

FRST302: Forest Genetics

Lecture 4.2 - Accelerating Tree Improvement II

Recap

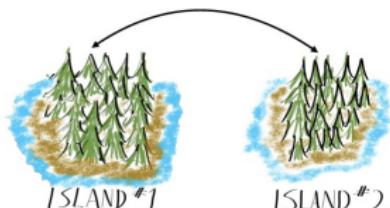
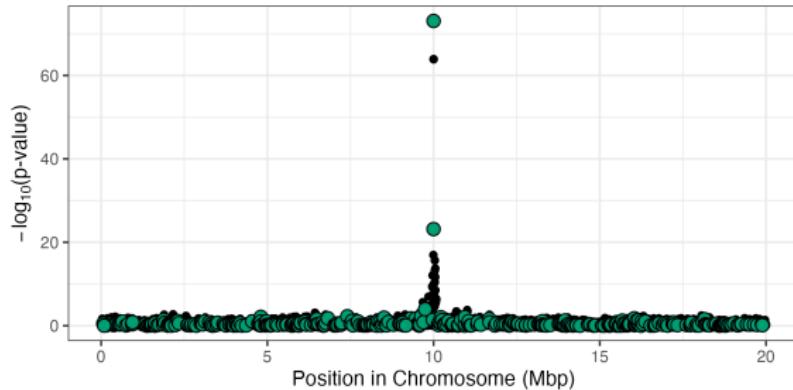
Association genetics is a powerful way to identify genes underlying important traits

Association genetics **is not** a silver bullet as there are multiple statistical issues

Good at identifying large effect loci

Now What?

We identified a locus that increases a carrier's height by 3m!

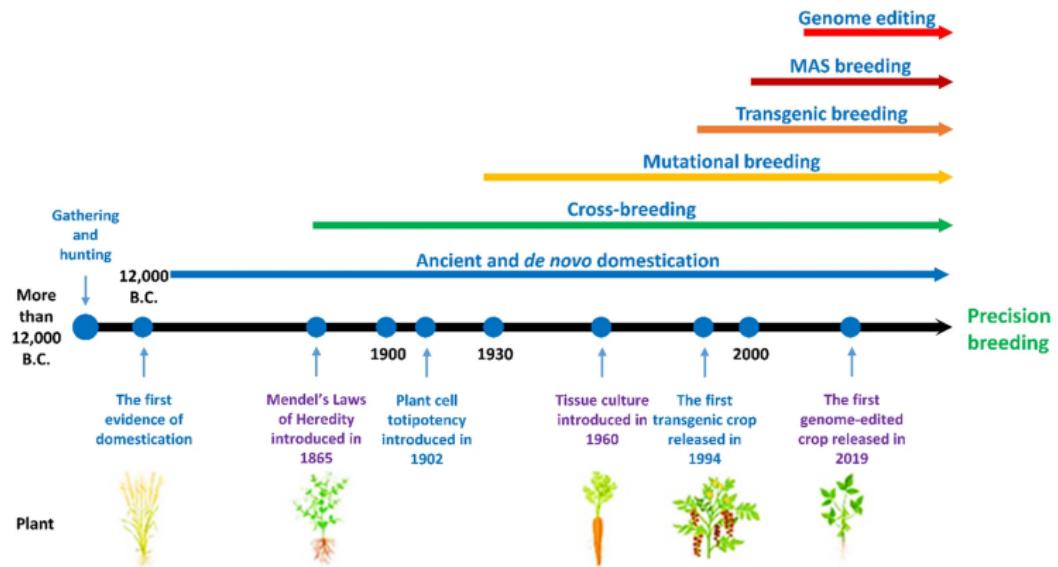


What are we going to do
with that information?

Overview for Today

- Introduction to genetic modification and genome editing
- Methods of genetic modification and genome editing
- Application of these in forest biology

Major Milestones in Plant Breeding



- 1 Domestication/traditional breeding
- 2 Genetics-informed breeding
- 3 Mutational breeding
- 4 Transgenic breeding
- 5 Genomic selection
- 6 Genome editing

Figure 1 from Van Vu et al. 2022 *Planta*

1. Domestication/Traditional Breeding

Humans have consciously (and unconsciously) altered the traits of many species of plants and animals for time out of mind

Artificial selection alters allele frequencies, leading to populations with a desired pattern of trait variation



