Genome Editing in Hazelnut: *Challenges and Prospectives*

ANSC 691

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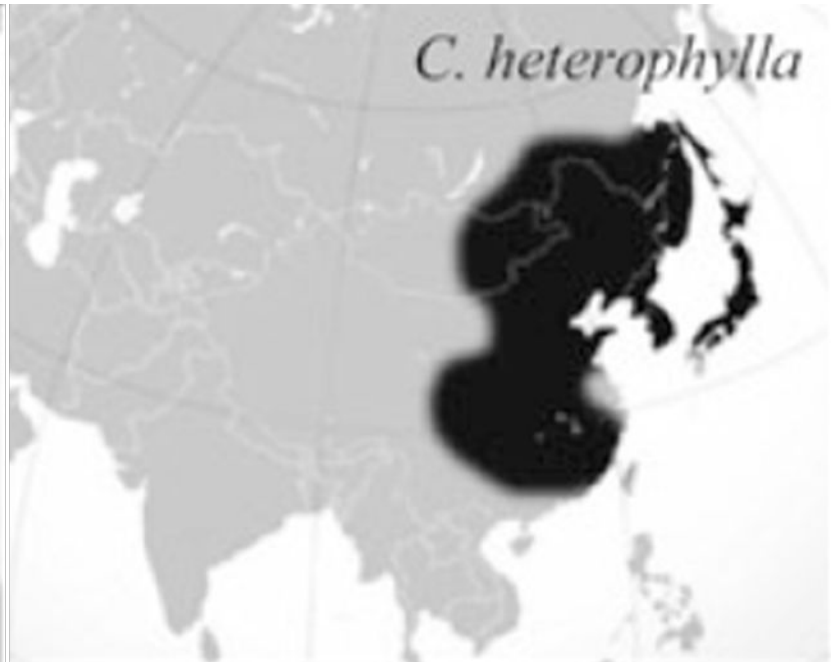
Presentation Map

- Hazelnut
- Gene Editing
- Challenges
- Prospectives
- Summary
- Questions

Hazelnut: What is it?

Hazelnut Around the World

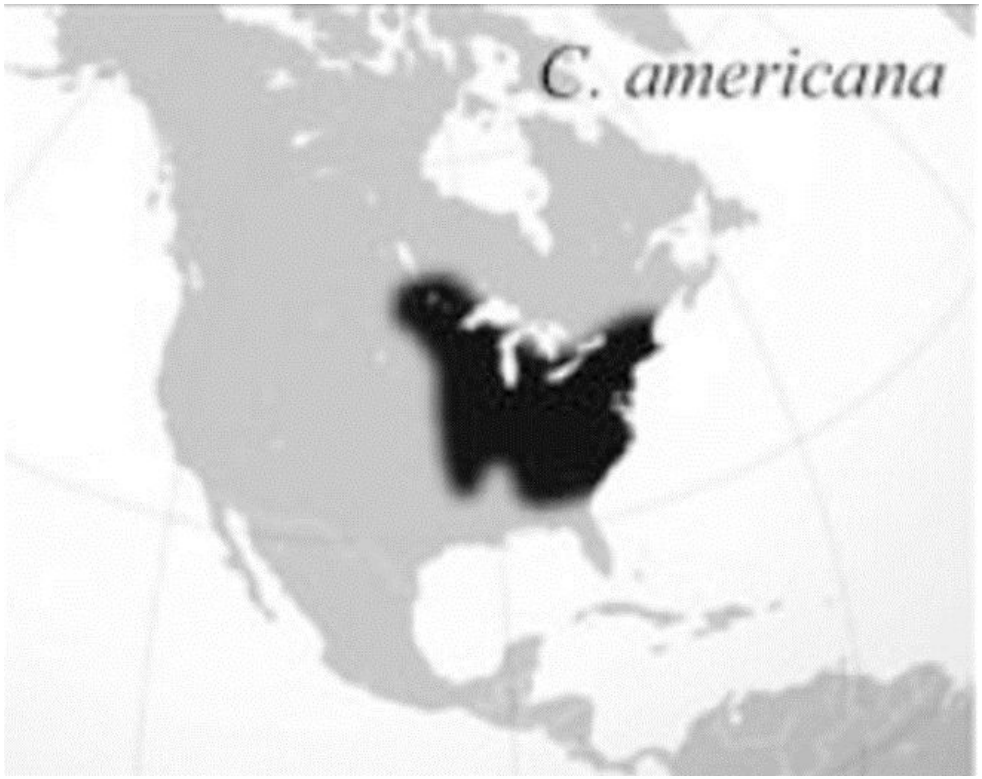


Source: Bassil et al. (2013)

