



Simplot Genome Edited Strawberry

Specialty Crops Risk Assessment Workshop

March 2026

Muffy Koch + SPS Regulatory Team



AGENDA

**Intro to Simplot Plant
Sciences**

Strawberry Biology

Line Development & Selection

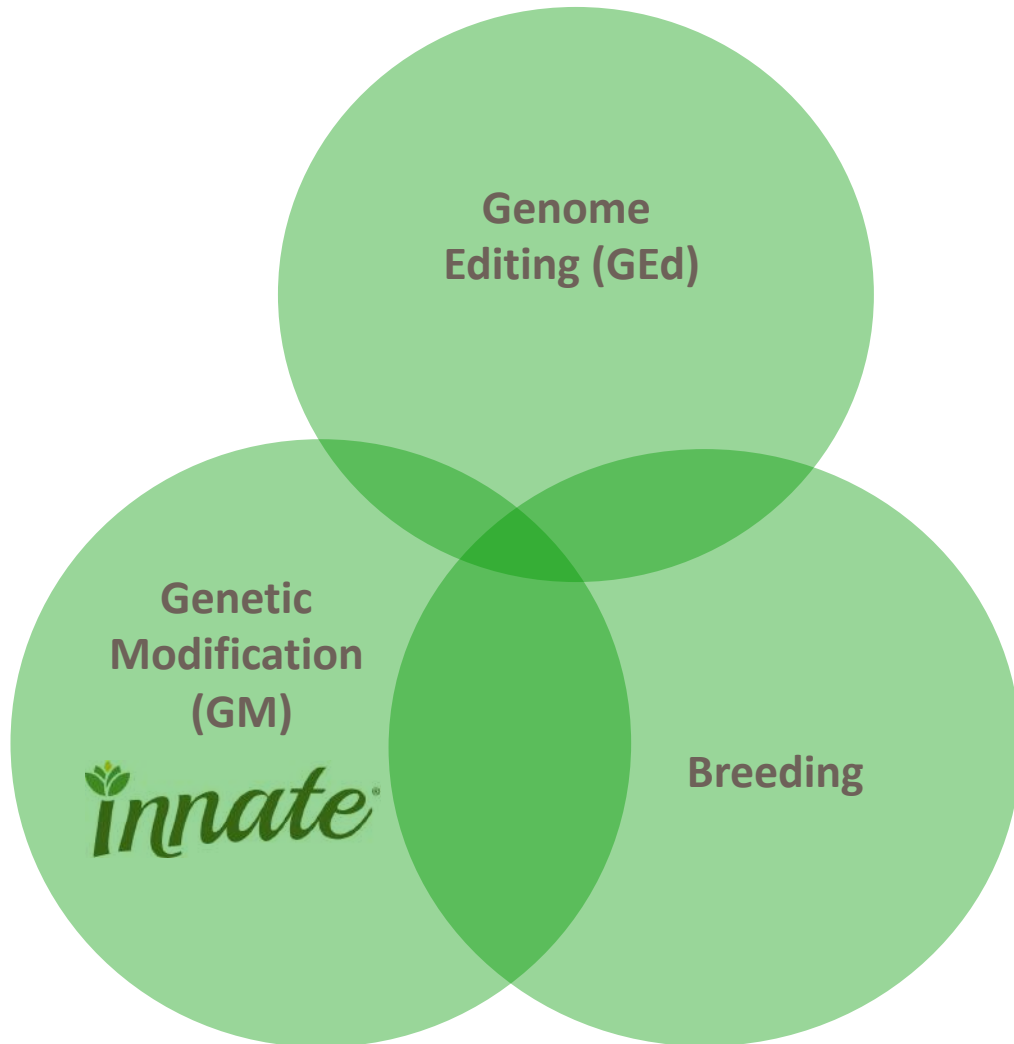
Product Assessment

**United States Regulatory
Strategy**

Intro to Simplot Plant Sciences



Simplot Plant Sciences



- Started in 2000, by Scott Simplot
- ~95 employees – R&D, Regulatory, Compliance, Agronomy, Commercial
- Division of the **Simplot Company**

Partnered with **Plant Science Genetics** who own the parent strawberry variety & sell planting material worldwide

