Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

Canada

Forage Breeding for the Future

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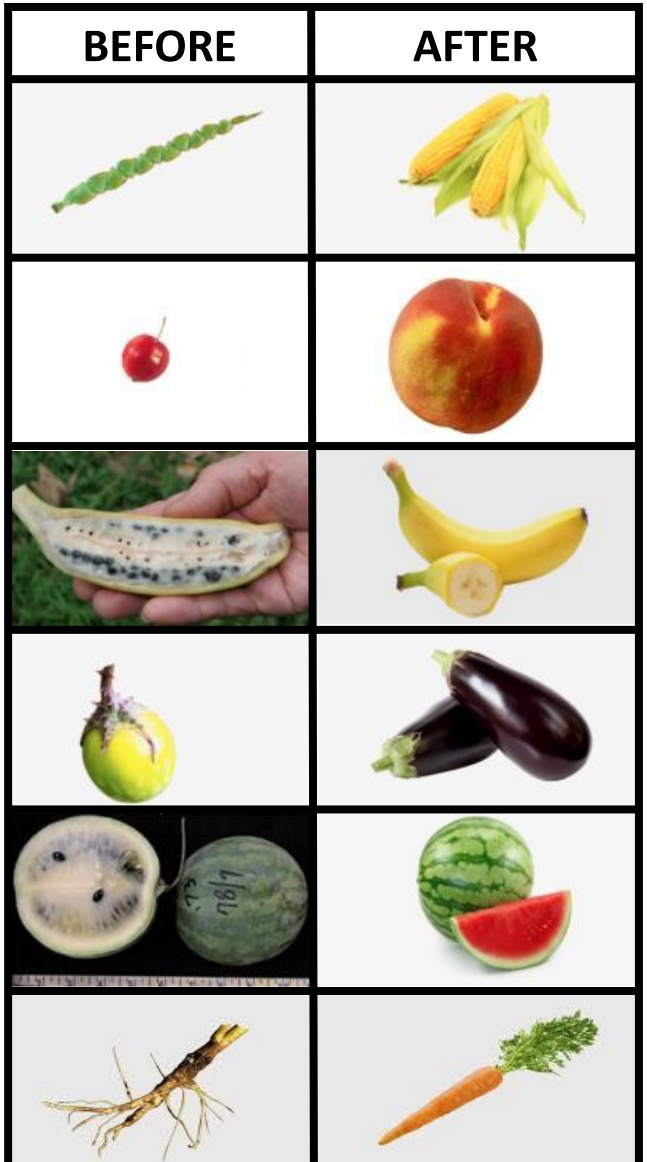
November 15, 2018

Plant breeding – a new thing?



Derived from: www.phytocode.org

- Around 13,000 years ago, humans began their own ‘breeding program’ that transformed wild plants into **domesticated** crops that better suited their needs

- The food that we grow and eat today did not always look and taste like it does now!

- Humans actively interfered with and manipulated crop evolution through selection
  - initially inadvertent
  - a very slow process
  - allowed improvement of numerous traits

New trait = genetic change

- Involved the ‘improvement’ of traits that were important at that time:
  - more robust plants
  - enlargement of fruits and/or grains
  - better flavour
  - synchronized flowering time
  - loss of seed dispersal

Source: blogs.ucdavis.edu

- Traits that need improving today are very different – driven by changing human needs and agricultural conditions

- Each new trait derives from a genetic change

Source: www.gmoanswers.com

We’ve been tweaking the genetics of our favourite crops for millennia!