Hazelnut: An Expanding Crop

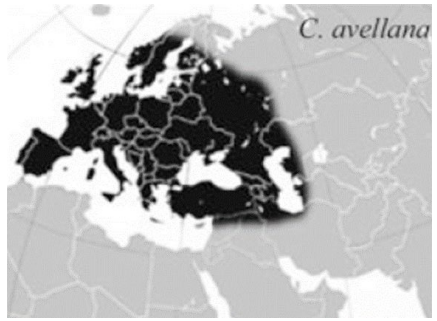
Hazelnut in North America: a challenge

Eastern Filbert Blight



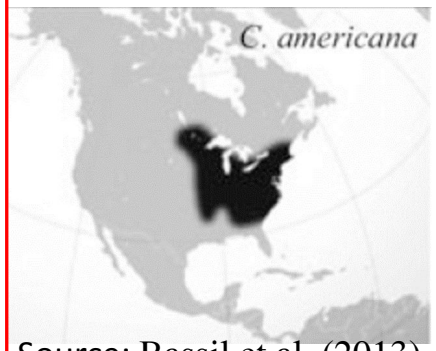
Source: Bassil et al. (2013)

Hazelnut Breeding: A slow process



Source: Bassil et al. (2013)

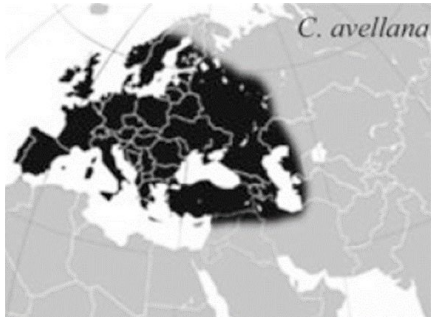
X



Source: Bassil et al. (2013)

Hybrid

X

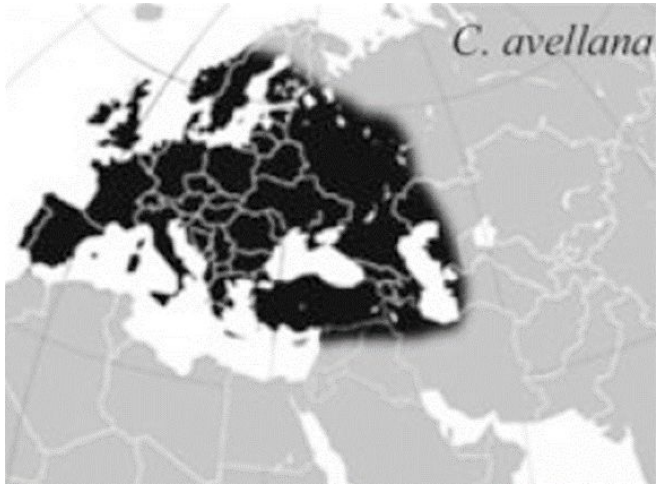


Source: Bassil et al. (2013)

- 8 years from cross to selection
- 17-18 years from cross to release of cultivar

There’s GOT to be a better way!

Gene Editing: A Solution



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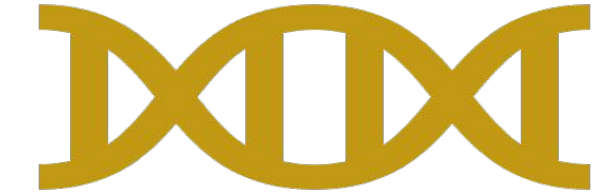
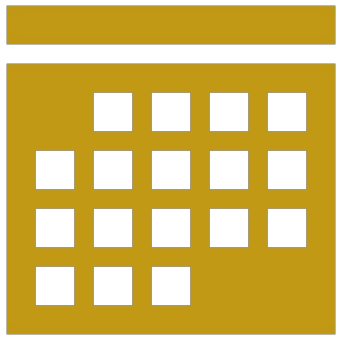


EFB Resistance Gene

What is Gene Editing?