var. *capitata*
cabbage



var. *acephala*
kale



var. *italica*
broccoli



var. *botrytis*
cauliflower



var. *alboglabra*
Chinese kale

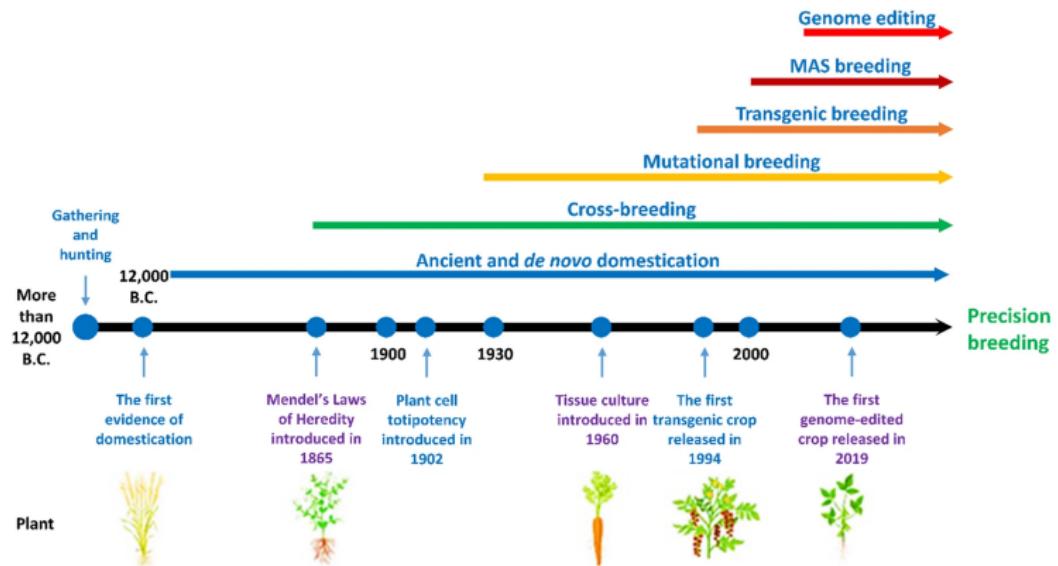


var. *gongylodes*
kohlrabi



var. *gemmifera*
brussels sprouts

Major Milestones in Plant Breeding



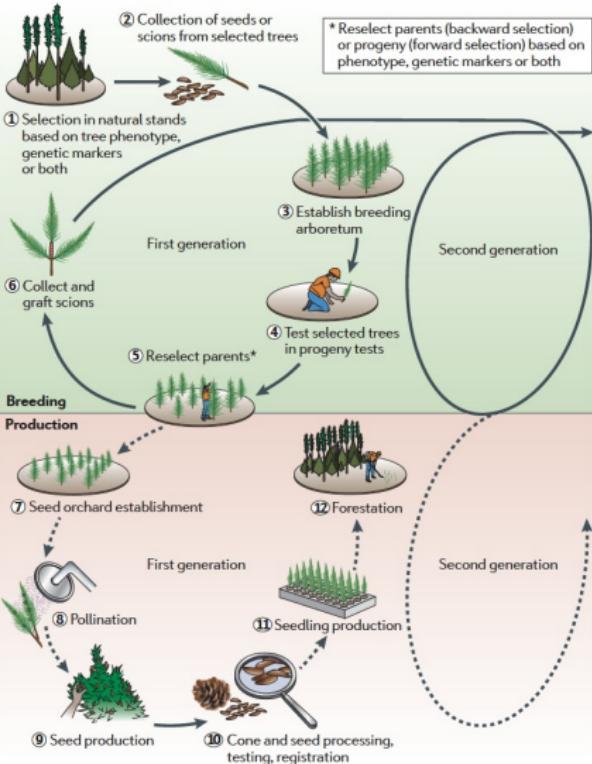
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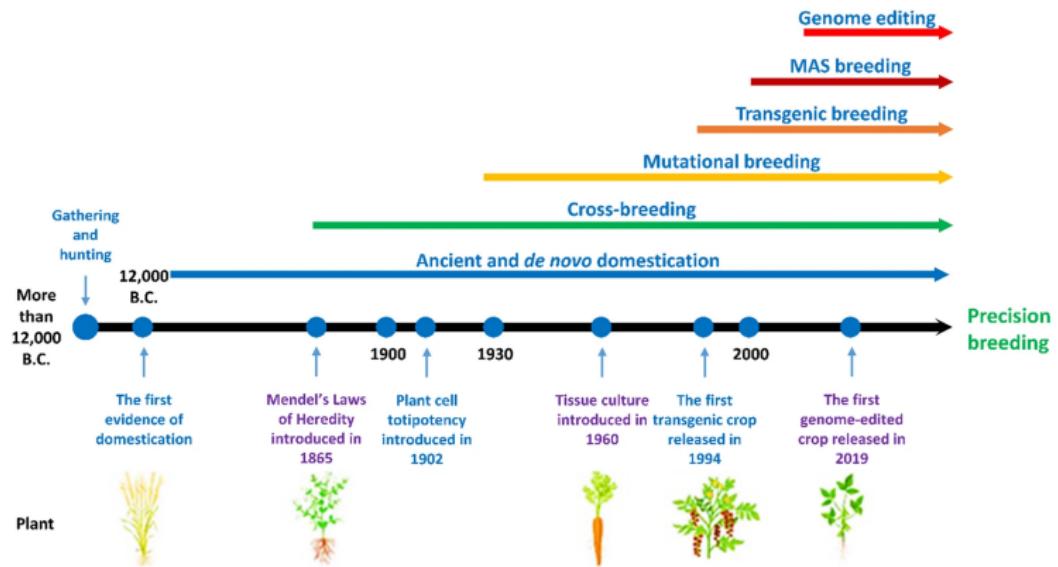
2. Genetics-informed breeding

Tried and true Each cycle takes from 20-30 years!

We already decided that this takes too long!



