2018 | THE ALMOND CONFERENCE

ALMOND BREEDING: IS THERE A ROLE FOR NEW GENETIC TECHNOLOGIES?

ROOM 312-313 | DECEMBER 4, 2018

California almonds® Almond Board of California

growing ADVANTAGE® The Almond Conference

AGENDA

- **Gabriele Ludwig**, Almond Board of California, moderator
- **Sebastian Saa**, Almond Board of California, moderator
- **Tom Gradziel**, UC Davis
- **Abhaya Dandekar**, UC Davis
- **Susan Jenkins**, UC Berkeley

ALMOND BREEDING: IS THERE A ROLE FOR NEW GENETIC TECHNOLOGIES?

Tom Gradziel

UC Davis

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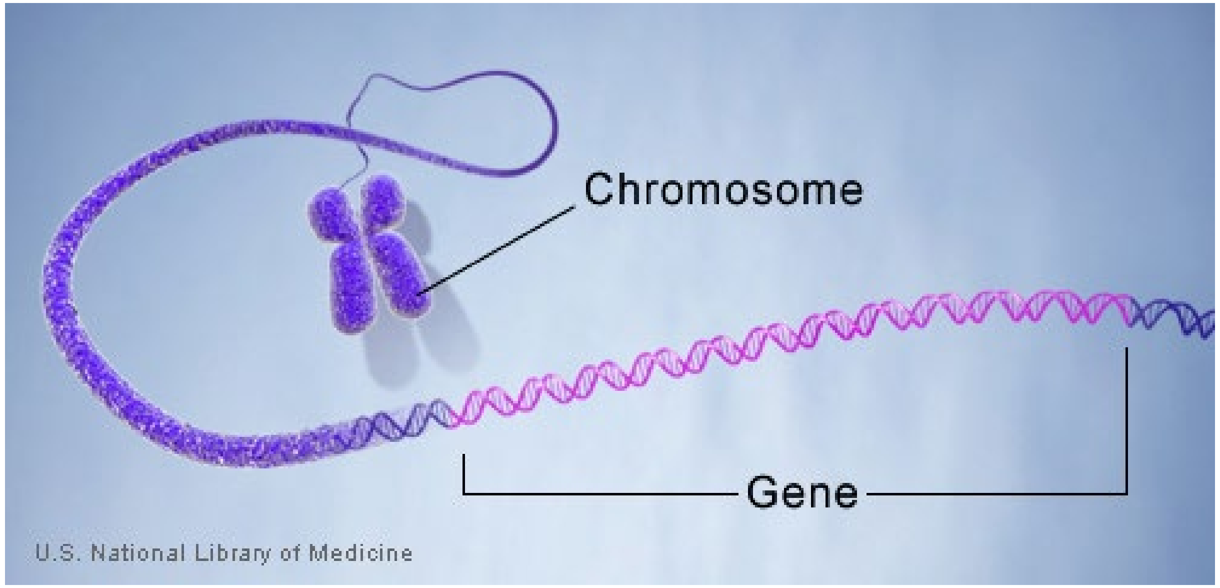
<u>Genetic engineering</u>:

benefits vs. risks

apprehensions vs.

opportunities



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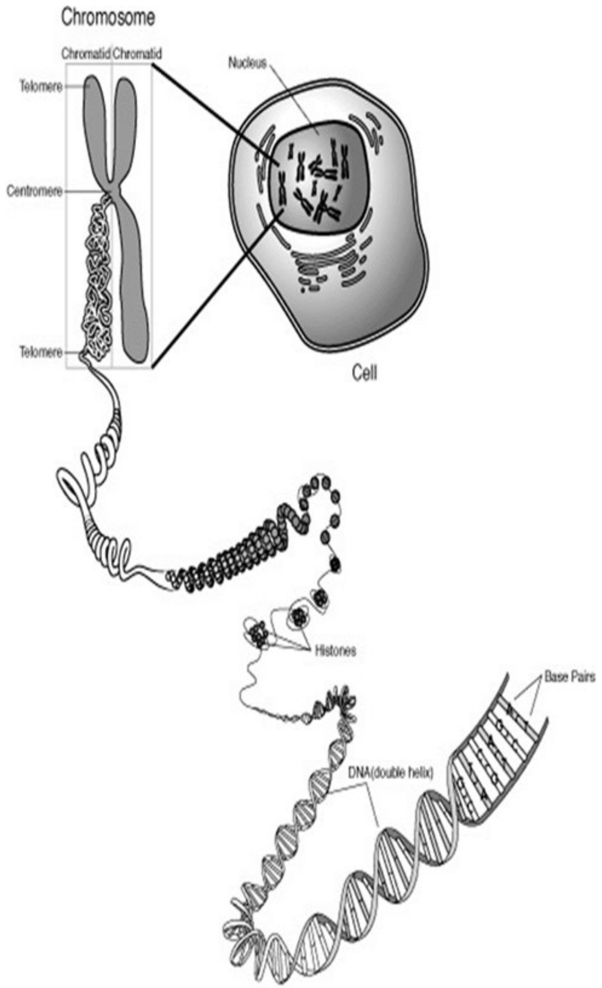
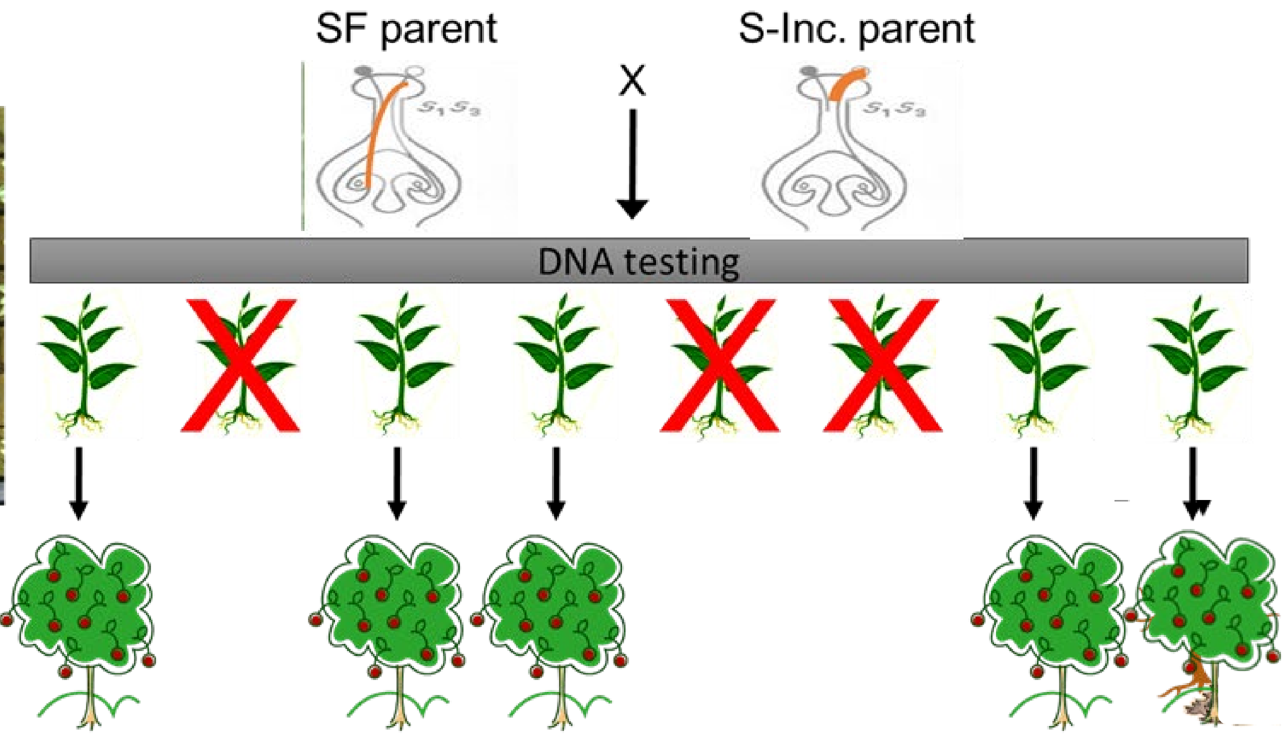
Genome sequencing



18.HORT35.Fresnedo-Ramirez
Nonpareil Genome Map and Annotation

Selection for Self-compatibility: DNA Marker-Based Approach

DNA test outcomes identify desired seedlings before field planting



RosBREED Combining Disease Resistance with Horticultural Quality in New Rosaceous Cultivars

