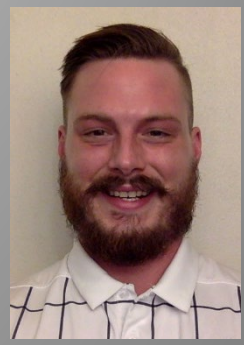
Time for CRISPR? A look at the options for creating novel ornamentals with gene editing and genetic engineering (GE) techniques

Steve Strauss and Ryan Contreras, Professors
Michael Nagle, PhD Candidate
Oregon State University

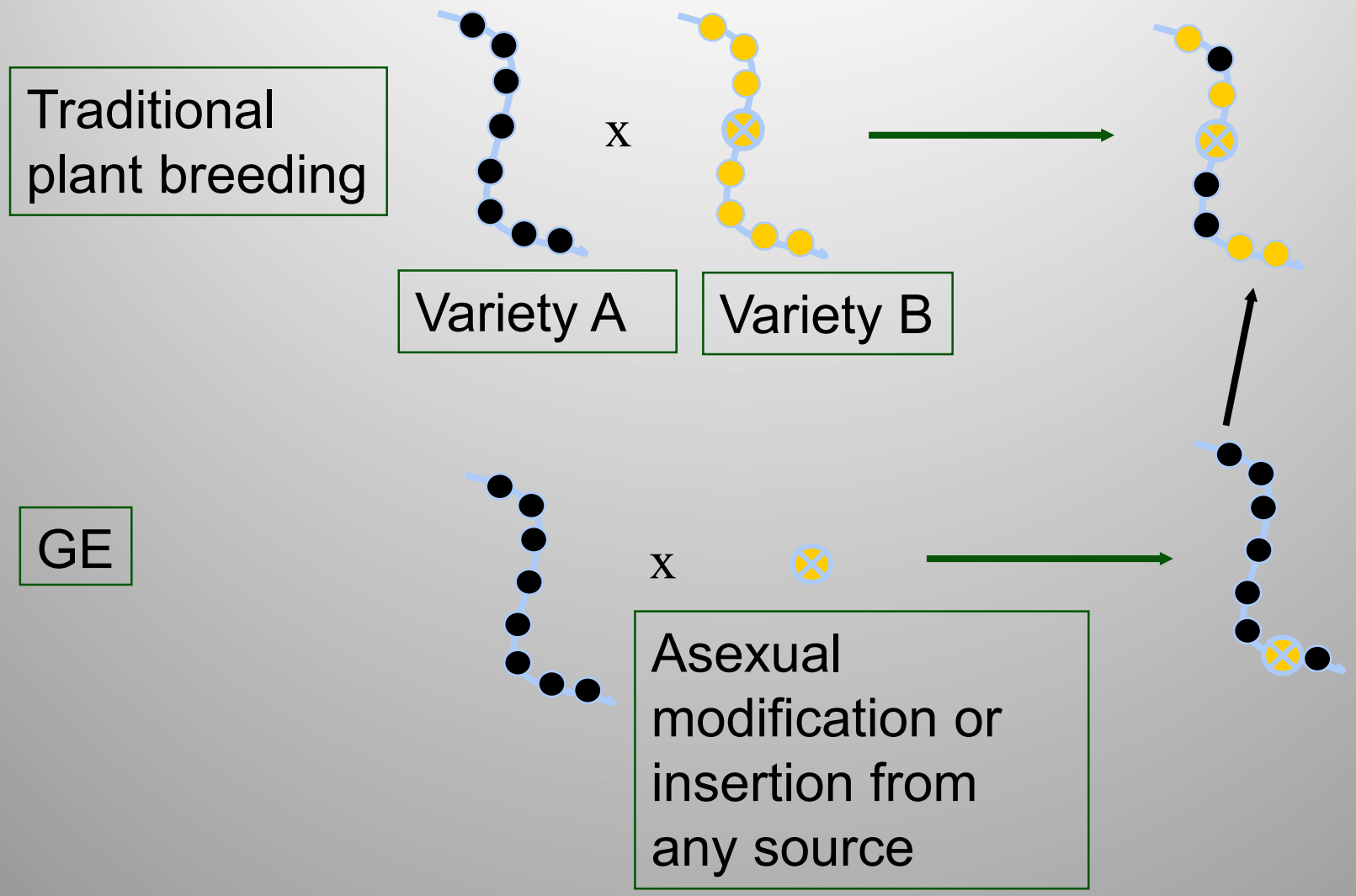
Far West Meeting – August 2019



Agenda

- Strauss
  - What are the technologies
  - Current and evolving regulatory system in USA
  - What are a few types of modified plants produced
- Contreras
  - How does ornamental breeding work, constraints thereof
  - What are some options for high value products
- Strauss – Concept for a university-industry consortium to do R&D and variety development
- Discussion – Moving forward?

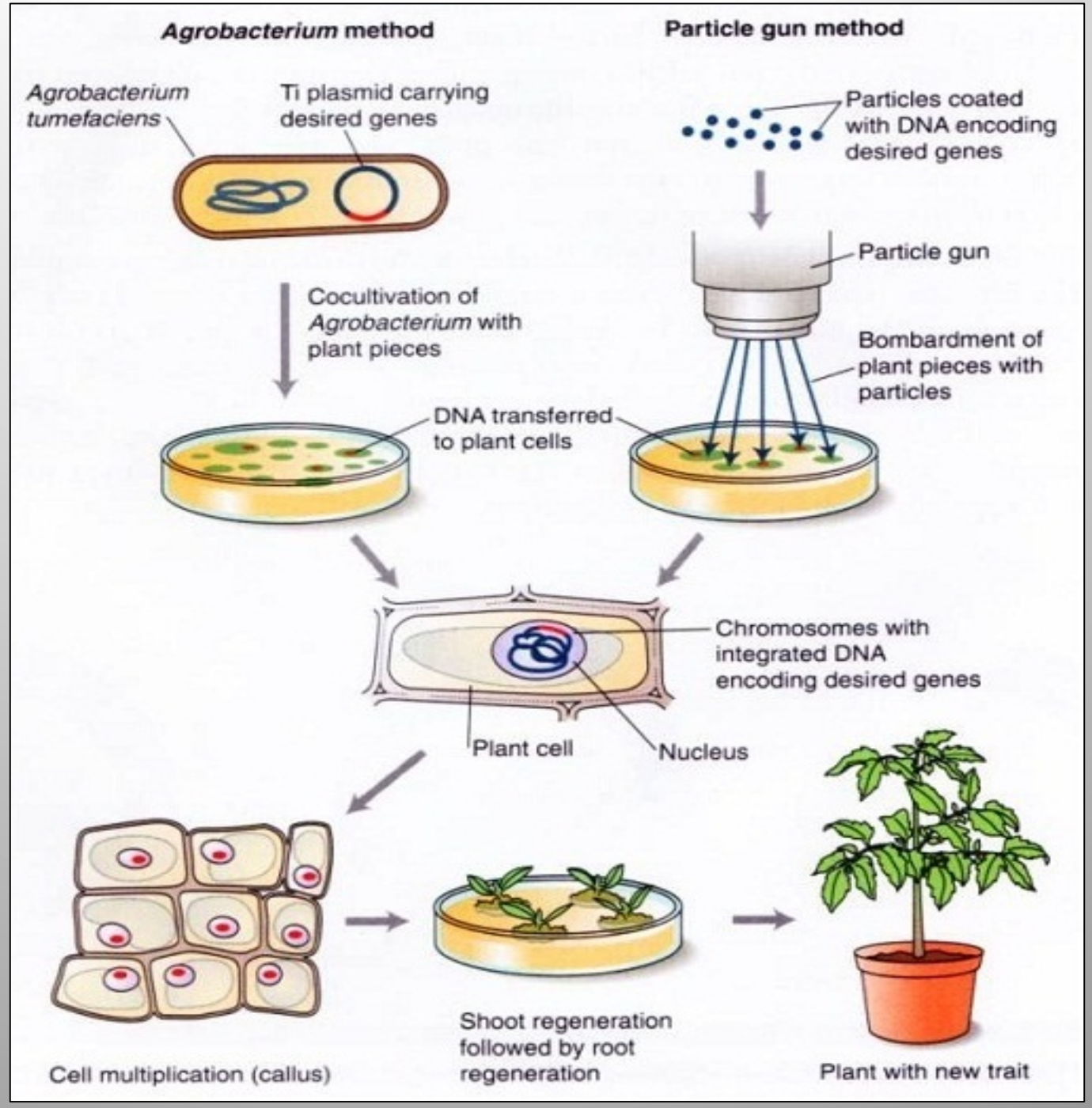
GE method (gene editing or engineering)



