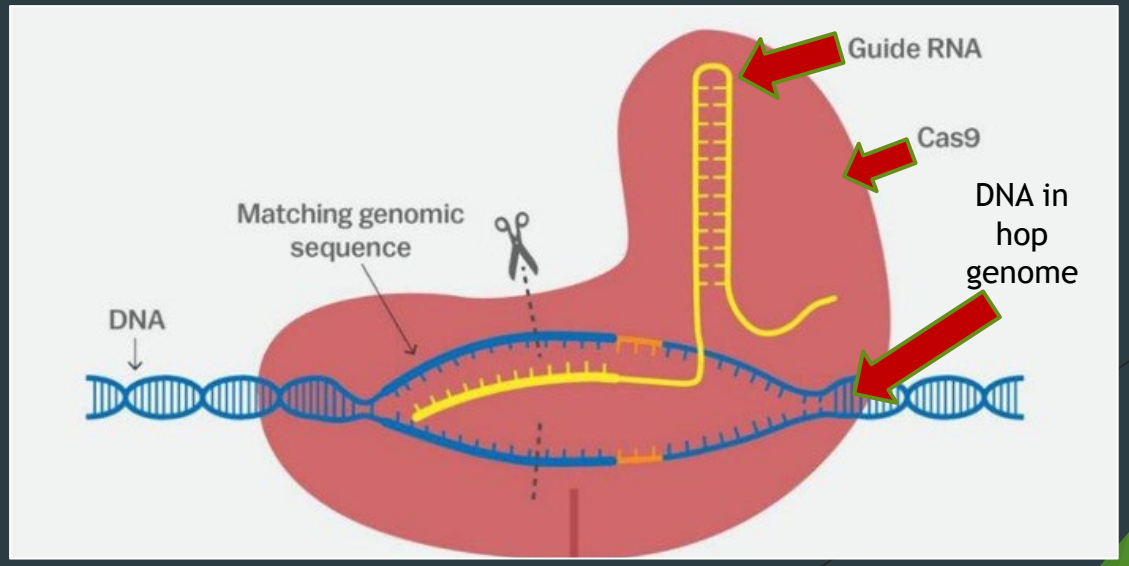


- Gene editing is using the package to deliver "machinery" that then changes genes *already inside the plant* to alter a trait

How CRISPR works at the molecular level

- CRISPR machinery consists of a DNA cutting component (Cas protein) and a sequence recognition component (guide RNA)

- CRISPR is a very customizable tool — to edit a new gene we need only to add a unique ~20-base sequence corresponding to guide RNA



Why use biotech in hop?

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




- Can be addressed by breeding hop varieties with improved traits that offer some protection from these pressures

The hop breeding cycle

- Breeding may take 8-15 years between an initial cross and commercial release of a new cultivar
- Deals with traits influenced by many genes
- Very difficult to incorporate new traits while maintaining aroma profile of prized cultivars