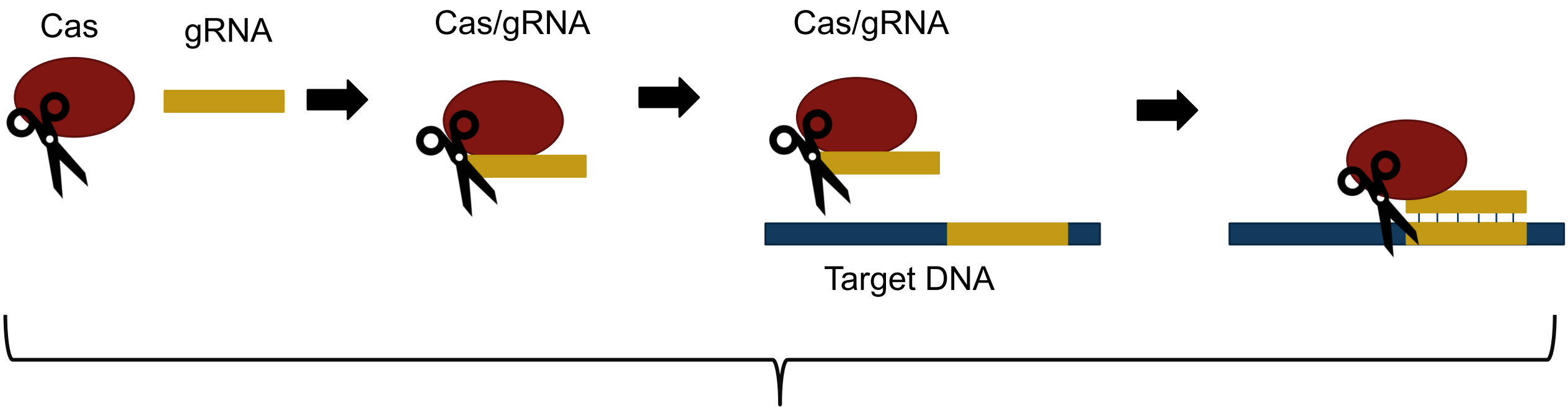
- Gene editing
  - Targeted alteration of a genetic sequence

- Genetic modification ≠ Gene editing
  - Random insertion of foreign genetic material



CRISPR: A powerful tool

- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats



Random insertion/deletions
Single nucleotide changes
Targeted insertions

Technical Requirements for CRISPR

Two things are needed to do CRISPR in a species:

1. A sequenced genome ☑
2. CRISPR Component Delivery
   1. DNA-Based
   2. DNA-Free

the plant journal

SEB
SOCIETY FOR EXPERIMENTAL BIOLOGY

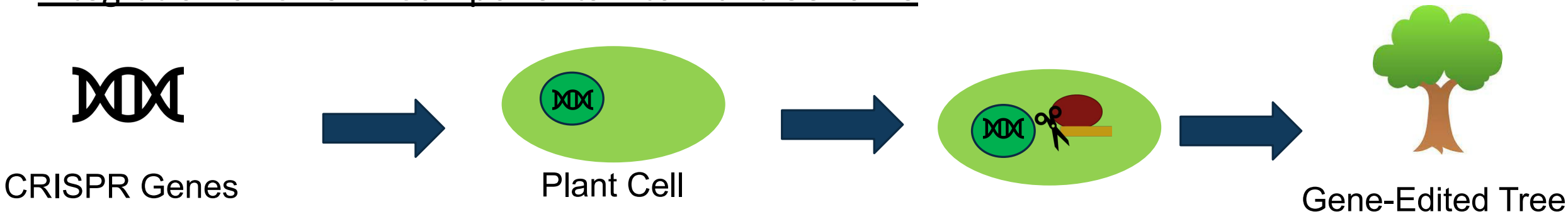
The Plant Journal (2020) doi: 10.1111/tpj.15099

A chromosome-scale genome assembly of European hazel (*Corylus avellana* L.) reveals targets for crop improvement