Strawberry Biology



Strawberry Biology

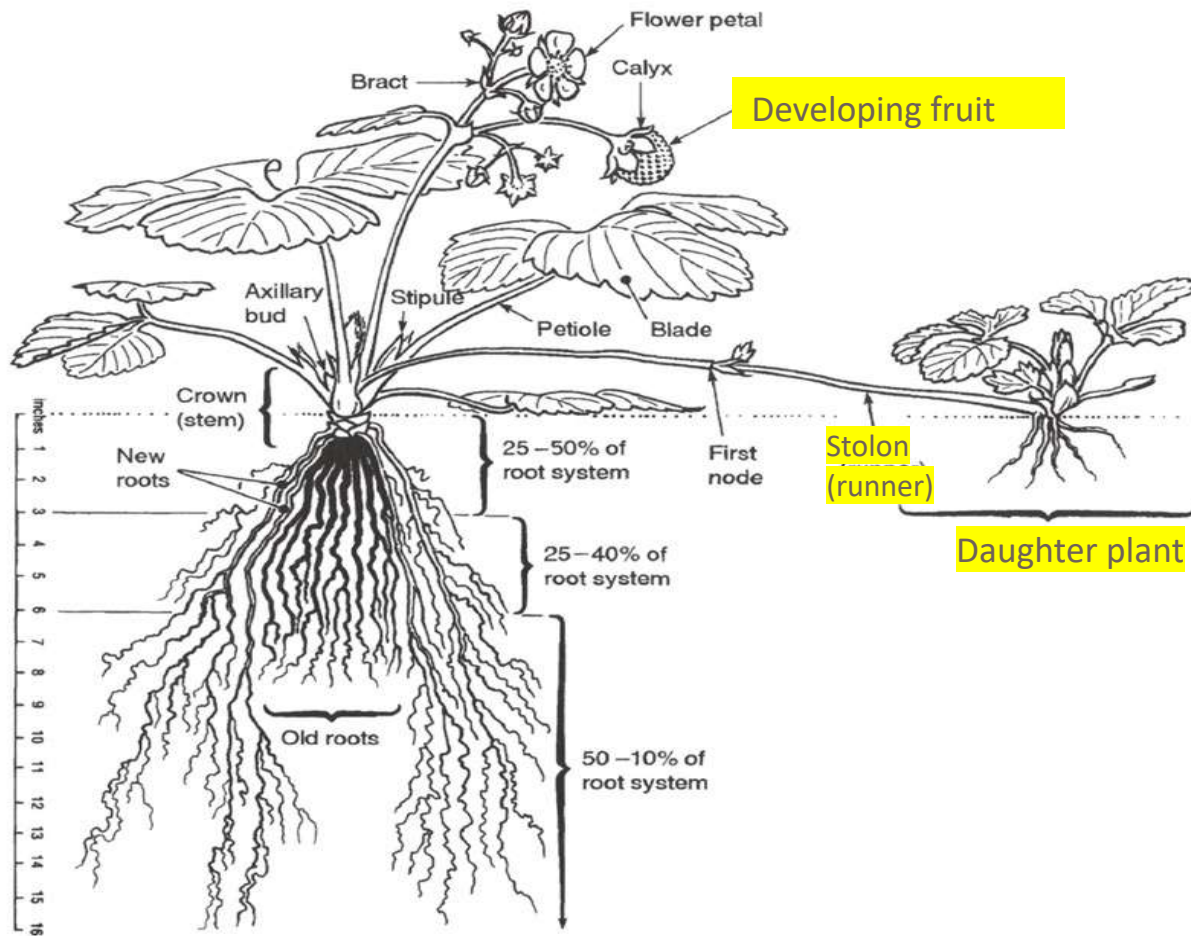
Taxonomy of Cultivated Strawberry

Kingdom	Plantae
- unranked	Angiosperms
- unranked	Eudicots
- unranked	Rosids
Order	Rosales
Family	Rosaceae
Subfamily	Rosoideae
Genus	<i>Fragaria</i>
Species	<i>× ananassa</i>



- Family Rosaceae
- Wild *Fragaria* spp occur across America, Europe, & Asia
- Cultivated strawberry: *Fragaria x ananassa* Duch.
 - Natural hybrid originated from cross between
 - *F. chiloensis* (Chile) X *F. virginiana* (North America)
- *F. x ananassa*
 - Octoploid (8x allopolyploid)
 - 4 diploid sub-genomes

Strawberry Propagation



- Vegetatively propagated
 - Producers buy & plant bare rooted plants
 - Nursery & fruit production is geographically segregated
 - True seed is used in breeding, but not used in commercial production



S4A GEd Strawberry: *Fragaria x ananassa* Duch.