Major Milestones in Plant Breeding

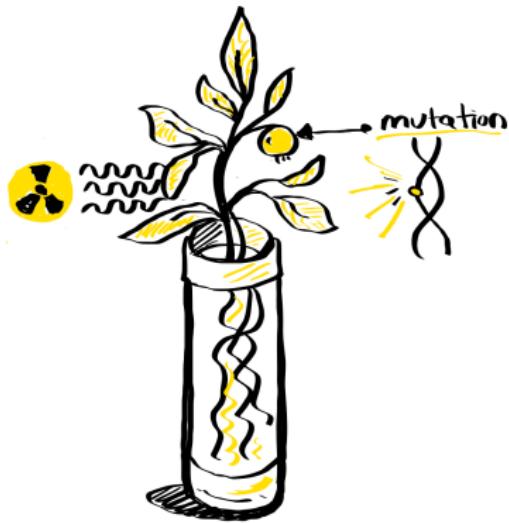


- 1 Domestication/traditional breeding
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Figure 1 from Van Vu et al. 2022 *Planta*

3. Mutational Breeding

Applying mutagenic agents to give rise to desirable traits (e.g. chemicals, X-Rays, even *cosmic rays*!!)

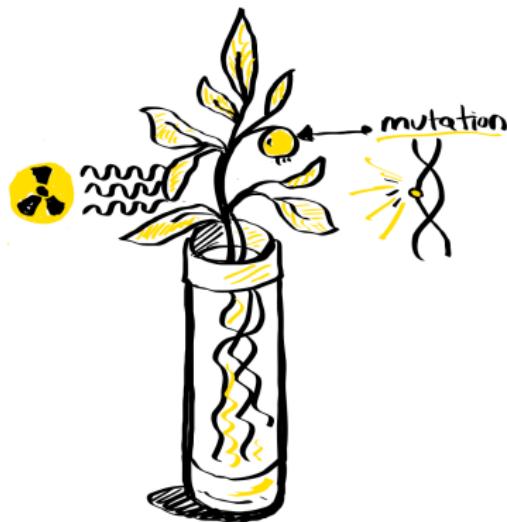


- Speeds up the natural process of mutation
- Randomly introduces mutation to plant genomes
- Widely used in agriculture



3. Mutational Breeding

Applying mutagenic agents to give rise to desirable traits (e.g. chemicals, X-Rays, even *cosmic rays*!!)



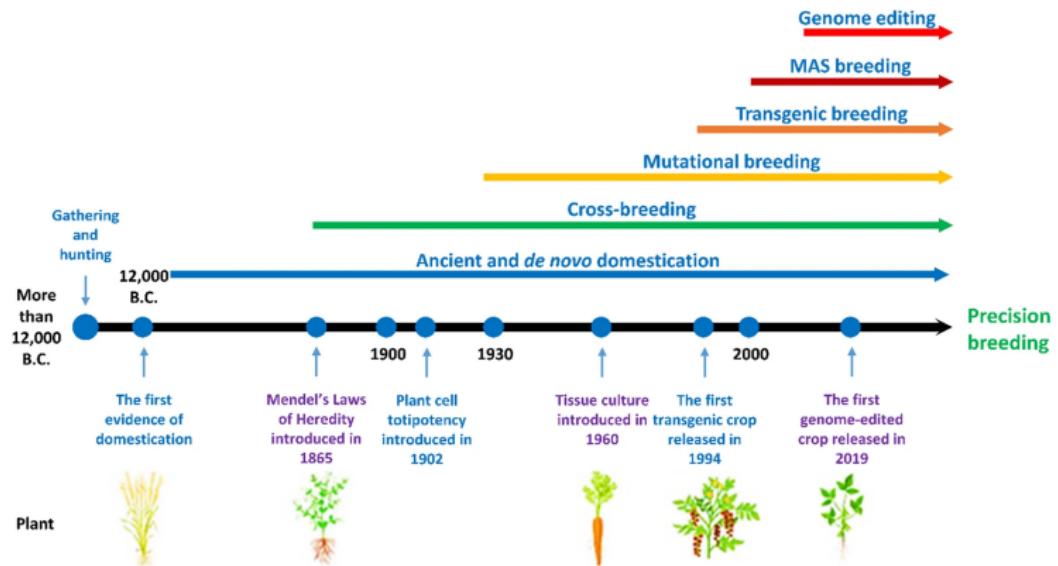
- Mutagenesis may give rise to useful mutations
- May lead to harmful ones in addition
- Useful mutants are backcrossed to utilize the new allele

Mutational breeding is not used in forest genetics

It would be far too slow

*At least as far as I can tell!

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Figure 1 from Van Vu et al. 2022 *Planta*

4. Transgenic Plant Breeding

Transgenesis: using genetic engineering techniques to introduce genes from other lineages, species (or even kingdoms!)



Transgenic poplar plantation; Figure 2 Häggman et al 2013 *Plant Biotech*

4. Transgenic Plant Breeding

Genetically Modified Organisms (GMOs) have been a hot topic for many years

- Food safety concerns
- Ethical concerns
- Ecological concerns

The definition of a GMO is still not clear to many people
E.g., does cloning and selective breeding lead to GMOs?



What is Genetic Modification?

Genetic modification (GM), or genetic engineering, is the direct manipulation of an organism's genome using biotechnology.