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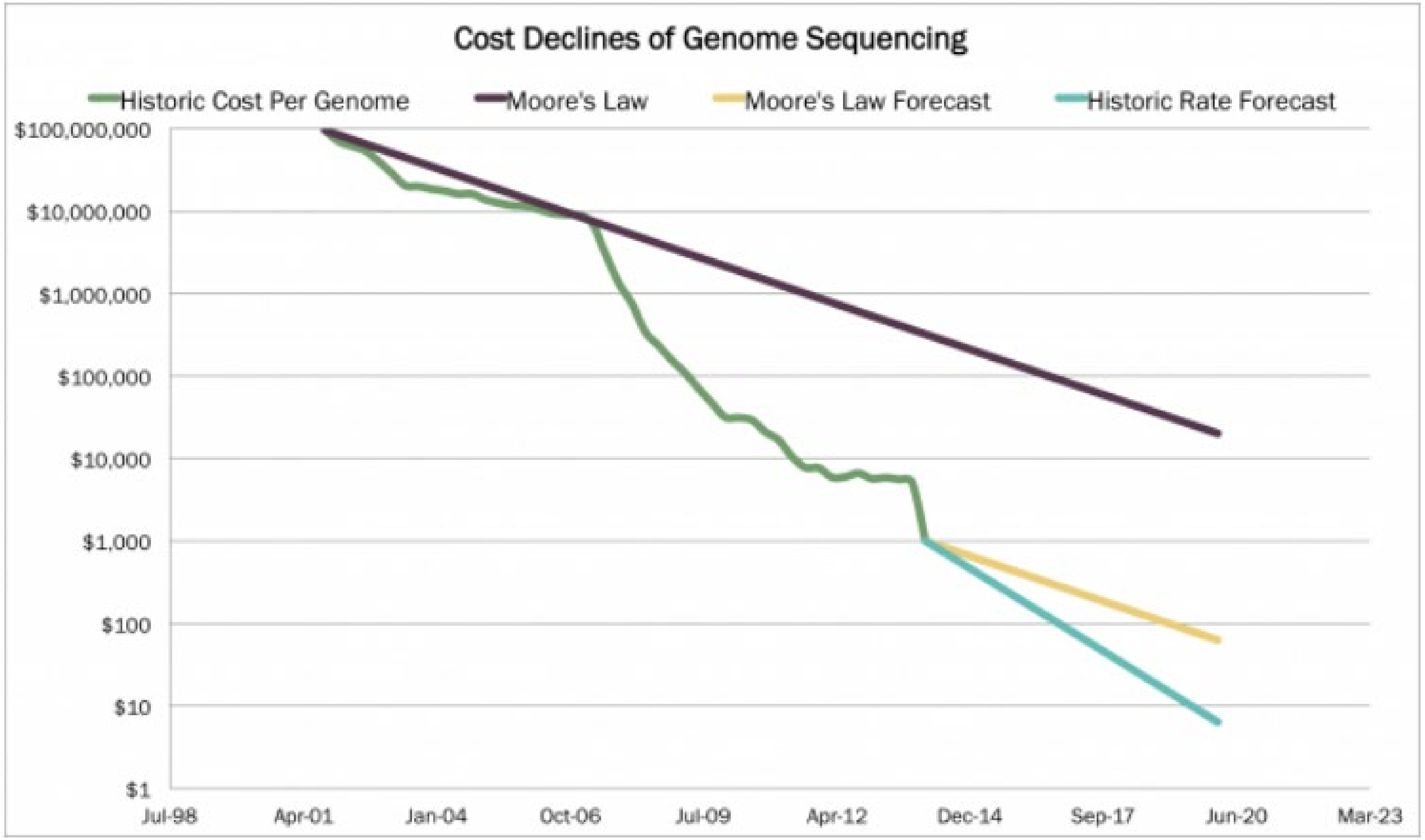
The first whole human genome sequencing cost roughly $2.7 billion in 2003. In 2006, the cost decreased to $300,000. In 2016, the cost decreased to $1,000.

Current whole genome costs as low as $700.



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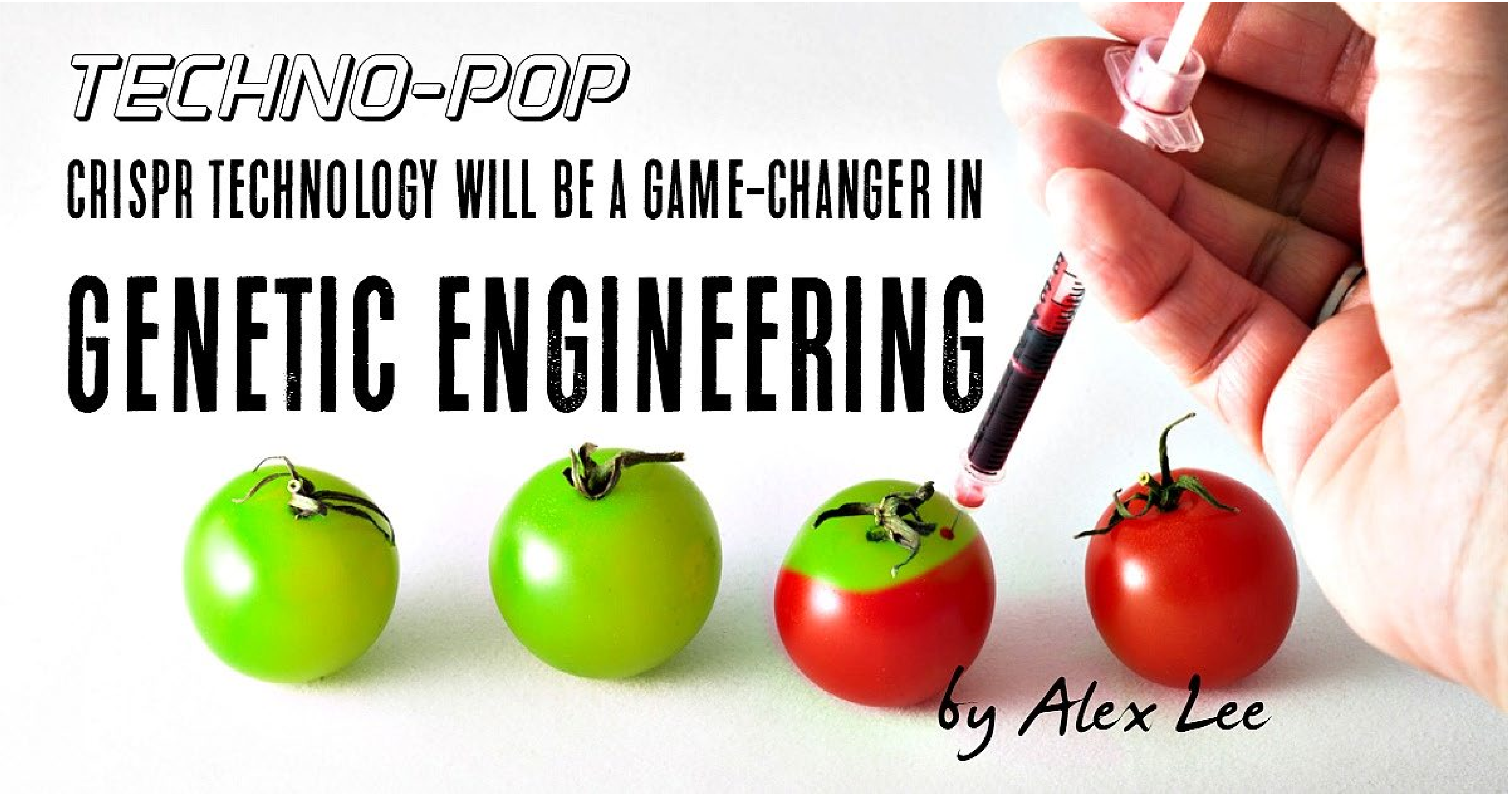
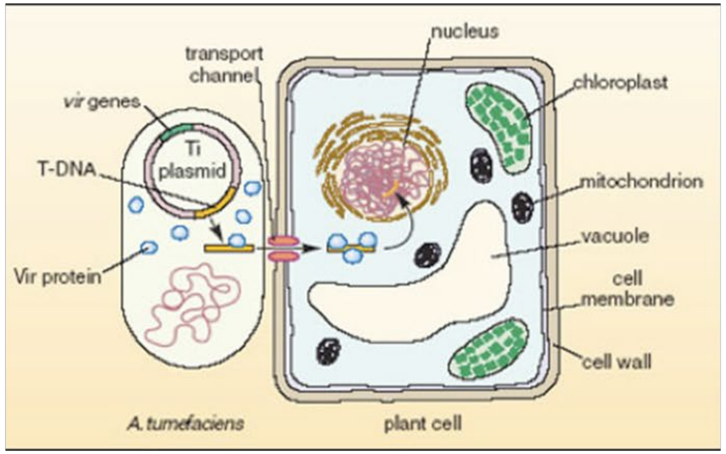
CRISPR: a revolution in the genetic manipulation of organisms.