Parent strawberry variety

- Plant Science Genetics (partner)
- Good flavor, color, and aroma
- LONG shelf life ~18 days
 - Shipping waste can be reduced from ~12% to ~2%
 - Storage waste can be reduced from ~20% to ~5%
- Winter short-day flower initiation
→ Dec-May fruit

Natural mutation / GEd results in remontancy in strawberries

- Adds summer long-day initiation
→ adds Apr-Nov fruit
- Enables year-round production of strawberries at 3 locations in the United States



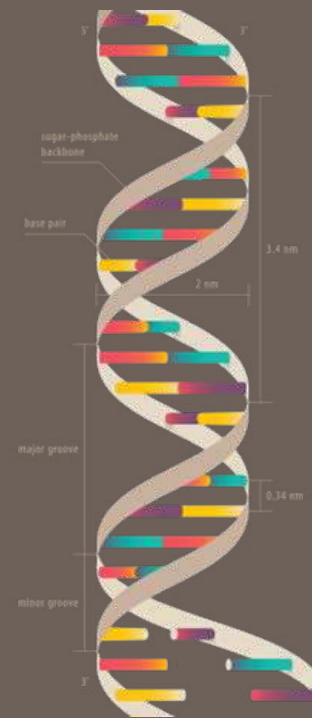
Parent Strawberry

Genome Edited Strawberry

Remontant trait
=> longer harvest season

Flowering + Berries

Line Development & Selection



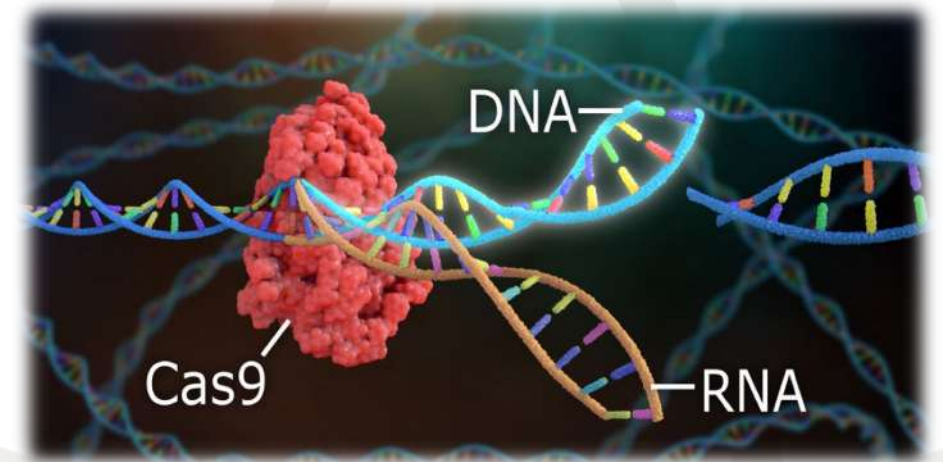
Target Gene



- *Gn1* (confidential) is conserved across plant families
 - Natural variation in *Gn1* homologs is important for domestication & crop improvement of annual crops including
 - Apple, maize, strawberry, etc.
 - Natural *Gn1* mutations found in remontant wild strawberry spp.
 - Commercial, remontant strawberry varieties
 - E.g., Ozark Beauty, Ogallala, Fort Laramie
- Identified *Gn1*, expression & role in the plant
- Only one function known
 - Not involved in metabolic pathways

Development of Remontant Strawberries

- Plasmid with CRISPR/Cas9 and a guide RNA (gRNA) designed specifically to target *Gn1*
 - gRNA specificity confirmed using two gRNA programs, Cas-designer & GuideScan
- *Agrobacterium* transformation optimized for transient expression of plasmid in strawberry cells
- *Gn1* alleles were edited by causing double stranded breaks at the target site
 - Allowed plant to repair the DNA naturally
 - Introduced edits in the sequence



Selection of Remontant Strawberry

- Two-step selection process:
 - Antibiotic to identify and discard shoots with plasmid DNA
 - Screenable markers to identify shoots with integrated plasmid backbone sequences
- After selection, edits screened using ddPCR
 - ONLY lines with targeted gene deletions and no integrated plasmid were kept
- *Agrobacterium* eliminated with abt



Selection of Remontant Strawberry, cont.