GM includes:

Transferring genes

within and across species

Adding new genes

Knocking out genes



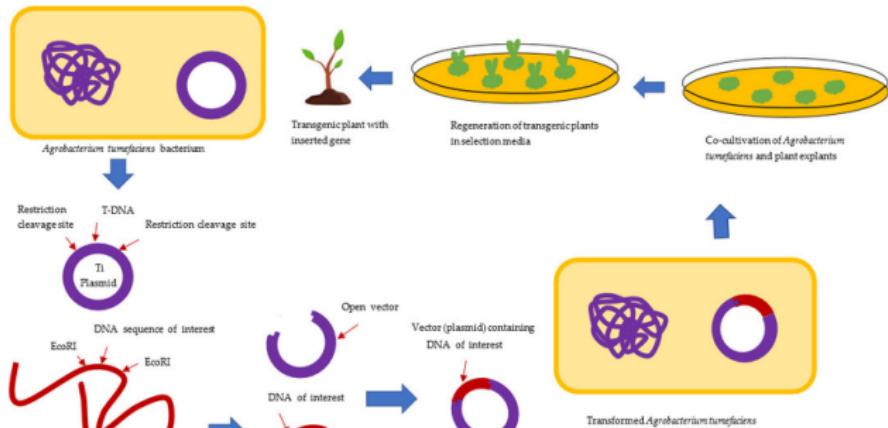
The world's first GM plant was produced in 1983

A gene from the bacterium *Agrobacterium tumefaciens* encoding an antimicrobial resistance protein was transferred to tobacco

How is GM Achieved in Plants?

There are several GM methods that are used in plants:

Agrobacterium tumefaciens

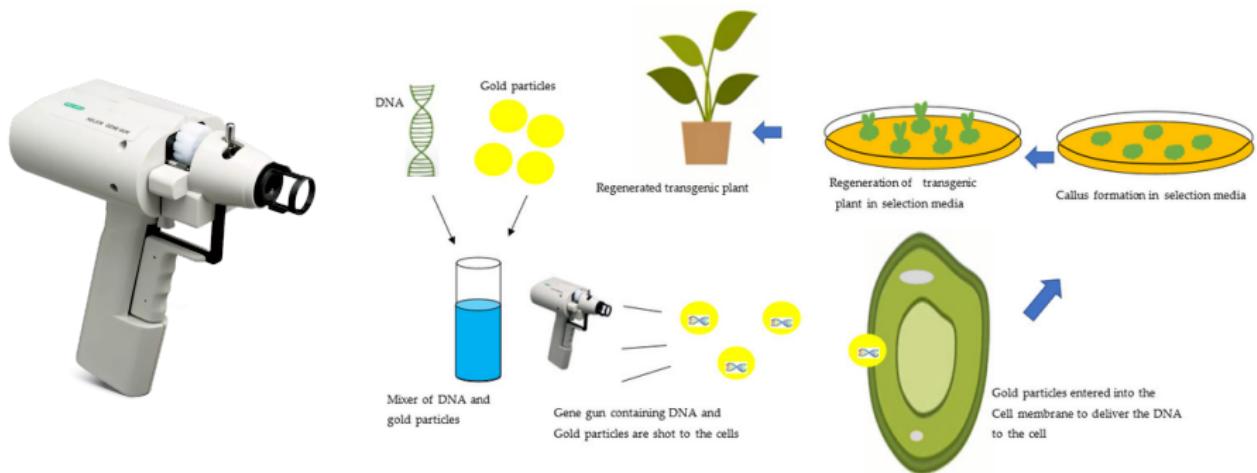


L-R: A crown gall caused by *A. tumefaciens*; Schematic from Ghimire et al. 2023 *Sustainability*

How is GM Achieved in Plants?

There are several GM methods that are used in plants:

Gene Gun (a.k.a. BioListics)



Put ethical, ecological and economic concerns aside for the moment...

What are some ways that GM could be used in forest trees?

Carbon Sequestration

Carbon capture and sequestration by growing trees is a so-called
"nature-based climate solution"

A core component of the carbon economy

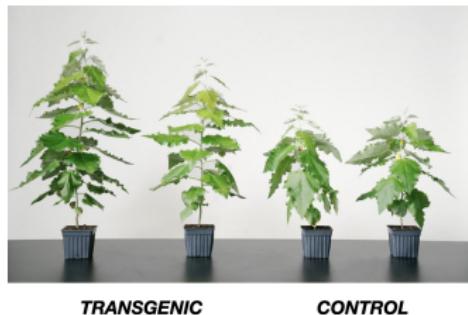


Engineering trees to better capture and store CO_2 seems like a
good goal

There is a *LOT* more that could be said about this topic

GM in Forest Trees: Example

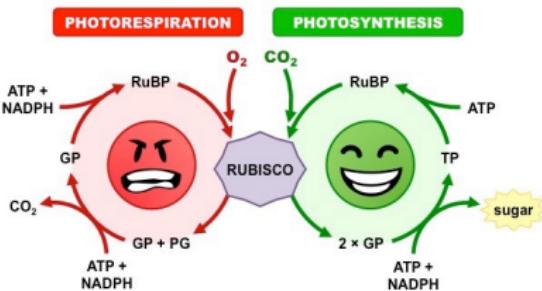
Living Carbon is a company based in California that creates
“bespoke carbon sequestration solutions”



Living Carbon has used GM to develop trees to more efficiently capture carbon

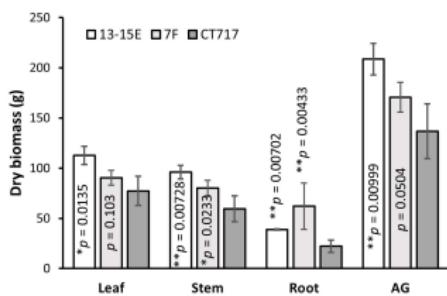
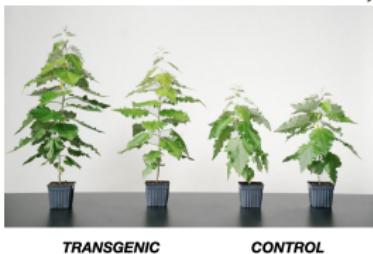
GM in Forest Trees: Example