http://thecasualobserver.co.za/genetic-engineering-genie-bottle/

Genetic transformation: old and new

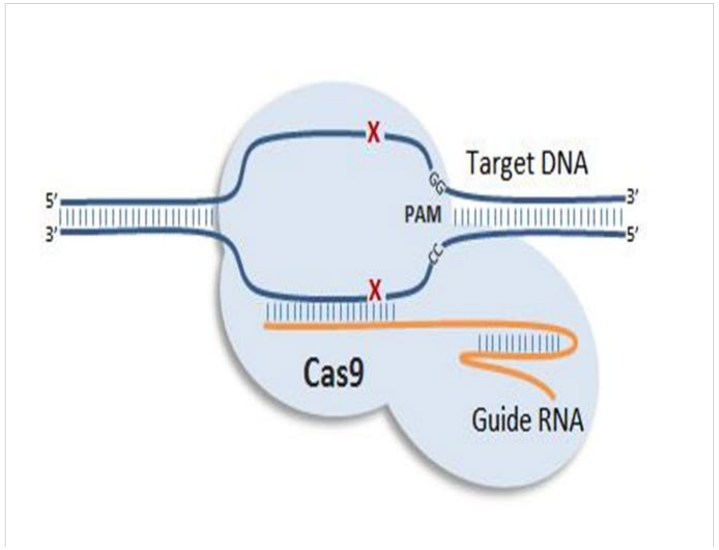


GMOs

Agrobacterium Transformation Mediated (think crown gall)