Agrobacterium-free plants potted in soil

- Propagated for planting material
- Lack of plasmid DNA confirmed by next-generation sequencing (NGS)

Evaluated in field trials

- 3 locations; 2 seasons
- Phenotype & agronomy
 - Flowering times
 - Fruit production

4 **S4A** lines selected for commercialization
in the U.S.



Product Assessment



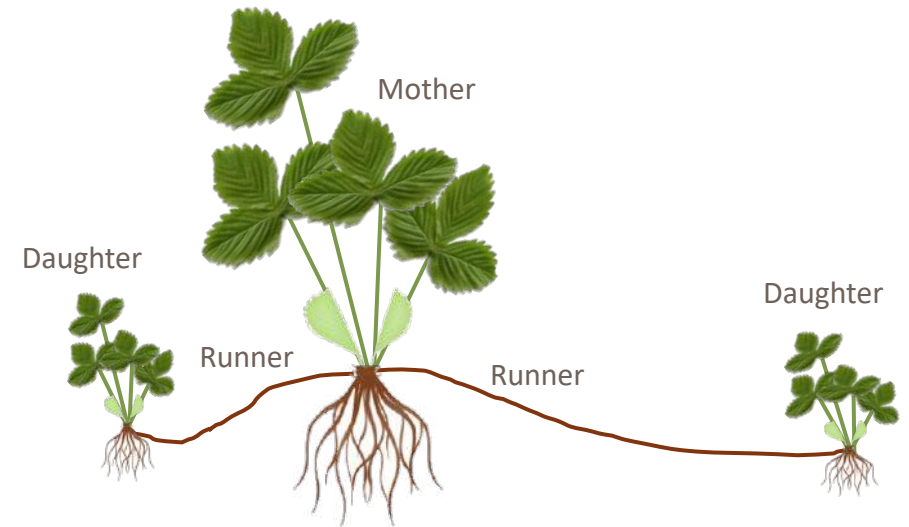
Molecular Characterization

Sequenced targeted alleles

- PCR amplification & nanopore sequencing
- Confirmed early termination (stop codon) in one or more alleles
 - Gene knock out

No foreign DNA integration

- Confirmed by PCR & DNA capture-based target enrichment using NGS



Food Quality Assessment – Allergen and Toxin Analysis



- We feel, based on the science, unless there is an identified potential impact, no allergen and toxin studies are needed
- It is unlikely that allergens or toxins would result from knock out of *Gn1*, nevertheless ...
- Allergen and Toxin Analysis
 - Novel ORF sequences in edited alleles compared to the COMPARE database for sequence identity to known allergens
 - No matches
 - *Gn1* not known to effect endogenous allergen (fra a)
- Toxin Analysis
 - Toxin similarity searches performed using the UniProtKB database
 - No matches
 - No known endogenous toxins in strawberry

Based on these findings, no new allergens or toxins are present in S4A strawberries

Food Assessment - Composition

Key nutrients in strawberry

Proximates and Fiber (7)		
Protein	Fat	Ash
Total Dietary Fiber	Carbohydrates	Calories
Moisture		
Vitamins (2)		
Vitamin B9	Vitamin C	
Minerals (2)		
Manganese	Potassium	
Sugar Profile (4)		
Fructose	Glucose	Sucrose
Total Sugars		

- We feel, based on the science, unless there is an identified potential impact, no composition studies are needed
- Composition & nutrition
 - Analytes important to the strawberry industry were measured
 - Mean values were all within combined literature ranges
 - *Gn1* KO strawberries are as nutritious as conventional strawberries
 - *Gn1* not known to affect endogenous metabolites or antinutrients
 - S4A strawberries will be grown for food & processed similarly to other strawberries
- Strawberry is not an animal feed