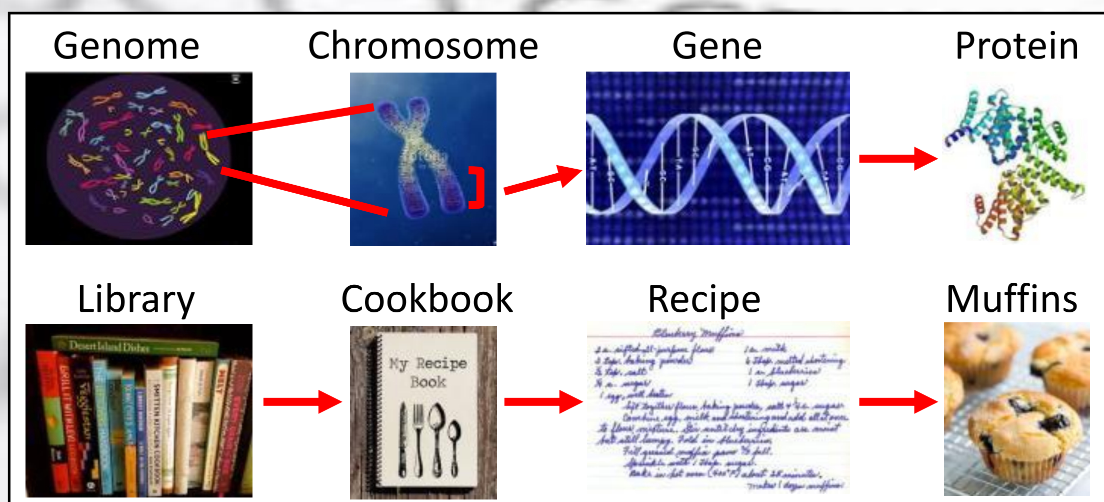
A little bit about genetics…..

- Every cell in a given individual contains the same DNA
  - made up of 'alphabet' of four nucleotides – A, T, C and G

- A sequence of these four 'letters' makes up a gene
  - only difference between different genes is the order of the four nucleotides
  - each gene has a different 'meaning' – they translate into different proteins, which are responsible for different traits

- Many genes are placed alongside one another to form chromosomes

- Living creatures have thousands of genes that together make up their genome



- A genetic change (mutation) can mean the substitution of one nucleotide for another, or bigger deletions, insertions or rearrangements – occur naturally!

- Effect of mutation = new trait

History of crop breeding

'Non-GM':

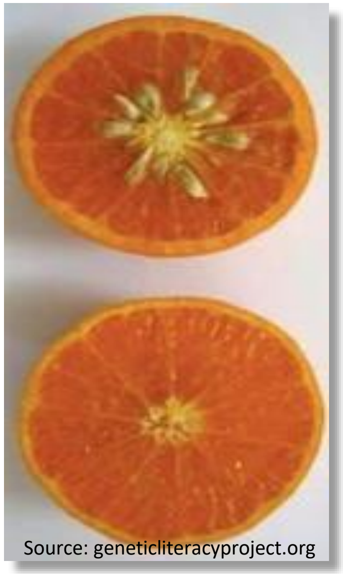
Selective breeding:
- ~ 13,000 years ago
- Select plants with naturally occurring mutations

Cross-breeding:
- ~ 300 years ago
- Cross plants to obtain desired genetic trait

Mutagenesis:
- ~ 80 years ago
- Use chemicals and radiation to induce mutations in plants

Almost everything we eat

e.g. pears, apples, grapefruit, rice, mint and some bananas


Source: geneticliteracyproject.org

'GM':

Transgenics/cisgenics/RNAi:
- ~ 35 years ago
- Transfer gene from any organism into crop

e.g. corn, cotton, papaya, soybeans, squash, canola, alfalfa


Source: npr.com

'GM'/'non-GM':

Genome editing:
- ~ 10 years ago
- Precise genetic changes

e.g. canola, more to come soon!


Source: semanticscholar.org

All of these measures, whether 'GM' or 'non-GM', involve genetic changes!