What it looks like

- GE = Direct modification of DNA
  - Vs. indirect modification in breeding
- Asexually modified, usually in somatic cells
  - Then <u>regenerated</u> into whole organisms, usually starting in Petri dishes

Overview of steps to create a GE plant

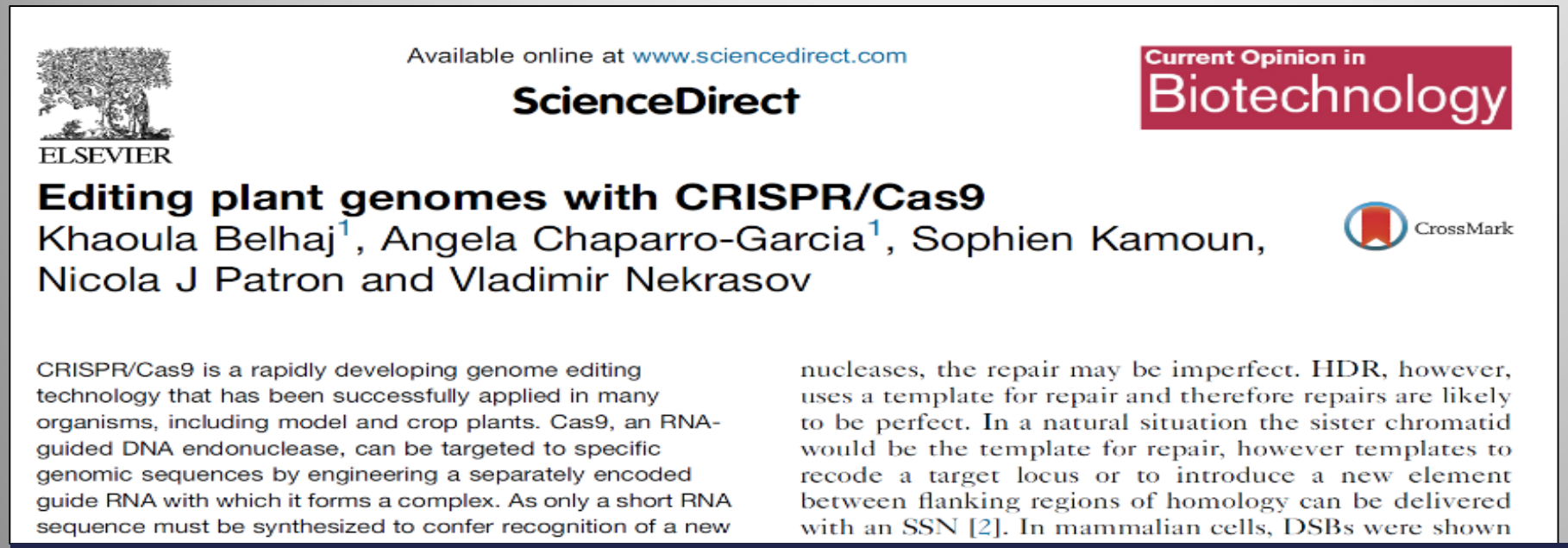


Gene editing

- ~ Specific, efficient modification of native genes
- CRISPR the main method out there
- Works pretty well everywhere!

A big deal for plants?

Ability to modify native genes efficiently -- The theoretical becomes practical



Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

ELSEVIER

Editing plant genomes with CRISPR/Cas9

Khaoula Belhaj[1], Angela Chaparro-Garcia[1], Sophien Kamoun, Nicola J Patron and Vladimir Nekrasov

CrossMark

CRISPR/Cas9 is a rapidly developing genome editing technology that has been successfully applied in many organisms, including model and crop plants. Cas9, an RNA-guided DNA endonuclease, can be targeted to specific genomic sequences by engineering a separately encoded guide RNA with which it forms a complex. As only a short RNA sequence must be synthesized to confer recognition of a new

nucleases, the repair may be imperfect. HDR, however, uses a template for repair and therefore repairs are likely to be perfect. In a natural situation the sister chromatid would be the template for repair, however templates to recode a target locus or to introduce a new element between flanking regions of homology can be delivered with an SSN [2]. In mammalian cells, DSBs were shown

"CRISPR/Cas9 is a game-changing technology that is poised to revolutionize basic research and plant breeding."

Science journalist Carl Zimmer explains CRISPR DNA editing in 90 seconds

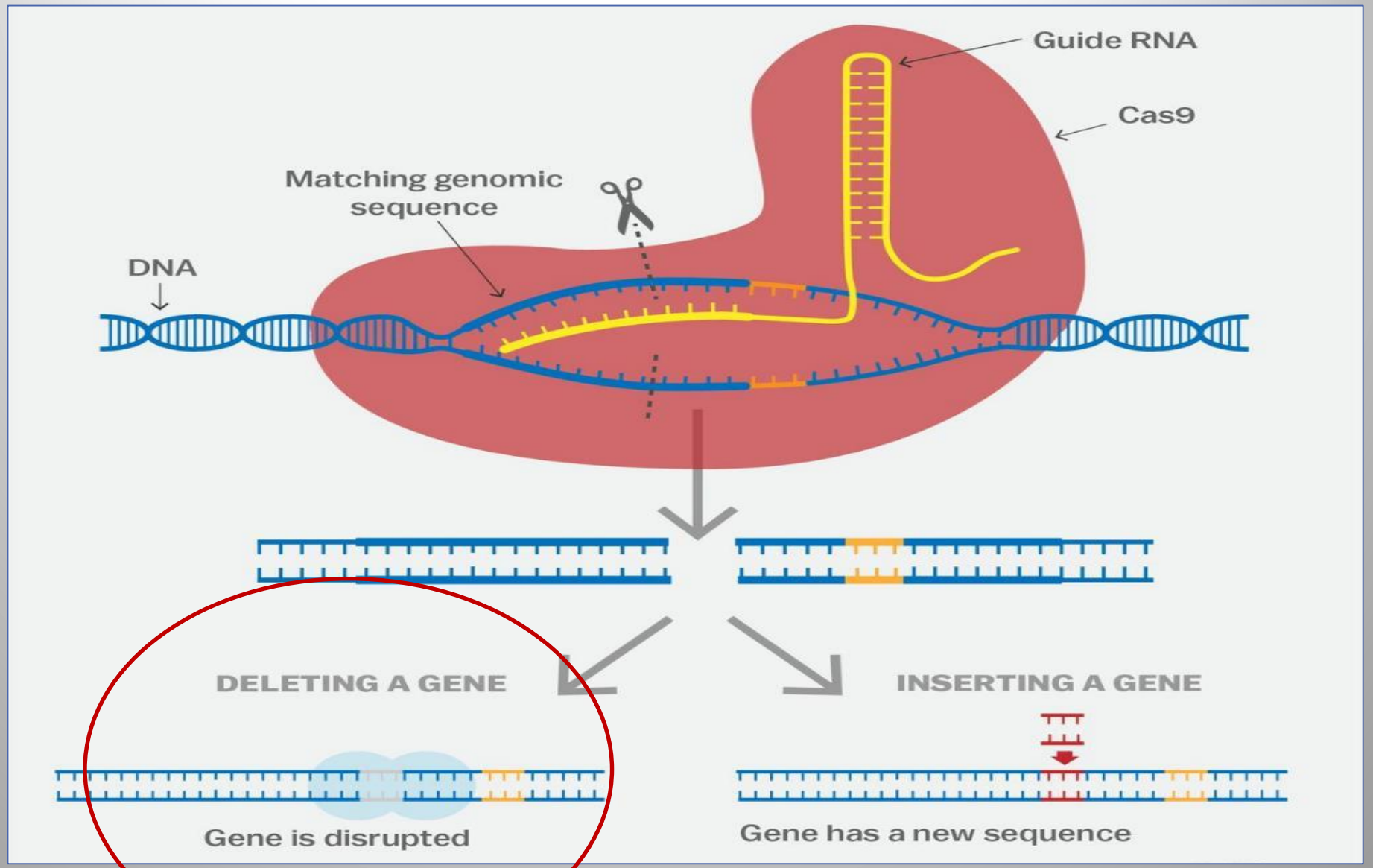
https://youtu.be/ZImVkl8QTW8

Sandman CRISPR !