Environmental Assessment – Internal Rationale



- Remontant trait exists in wild strawberries & cultivated varieties
- Cultivation of these varieties & proximity to wild species has not caused environmental damage
- Commercial strawberries are not considered weedy, invasive, or persistent
- Outcrossing occurs rarely & impact is negligible
- Impact of S4A cultivation on biodiversity is no different to existing cultivation

Based on this analysis, S4A strawberries do not have any different environmental impacts compared to conventional varieties

United States Regulatory Strategy



USDA, Biotechnology Regulatory Service



2020

- June: AIR letter submitted for GEed to develop remontant strawberry varieties
- August: Regulatory response: “... the USDA does not consider your genome edited strawberries to be regulated pursuant to 7 CFR Part 340.” (AIR letter to Simplot)
- Content, 9pp
 - Intended phenotype
 - Development methods: *Agro* transformation with transient expression; RNP transfection
 - Table of genetic elements in construct
 - Rationale for not regulated:
 - No plant pest sequences remain in the plant
 - No increased weed potential or plant pest risk

FDA, Voluntary Premarket Meeting

December 2024

- Information presented:
 - No significant homology to known allergens or toxins (not necessary for this trait)
 - No increase in levels of potentially harmful endogenous allergens or antinutrients
 - No change in the nutritional value of the food
 - No change to how the plant is used for food or feed
 - No introduction of new genetic elements



EPA – M009

Sept 2024

- M009 non-FIFRA-regulated determination request (exemption from regulation) based on
 - Title 7 of the U.S. Code, § 136, definition of ‘pesticide’
- Simplot believes the target **gene** (*Gn1*) or **edit** in *Gn1* do not meet criteria for a PIP or a PIP plant growth regulator, because the *Gn1* gene product:
 - Does not prevent, destroy, repel, or mitigate a pest
 - Is not a known toxicant or toxic in concentrations found in the plant
- Simplot does not consider the remontancy **trait** in strawberry to require a FIFRA registration because **trait**:
 - Does not mitigate a pest
 - Does not alter plant behavior from what already exists in wild and cultivated strawberry plants



Summary



Conventional Breeding vs. Simplot's GEd Development



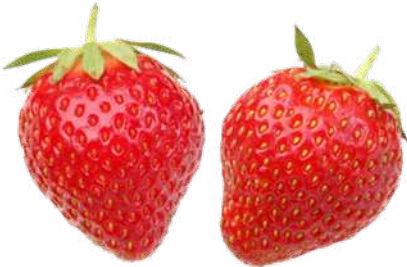
Molecular, bioinformatics & composition screening

- Not necessary for GEd determinations (same as conventional)
- These tests are available to us, but are expensive
 - Only necessary if GEd traits has an identified potential impact

Data collected	Conventional Breeding	Gene editing S4A
Agronomic characteristics	✓	✓
• Yield	✓	✓
• Growing requirements	✓	✓
Phenotypic characteristics	✓	✓
• Plant growth	✓	✓
• Desirable fruit attributes	✓	✓
Molecular screening		✓
• Sequence of targeted alleles		✓
• No foreign DNA integration		✓
• No new allergens or toxins		✓
Composition		✓
Post-harvest monitoring		✓
• Stewarding the product	✓	✓

Summary: Remontant S4A Strawberry

Remontant Strawberry S4A	
Editing tool	CRISPR-Cas9
Target gene	<i>Gn1</i>
Type of edit	Gene knock out; loss of function
Phenotype	Remontancy



Food Quality	S4A
Allergens, Toxins, Antinutrients	
• Endogenous toxins	None
• Endogenous allergens	Fra a
• Antinutrients	No
• Homology to known allergens/toxins	No
Nutrition & composition	Unaltered
• <i>Gn1</i> involved in a metabolic pathway	No
Changes in food production & use	No
Foreign DNA in final product	No
S4A is as nutritious as conventional strawberries	

Environmental Assessment	S4A
Novel trait in the population	No
Outcrossing	No harm
Weedier	No
More persistent	No
More invasive	No
Biodiversity impact	None
S4A is not a plant pest & will have the same environmental impact as commercially cultivated strawberries	



Thank You