Let’s talk about GMOs……

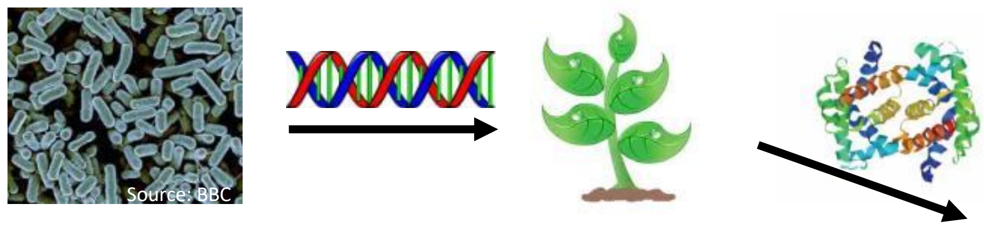
- Many misunderstandings and misconceptions about what GMOs actually are.

- Umbrella term that covers a range of very different molecular breeding technologies, which in some cases yield genetic changes that are no different than what you could achieve using conventional breeding (‘non-GM’).

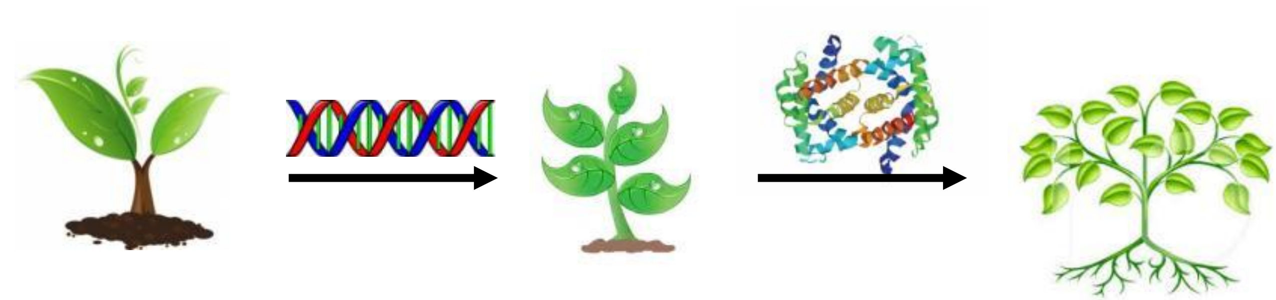
Yet another unauthorized experiment in genetically-modified food

Transgenic, cisgenic, RNAi (GM)

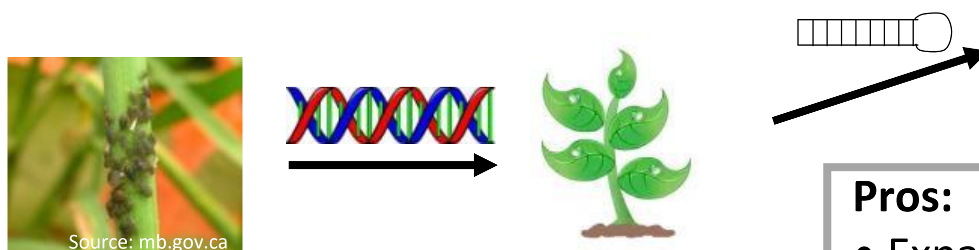
Transgenic
'Foreign' protein-coding gene



Cisgenic
Protein-coding gene from close relative



RNAi
Non-protein-coding DNA



These methods of achieving genetic changes are very different!

Should be considered based on target gene, source, and whether it produces a new protein (toxicity, allergenicity)