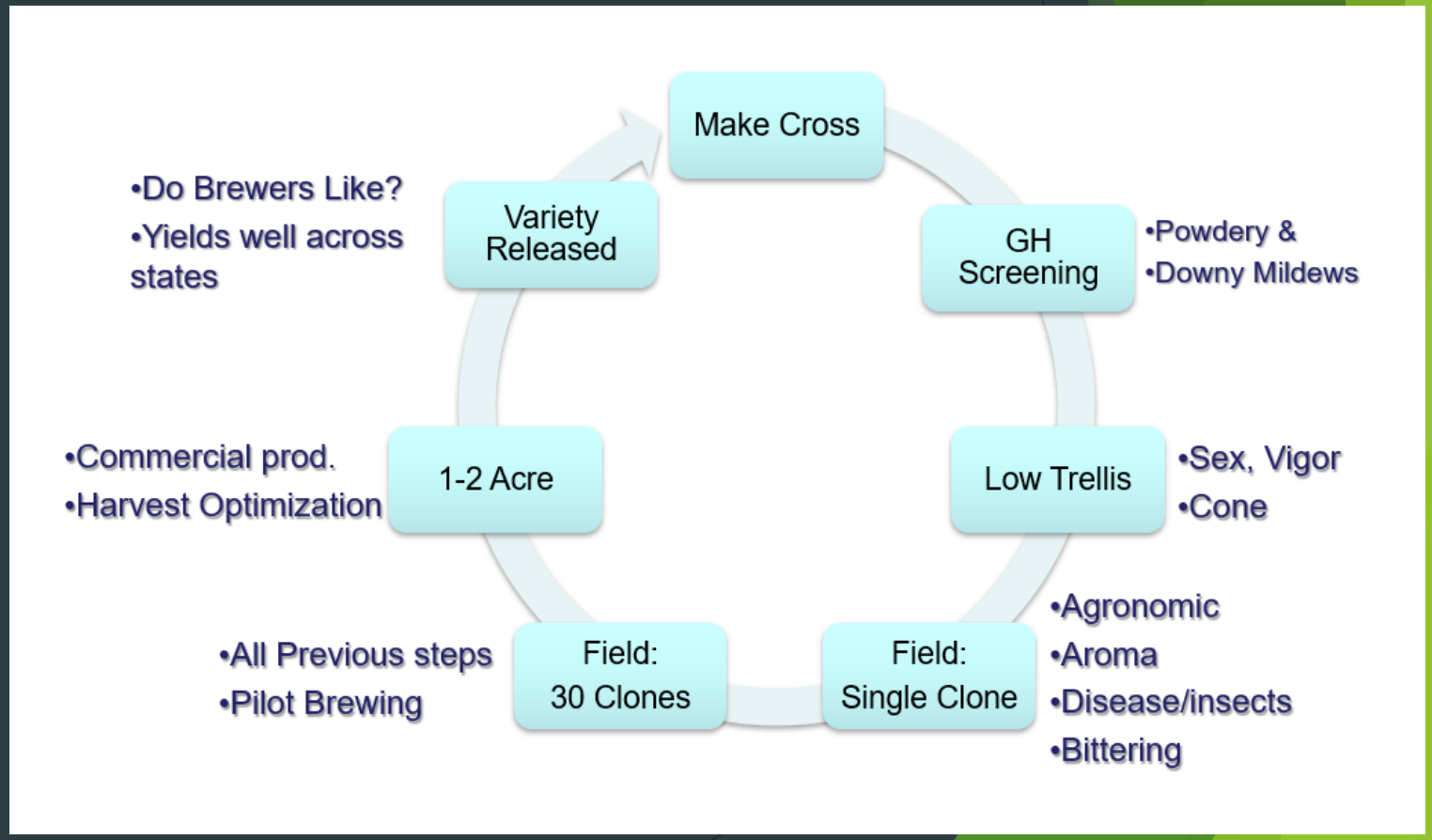
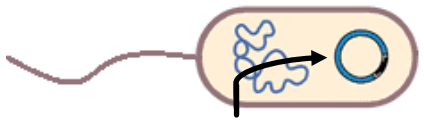


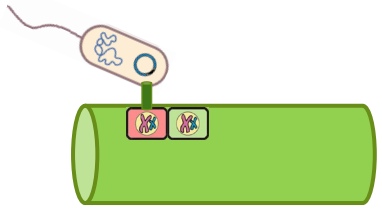
Figure courtesy of John Henning, USDA

Transforming plants with *Agrobacterium*

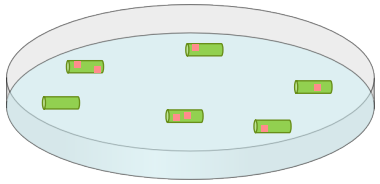
A selected gene sequence is inserted into an *Agrobacterium* cell