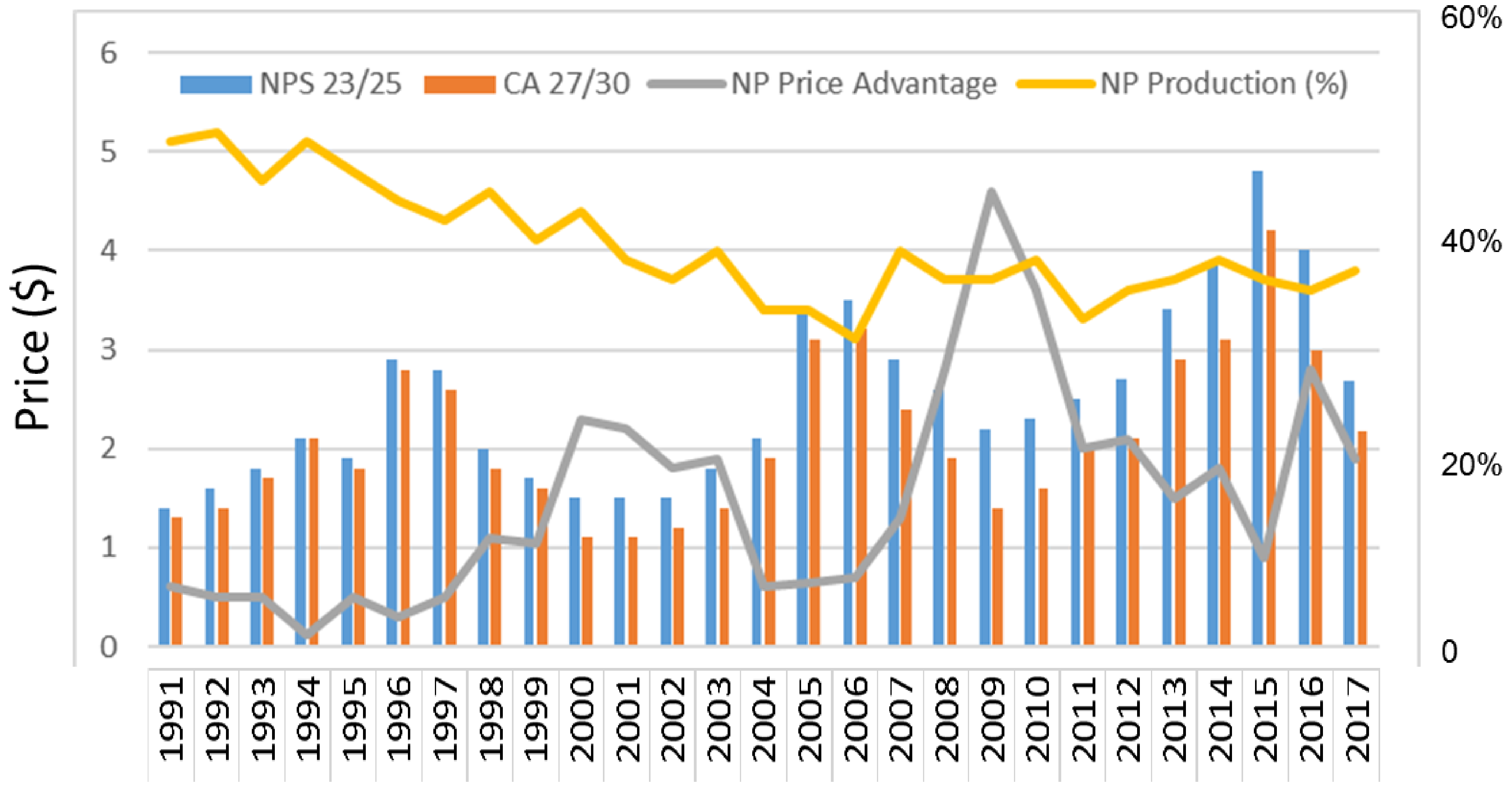
- **Plant regeneration??**

CRISPR/Cas9 System for Mutagenesis and Genome Editing

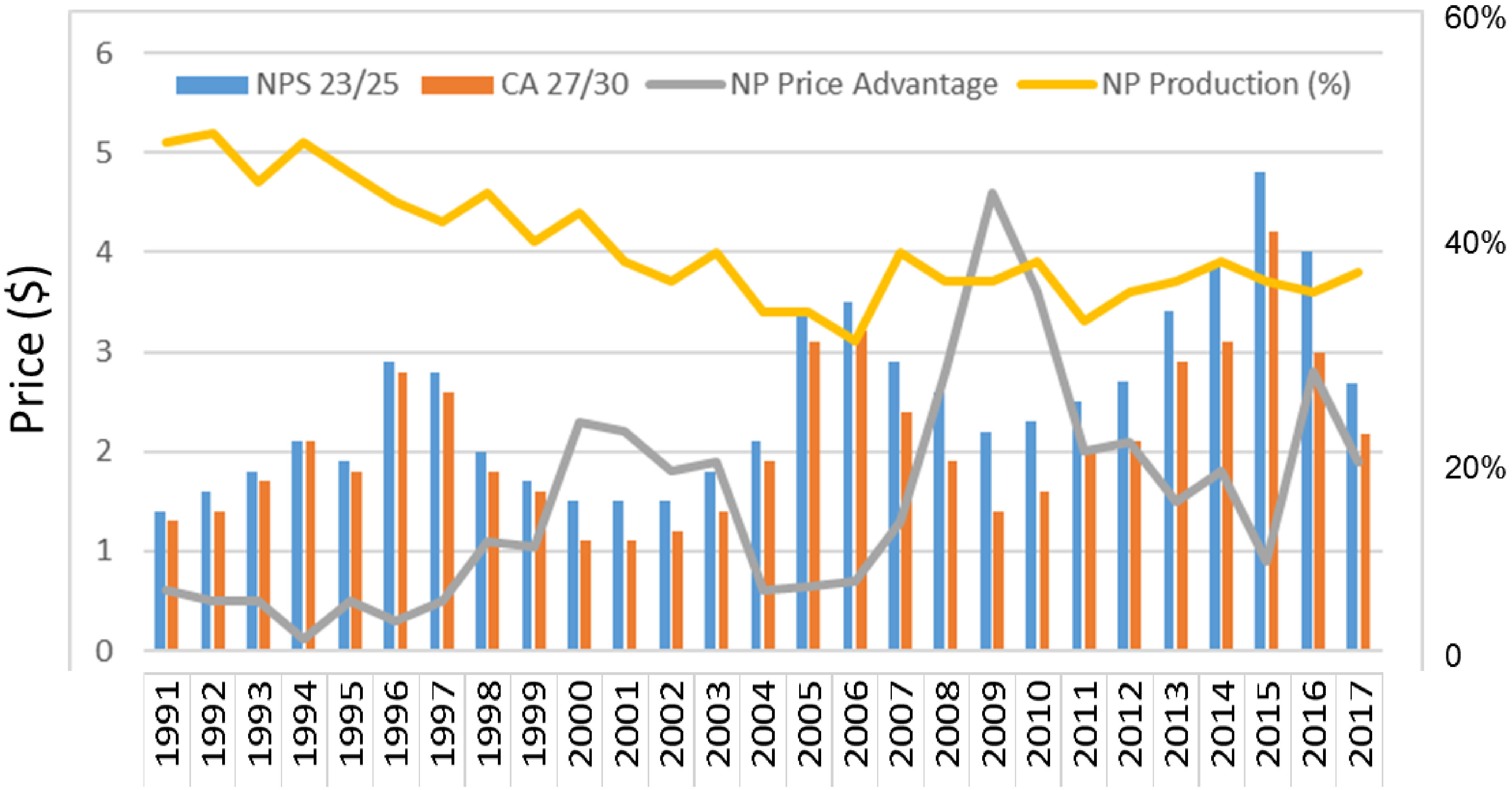


18.HORT29.Dandekar
Almond Cellular Tissue Regeneration

Nonpareil almond dominates the California industry



Genetically engineer a Self-fruitful Nonpareil



Self-incompatibility is controlled by a major effect gene.
We know the gene.
We know its sequence.

```
Sf  Tuono (a)  VQQWPPTTCRFSGK-PSNNRRPLPIFTIRGVWPSNYSNPRMRSNCTGSQFKKILS-PRLRSKLERAWPDVESGNDTKFWEDEWNKHGKCSEQTLNQMQYFERSHQMW
Sf  Tuono (b)  VQQWPPTNCRVRIKRPCSNPRPLQYFTIHGLWPSNYSNPTKPSNCNGSQFNFTKVSPKMRVKLKRSWPDVESGNDTRFWEGEWNKHGTCSEGSLNQMQYFERSHEMW
Sf  Tuono (c)  VQQWPPTTCRFSGK-PSNNRRPLPIFTIRGIWPSNYSNPRMRSNCTGSQFKKILS-PRLRSKLERAWPDVESGNDTKFWEDEWNKHGKCSEQTLNQMQYFERSHQMW
Sfa Ponç       VQQWPPTTCRFSGK-PSNNRRPLPIFTIHGIWPSNYSNPRMRSNCTGSQFKKILS-PRLRSKLERAWPDVESGNDTKFWEDEWNKHGKCSEQTLNQMQYFERSHQMW
Sf  Cambra     VQQWPPTTCRFSGK-PSNNRRPLPIFTIHGIWPSNYSNPRMRSNCTGSQFKKILS-PRLRSKLERAWPDVESGNDTKFWEDEWNKHGKCSEQTLNQMQYFERSHQMW
S30 F.G.Grande VQQWPPTTCRFSGK-PSNNRRPLPIFTIHGIWPSNYSNPRMRSNCTGSQFKKILS-PRLRSKLERAWPDVESGNDTKFWEDEWNKHGKCSEQTLNQMQYFERSHQMW
Sf  Lauranne   VQQWPPTTCRFSGK-PSNNRRPLPIFTIHGIWPSNYSNPRMRSNCTGSQFKKILS-PRLRSKLERAWPDVESGNDTKFWEDEWNKHGKCSEQTLNQMQYFERSHQMW
                 C1                    C2                      RHV                           C3

Sf  Tuono (a)  SSFNITNILEKASIVPNATQTWTYSDILSPIKAATQRIPLLRCKGNPQRQAKSQPKNRGKSQPKSQATTQLLHEVV
Sf  Tuono (b)  YSFNITEILKNASIVPHPTQTWKYSDIVAPIKTATKRTPVLRCKPDP------------AQNKSGPKTQLLHEVV