- In plant cells, chloroplasts carry out photosynthesis
- The enzyme at the heart of photosynthesis can also carry out photorespiration to recycle toxic by-products of photosynthesis
- **Photorespiration can reduce the efficiency of the conversion of light into biomass by 20-50%**



GM in Forest Trees: Example

Living Carbon has produced transgenic hybrid poplar (*Populus tremula* × *Populus alba*)



- Introduced a gene encoding a small interfering RNA
- Silencing a gene involved in photorespiration
- Transgenic plants accumulated up to 53% more above-ground biomass than controls
- Results varied by transgenic line

Limitations of GM

- The insertion of a new gene may not occur
- No control on targeting DNA locations
- One gene at a time
- Expensive and slow
- Under strict regulation



Homer introduced tobacco genes into tomato with a radiation-based procedure to produce his ToMacco

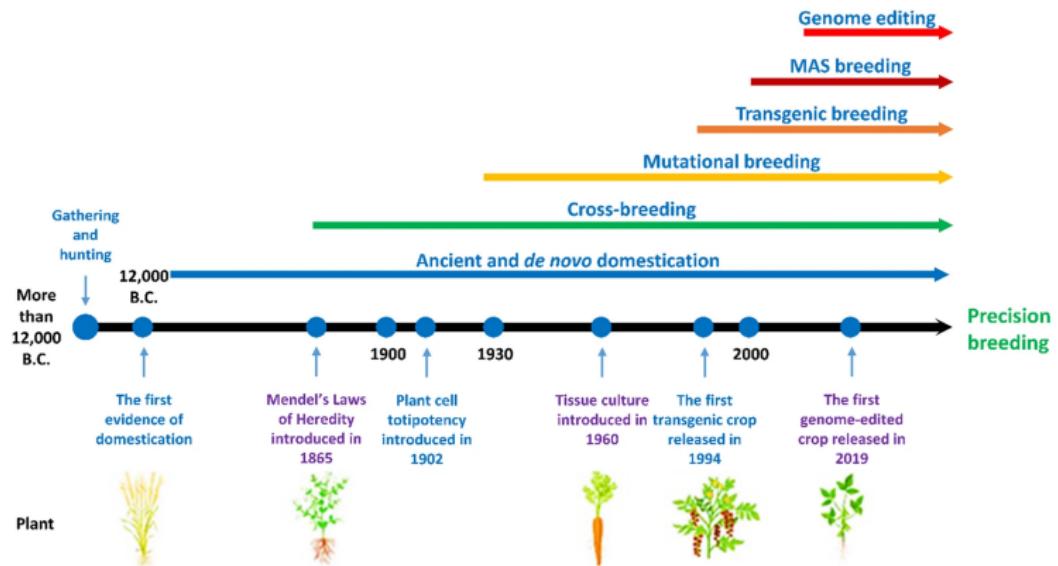
Questions?

Jennifer Doudna and Emmanuelle Charpentier



Do you know what they won the Nobel prize for?

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Genome Editing

- Genome editing is a type of genetic engineering using “molecular scissors”
- These “scissors” create site-specific double-strand breaks **at desired locations** in the genome.
- The induced double-strand breaks are repaired with errors, resulting in targeted mutations ('edits').

Genome Editing

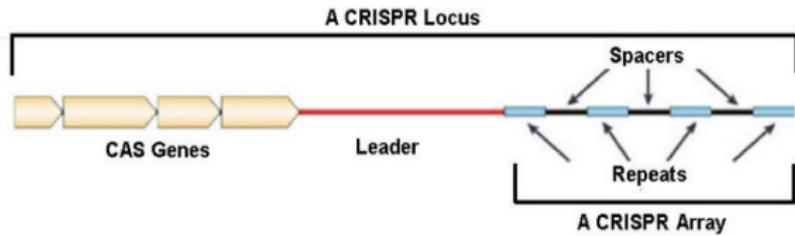
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Limitations of GM not shared by genome editing

- ~~The insertion of a new gene may not occur~~
- ~~No control on targeting DNA locations~~
- ~~One gene at a time~~
- ~~Expensive and slow~~
- Under strict regulation

CRISPR-Cas9

Genome editing is typically achieved using the CRISPR/Cas system



Clustered Regularly Interspaced Short Palindromic Repeats

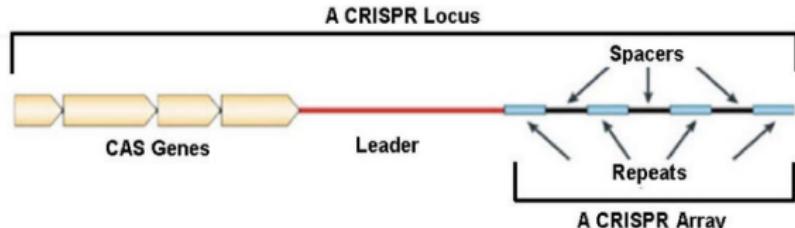
The CRISPR/Cas system is a bacterial immune system that confers resistance to foreign genetic elements and provides a form of acquired immunity

