Excluded methods discussion

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Consumers Union

NOSB meeting

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Codex Alimentarius

- **Food safety standard setting organization of the United Nations. Joint World Health Organization (WHO) and Food and Agriculture Organization (FAO)**
- **Set up in 1963 to help developing countries with range of voluntary standards, guidelines and recommendations associated with food safety**
- **1996 Uruguay Round of General Agreement on Tariffs and Trade sets up World Trade Organization (WTO)**
- **Codex standards, guidelines and recommendations considered "trade legal," and are referenced by WTO**

Codex Alimentarius

- **Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology (2000 – 2003; 2005-2008)**
- **Hosted by Japan**
- **Developed 4 key documents:**
- **CAC/GL 44 Principles for Risk Analysis of Foods Derived from Modern Biotechnology (2003)**
- **CAC/GL 45 Guideline for the Conduct of Food Safety Assessment of Foods Derived from Modern Biotechnology (2003, 2008)**
- **CAC/GL 46 Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms (2003)**
- **CAC/GL 68 Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals (2008)**

Codex Alimentarius: Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44—2003)

- "8. ***"Modern Biotechnology"*** means the application of:
  - i) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
  - ii) Fusion of cells beyond the taxonomic family,
- that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection" (Para 8, CAC/GL 44-2003)

Codex Alimentarius

- Technologies included under the Codex definition of "modern biotechnology," aka GE or GM:
  - Cisgenic, intragenic
  - Reverse breeding
  - ODM (oligonucleotide directed mutagenesis)
  - TALEN (transcription activator-like effector nucleases)
  - Meganucleases
  - ZFN (zinc finger nucleases)
  - CRISPR/Cas (clustered, regularly interspaced, short palindromic repeats)
  - RNAi
- Not included under Codex definition:
  - TILLING (targeting induced local leisons in genomes)

Osakabe Y and K Osakabe. 2015. Genome editing with engineered nucleases in plants. *Plant Cell Physiology* 56(3): 389-400

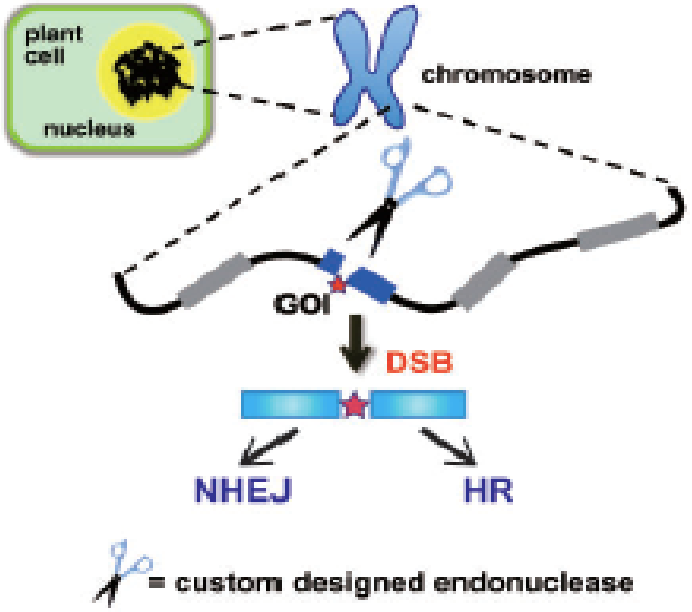

Fig. 1 Targeted genome editing in plants using engineered nucleases ('GEEN'). Engineered nucleases are used to induce a double-stranded DNA break (DSB) at a specified locus of the gene of interest (GOI). DSBs are repaired by either non-homologous end-joining (NHEJ) or homologous recombination (HR).