Sf  Tuono (c)  SSFNITNILEKASIVPNATQTWTYSDILSPIKAATQRIPLLRCKGNPQRQAKSQPKNRGKSQPKSQATTQLLHEVV
Sfa Ponç       SSFNITNILEKASIVPNATQTWTYSDILSPIKAATQRIPLLRCKGNPQRQAKSQPKNRGKSQPKSQATTQLLHEVV
Sf  Cambra     SSFNITNILEKASIVPNATQTWTYSDILSPIKAATQRIPLLRCKGNPQRQAKSQPKNRGKSQPKSQATTQLLHEVV
S30 F.G.Grande SSFNITNILEKASIVPNATQTWTYSDILSPIKAATQRIPLLRCKGNPQRQAKSQPKNRGKSQPKSQATTQLLHEVV
Sf  Lauranne   SSFNITNILEKASIVPNATQTWTYSDILSPIKAATQRIPLLRCKGNPQRQAKSQPKNRGKSQPKSQATTQLLHEVV
                   RC4                                                        C5
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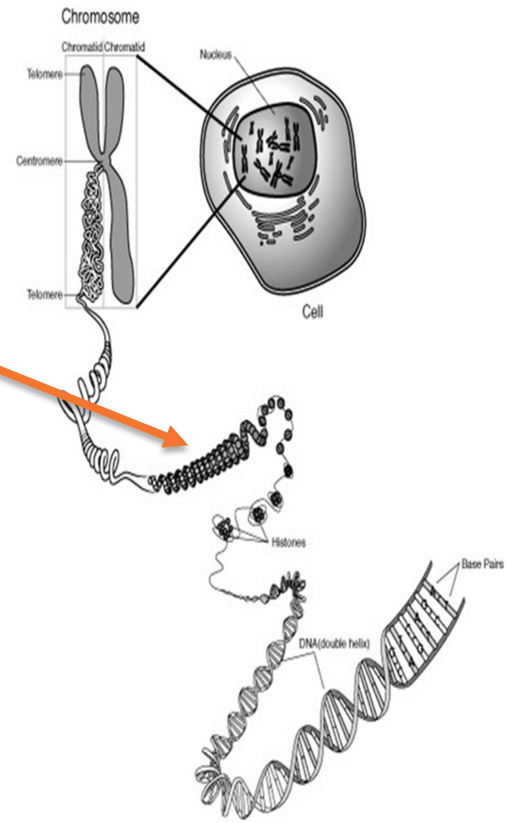
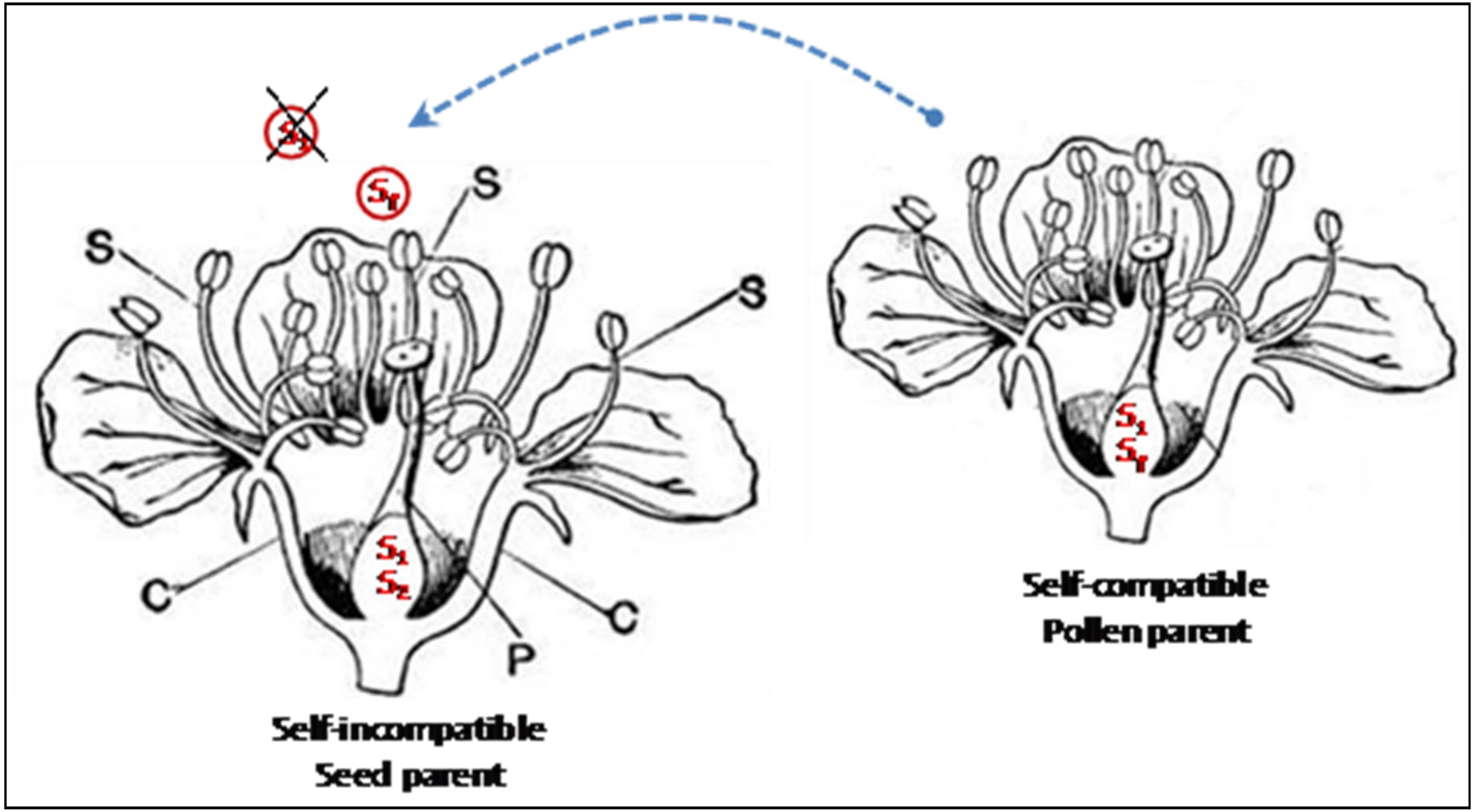


A classic "silver bullet" solution.

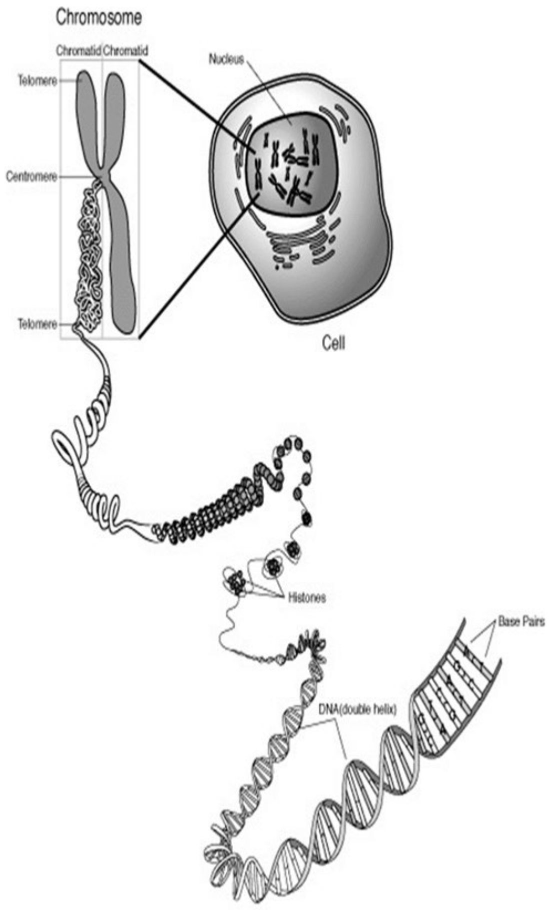
ABC funded A.Dandekar
Almond self-incompatibility

Self-fruitfulness as a result of Self-incompatibility and flower structure promoting high rates of self-pollination.

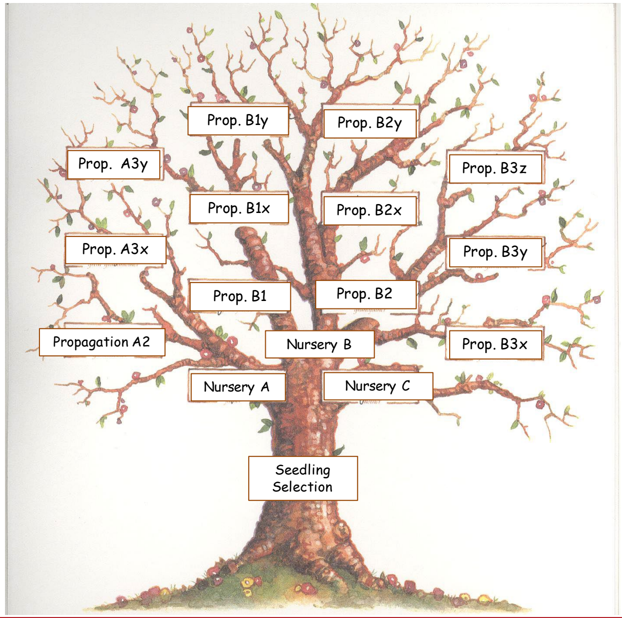


Control of self-pollination is very complex both developmentally and genetically.

Epigenetics:
what we don't know.



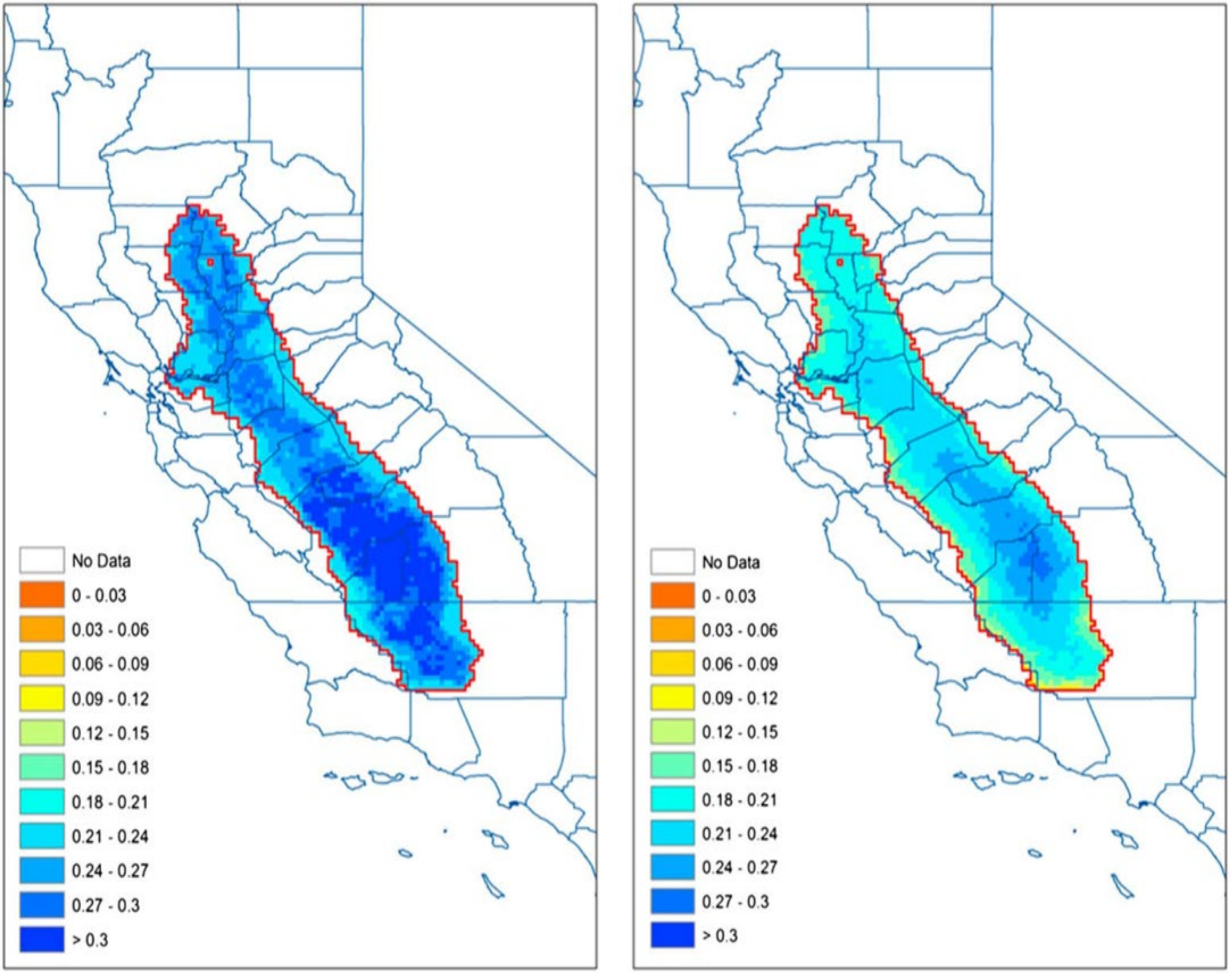
18.HORT34.Sudarshana
Genomic Approaches
to Noninfectious Bud Failure



As we know,
There are known knowns.
There are things we know we know.
We also know
There are known unknowns.
That is to say
We know there are some things
We do not know.
But there are also unknown unknowns,
The ones we don't know
We don't know.

—Donald Rumsfeld. Feb. 12, 2002, Department of Defense news briefing