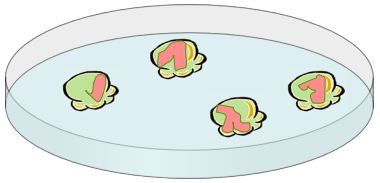
Agrobacterium delivers target gene to plant cells



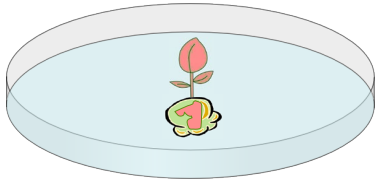
When plant tissue is initially plated, relatively few cells are modified with the selected gene



As callus forms, cells that have the gene become more abundant through selection



“Transformed” shoots are formed from populations of cells with the selected gene

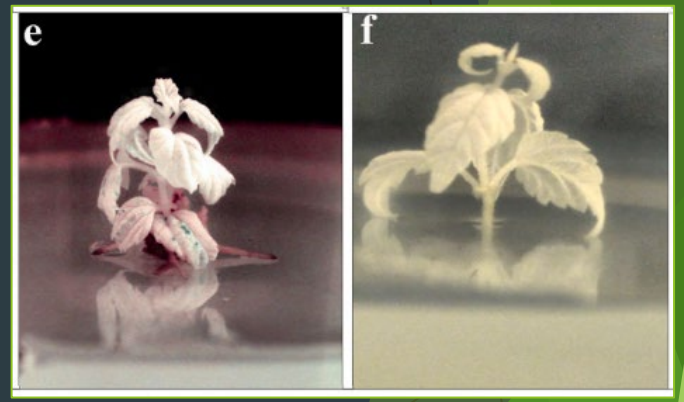


Transformed shoots can be rooted and grown into a plant that is now permanently modified

Prior work in hop transformation

- A few labs have reported successful transformation of hop, all in European cultivars
- The first report of successful gene editing in hop was published by a Czech group in 2021
- This is encouraging, but methods need to be adapted to each different cultivar
- We were awarded a USDA-NIFA grant in 2021 to develop transformation/gene editing methods in US hop varieties

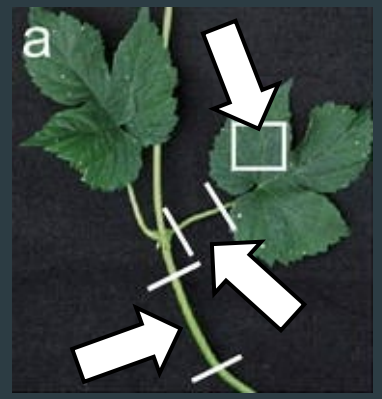
Transgenic shoot of 'Tettnanger' (Arias & Weber, 2013)



Gene edited hop plants for *PDS* gene (proof-of-concept) (Awasthi et al., 2021)

Roadmap to establishing a tissue culture-based gene transfer system

- Regeneration
  - Which cultivars will regenerate?
  - Media composition
    - Hormones
    - Sugars
    - Macronutrients
  - Starting plant tissue type
  - Lighting conditions

