Precision Plant Breeding With CRISPR Genome Editing: Opportunities and Challenges

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University of Florida
Current Challenges

7.7 B → 8.5 B by 2030 → 9.7 B by 2050

Urban sprawl encroaches rapidly on farmland

Hunger
Current Challenges

Extreme Weather/ Climatic Change

Flood

A home is surrounded by floodwaters from Tropical Storm Harvey in Spring, Texas

Drought

A Texas State Park police officer walked across the lake bed of O.C. Fisher Lake in San Angelo, Texas. 2012
Current Challenges

Extreme Weather/ Climatic Change

Heat Wave, July 2018

Tornado
Current Challenges

Extreme Weather/ Climatic Change

Ice covers the Lake Michigan shoreline on January 30 in Chicago 2019

Temperature increase at North Pole
Current Challenges

Extreme Weather/ Climatic Change

Hurricane

Devastated buildings damaged by the Hurricane Irma, 2017

Wild Fire

Wildfire on the hillside in Burbank, Calif. 2018
Objectives of Plant Breeding

- Primary objective is to increase crop *yield* and improve *quality* of crop produce

Weed Control

Integrated Pest Management

Herbicide resistant Soybean

Insect resistant corn (top)

Objectives of Plant Breeding

• Primary objective is to increase crop *yield* and improve *quality* of crop produce

- Solution for limiting resources (e.g. P)
- Reduce energy and cost
- Reduce environmental impact

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Fertilizer Use Efficiency (N, P, K)

Water Use Efficiency

Soybean plants subjected to drought stress

Plant Domestication

- Plant domestication: selection by nature power

Desired traits: Larger grain, high yield, less bitterness
Plant Domestication
Plant Domestication
Hybridization Breeding: Basic Concepts

Human → Cell → Nucleus (black region - genes) → DNA helix

Skin color gene:

ATGACGGATCAGCGGCAAGCGGAATTGGCGGACATA
TACTGCTTAGTCGGCGTTGCGTTAACCCTGTGTTAT
Hybridization Breeding

Hybridization breeding Depends on genetic variation
Crossing Different Parents to Create New Variations
Hybridization Breeding

The pug and beagle have been bred to produce the ‘puggle’, a mixed breed with both pug and beagle traits.

• Introduce desirable traits from one parent to another
• Selection based on morphological characteres
• With artificial intervention
Hybridizations Breeding: Backcrossing to Clean Up Genome
Hybridizations Breeding: Backcrossing to Clean Up Genome

100% A

<table>
<thead>
<tr>
<th>F1</th>
<th>BC1</th>
<th>BC2</th>
<th>BC3</th>
<th>BC4</th>
</tr>
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<tbody>
<tr>
<td>50%:50%</td>
<td>25%:75%</td>
<td>12.5%:87.5%</td>
<td>6.75%:93.25%</td>
<td>3.38%:96.62%</td>
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</tbody>
</table>

Tolerant to drought
Lower quality plant

Sensitive to drought
Higher quality plant

GOAL
100% high quality plant + drought tolerance
Linkage Drag: Nightmare for Traditional Plant Breeders

Never has progenies with good flavor and better postharvest quality, disease resistant tomato.
Pros and Cons of Hybridization Breeding

Pros:
• No requirement for knowing genetic and genomic background
• Not regulated by USDA, ecological, environmental friendly
• Straightforward phenotypic selection, technically easy

Cons:
• Heavily relies on genetic variation, may not exist in nature
• Time and labor consuming compatibility, embryo lethality
• Quantitative traits are hard for selection
• Linkage drag
Mutation Breeding

- Spontaneous mutations: continuous source of natural genetic variation
- Traditional mutagenesis: inducing mutations by radiation or chemical mutagens
  - Large population of mutagenized plants needed (5,000-10,000)
Traditional Mutagenesis

Population of plants with genome-wide mutations (e.g. 17,000x)

Select plants with mutations in targeted gene

Further crossing to remove undesirable mutations and to obtain optimal varieties
Examples of Mutation Breeding

- Seedless orange
- Salt Tolerant Rice
- Diverse colorful flowers
- Foliage with different leaf pattern
- Bad mutations
Pros and Cons of Mutation Breeding

Pros:
• Induction of desirable mutant that is not present in natural plant materials
• Not regulated by USDA, ecologically, environmentally friendly
• Straightforward phenotypic selection, technically easy

Cons:
• generally random and unpredictable
• “good” mutations come with “bad” mutations
• Need large mutant pool to identify “good” one
• Costly and Slow
Molecular Marker Assisted Selection Breeding

- Marker assisted selection refers to the use of DNA markers that are tightly-linked to target loci.