Osakabe Y and K Osakabe. 2015. Genome editing with engineered nucleases in plants. *Plant Cell Physiology* 56(3): 389-400

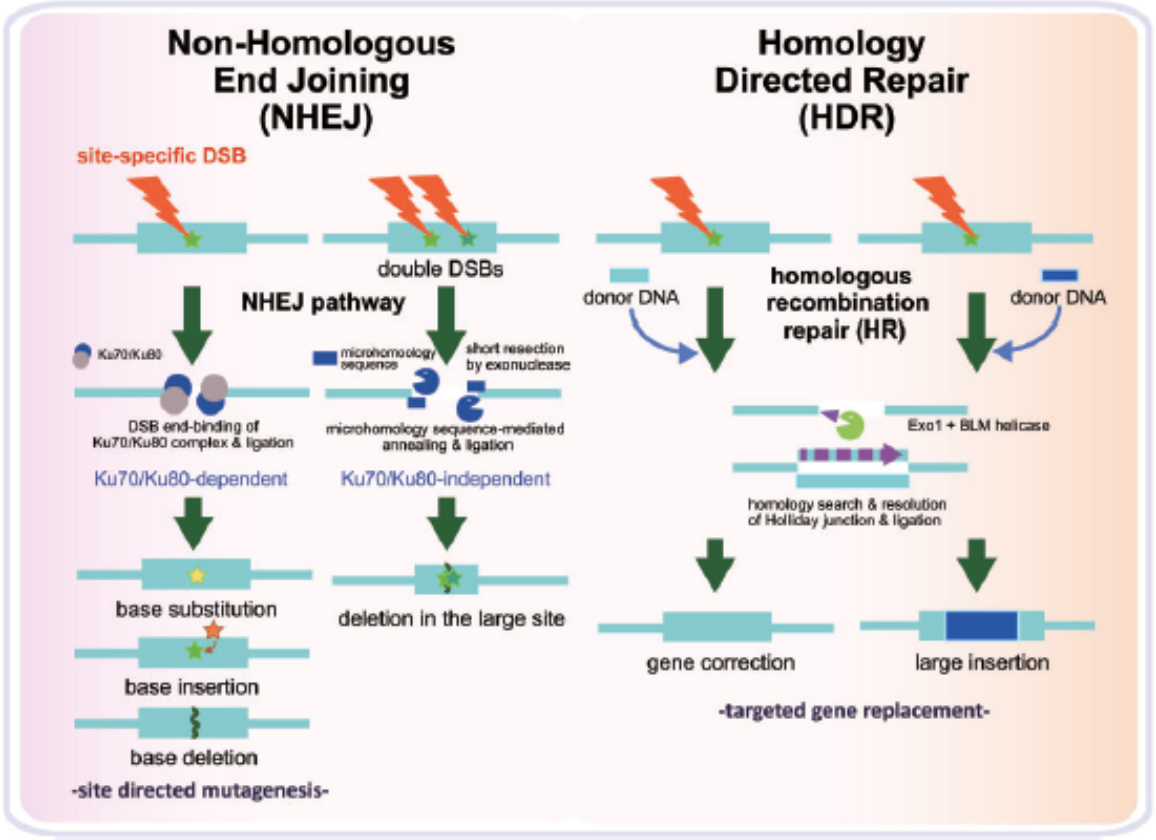

Fig. 2 DNA repair pathways involved in the repair of DSBs generated by engineered nucleases. The NHEJ-mediated pathway leads to the generation of variable insertion or deletion mutations and can be used for site-directed mutagenesis. HR with double-stranded donor DNAs leads to the creation of precise nucleotide substitutions or insertions and is used for targeted gene replacement.

ODM (oligonucleotide directed mutagenesis)

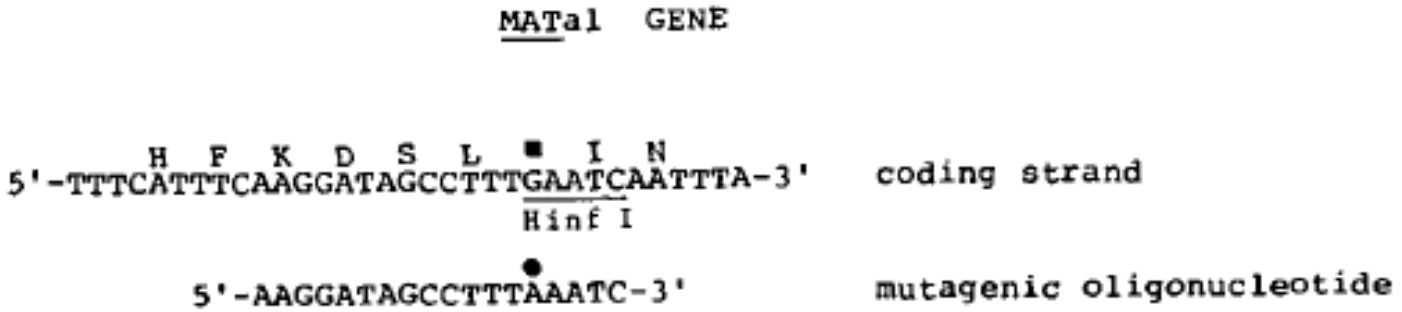

Figure 2. The DNA sequence of the coding strand of MATa1 in the region containing the inframe TGA codon. Below is shown the sequence of the oligonucleotide synthesized to change the TGA to TAA. The mutation destroys the Hinf I site in this region (underlined).