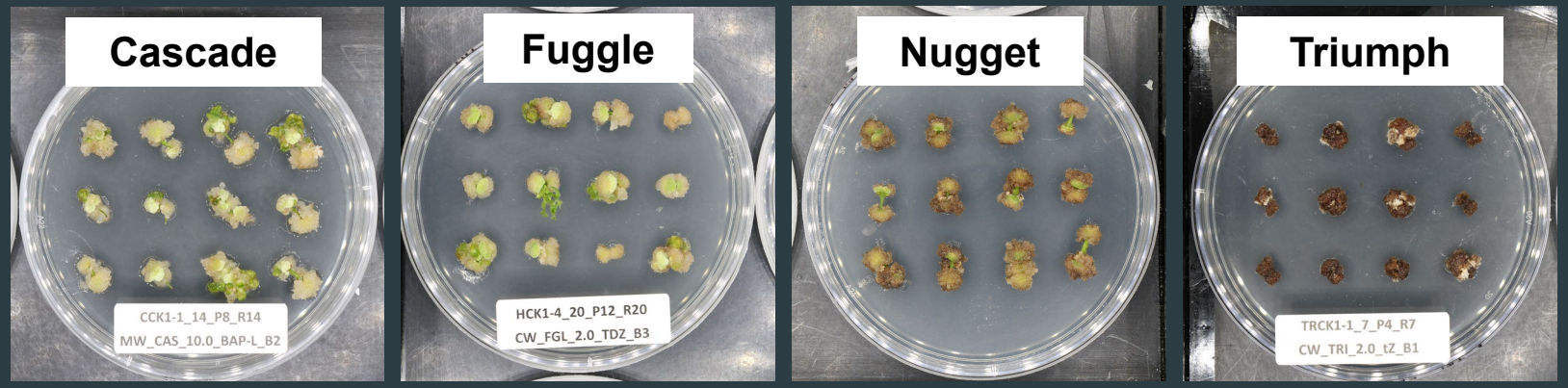


Horlemann et al., 2003

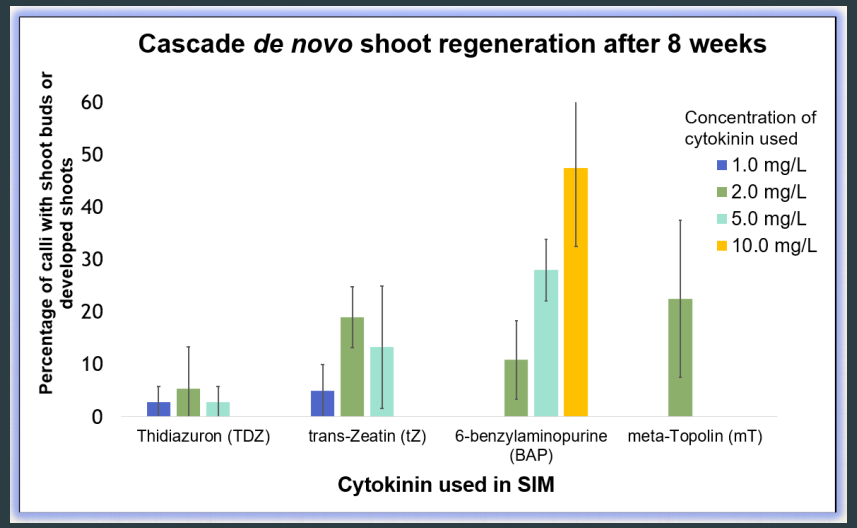
- Transformation
  - Which strains of *Agrobacterium* to use
  - How much *Agrobacterium* inoculum to use
  - Which visual and selectable marker genes to use
  - Starting plant tissue type
  - Techniques to help *Agrobacterium* deliver DNA to more cells