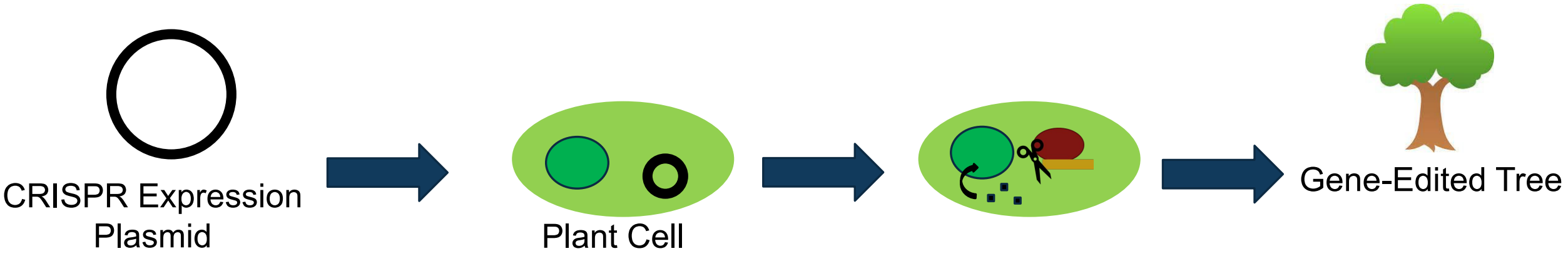
Stuart J. Lucas[1,*], Kadriye Kahraman[1,2], Bihter Avşar[1,2], Richard J.A. Buggs[3,4] and Ipek Bilge[1,2]