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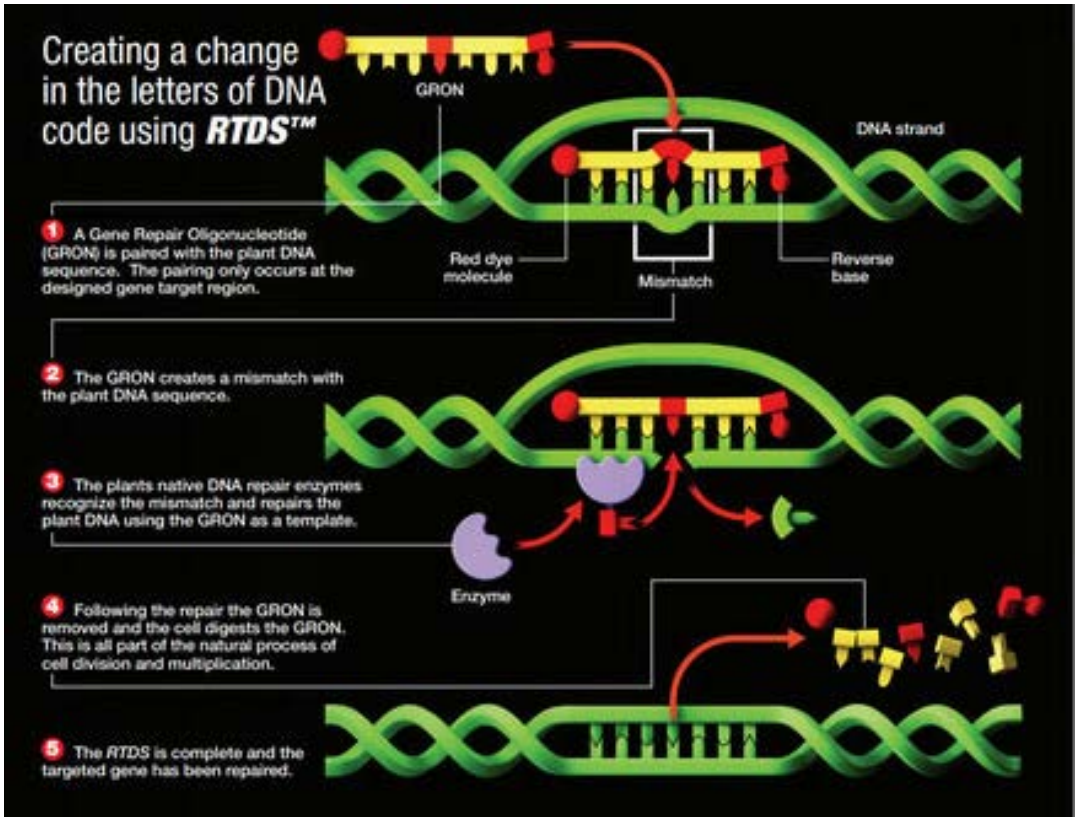

Creating a change in the letters of DNA code using RTDS™
GRON
DNA strand
1 A Gene Repair Oligonucleotide (GRON) is paired with the plant DNA sequence. The pairing only occurs at the designed gene target region.
Red dye molecule
Mismatch
Reverse base
2 The GRON creates a mismatch with the plant DNA sequence.
3 The plants native DNA repair enzymes recognize the mismatch and repairs the plant DNA using the GRON as a template.
Enzyme
4 Following the repair the GRON is removed and the cell digests the GRON. This is all part of the natural process of cell division and multiplication.
5 The RTDS is complete and the targeted gene has been repaired.