Experiments testing regeneration

- Screened for shoot regeneration capacity in several public hop cultivars

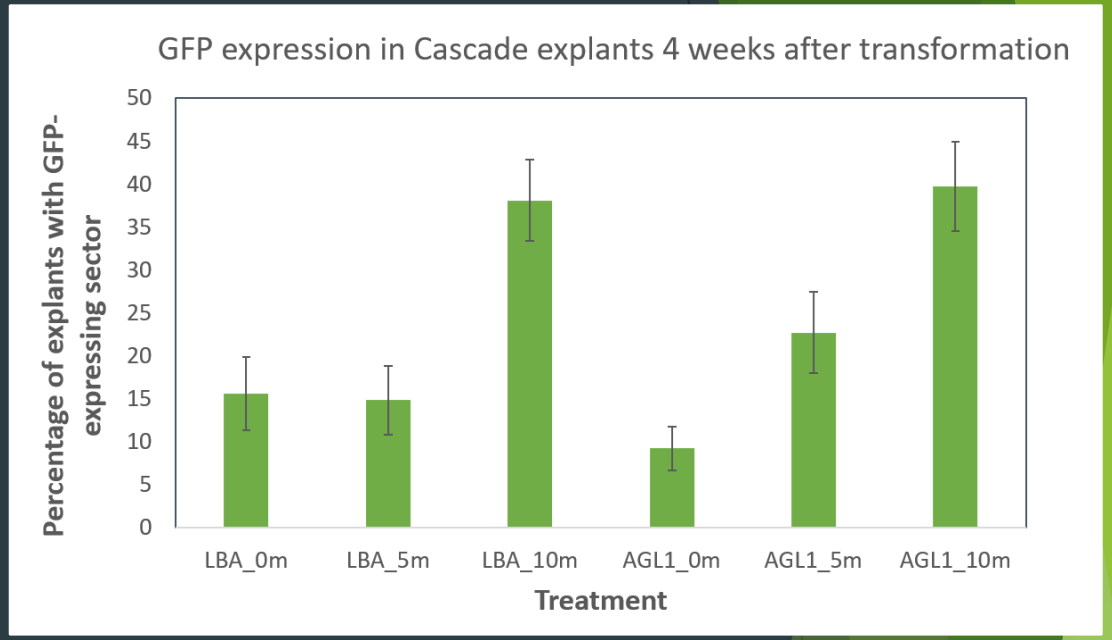


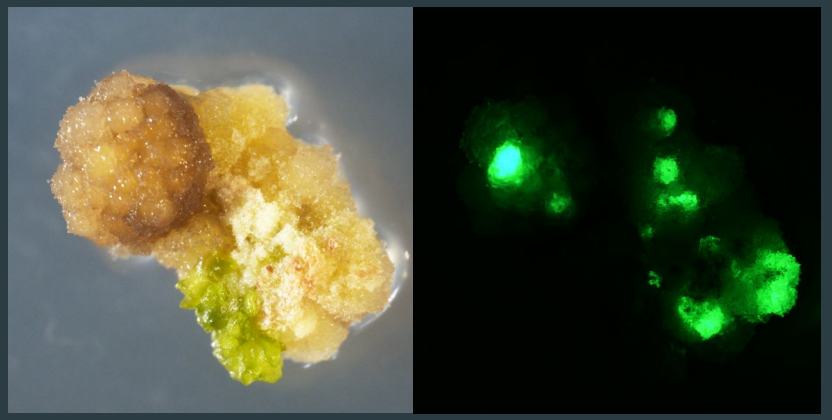
- Optimization for media hormone content in individual cultivars

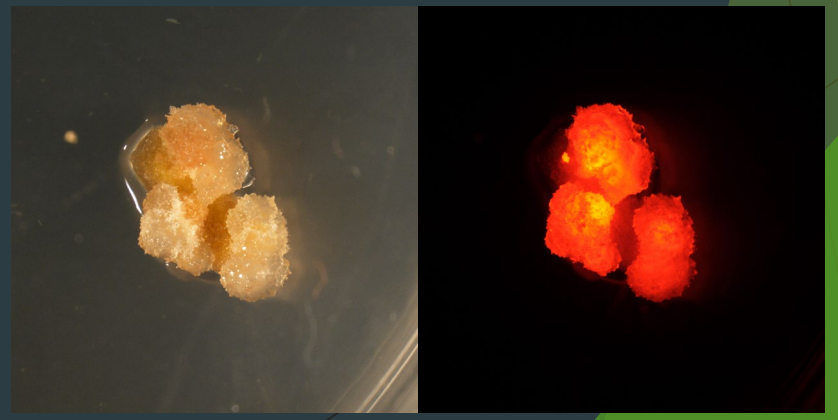


Experiments testing transformation

- Using visual marker gene expression as a basis to see how well gene delivery worked
- *Agrobacterium* strains compared
- Several methods to try to increase gene delivery from *Agrobacterium*
- Compared utility of different marker genes