CRISPR Component Delivery: DNA-Based

Integration of CRISPR Components into Plant Genome

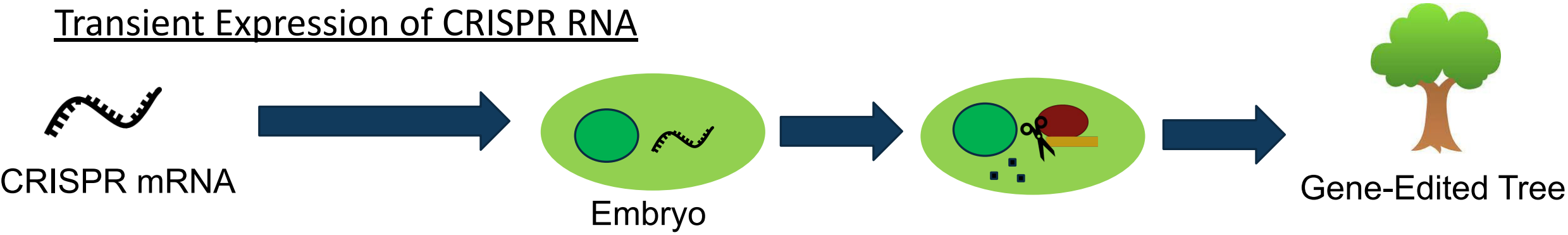


Transient Expression of CRISPR DNA-Plasmid

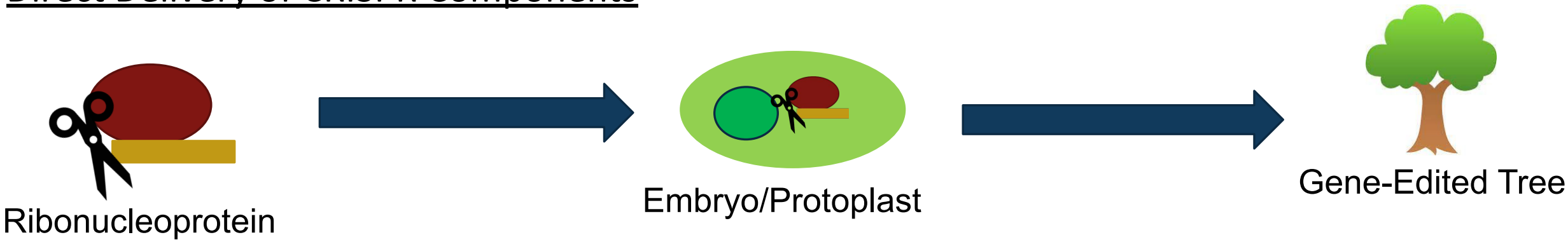


CRISPR Component Delivery: DNA-Free

Transient Expression of CRISPR RNA



Direct Delivery of CRISPR Components



Challenges

Regulatory

Technical

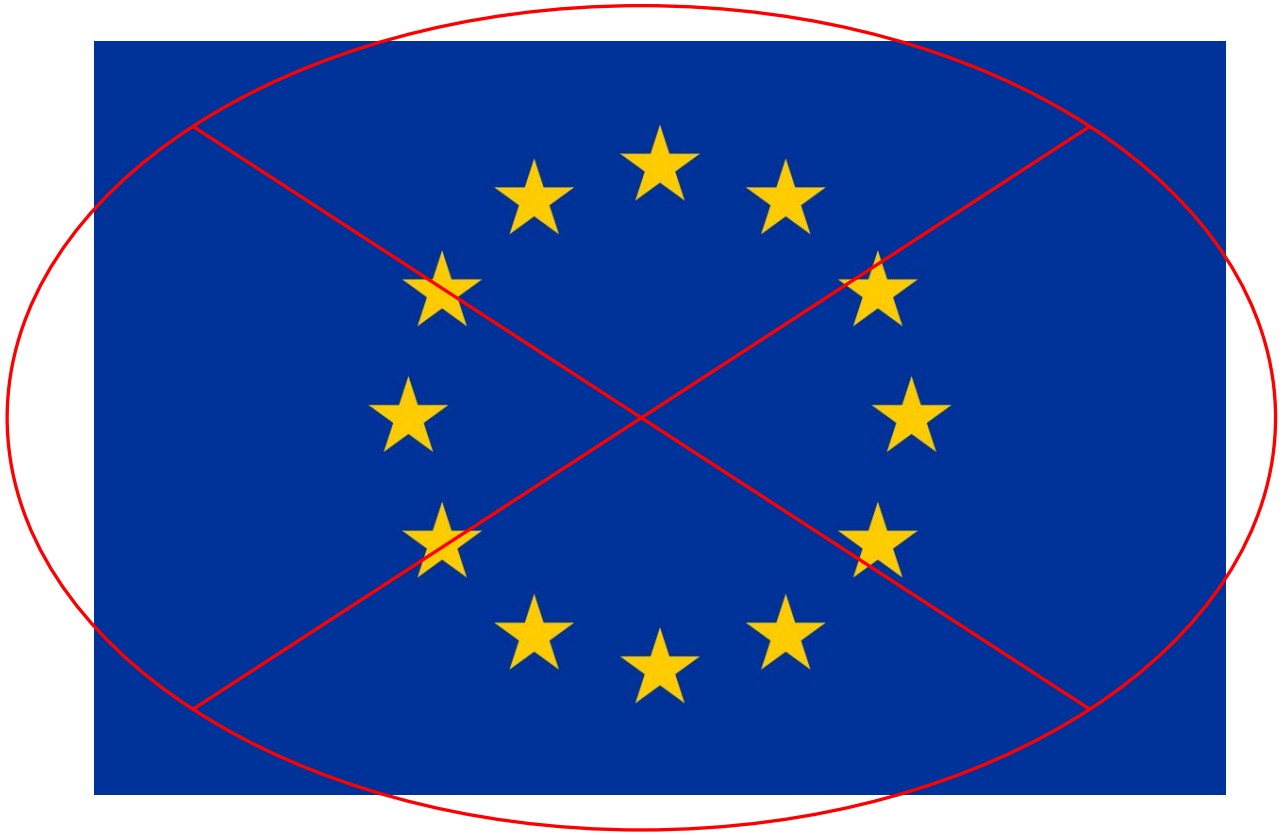
Regulation

- Regulation should be avoided for niche crops (Bullock et al., 2021)

- Processed based regulation
  - Europe
  - Any engineering by humans is regulated (Entine et al., 2021)

- Result based regulation
  - North America
  - Regulation can be avoided

GE Hazelnut Incompatible with Europe



Avoiding Regulation in the USA

United States Department of Agriculture

- A single deletion of any size/random insertion is made; OR
- A single base pair is changed; OR
- A single piece of DNA, from a sexually compatible organism, is inserted (Entine et al., 2021)

Food and Drug Administration

- The product is not significantly different from what is already on the market (Entine et al., 2021)