Osakabe Y and K Osakabe. 2015. Genome editing with engineered nucleases in plants. *Plant Cell Physiology* 56(3): 389-400

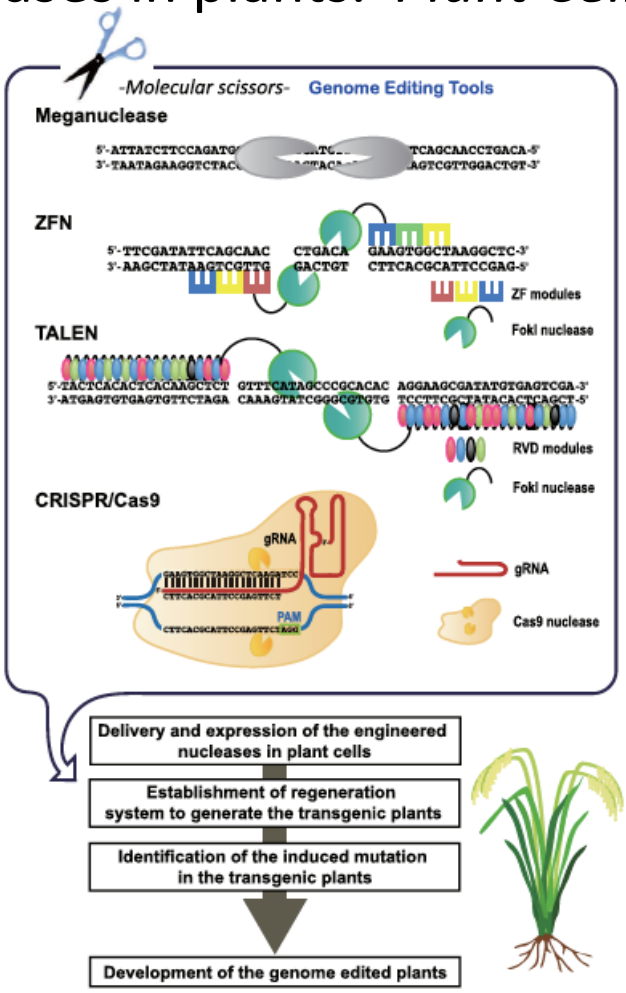
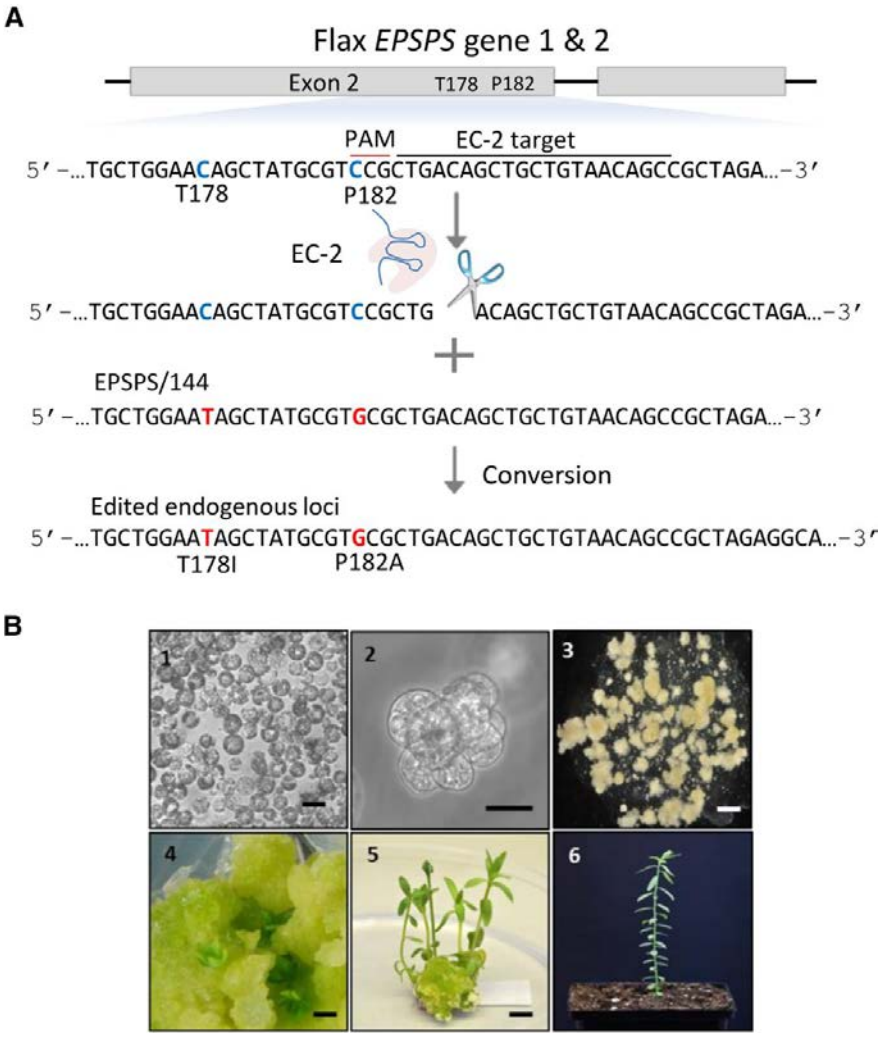