Pros:
- Expand gene pool
- Fast and easy
- Specific

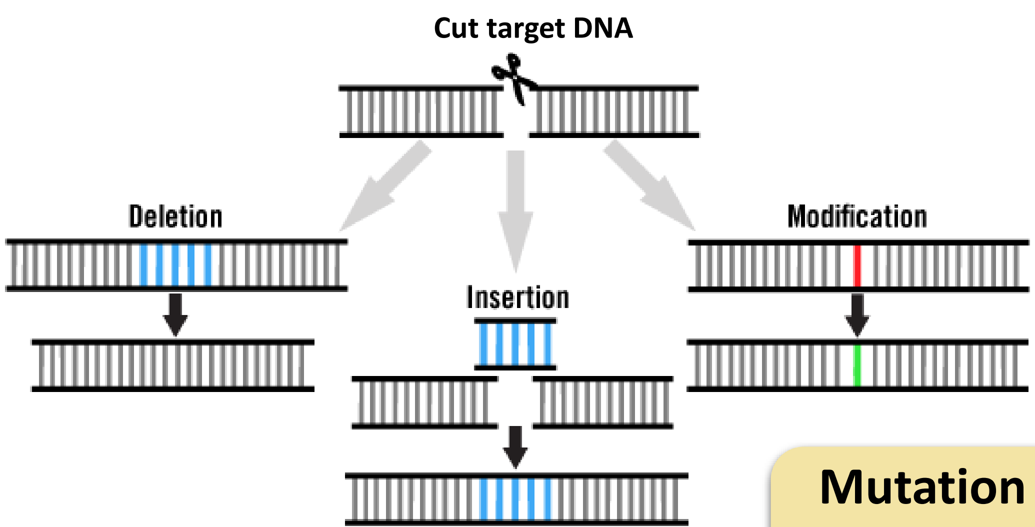
Cons:
- Acceptance low (GM)
- Safety/environment

Genome editing (GM/non-GM)

A new breeding technology – a tool that has the potential to take breeding to a new level

Precision 'gene surgery'

- Introduce protein that acts as molecular scissors and is targeted to a specific genetic site



Source: mirusbio.com

- Cuts DNA at a precise, pre-determined location

- Cell itself repairs the cut, resulting in small mutation

Target gene is 'knocked out'

Mutation can be indistinguishable from that occurring naturally or as a result of induced mutagenesis (which is 'non-GM')

Genome editing: GM or not?

Increasing number of crop species altered using genome editing that the USDA has stated will not be regulated

Source: Pacher and Puchta (2017) Plant J 90:819-833

Canola:
- herbicide resistant (Cibus)
  - *already on market in Canada and USA*

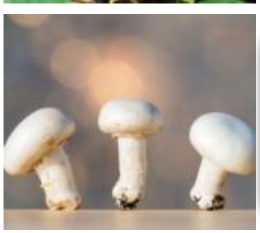
Rice:
- disease resistance (Iowa State U)

Alfalfa:
- low-lignin (Calyxt)

Soybean:
- high oleic acid (Calyxt)
- low linoleic acid (Calyxt)
- stress tolerant (USDA)

Wheat:
- PM resistance (Calyxt)

Tobacco:
- low-nicotine (NCSU)



Mushroom:
- non-browning (Penn State)

Potato:
- non-browning (Calyxt)
- low acrylamide (Calyxt)

Green foxtail:
- late flowering (Donald Danforth Plant Science Center)

Corn:
- waxy (Dupont)
- high yield (Benson Hill)
- low phytate (Dow)
- disease resistant (Dupont)

Camelina:
- high oil (Yield10)

Flax:
- herbicide resistant (Cibus)
  - *expected to be on market by 2019*

Genome editing: GM or not?

Globally, things are less clear, but discussions are ongoing

Lack of cohesion in terms of regulatory policies surrounding gene edited crops:

- US, countries in South America, Japan will not regulate, not ‘GM’
- EU will regulate, are ‘GM’
- Canada regulates depending on the trait, could be ‘PNT’
- Many countries are still undecided

What constitutes a ‘GM’ crop?????