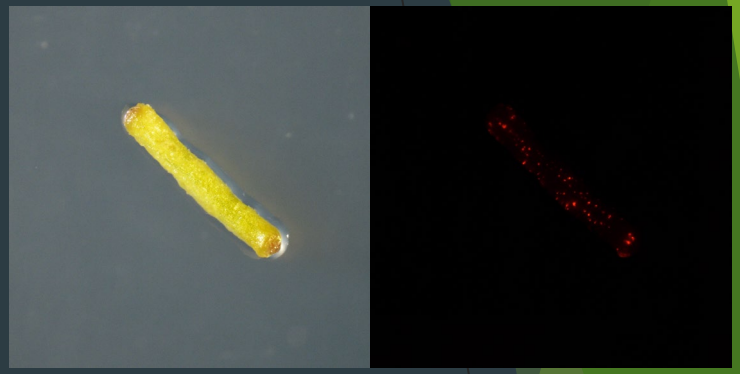


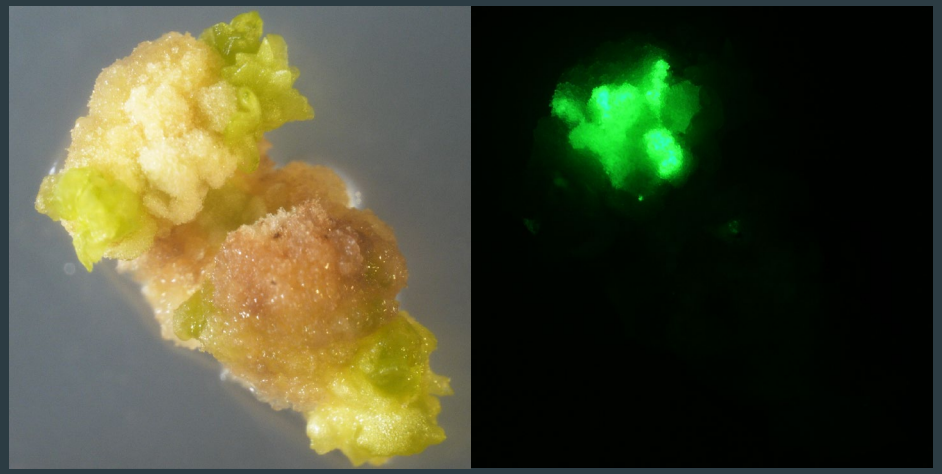


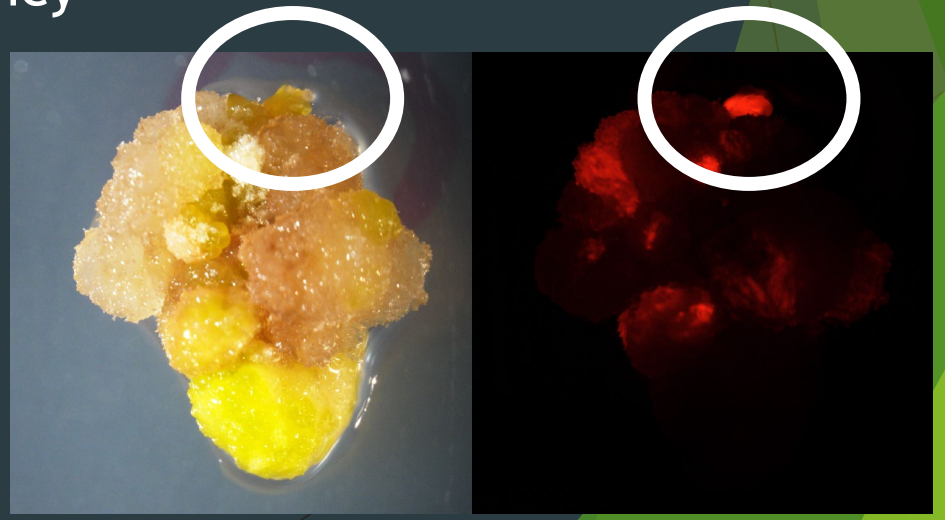


Status of our *in vitro* transformation system

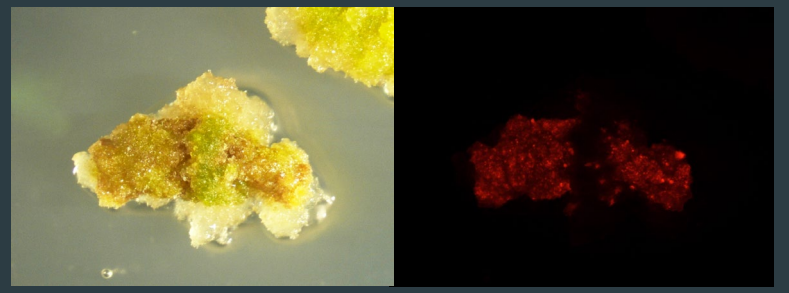
- Working primarily with Cascade and Fuggle
- Gene delivery to stem tissue is low
- Need to optimize selection to balance regeneration and transformed tissue
- Testing more methods to boost transformation and further improve regeneration efficiency

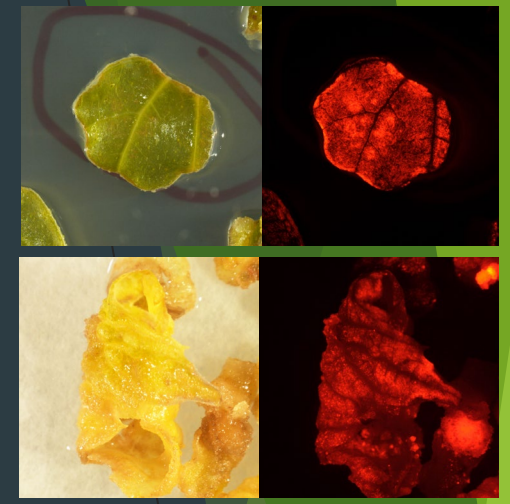




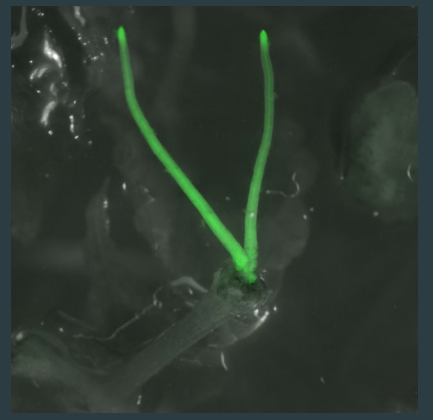


Some promising directions to explore





- Plant tissues other than stem might be better-suited for transformation



- An alternative approach using “hairy roots”

- Development (“DEV”) genes to facilitate transformation

- *In planta* transformation methods – still a very new technology, but would allow us to bypass tissue culture

What traits could be addressed with genetic engineering/editing?

Traits benefitting growers:

- Disease resistance traits specific to common pathogens
- Plant height
- Flowering time—early or later-maturing varieties

Traits benefitting brewers/end consumers:

- Storage stability
- Altered bittering and aroma qualities
- Medicinal compound production?

Prerequisites for applying gene editing to improve agricultural traits

- A viable transformation system in the cultivar we want to improve
- Genome sequencing data