Fig. 3 Current methods in GEEN and an overview of the workflow of plant genome editing. Homing endonucleases/meganucleases (EMNs) recognize long (~20 bp) DNA sequences. *Fok*I nuclease is used as the DNA cleavage domain in ZFN, which bind the target DNAs via engineered C2H2-zinc finger (ZF) domains and in TALEN that recognize the targets by the engineered TALEs composed of RVD domains derived from the plant pathogen *Xanthomonas*. The CRISPR/Cas9 system utilizes RNA-guided engineered nucleases (RGNs) that use a short guide RNA (gRNA) to recognize DNA sequences at the target sites.

Approach used to target the EPSPS loci in flax.



Noel J. Sauer et al. Plant Physiol. 2016;170:1917-1928

Lee J et al. 2016. Designed nucleases for targeted genome editing. *Plant Biotechnology Journal* 14: 448-462.

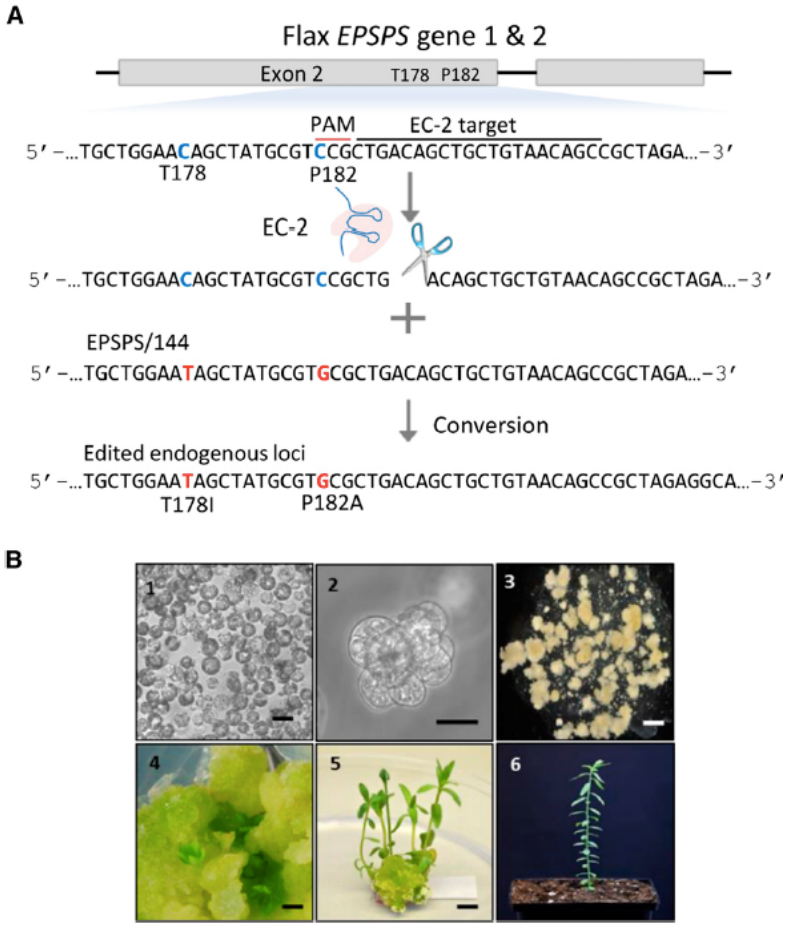
Table 1 Comparison of three classes of designed nucleases