https://youtu.be/k99bMtg4zRk

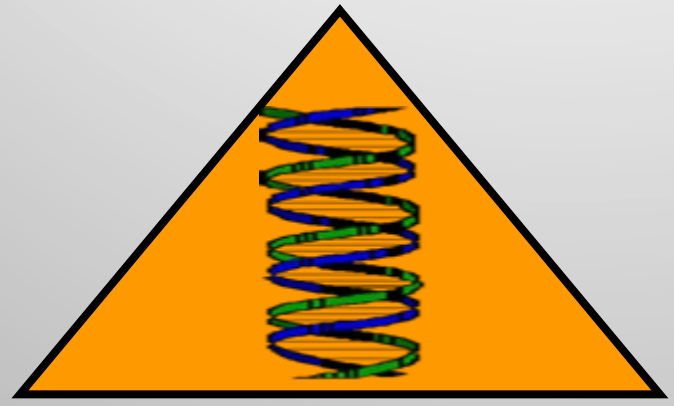
Overview of CRISPR gene edit machinery

Two parts: Nuclease and guide RNAs to direct it in genome



The GMO regulatory triangle in the USA

USDA – US Deptartment of Agriculture



EPA - Environmental Protection Agency

FDA - Food & Drug Administration

Large changes to regulations on GE crops on the horizon at USDA

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

7 CFR Parts 340 and 372

[Docket No. APHIS–2018–0034]

RIN 0579–AE47

Movement of Certain Genetically Engineered Organisms

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Proposed rule.

SUMMARY: We are proposing to revise our regulations regarding the movement (importation, interstate movement, and

Regulatory Services, APHIS, 4700 River Road Unit 98, Riverdale, MD 20737–1238; (301) 851–3944.

SUPPLEMENTARY INFORMATION:

Background

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) administers the regulations in 7 CFR part 340, ''Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests'' (referred to below as the regulations).

These regulations govern the introduction (importation, interstate movement, or release into the

vector agent [2] is a plan
Administrator has rea
GE organism is a plan
is defined in current §
living stage (including
dormant forms) of ins
nematodes, slugs, snai
other invertebrate ani
fungi, other parasitic
reproductive parts the
any organisms similar
any of the foregoing; c
agents or substances,
or indirectly injure or
damage in or to any p
thereof, or any proces
manufactured, or othe
plants.'' For a GE orga
regulated article to be
permit authorizing the

Product not GE process, gene editing and many other potential exemptions - I

- **Process regulation dominates today**: Each insertion regulated today regardless of whether genes and mechanisms the same or give the same trait – radical change proposed to **product based regulations**
- "The approach we are proposing would differ from the current regulatory framework in that regulatory efforts would focus on the **properties** of the GE organism itself rather than on the method used to produce it."
- "…modified GE plants would not be regulated or subject to a regulatory status review in accordance with § 340.4, if: • The genetic modification is solely a deletion of any size…"

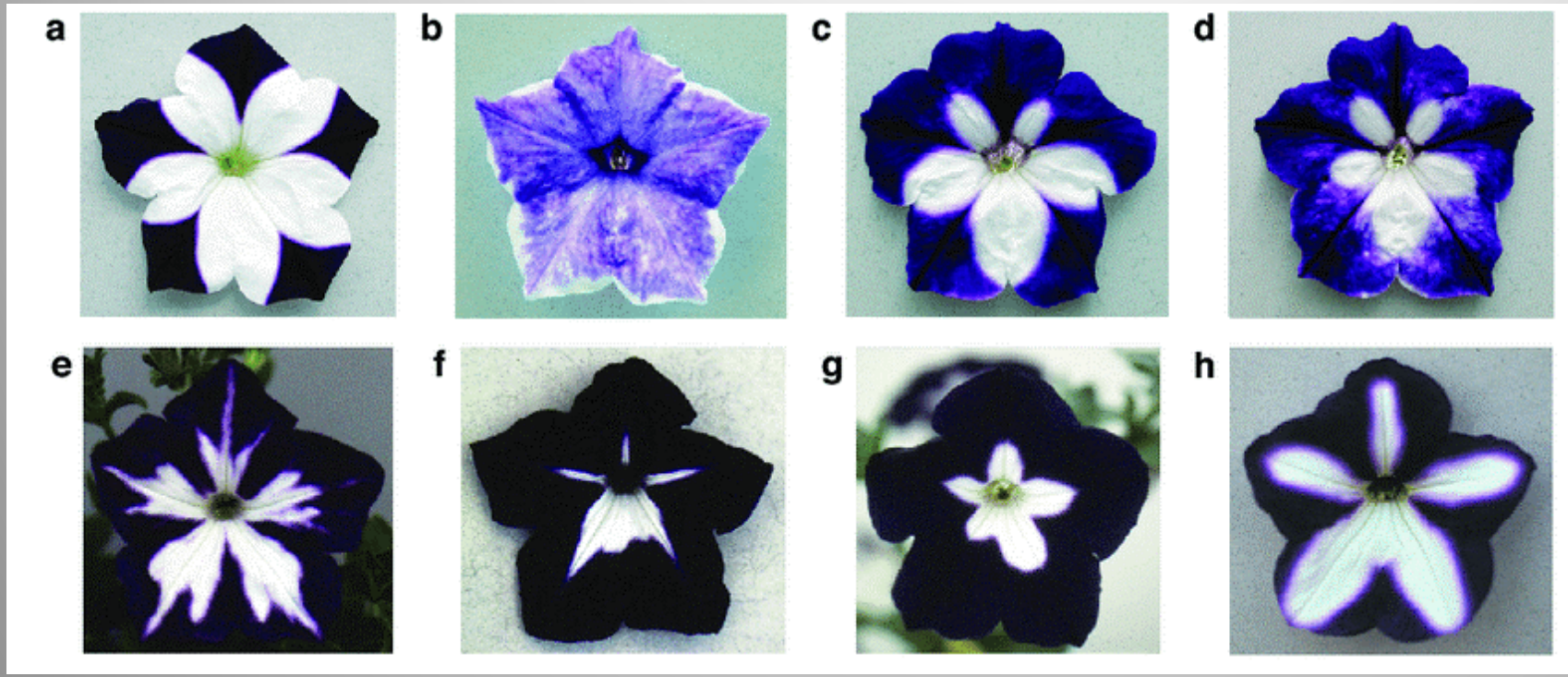
Product not GE process, gene editing and many other potential exemptions - II

- "…would **exempt** GE plants with plant-trait-mechanism of action (MOA) combinations that we have already evaluated by conducting a regulatory status review.."
- Full contained **field trials** a serious hurdle today..
- "APHIS considers information from field tests to be unnecessary, in most cases, for a determination of regulatory status under the proposed regulations."

What kinds of traits are possible and of ornamental interest ?