HopBase 

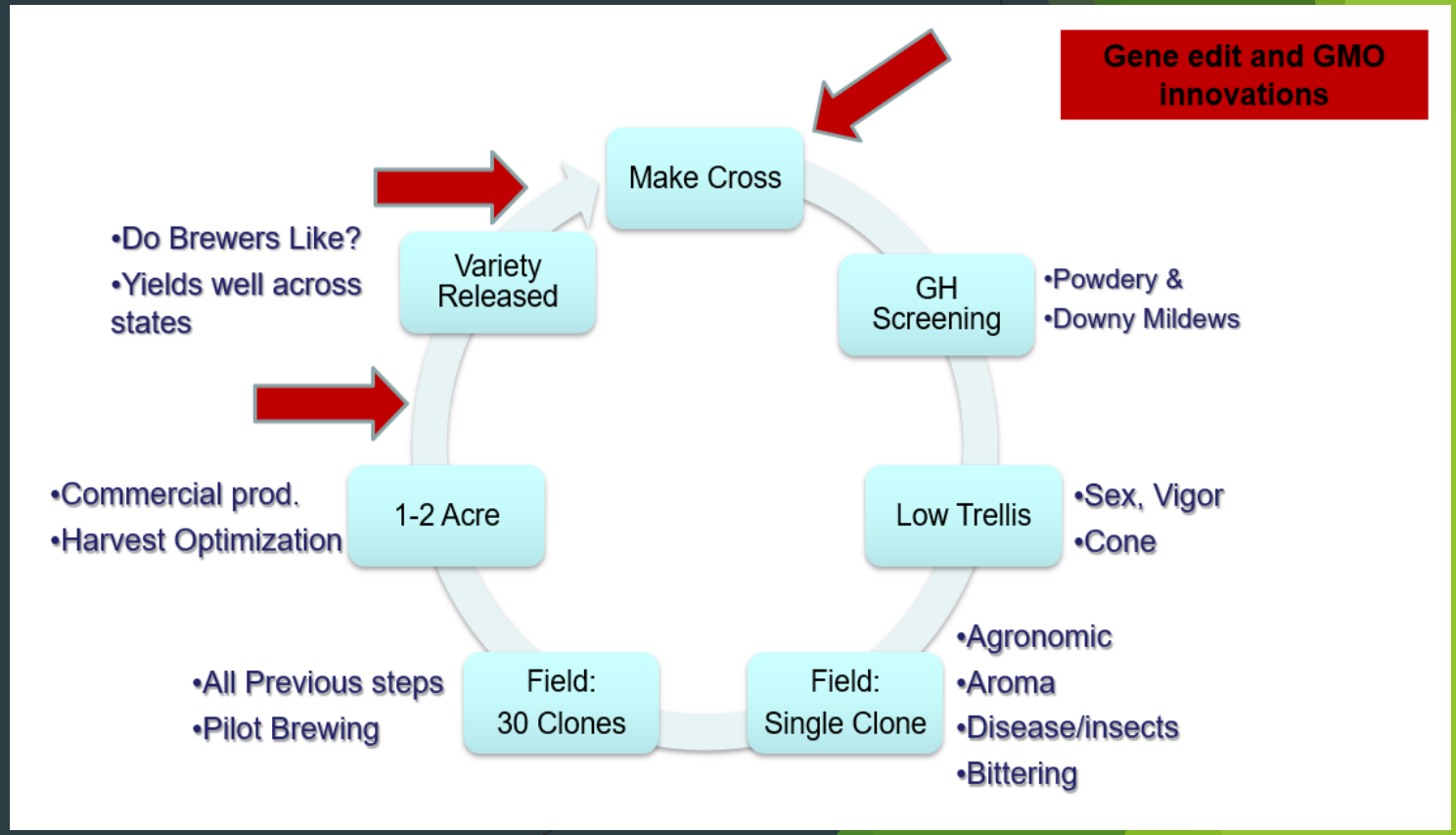
- Genes → Traits information – genetics research

Summary

- Transformation techniques are the bottleneck for implementing gene editing in hop
- It is challenging, but the potential benefits are worth it
- Need to have more gene functional data for gene editing to be of most benefit
  - CRISPR can enable genetics research, helping to speed up discoveries

Summary

- GE is not something that can substitute for breeding, but it can:
  - Complement it – fill in gaps with respect to specific things that would be difficult to address through breeding
  - Support it – be used as a research tool to uncover gene functional information to inform breeders



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R&D collaboration

We are seeking opportunities for partnership with industry groups to continue developing these tools in hop

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