- Assumption: DNA markers can reliably predict phenotype.
Pros and Cons of MAS Breeding

Pros:
• Similar to traditional breeding, not regulated by USDA
• Accelerating breeding process
• Easier for stacking multiple traits within the same cultivar

Cons:
• Must know genomic and genetic background
• Very costly
• False markers
Genetic Engineering

GMO ...

THE KILLING FIELDS

DO YOU KNOW WHAT THIS STUFF CAN DO TO YOU?

YES, HELP KEEP ME ALIVE.

Hey Look!
My Genetically modified fruit bit me back!

Frankenfoods are FUN FOODS!
Are these crops GMOs?

- Soybean
- Safflower
- Chicory
- Flax seeds
- Alfalfa
- Canola
- Creep Bentgrass
- Tobacco
- Polar
- Plum
- Eucalyptus
Are these crops GMOs?

- Rice
- Soybean
- Safflower
- Chicory
- Flax seeds
- Alfalfa
- Canola
- Creep Bentgrass
- Tobacco
- Polar
- Plum
- Eucalyptus
How many approved GMOs in U.S.?

203 approved Varieties for 21 crop species
## What Traits Were Modified?

<table>
<thead>
<tr>
<th>Traits</th>
<th>Crops</th>
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<tbody>
<tr>
<td>Altered lignin production</td>
<td>Alfalfa</td>
</tr>
<tr>
<td>Non-browning</td>
<td>Apple</td>
</tr>
<tr>
<td>Modified fatty acid</td>
<td>Canola</td>
</tr>
<tr>
<td><strong>Herbicide Tolerance</strong></td>
<td>Alfalfa, Canola, Chicory, cotton, creeping bentgrass, flax, corn, rice, soybean, sugar beet, wheat</td>
</tr>
<tr>
<td>Male sterility</td>
<td>Alfalfa, Chicory, corn</td>
</tr>
<tr>
<td><strong>insect-resistance</strong></td>
<td>Cotton, corn, potato, rice, soybean, sugar cane, tomato</td>
</tr>
<tr>
<td>Nutrition</td>
<td>Canola, corn, potato, rice, soybean</td>
</tr>
<tr>
<td>Drought tolerance</td>
<td>Corn</td>
</tr>
<tr>
<td>Delayed ripening</td>
<td>Melon, tomato</td>
</tr>
<tr>
<td>disease resistance</td>
<td>Papaya, potato, squash</td>
</tr>
<tr>
<td>Modified flower color</td>
<td>rose</td>
</tr>
<tr>
<td>Yield</td>
<td>soybean</td>
</tr>
<tr>
<td>Nicotine reduction</td>
<td>tobacco</td>
</tr>
</tbody>
</table>
GMO Examples

Developed by Australia
Approved in Australia, Japan, European Union

Modified color (genes from petunia)
Herbicide resistance (mutated gene from tobacco)
GMO Examples

Susceptible plants

Ringspot virus resistant papaya

Conventional Apple Variety

Arctic™ Apple Variety

Bt-corn

Non-GMO

Top 10 Omega 3 Foods To Add To Your Diet

Innate™ Conventional
Stopping Citrus Greening

EPA approves field trials of disease-resistant GMO citrus trees

Biotechnologia Sí | November 16, 2016

Stopping Citrus Greening

American chestnuts were once a dominant tree, and a major source of food, in the forests of eastern North America. Today, chestnuts are nearly extinct due to a fungus that was accidentally introduced from China in the 1950s.

To save iconic American chestnut, researchers plan introduction of genetically engineered tree into the wild

By Gabriel Popkin | Aug. 26, 2018 | 12:30 PM
Development Of Glowing Ornamental Plants

LED(365nm) UV light

WT  eGFP  eYGFPuv

LED (365 nm)  w/o emission filter

Bright-field  Dark-field

Scientific Reports (2018) 8:16556
### Table 1. Global Area of Biotech Crops, the First 21 Years, 1996 to 2016