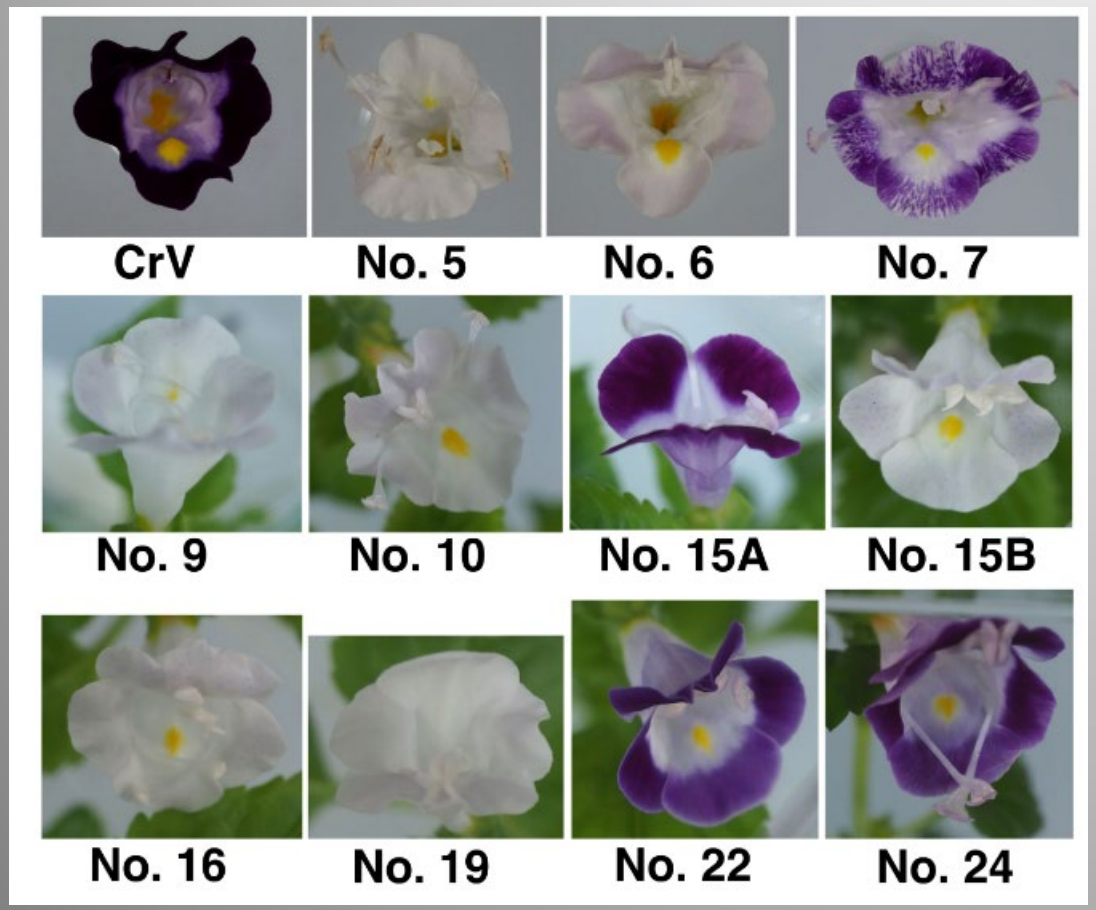
- Modifications to leaf and flower color
- Modification to plant form, semi-dwarfism
- Modifications to flowering/fertility: Containment of exotics, messiness of fruit, allergenicity
  - Also more flowers, rapid breeding
- Modification of flower/fruit longevity: Ripening, senescence control
- Pest resistance
- Enhanced transformation capability

Overexpression of pigment genes – led to variegated patterns in petunia



https://doi.org/10.1007/BF00040715

CRISPR/Cas9-mediated knockout of an anthocyanin synthesis gene in *Torenia* (wishbone flower) produces white or variegated flowers



Nishihara *et al. BMC Plant Biology* (2018) 18:331
https://doi.org/10.1186/s12870-018-1539-3

BMC Plant Biology

RESEARCH ARTICLE Open Access

Application of the CRISPR/Cas9 system for modification of flower color in *Torenia fournieri*

Masahiro Nishihara[1*], Atsumi Higuchi[1], Aiko Watanabe[1] and Keisuke Tasaki[1,2]

Increasingly deep purple hues from Florigene Mooncarnation

FLORIGENE® Moontea™

FLORIGENE® Moontea™ has a dark purple tone, before opening it may appear to be a purple burgundy and as it opens, it texture becomes a purple velvet color.

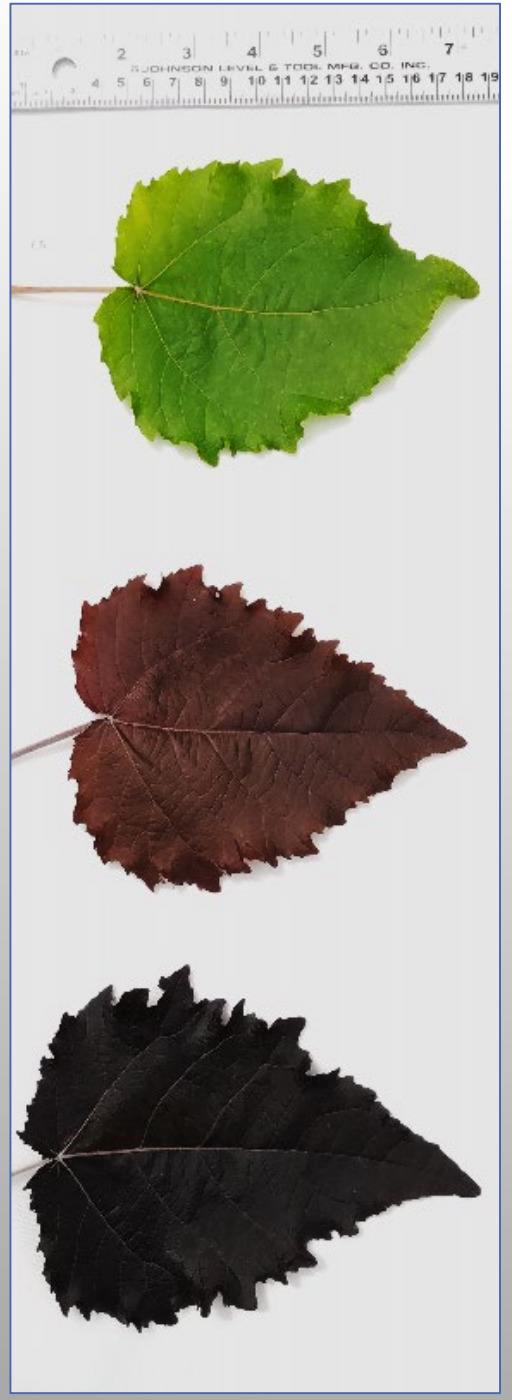
Purple tomatoes with high anthocyanin – a pigment and antioxidant

https://www.jic.ac.uk/purple-tomatoes/

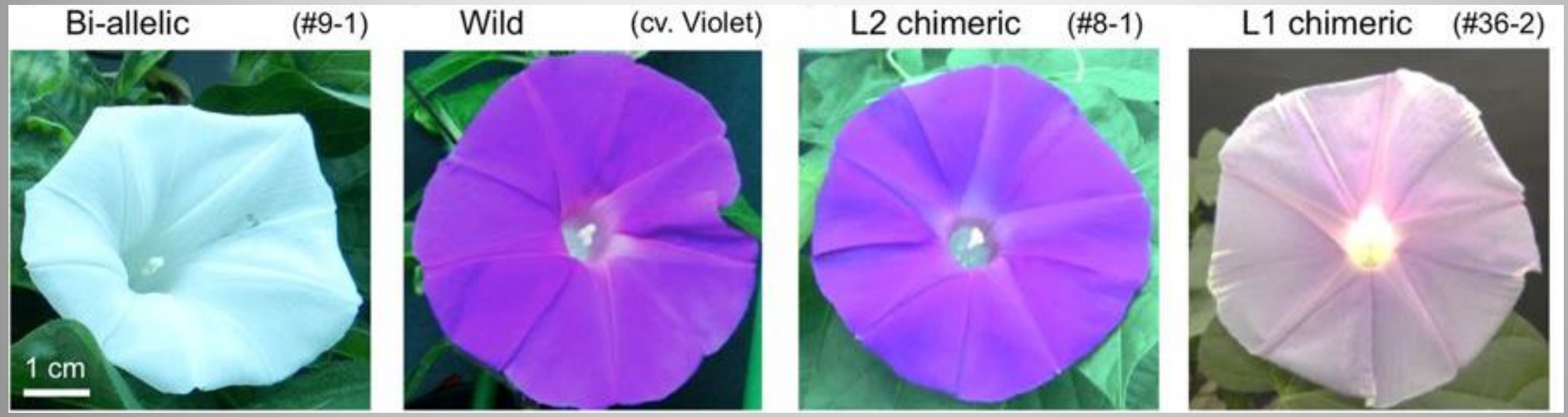
John Innes Centre
Unlocking Nature's Diversity