One known is that the future is largely unknown.



1981–1999 2001–2012

- **Loss of Valley fog**
- **Decrease in winter chilling**
- **Decrease in water quantity/quality**
- **Air quality**
- **Regulation of PM5, (harvest dust)**

- **Few "silver bullet" solutions**

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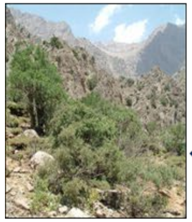
Cultivar and rootstock breeding:
new solutions to new (and old) problems

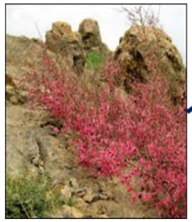
Novel problems => Novel genetic solutions

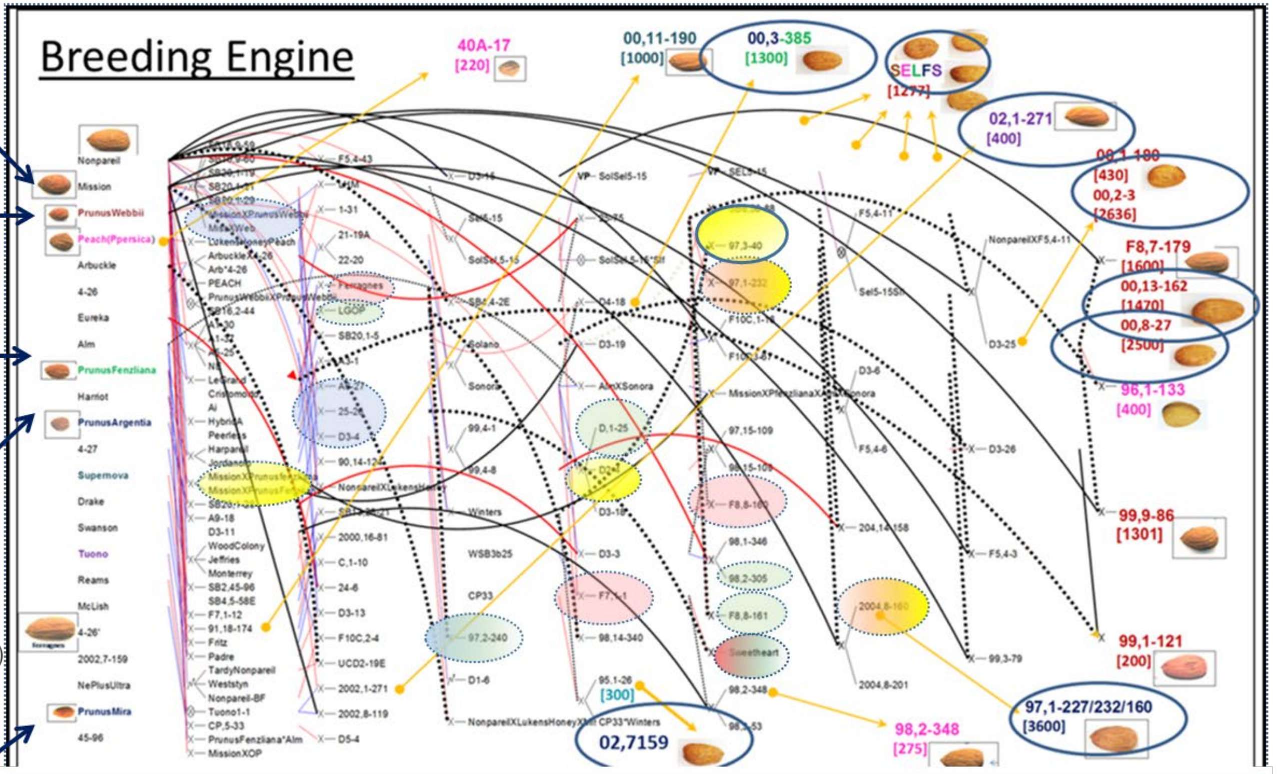

P. webbii (Iran)


P. persica (Korea)


P. fenzliana (Syria)


P. argentea (Turkey)


P. mira



Disease Pest Drought Markets

18.HORT1.Gradziel
Almond Variety Development

18.HORT10.Gradziel
Almond Rootstock Development

18.HORT2.Lampinen/UCCE
Field Evaluation Almond Varieties

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Advanced breeding selections

-ability to handle complex genetics

-assessing opportunities/risks,

-juvenile-so amenable to transformation/regeneration

Catch-frame harvest with field de-hulling

18.HORT1.Gradziel
Almond Variety Development

18.HORT10.Gradziel
Almond Rootstock Development

18.HORT2.Lampinen/UCCE
Field Evaluation Almond Varieties

Selection-Chico	Avg. Rating
UCD1-271	4.5
UCD8-201	4.5
Sweetheart	4
UCD18-20	4
UCD8-27	4
Winters	4
2-19E Kester	3.5
UCD8-160	3.5
Nonpareil	3
UCD1-16	3
UCD1-232	2.5
UCD7-159	2.5
UCD3-40	2
UCD1-6 Rootstock	1
UCD3-53 Rootstock	0

Water use efficiency for deficit irrigation (Shoots cut at 100+F & held for 24h)