Here's a simple video overview:

https://youtu.be/6tw_JVz_IEc?si=_H2TrKqr5rSOpEL

CRISPR-Cas9

Genome editing is typically achieved using the CRISPR/Cas system



Clustered Regularly Interspaced Short Palindromic Repeats

A CRISPR locus:

- Encodes the Cas9 protein (the “scissors”)
- Transcribes a guide RNA to find the target

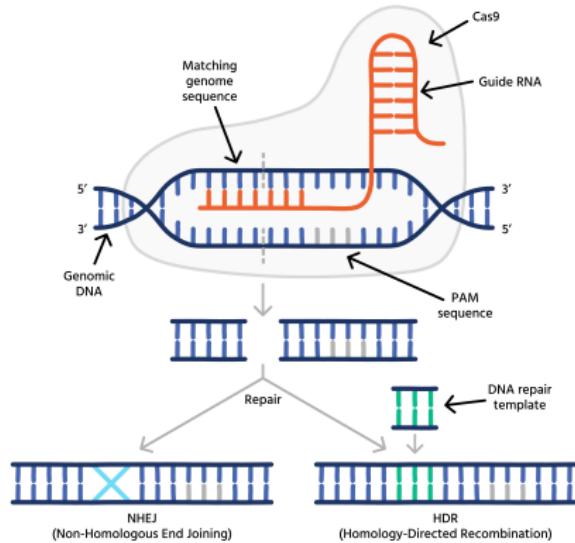
The two components form a complex to find and destroy the target DNA

Here's a simple video overview:

https://youtu.be/6tw_JVz_IEc?si=_H2TrKqr5rSOpEL

CRISPR and Gene Editing

- CRISPR/Cas9 induces a double strand break at a specific DNA sequence
- Adding a synthetic “guide” can direct the system to make arbitrary cuts
- Add a custom exogenous DNA template for DNA repair to incorporate into the host’s genome
- Can be multiplexed, editing numerous gene regions at once



By *editing* DNA rather than inserting it, a wider range of changes are made possible

Unintended Side Effects

Genome editing with CRISPR/Cas9 can cause DNA deletions and rearrangements near the target site on the genome

A problem for clinical applications, maybe less so for in the context of plant breeding

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NEWS | 16 July 2018

CRISPR gene editing produces unwanted DNA deletions

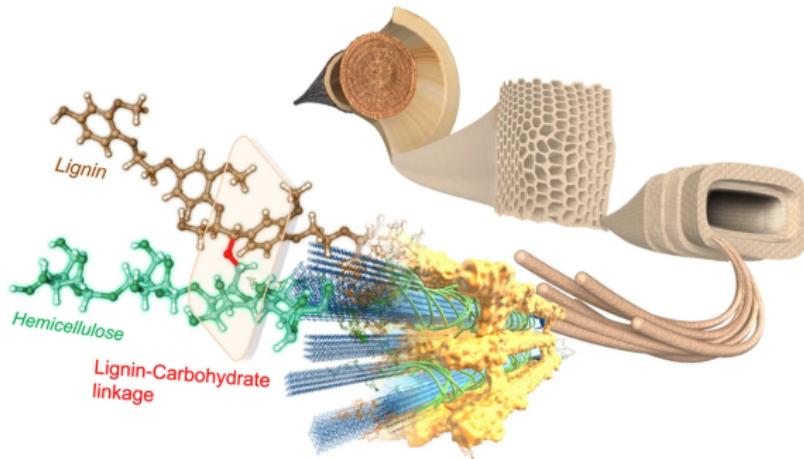
DNA-cutting enzyme used for genetic modification can create large deletions and shuffle genes.

Individuals with deleterious side effects from CRISPR could simply be removed from circulation

Put ethical, ecological and economic concerns aside for the moment...

What are some ways that genome editing be used in forest trees?

Lignin Removal



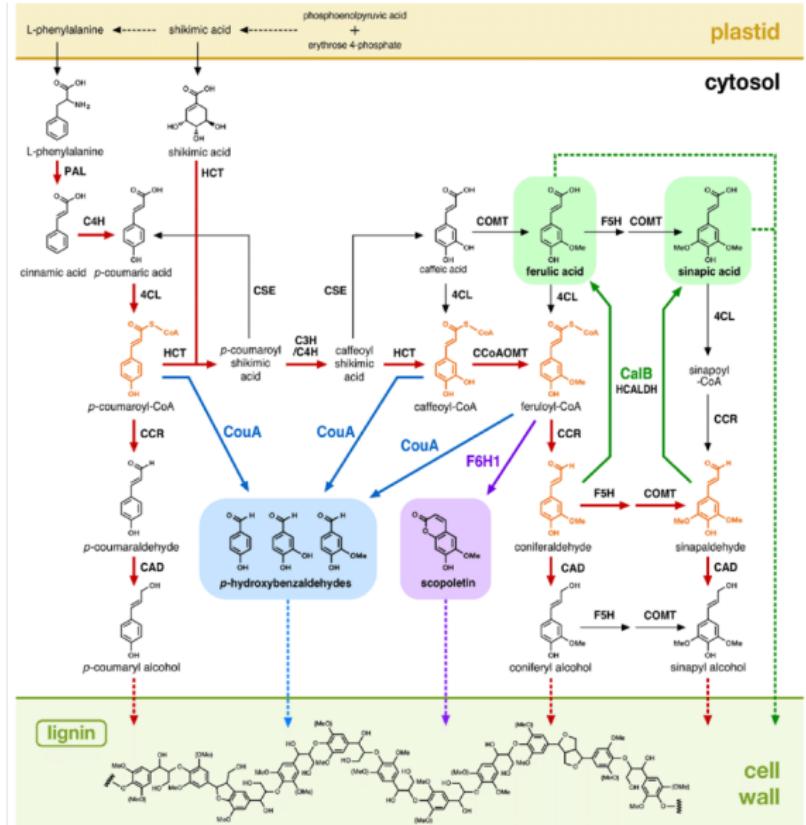
Lignin is one of the most abundant polymers on earth