Cost and time for de-regulation of a ‘GM’ crop is prohibitive, and therefore often only large corporations are capable of commercialization

It is essential that we develop coordinated, efficient, fact-based and product-focused regulatory systems to avoid loss of innovation in agriculture and delays in the diffusion of these new technologies

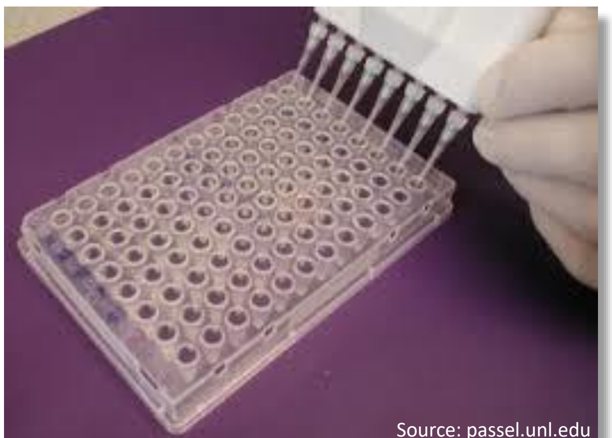
How do we apply this to forages?

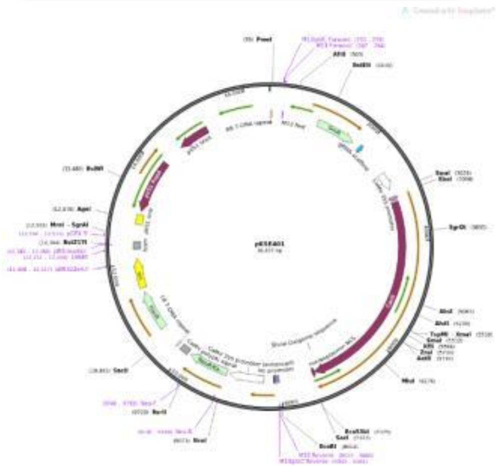

Source: hindawa.com

We apply molecular genetic techniques and biotechnology to advance the pace and ease of forage improvement

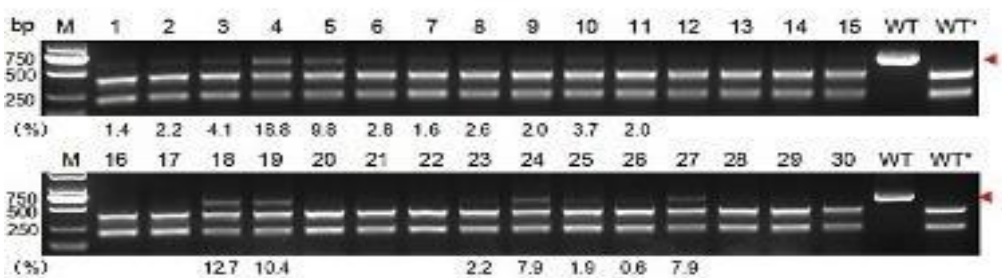
Perennial legumes
- **Alfalfa**
- **Sainfoin**


Source: dreamstimecom


Source: passel.unl.edu



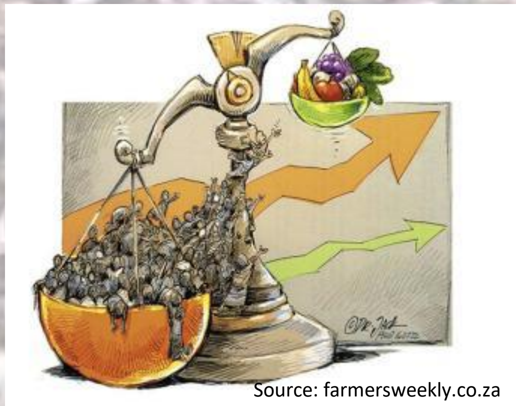

Source: naturesseed.com



Source: Cai et al. 2015. PLOS One 10:e0136064

Forage traits for today

1) Population growth



Estimated that we need to increase food production by 70% in the next 40 years to keep up with population growth

Source: fao.org

- Improve biomass yields
- Improve stress tolerance
- Increase leaf lipid content
- Improve digestibility
- Improve pest resistance
- Improve disease resistance
- Improve nutrition
- Reduce health issues
- Reduce fertilizer use