”True Blue” chrysanthemums by Suntory

Leaf color modifications (poplar from Strauss laboratory)



CRISPR of anthocyanin synthesis genes in morning glory



SCIENTIFIC REPORTS

OPEN

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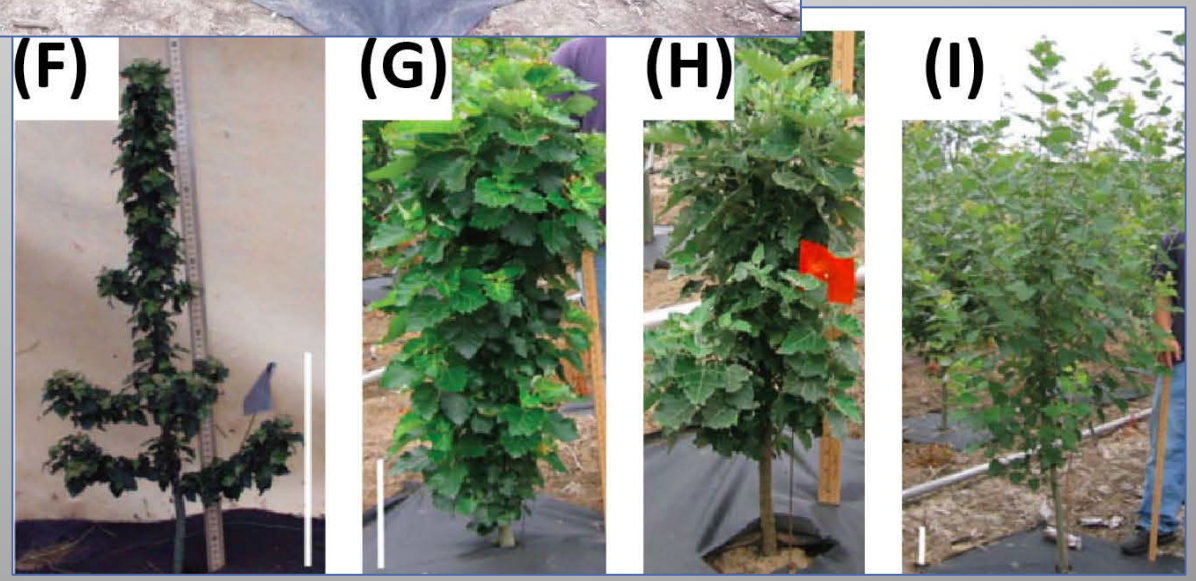
Published online: 30 August 2017

CRISPR/Cas9-mediated mutagenesis of the *dihydroflavonol-4-reductase-B (DFR-B)* locus in the Japanese morning glory *Ipomoea (Pharbitis) nil*

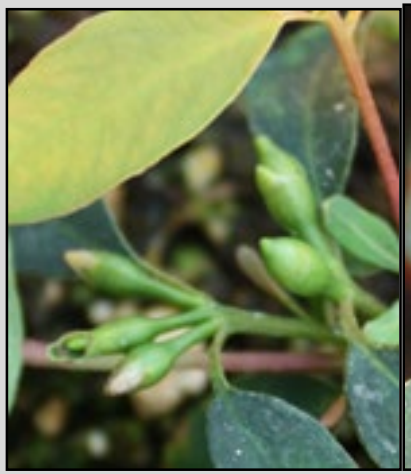
Kenta Watanabe[1], Anna Kobayashi[2], Masaki Endo[3], Kimiyo Sage-Ono[4], Seiichi Toki[3,5,6] & Michiyuki Ono[1,2,4]

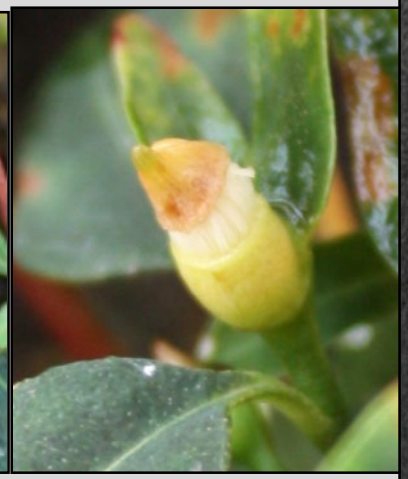
Modification to form and stature with semi-dwarfism genes (poplar from Strauss laboratory)

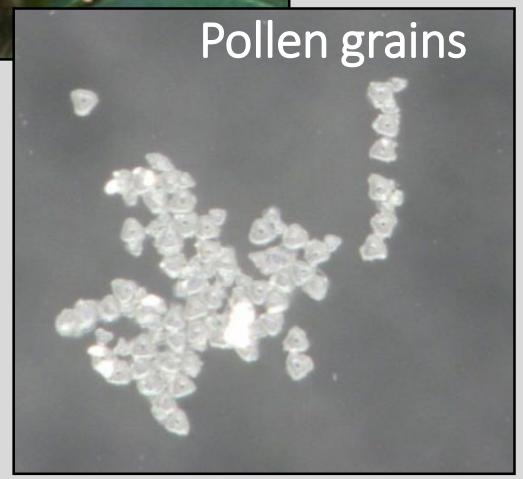


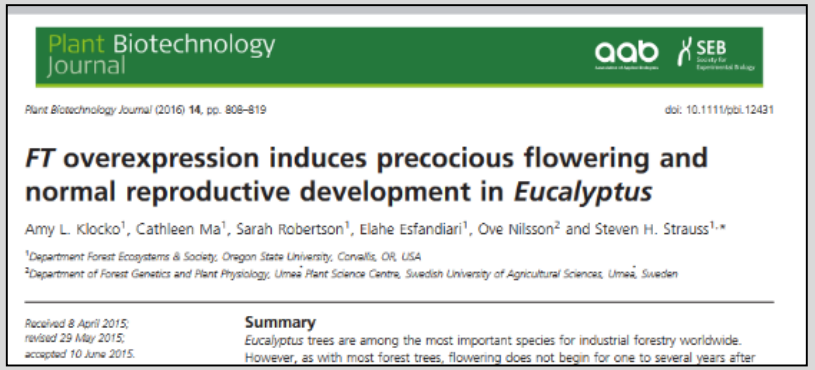
Early, intense flowering and reduced stature in many tree species

(Eucalypts in Strauss laboratory)









Plant Biotechnology Journal

Plant Biotechnology Journal (2016) 14, pp. 808–819 doi: 10.1111/pbi.12431

FT overexpression induces precocious flowering and normal reproductive development in *Eucalyptus*

Amy L. Klocko[1], Cathleen Ma[1], Sarah Robertson[1], Elahe Esfandiari[1], Ove Nilsson[2] and Steven H. Strauss[1,*]

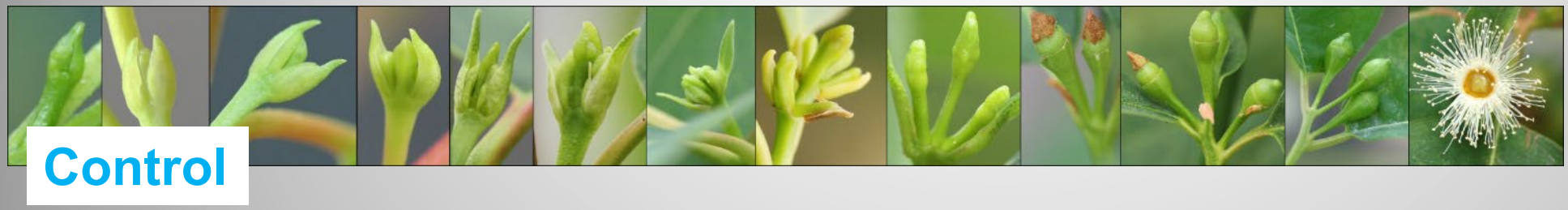
[1]Department Forest Ecosystems & Society, Oregon State University, Corvallis, OR, USA
[2]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden

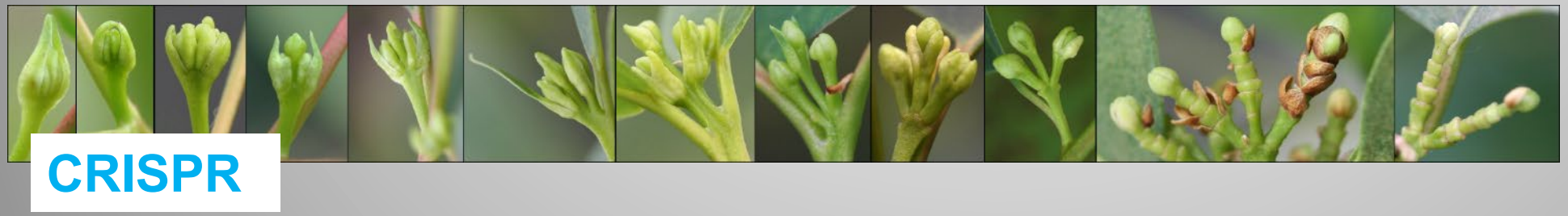
Received 8 April 2015;
revised 29 May 2015;
accepted 10 June 2015.