Removal of lignin from pulp is a key part of paper manufacture

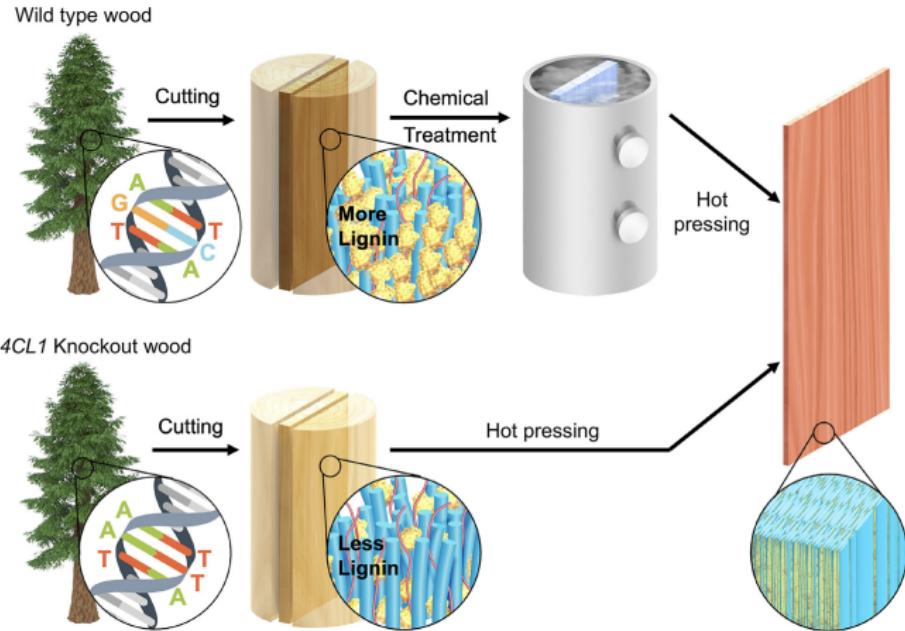
High density engineered wood can be made from wood that has been treated for reduced lignin

The process of delignification consumes energy and generates toxic byproducts

Lignin Biosynthesis

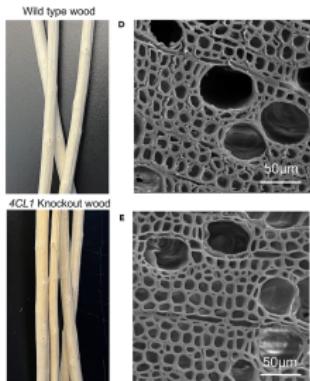


Genome Editing to Reduce Lignin Content



The strength exhibited by this engineered wood is similar to that of aluminium used in aircraft wings!

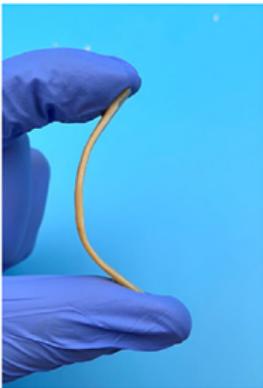
Genome Editing to Reduce Lignin Content in Poplar