- The introduced trait already exists in the environment (Entine et al., 2021)

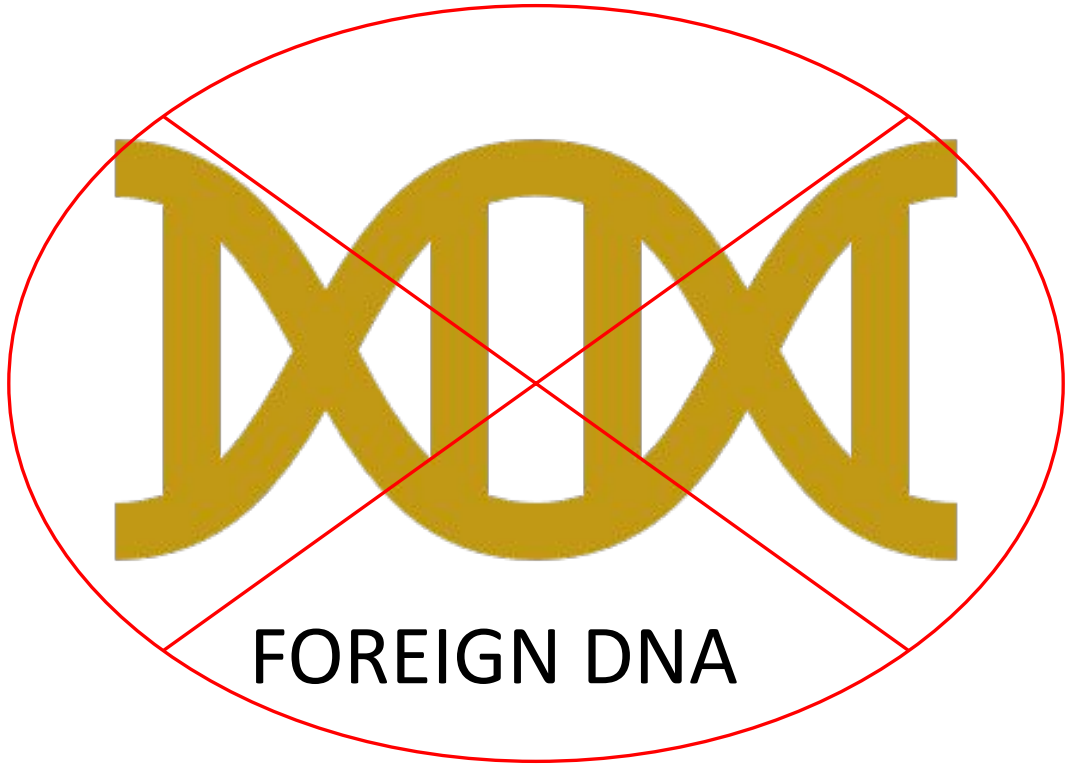
Avoiding Regulation in Canada

Health Canada

- Current Regulation (Entine et al., 2021)
  - Case-by-case determination
  - Irrespective of technology

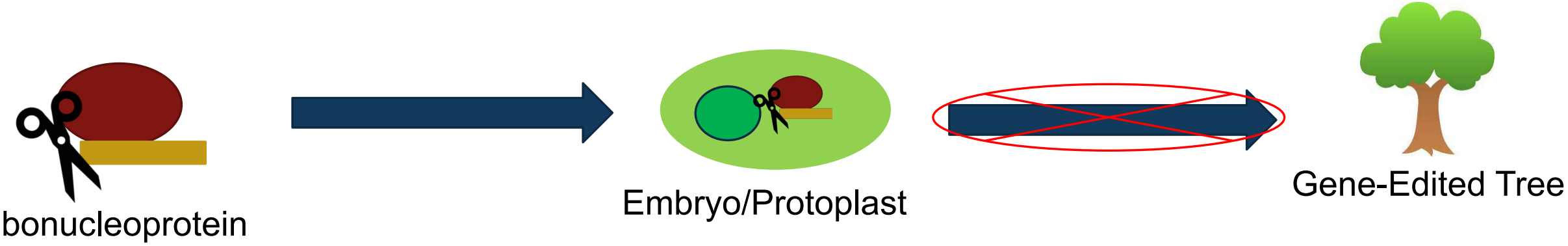
- Review of ‘safe’ gene-editing practices underway
  - Expected update: 2021