	ZFN	TALEN	RGEN (CRISPR/Cas9)
Recognition site	18–36 bp per ZFN pair	30–40 bp per TALEN pair	22 bp (20-bp guide sequence + 2-bp protospacer adjacent motif (PAM) from *Streptococcus pyogenes*)
Restriction in target site	G-rich	Start with T	End with an NGG (NAG: lower activity) sequence
Success rate	Low	High	High
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)
Ease of engineering	Difficult	Moderate	Easy
Ease of multiplexing	Low	Low	High

ZFN, zinc-finger nuclease; TALEN, transcription activator-like effector nuclease; RGEN, RNA-guided engineered nuclease; CRISPR, clustered regularly interspaced short palindromic repeat; Cas9, CRISPR-associated protein 9; sg RNA, single-chain guide RNA.

Sauer NJ et al. 2016. Oligonucleotide-Mediated Genome Editing Provides Precision and Function to Engineered Nucleases and Antibiotics in Plants. *Plant Physiology* 170: 1917-1928

Figure 7. Approach used to target the EPSPS loci in flax. A, Target region of the *EPSPS* loci Thr-178 and Pro-182 of exon 2. The EC-2 protospacer is shown as a black line, and the PAM is shown as a red line. The nucleotides within the codons targeted for edit are in blue (ACA and CCG), and edited nucleotides are in red (ATA and GCG). The CCG→GCG edit disrupts the PAM, minimizing EC-2 activity on an edited gene. B, Stages in the flax genome-editing workflow. Image 1, Protoplasts (bar = 10 μm); image 2, microcolony at 3 weeks (bar = 50 μm); image 3, microcalli at 7 weeks (bar = 100 μm); image 4, shoot initiation from callus (bar = 0.5 cm); image 5, regenerated shoots (bar = 0.5 cm); and image 6, regenerated plant in soil.



FASTRACK BREEDING

Breeding, carried out in the field, is affected by climate, diseases, and insect pests.
Not every year is successful.
Due to this year's cold spring temperatures we lost most of our hybridizations.

Pollination

Protecting pollinations

Field planting seedlings

THE PROBLEM:

- Fruit trees like peach, plum, apricot and cherry, need to grow for at least three years and sometimes more than seven years before they flower and fruit.

- That means for a breeder to improve a trait such as disease resistance, it is necessary to wait 3-7 years to see the results of each cross. Several to many generations are needed to produce an improved variety.

- In practice it can take anywhere from 15 -20 years to produce a new variety, longer when introgressing traits from unimproved genotypes.

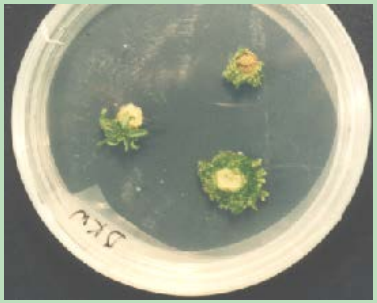
One solution:
Hire younger fruit tree breeders

Another Solution – FASTRACK

A breeding system that utilizes GE for producing generation cycles of one year or less but does not produce a GE final product.

We inserted the Flowering locus T gene from poplar trees into plum. The results were *striking*.

Plum transformation with early flowering (FT) gene from poplar: **July year 1**



Harvesting of fully ripe plum fruits with viable seeds in the greenhouse: **May year 2 (10 months)**