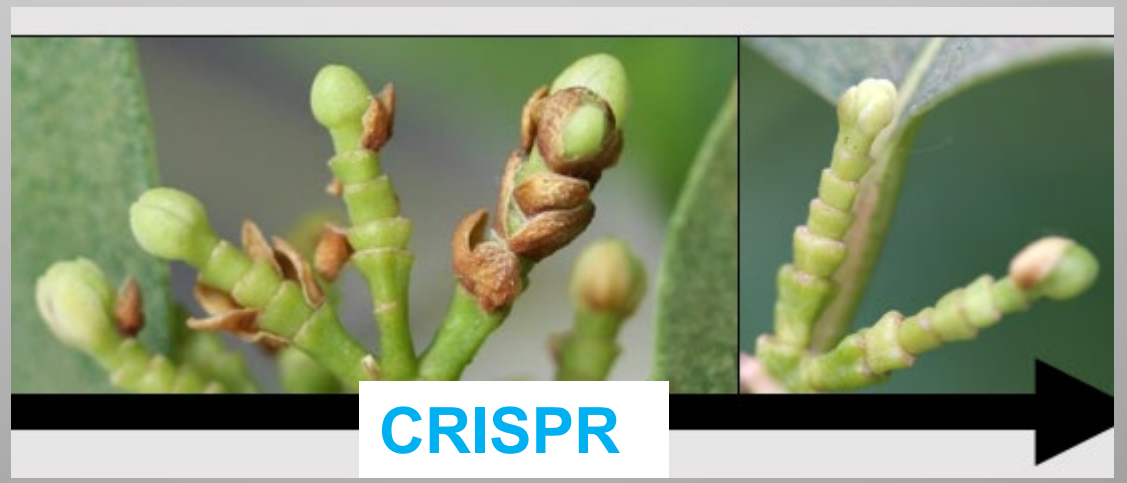
Summary

Eucalyptus trees are among the most important species for industrial forestry worldwide. However, as with most forest trees, flowering does not begin for one to several years after

CRISPR against floral genes – sterility to avoid invasiveness problems (Eucalyptus)







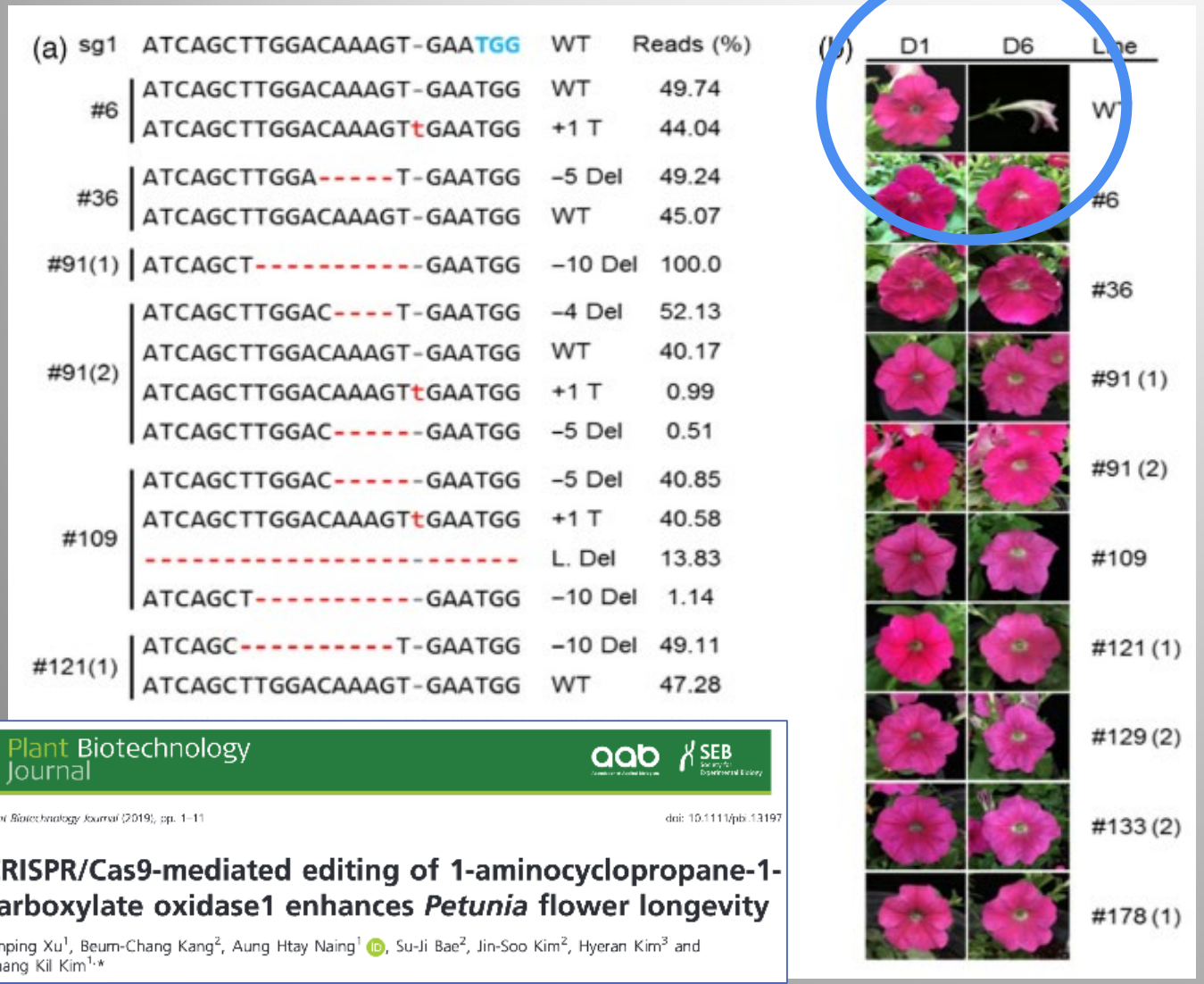
No detectable effects of CRISPR knockout plants on vegetative growth in greenhouse

Ripening delay: Florigene long-life carnations by ethylene suppression (2005)

Comparison of three carnation lines show RNAi can replace STS (silver thiosulfate) treatment – an ethylene inhibitor to delay ripening

Tanaka, Y. 2005. Genetic engineering in floriculture. *Plant cell, tissue and organ culture*, *80*(1), pp.1-24.

CRISPR a better means to reduce ethylene production?