Summary.

- **Powerful genome sequencing is ridiculously cheap.**
  - **Largely limited to "silver bullet" analysis.**
- **New CRISPR technologies are mind-boggling powerful.**
  - **Largely limited to "silver bullet" solutions.**
- **UCD almond cultivar and rootstock breeding now reaping benefits from long-term ABC funded complementary genetic improvement strategies.**
  - **Complex germplasm facilitates genome sequencing.**
  - **Juvenility in new selections facilitates genetic engineering/regeneration**
  - **New genetic technologies greatly facilitate breeding.**
    - **The challenge is anticipating the 'Unknown unknowns'.**

ALMOND BREEDING: IS THERE A ROLE FOR NEW GENETIC TECHNOLOGIES?

Abhaya M. Dandekar

Plant Sciences Department; UC Davis

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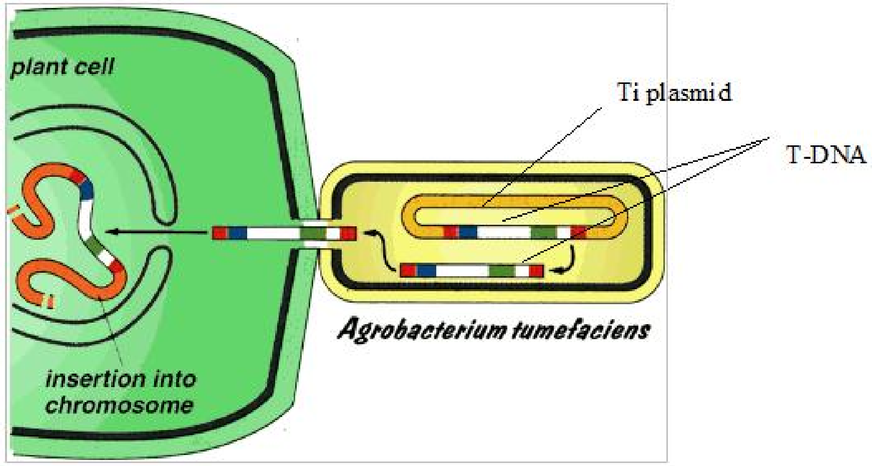
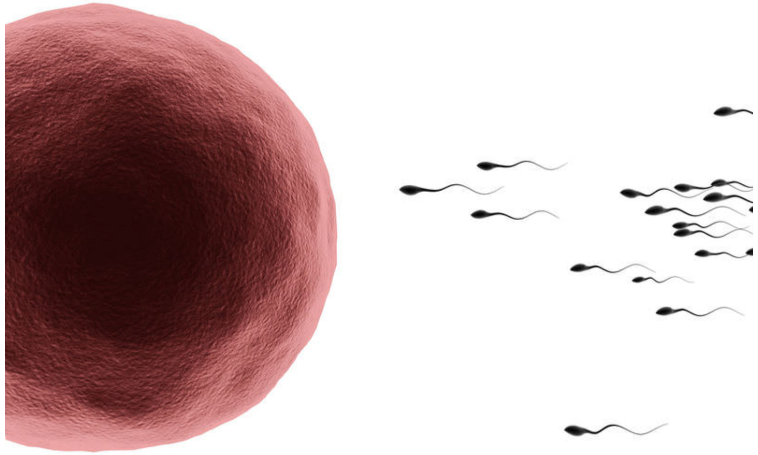
Translating New Breeding Technologies

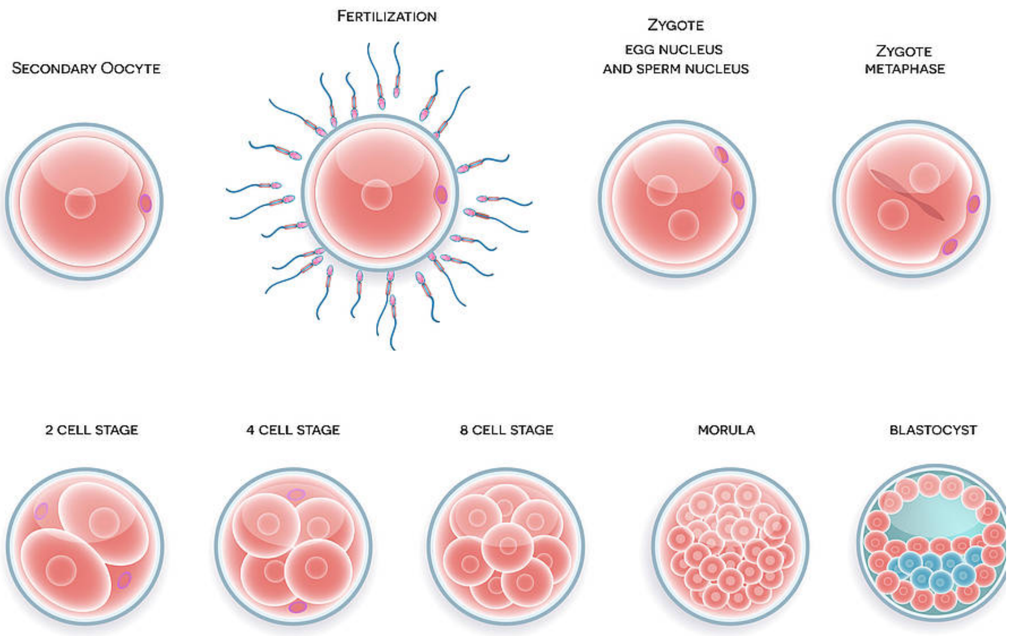
- <u>Traditional Breeding</u>
- "The Sledge Hammer"
  - Sexual
  - Narrow germplasm
  - Too many side effects

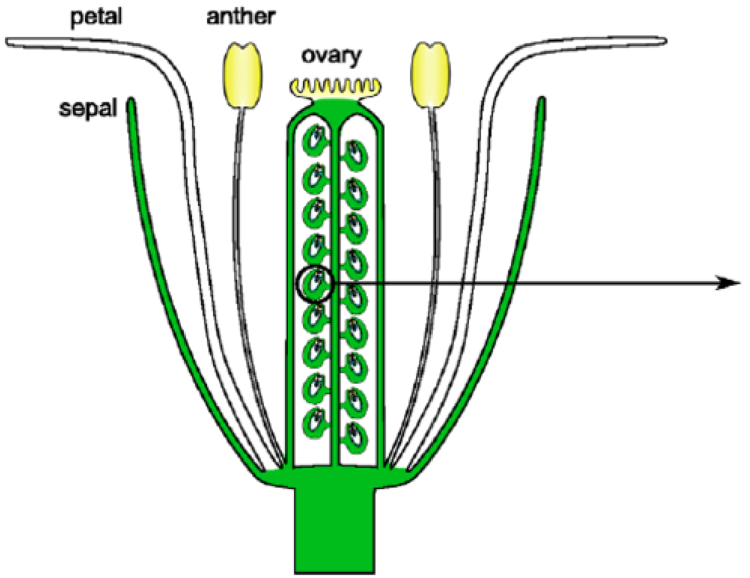


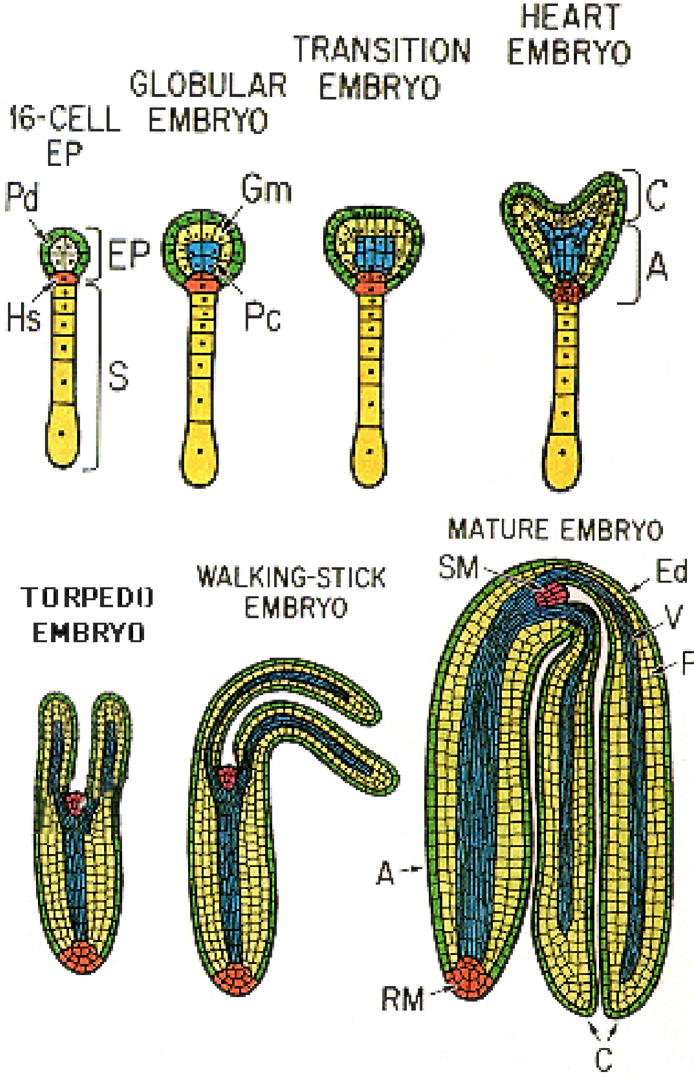


- <u>New Breeding Technologies</u>
- "The Scalpel"
  - Asexual
  - Infinite germplasm
  - Few side effects

Step 1: Need Totipotent Stem Cells

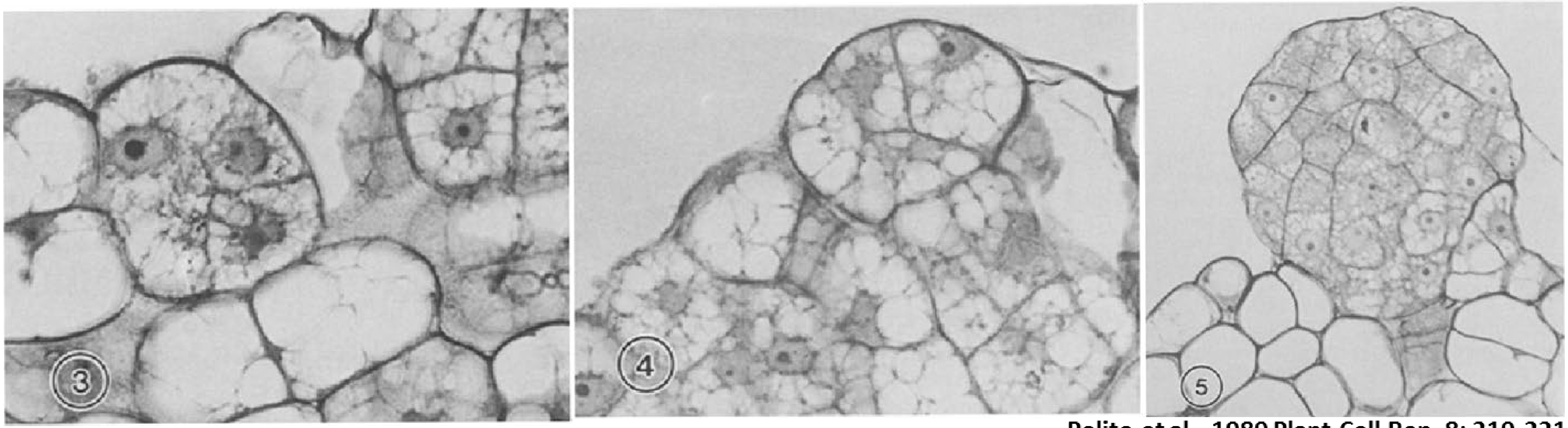






- A single stem cell has the wisdom to make the whole organism

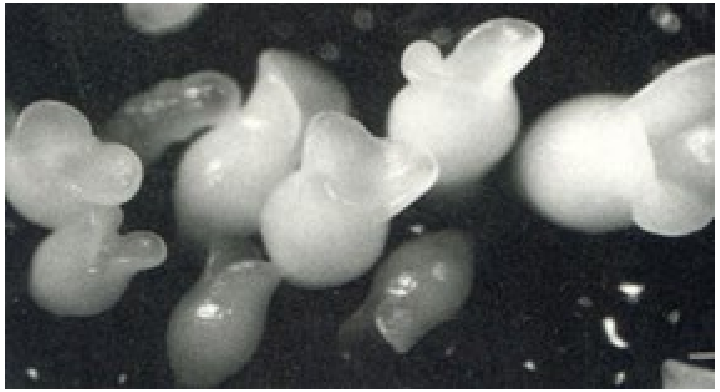
Embryonic Stem Cells: Walnut



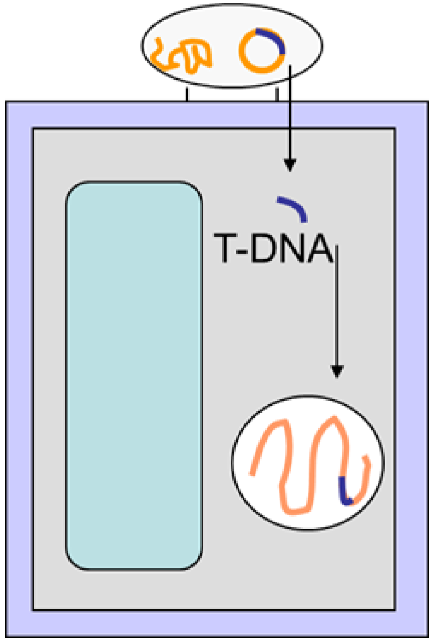