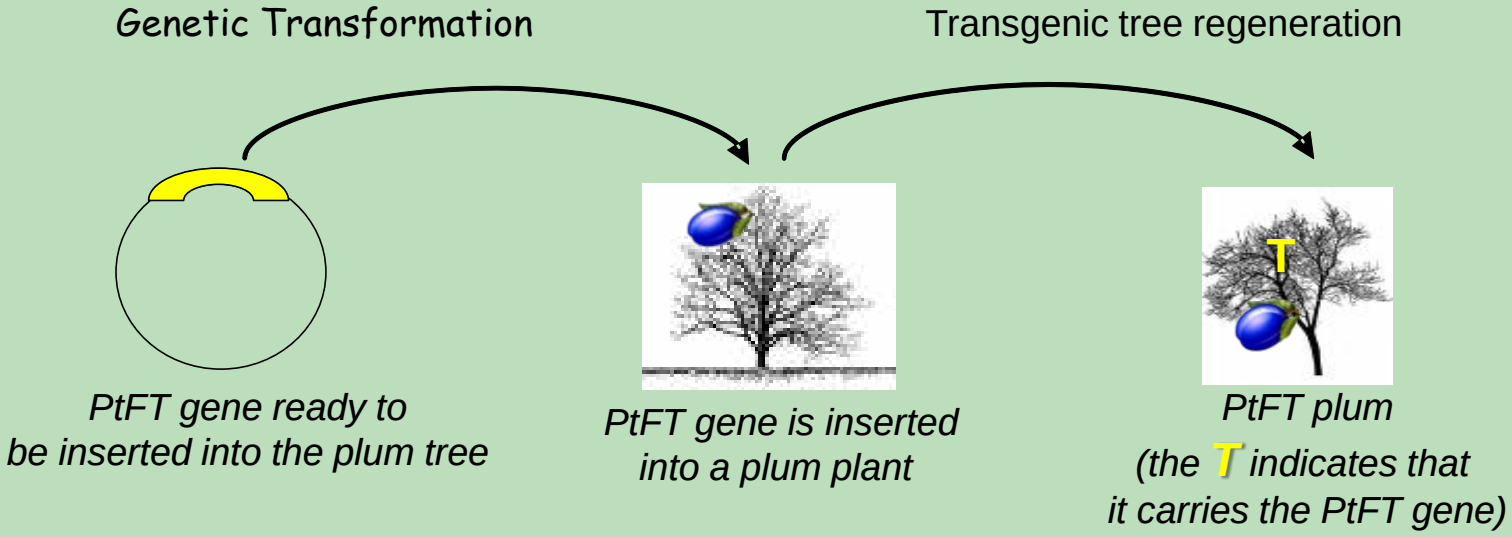
One year from gene transfer to ripe fruit!

Then, from seed to seed in less than 1 year

Instead of 4 years or more

How does it work?

The PtFT gene is inserted into a parent plum line through genetic engineering.



An early flowering plum parent tree is produced that can flower and fruit within a year.

Conventional Breeding program with 4yr/ generation cycle

Using PtFT early flowering Plums for breeding disease resistant plum varieties

FasTrack Breeding program

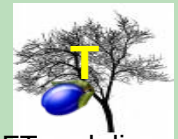
PtFT plum

X

'disease resistant' plum

Markers?

25% of seedlings PtFT and disease resistant – select for fruit traits



PtFT and disease resistant

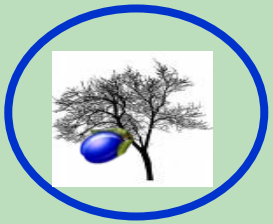
back X cross

High fruit quality plum

Markers?

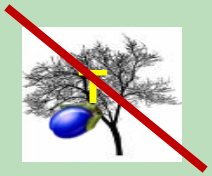
25% of seedlings PtFT and disease resistant – select for fruit traits

Two additional backcrosses or modified backcrosses with high quality types



disease resistant high quality (not GE)

PtFT+ disease resistant



PtFT + disease susceptible

disease susceptible

20 years for 3 BC

8 years for 3 BC

Now must wait 4 years for first fruit to check for variety potential.
Years of conventional backcross breeding have been saved.

Early Flowering clones in the greenhouse

Simultaneous Development of Flowers and Fruit

Six month old early flowering & normal fruiting transgenic plum

‘FasTrack’ Breeding Advantages

‘FASTRACK’

- 1 year generation time
- can make crosses and produce fruit year-round
- avoids winter and spring injury in the field
- reduced insect and disease pressures

CONVENTIONAL

- 4-6 year generation time
- one pollination/fruiting per year
- subject to seasonal climate extremes
- disease, insect, and weed pressures

The final products of FasTrack breeding not containing the transgenes are not considered different from those bred conventionally and could not be distinguished from conventional cultivars.

One reason why we need more rapid tree breeding - rapid influx of new pests and diseases

Citrus greening

Chestnut blight

Plum pox virus

Dutch elm disease

Hemlock woolly adelgid

African Fig Fly

Pine Shoot Beetle

Spotted Wing Drosophila

Emerald ash borer

Oriental fruit fly

Asian longhorn beetle

White pine blister rust

Citrus canker

Brown marmorated stink bug