(a)

sg1	ATCAGCTTGGACAAAGT-GAATGG	WT	Reads (%)
#6	ATCAGCTTGGACAAAGT-GAATGG	WT	49.74
	ATCAGCTTGGACAAAGTtGAATGG	+1 T	44.04
#36	ATCAGCTTGGA-----T-GAATGG	−5 Del	49.24
	ATCAGCTTGGACAAAGT-GAATGG	WT	45.07
#91(1)	ATCAGCT----------GAATGG	−10 Del	100.0
#91(2)	ATCAGCTTGGAC----T-GAATGG	−4 Del	52.13
	ATCAGCTTGGACAAAGT-GAATGG	WT	40.17
	ATCAGCTTGGACAAAGTtGAATGG	+1 T	0.99
	ATCAGCTTGGAC------GAATGG	−5 Del	0.51
#109	ATCAGCTTGGAC------GAATGG	−5 Del	40.85
	ATCAGCTTGGACAAAGTtGAATGG	+1 T	40.58
	----------	L. Del	13.83
	ATCAGCT----------GAATGG	−10 Del	1.14
#121(1)	ATCAGC----------T-GAATGG	−10 Del	49.11
	ATCAGCTTGGACAAAGT-GAATGG	WT	47.28

(b)

Plant Biotechnology Journal

Plant Biotechnology Journal (2019), pp. 1–11 doi: 10.1111/pbi.13197

CRISPR/Cas9-mediated editing of 1-aminocyclopropane-1-carboxylate oxidase1 enhances *Petunia* flower longevity

Junping Xu[1], Beum-Chang Kang[2], Aung Htay Naing[1], Su-Ji Bae[2], Jin-Soo Kim[2], Hyeran Kim[3] and Chang Kil Kim[1,*]

Regeneration and transformation systems exist for diverse economically important horticultural trees/shrubs – but also much to be done

Scientific name	Common name	Annual sales (2014 USDA census)	Transformation reported?
Acer platanoides	Norway maple	$13.5 M	N
Syringa reticulata	Japanese lilac	$20.4 M *(all lilacs)	N
Lingustrum sinense	Chinese privet	$22.6 M *(all privets)	N
Rhododendron spp.	Azaleas	$92.8 M	Y
Hydrangea spp.	Hydrangea	$30.1 M	Y
Lagerstroemia spp.	Crapemyrtle	$66.0 M	Y
Fraxinus spp.	Ash	$9.9 M	Y

Sales data from United States Department of Agriculture
bi-annual Census of Horticultural Specialties (last reported 2014)

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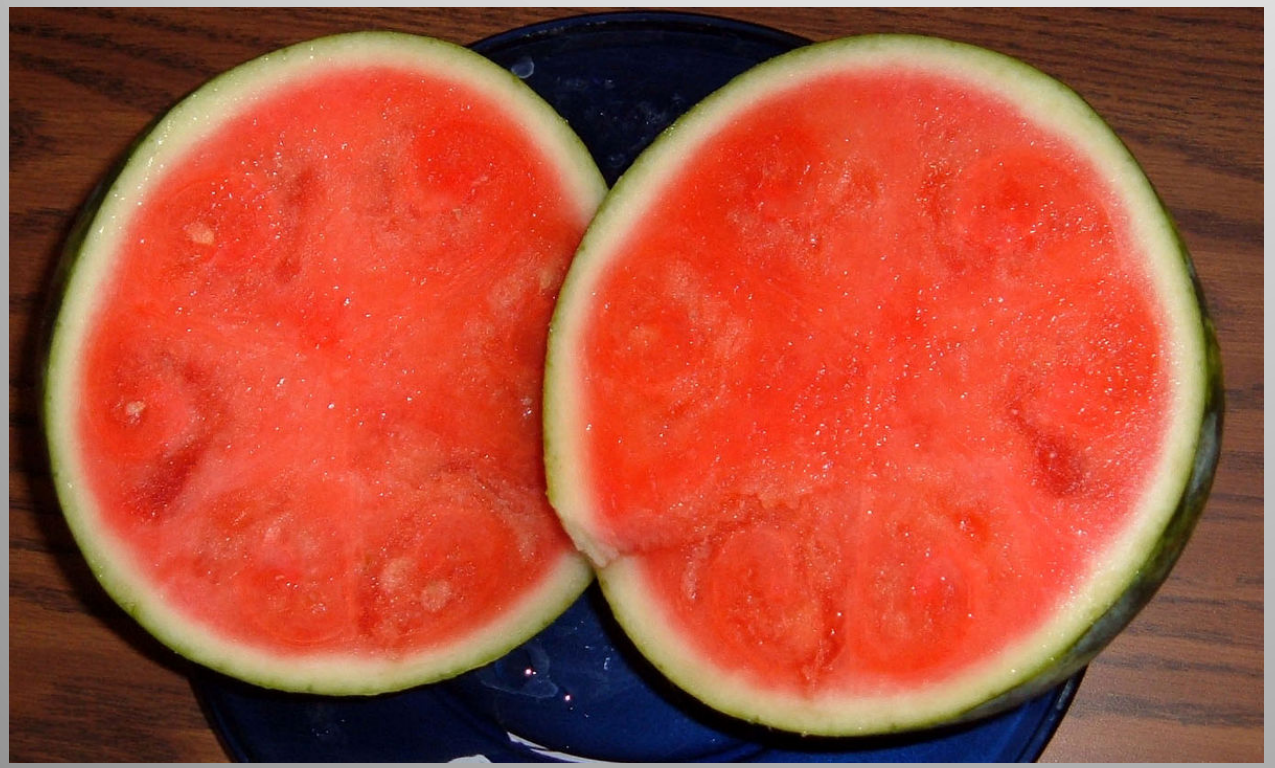
Classical Breeding vs. Biotechnology

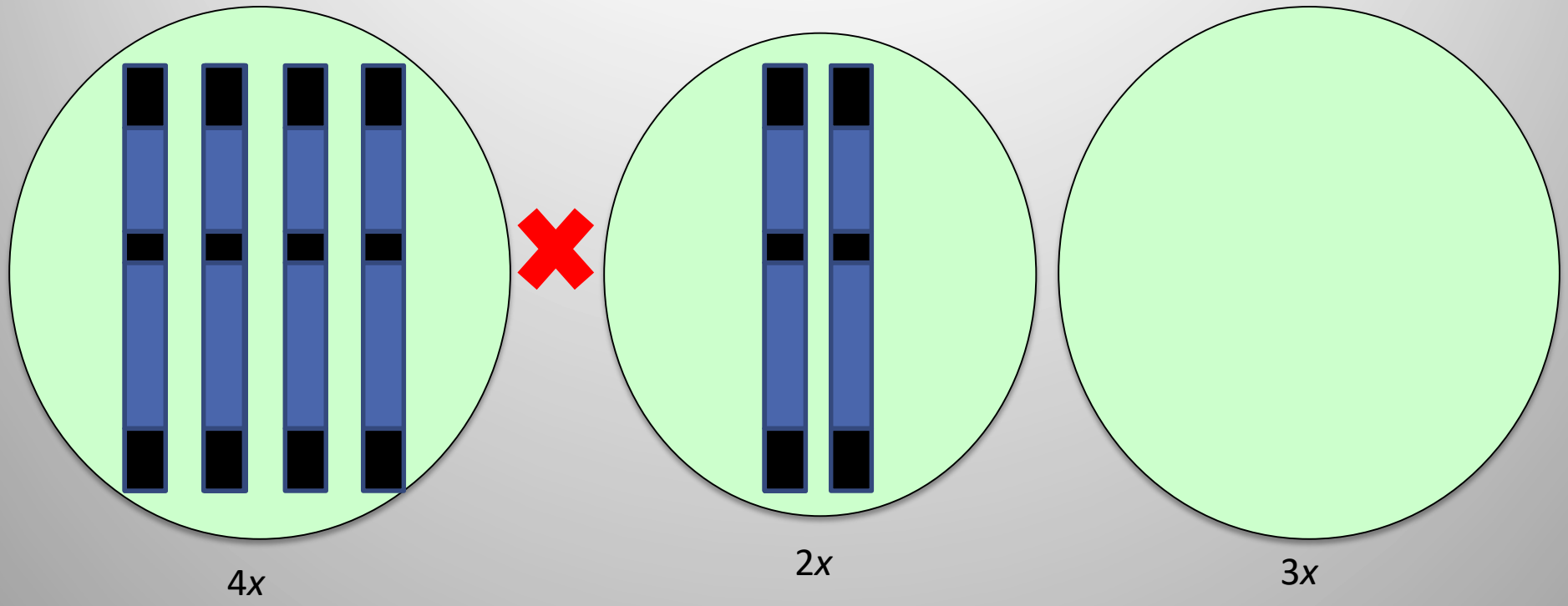
- What do we do now?
  - ***Sterility***
  - Novel phenotypes (color, fragrance, form)
  - Disease resistance
  - Improved production (rooting, container performance)
- What is possible and what is just daydreaming?