2) Climate change

In warm regions, crop yields drop ~3-5% with every 1^{o}C increase in temperature

Source: Burney and Ramanathan 2014

We need to come up with ways to grow more forages that are more efficient with better nutrition on less land in a harsher environment – in a sustainable and safe manner

Finding new sources of genetic variation

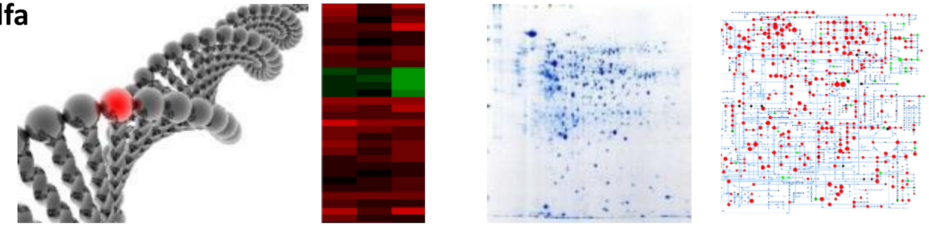
1) **Identify candidate genes that when modulated might elicit the desired trait**
   - from previous studies in other plant species
   - from alfalfa's wild relatives, which although not agronomically useful, often possess certain beneficial traits

Drought treatment:

Wild relatives **Cultivated alfalfa**

Assess plants for traits of interest
- drought tolerance
- salt tolerance
- pest resistance

Use comparative 'omics' approaches to identify candidate genes
- find out what the genetic basis of these beneficial traits are

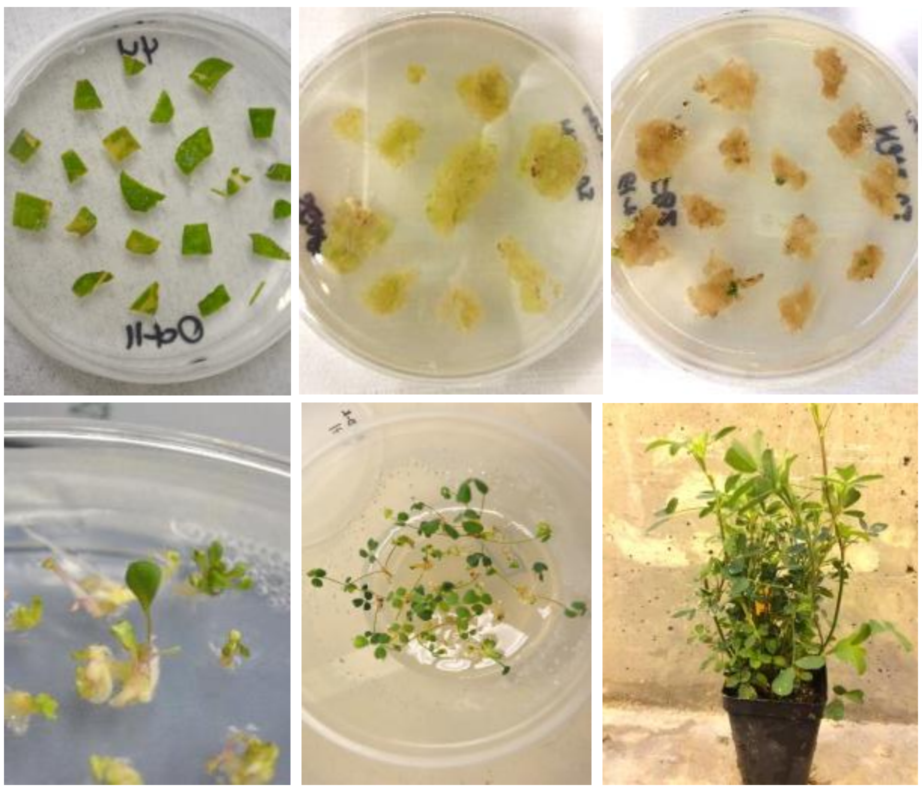


Genomics DNA | **Transcriptomics** RNA | **Proteomics** Protein | **Metabolomics** Metabolites