No Foreign DNA: They Key to Avoiding Regulation



Technical Challenges: DNA-Free CRISPR

Direct Delivery of CRISPR Components

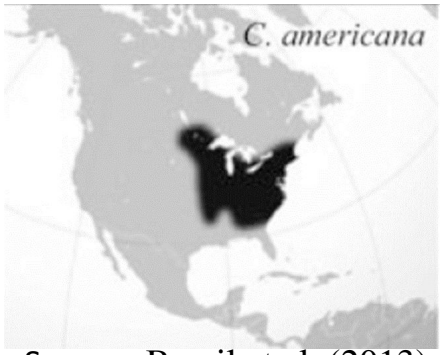


Hazelnut ***cannot*** be regenerated from single cells in-vitro

Prospectives: GE Hazelnut & North America

- North America produces ~3.5% of the global hazelnut supply (Tridge, 2021)
- North America consumes ~ 7% of total global imports (Tridge, 2021)
- GE Hazelnut can support the expanding North American Market

A Road to Gene Editing in Hazelnut

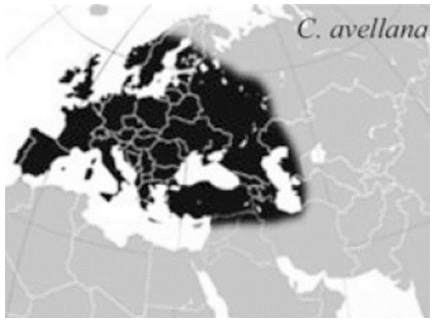


Source: Bassil et al. (2013)

EFB Resistance Gene

DNA-Free CRISPR

Targeted Insertion

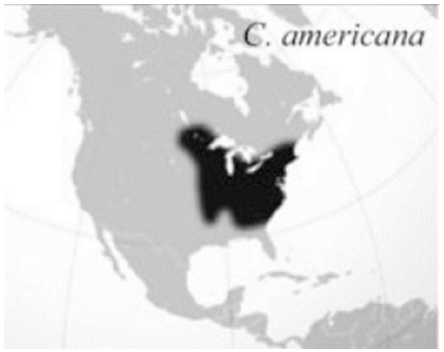


Source: Bassil et al. (2013)

Protoplast

EFB Resistant Tree

Regulation of Gene Edited Hazelnut Can be Avoided



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EFB Resistance Gene

DNA-Free CRISPR

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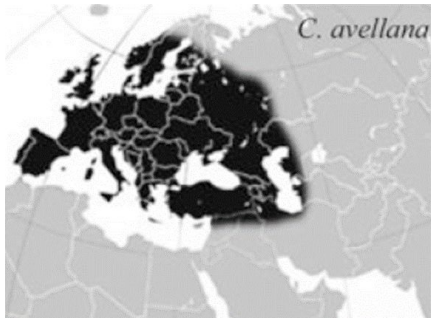

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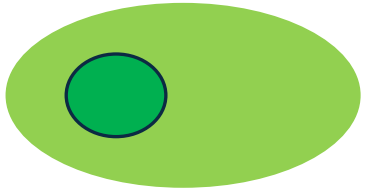


Protoplast

Gene-Edited Tree

USDA ✓

- A single deletion of any size/random insertion is made
- A single base pair is changed; OR
- A single piece of DNA, from a sexually compatible organism, is inserted

FDA ✓

- Product is not significantly Different

EPA ✓

- Trait already exists in environment

Regeneration of Hazelnut Protoplasts: Conceivable

Theor. Appl. Genet. 62, 171–176 (1982)

Plant Regeneration from *Citrus* Protoplasts: Variability in Methodological Requirements Among Cultivars and Species*