Polito et al., 1989 Plant Cell Rep. 8: 219-221

- The epidermal layer in developing walnut embryos have stem cells
- These stem cell naturally differentiate to form embryos
- These embryos can be germinated to make plants

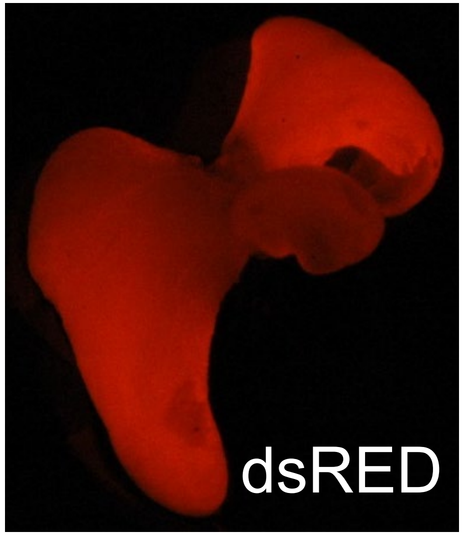
Step 2: Gene Delivery into stem cells and regeneration of transgenic plants



Somatic Embryo Propagation



Walnut Embryo stem cell

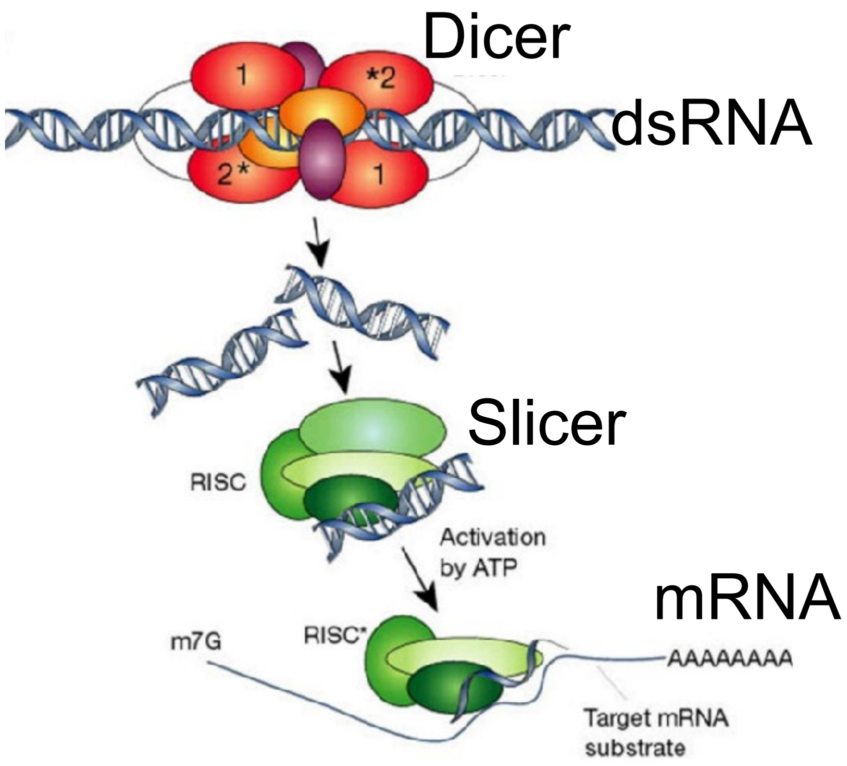


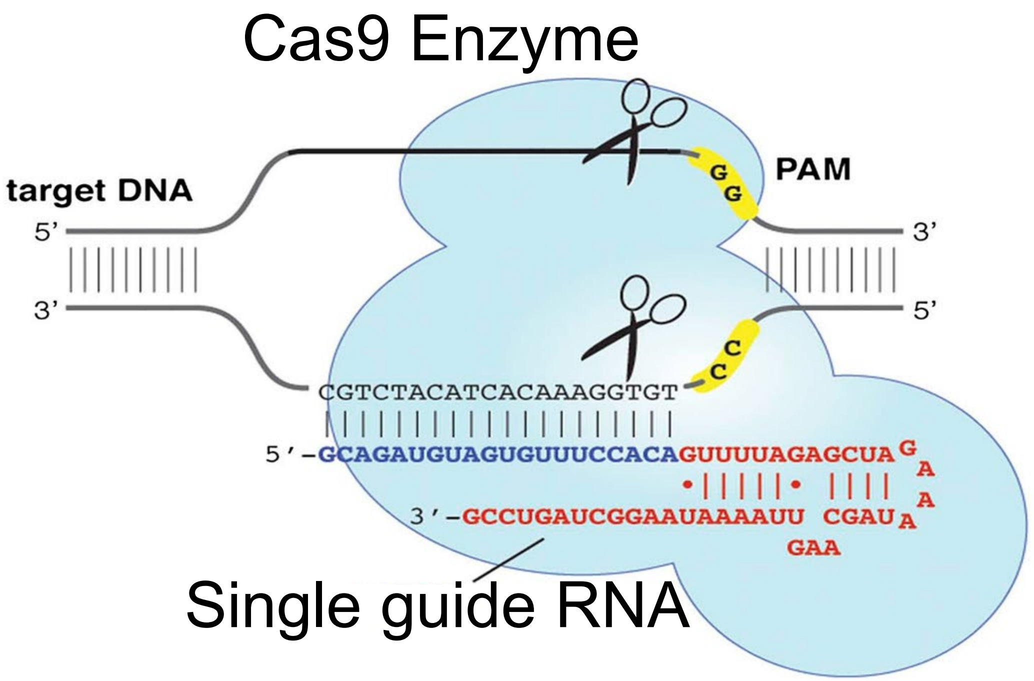
Selection

Germination

- Walnut somatic embryo stem cells regenerate into scion/rootstock plants
- *Agrobacterium*-mediated transformation of somatic embryos delivers new genetic information into embryo stem cells that regenerate into transgenic rootstocks

Step 3: Deploying gene editing components





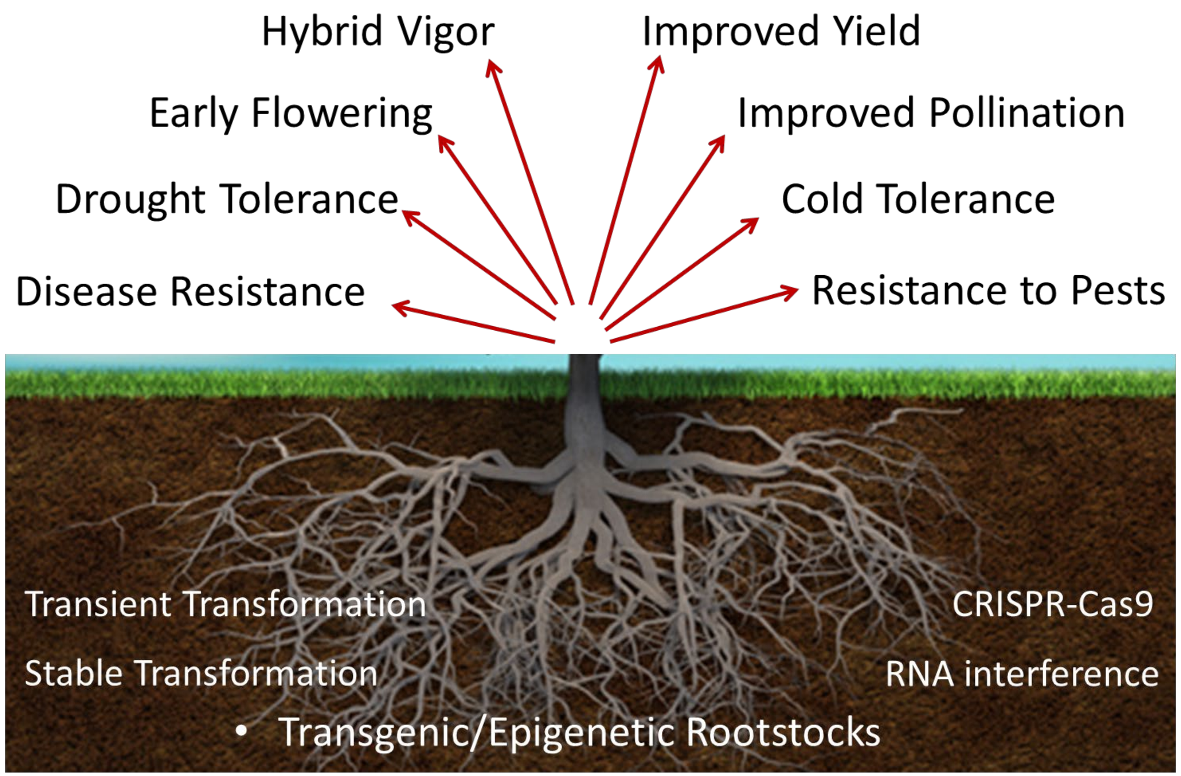
PDS Knockout

- RNAi Functional Knockout
  - dsRNA Trigger transgene
  - Dicer & Slicer onboard
- CRISPR Genome Editing
  - Cas9 transgene
  - sgRNA transgene

Step 4: Solving the Crown Gall Disease Problem

- Strains that make tumors are ubiquitous
- Tumors provide a food source stimulating pathogen populations
- Tumors disrupt the vascular system
  - Reducing nutrient flow
  - Plants get stressed
  - Reduce yield and quality
  - Orchards cannot be replanted

Engineered Rootstocks: Low prolife entry point for new breeding technologies



- Sustainable root system to graft any scion cultivar (nonGMO)
- Stack many traits in a single rootstock

Thank you!

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