Validating candidate gene function

2) Validate the function of identified candidate genes (or those identified previously in other plant species) using transgenic and RNAi approaches

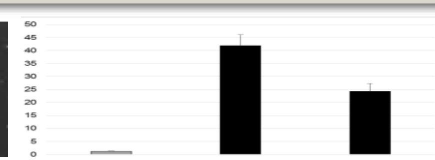
- proof-of-concept work



Introduce genetic cassette into alfalfa

- use *Agrobacterium*-mediated transformation

Confirm presence of genetic cassette



Assess plants for trait of interest

Developing germplasm – genome editing

3) Use genetic knowledge to generate improved alfalfa germplasm

Use CRISPR-Cas9-mediated genome editing

1) Can we increase efficiency?

2) Can it be used directly in adapted cultivars?

3) Can we achieve it without the initial use of a transgene?

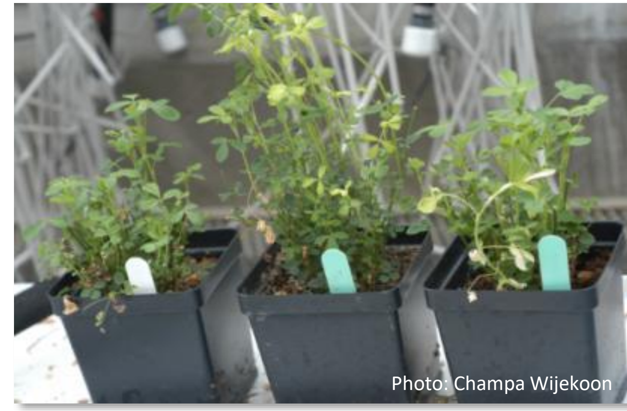
Source: Li et al. 2016. Front Plant Sci 7:12217

Knock-out gene of interest to elicit desired trait

Developing germplasm - TILLING

Screen candidate genes for mutations in mutagenized alfalfa population (non-GM)

Targeting Induced Local Lesions IN Genomes (TILLING)



First step is to generate a large mutagenized population (3000-5000 plants)