Wild type wood

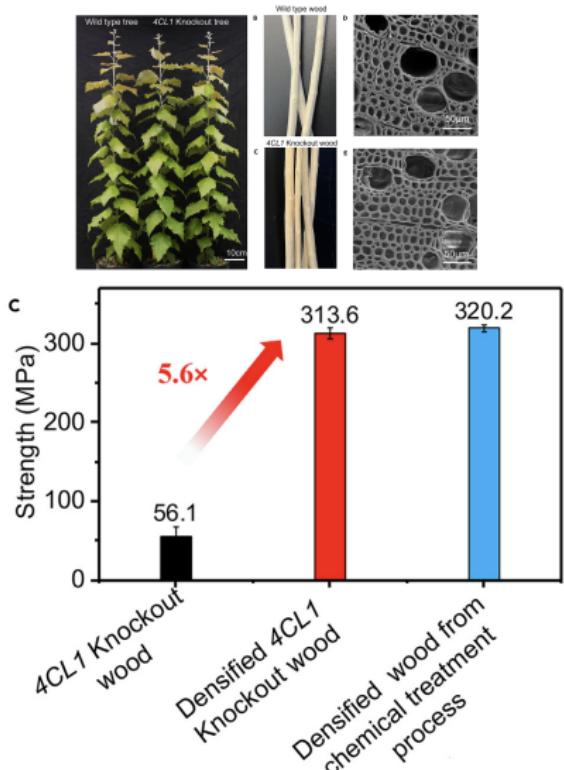


4CL1 Knockout wood



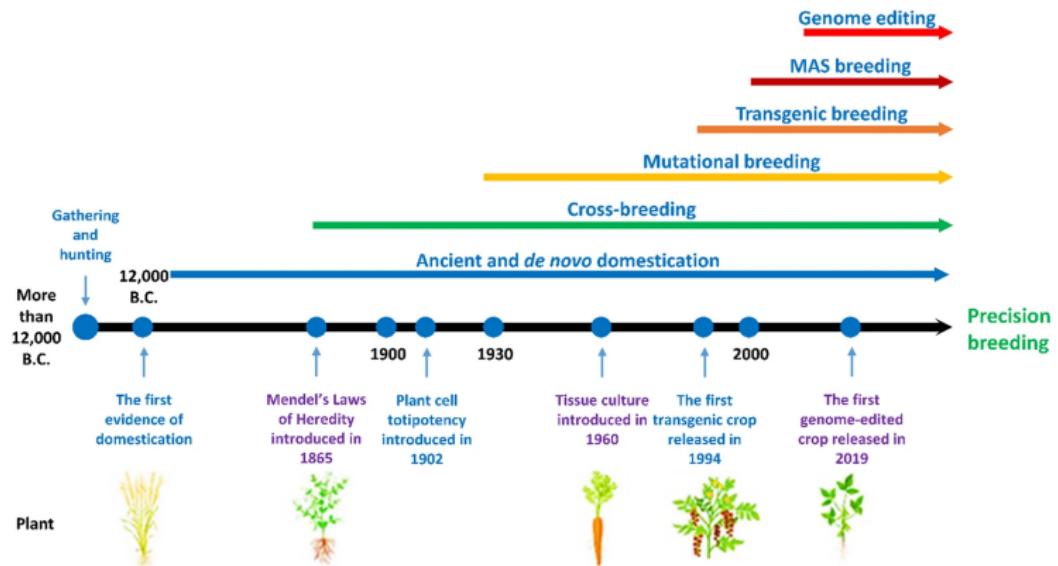
- Liu et al (2024) used genome editing to knockout 4CL1 (a core gene involved in Lignin biosynthesis)
- 4CL1 knockouts had many similar properties to wild-type trees
- *BUT*, with a 12.8% reduction in lignin content!

Genome Editing to Reduce Lignin Content in Poplar



- Densified wood from 4CL1 knockout individuals had similar tensile strength to wild-type trees
- **WITHOUT** the delignification step
- Imagine the possibilities!!

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Comparing Different Approaches



Selective Breeding

Mutation Breeding

Transgenic Breeding

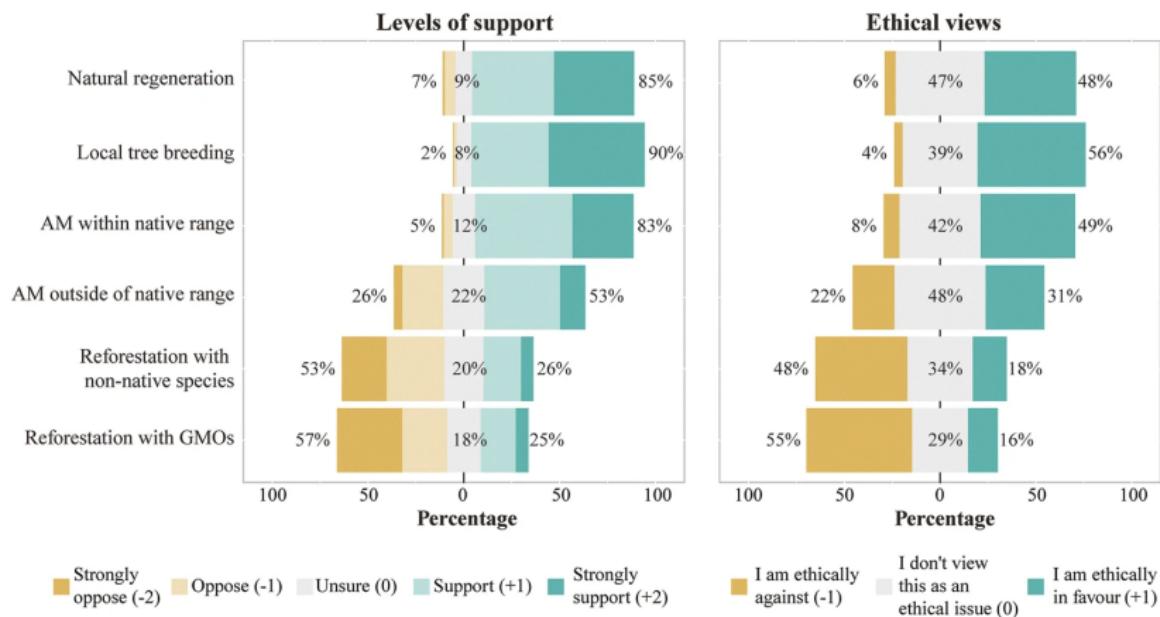
Genome Editing

Think about the different approaches:

- How long do they take?
- What steps are involved?
- How would the outcomes vary?

- Are different traits more/less amenable to different methods?
- How long do they take?
- Do you think people would have ethical concerns about the different methods?

The Public's Views



Learning Outcomes

- Introduction to genetic modification and genome editing
- Methods of genetic modification and genome editing
- Application of these in forest biology

A Question to Think About...

Both transgenesis and genome editing require us to choose specific genetic changes to make (e.g. gene to introduce, DNA to alter)

In the last lecture we found that in many cases we don't know the specific polymorphisms that underly genetic variation

How could you use genetic/genomic information to accelerate breeding even if you don't know the genes involved?