A. Vardi and P. Spiegel-Roy

Plant Cell Reports (1998) 17: 201–205

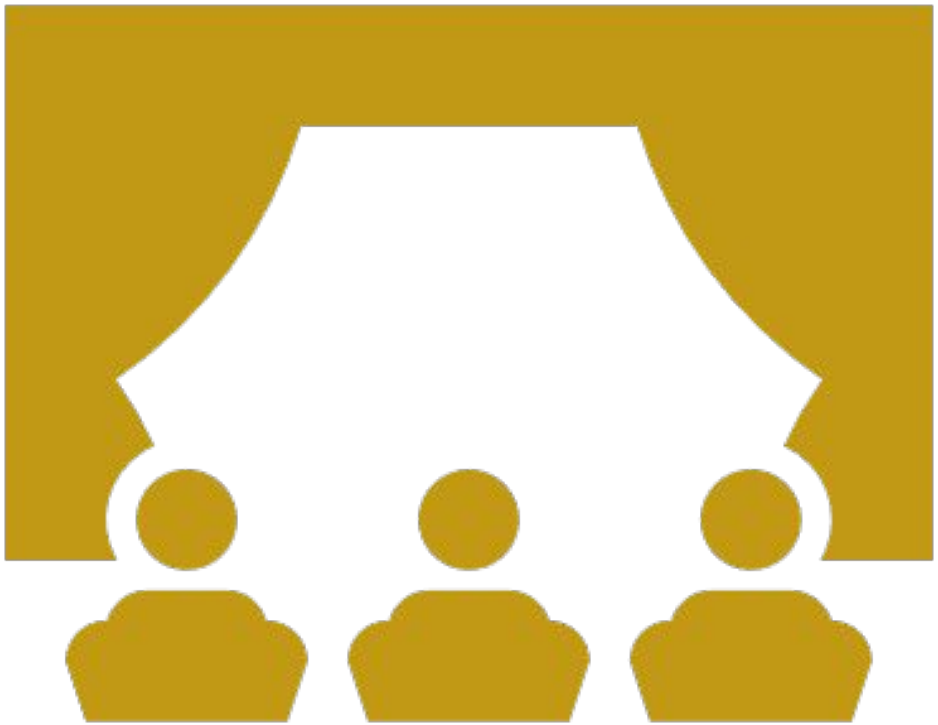
J. Qiao · H. Kuroda · T. Hayashi · F. Sakai

Efficient plantlet regeneration from protoplasts isolated from suspension cultures of poplar (*Populus alba* L.)

Summary: More Groundwork Needed

- Hazelnut is an expanding crop in North America
- Hazelnut breeding takes decades to produce elite varieties with novel traits
- Gene editing is an economically feasible tool to facilitate hazelnut breeding
- Gene edited Hazelnut must escape regulation
- Gene edited Hazelnut can escape regulation in North America
- DNA Free CRISPR should be used
- Regeneration of hazelnut from protoplasts/embryos must be established

Thanks for Listening!



Questions

References

Bassil, N.V., Boccacci, P., Botta, R., *et al*. (2013) Nuclear and Chloroplast Microsatellite Markers to Assess Genetic Diversity and Evolution in Hazelnut Species, Hybrids and Cultivars. Genetic Resourse and Crop Evolution 60:543–568.

Bullock, D.W., Wilson, W.W., Neadeau, J. (2021). Gene Editing Versus Genetic Modification in the Research and Development of New Crop Traits: An Economic Comparison. *American Journal of Agricultural Economics*. https://doi.org/10.1111/ajae.12201

Entine, J., Felipe, M.S.S., Groenewald, JH. *et al.* (2021).Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world. *Transgenic Research* https://doi.org/10.1007/s11248-021-00257-8

Lucas SJ, Kahraman K, Avşar B, Buggs RJA, Bilge I. (2021) A chromosome-scale genome assembly of European hazel (Corylus avellana L.) reveals targets for crop improvement. *the Plant Journal* 105(5):1413-1430. doi: 10.1111/tpj.15099.

Qiao, J., Kuroda, H., Hayashi, T. *et al.* (1998). Efficient plantlet regeneration from protoplasts isolated from suspension cultures of poplar (Populus alba L.). *Plant Cell Reports* 17**,** 201–205. https://doi.org/10.1007/s002990050378

Tridge. (2021, July). *Hazelnut suppliers, wholesale prices and global market information*. https://www.tridge.com/intelligences/hazelnut-in-shell

Vardi, A., Spiegel-Roy, P. & Galun, E. (1982). Plant regeneration from *Citrus* protoplasts: Variability in methodological requirements among cultivars and species. *Theoretical and Applied Genetics* 62**,** 171–176. https://doi.org/10.1007/BF00293354