- we use ethyl methanesulfonate (EMS) as the mutagen

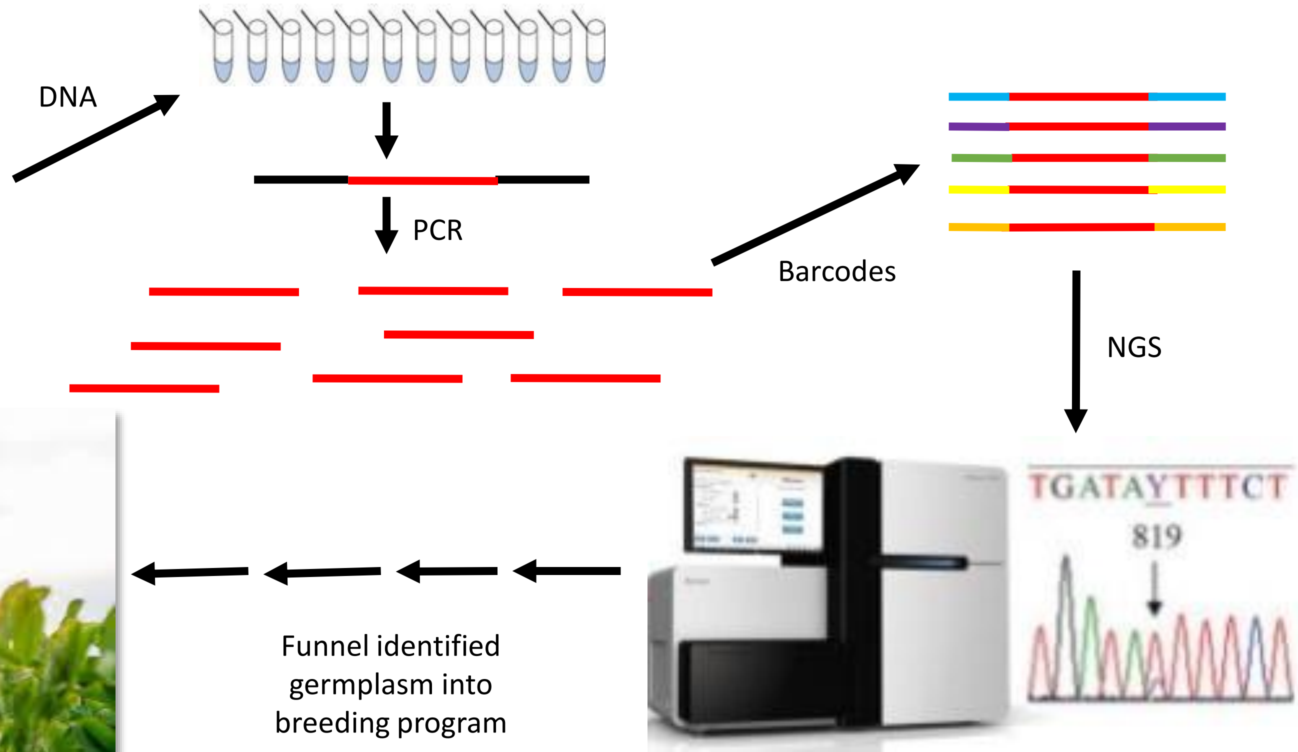
Treat plant tissue with chemical or physical mutagen

In those that survive, often induces hundreds of random, unknown mutations

If you're lucky, one will have the mutation you're looking for

Developing germplasm - TILLING

Photo: Rebecca Orlando



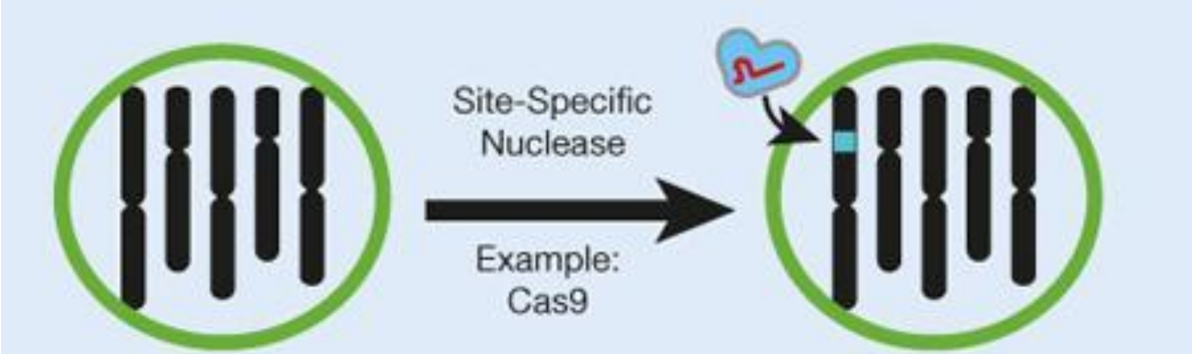
- **Self-incompatibility of alfalfa makes this a bit more challenging**
  - however, it's being done in other self-incompatible plants, including radish and forage grasses

Kohzuma et al. 2017. Breed Sci 67:268
Manzanares et al. 2016. N Biotechnol 25:594

Mutagenesis vs. genome editing

Induced mutagenesis (non-GM):

Genome editing (GM or non-GM?):



Source: Shih 2017 CEP

Source: illinoiswildflowers.info

Together with conventional breeding, molecular breeding technologies have the potential to be instrumental in a sustainable future of forage and livestock production

Source: wikihow.com

Acknowledgments

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Thank you!