Sterility induction by chromosomal modification

- Change ploidy (chromosome number)
- Induced polyploidy then backcross



How triploids are produced and lead to sterility



Sterility: an example from maple

- *Acer ginnala*
- Started work in 2012
- Tetraploids (4x) confirmed 2013
- 4x flowered 2015
- First triploids flowered this year

Sterility: another example from maple

- Norway maples started 2011
- No triploids have flowered
- The moral: it takes time
- Extensive testing
- *Not regulated*

Sterility: Triploidy is not perfect

Group	Fruit set (%)	Seeds per fruit	Germ-ination (%)	Seedlings per flower	Relative fertility (%)
A (3x)	5	1.5	8.8	0.006	0.74
B (3x)	22.4	2.5	18.4	0.109	13.59
C (2x)	22.5	3.4	39.8	0.353	44.35

Phillips et al. 2016 HortScience 51(8):968-971.

Sterility: A Final Word

- It is possible through traditional breeding
  - In most cases (some barriers to 3x plants)
- Requires extensive testing – but all plants do for release
- Not always “sure thing” but once confirmed, should be reliable
- Biotechnology approach is “proven” by comparison but also require testing to select best products
  - Regeneration system and regulation are downsides

Novel Phenotypes

- Some very possible
- Combine existing traits
- Induce knockouts
- Grow lots of seedlings

Novel Phenotypes

Novel Phenotypes

Potential Value?

- Lilacs total $20.4 million annually
- What is the potential value for a tree lilac with purple leaves?
- How about pink/red/purple flowers?
- Every grower I have talked to indicated they would adopt…
- Perhaps 15% expansion of the market? 20%?
  - $4-5 million seems reasonable for such a novel product

Potential Value?

- Norway maple is down as much as 95% according to some growers – largely due to sterility
- STILL has a value of $13.5 million
- Continued decline of ash leaves room for more maple production
- Could we double production with introduction of sterile cultivar(s)?
- $10 million seems reasonable to me

Advantage of biotechnology

- Retain genotype of preferred cultivar(s)
- No seedling generation
- Performance, phenotype, propagation, etc. all maintained from cultivar(s) of choice

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Summary of some key points

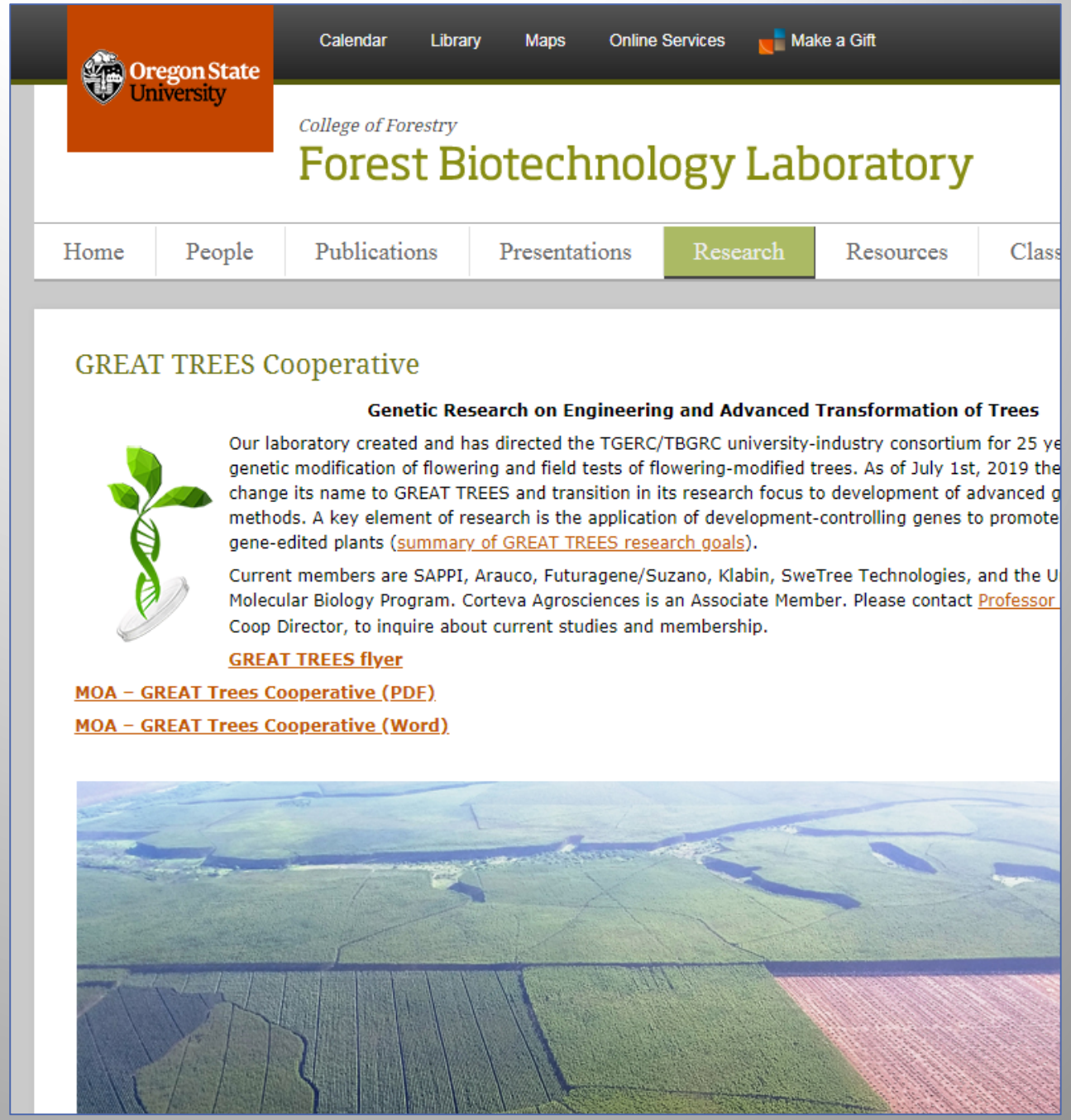
- Science has identified many genes and technologies important to our industry
- Many traits and options – what makes sense will vary widely from species to species and place to place
- Need to develop transformation/regeneration methods that can work in many species – very little research is underway
- Gene editing is very efficient and likely to be unregulated or lightly regulated in the USA and many other countries
- Biotech methods retain varietal integrity – can fix or tweak proven and favorite genotypes

Do we wish to move forward? Some key questions

- Do we in the west wish to lead in this area?
- How to mobilize an effort with many technical and social uncertainties, and significant and long term costs from research and variety development?
- Should we use our historically strong industry-university collaborations to organize, enable?
- OSU is very interested in seeing its science capabilities used for direct social good by collaboration with private sector – an "eager" partner
- Incubate as university consortium, with spin-offs to come as work matures?

A Coop model?

OSU GREAT TREES Coop, 25+ years of operation at OSU



http://people.forestry.oregonstate.edu/steve-strauss/great-trees-cooperative

Key Coop elements - I

- OSU leads on science and organization, contributes laboratory and leadership salary
  - Leveraging of federal grants a key element – often far exceeding industry contributions (~40:1 for Strauss laboratory)
- Industry members contributes to research costs, usually at reduced overhead rates, and provides in-kind aid (nursery, field trials)
- Joint decisions on goals, methods, patents, releases of improved materials

Key Coop elements - II

- Industry members obtain free or reduced royalties for patent licenses, may obtain part of royalties from others
- Regular reviews of plans, progress
- Outreach and education efforts likely an important part of effort given pubic biotech concerns

General work plan

- Identify cooperators and work structure/agreement
- Choose high value, tractable target varieties and traits
- Choose intellectual property and regulatory paths
- Develop genetic modification – regeneration systems
- Produce variety of gene edited plants for lab, greenhouse, and collaborative field testing – choose one or two for advanced testing, plant patent consideration
- Develop needed background information for public and education

Thoughts?

Interested?

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