<table>
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<tr>
<th>Year</th>
<th>Hectares (million)</th>
<th>Acres (million)</th>
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<td>1996</td>
<td>1.7</td>
<td>4.2</td>
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<tr>
<td>1997</td>
<td>11.0</td>
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<tr>
<td>1998</td>
<td>27.8</td>
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<td>1999</td>
<td>39.9</td>
<td>98.6</td>
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<td>2000</td>
<td>44.2</td>
<td>109.2</td>
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<tr>
<td>2001</td>
<td>52.6</td>
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<tr>
<td>2002</td>
<td>58.7</td>
<td>145.0</td>
</tr>
<tr>
<td>2003</td>
<td>67.7</td>
<td>167.3</td>
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<tr>
<td>2004</td>
<td>81.0</td>
<td>200.2</td>
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<tr>
<td>2005</td>
<td>90.0</td>
<td>222.4</td>
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<td>2006</td>
<td>102.0</td>
<td>252.0</td>
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<tr>
<td>2007</td>
<td>114.3</td>
<td>282.4</td>
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<tr>
<td>2008</td>
<td>125.0</td>
<td>308.9</td>
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<td>2009</td>
<td>134.0</td>
<td>331.1</td>
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<tr>
<td>2010</td>
<td>148.0</td>
<td>365.7</td>
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<tr>
<td>2011</td>
<td>160.0</td>
<td>395.4</td>
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<tr>
<td>2012</td>
<td>170.3</td>
<td>420.8</td>
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<td>2013</td>
<td>175.2</td>
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<tr>
<td>2014</td>
<td>181.5</td>
<td>448.5</td>
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<tr>
<td>2015</td>
<td>179.7</td>
<td>444.0</td>
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<tr>
<td>2016</td>
<td>185.1</td>
<td>457.4</td>
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<tr>
<td>Total</td>
<td>2,149.7</td>
<td>5,312.0</td>
</tr>
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</table>

Figure 1. Global Area of Biotech Crops, 1996 to 2016: Industrial and Developing Countries (Million Hectares)

Source: ISAAA, 2016
In 2016, global area of biotech crops was 185.1 million hectares, representing an increase of 3% from 2015, equivalent to 5.4 million hectares.

How Are GMOs Created?

1. Cut out the gene.
2. Insert gene into a vector with a selectable antibiotic resistance marker gene.

Agrobacterium-mediated transformation

Co-cultivation of Agrobacterium with plant tissues

Load bullet into gene gun.

Insect resistant tomato
Pros and Cons of Genetic Engineering

Pros:
• Fast way to verify gene function
• Precisely modify crop productivity and quality

Cons:
• Necessary to know gene function
• Very costly, complicated procedures
• Heavily regulated by USDA
CRISPR Loci induce acquired immunity in Bacteria against the virus infection or plasmid transfer

Horvath and Barrangou, 2010, Science: 167-170
Features of CRISPR/Cas9 Genome Editing

• High precision, High efficiency, Broad application
• Procedures are identical to genetic modification
• Final products are similar to traditional breeding
**High Precision:** no or less non-targeted mutations

**Traditional Mutagenesis Vs Targeted Mutagenesis**

Population of plants with genome-wide mutations (e.g. 17,000x)  

A few plants with targeted mutations

Traditional mutagenesis  

Targeted mutagenesis
How CRISPR/CAS9 Can Create Precise Mutation?

- Can recognize the unique location
- Cas9: one protein
- Guided sequences

DNA Bind → Cut

Gene disruption: Turn off
Gene correction: Turn on

Repair template
Turn Off or Turn On

Disease susceptible cultivar

Disease resistant cultivar

Genes

Disease sensitive gene

Disease resistant gene

Genes

Genes

Genes

Genes

Genes

Genes

Genes

Genes

Genes

Genes
Precision

ALS2 gene editing for herbicide resistant corn

- Ability to target ALS2, but not sister ALS1, for herbicide resistance in corn
High Efficiency: Multi-Targeting

Simultaneous targeting of multiple genes

In rice, more than 8 targets were successfully targeted.
High Efficiency: Time

Selection and plant regeneration: 2-3 months

Growth to maturity and seed set in controlled environment: 3-4 months
High Efficiency: Time

- Significantly reduce the number of crossing cycles
- Significantly reduce plant number for each generation
High Efficiency: Time

Year: 1 2 3 4 5 6 7 8 9 10 11 12 13 14

- **EDITING**
  - 1st commercial sales in year 4

- **BREEDING**
  - 1st commercial sales in year 9

- **TRANSGENICS**
  - 1st commercial sales in year 13-20

UF/IFAS
UNIVERSITY of FLORIDA
MLO1 gene, controlling powder mildew sensitivity, has been identified in wheat, barley, rice, tomato, petunia, tobacco, eggplant, cucumber, squash, melon, strawberry, lettuce, orange, and more.

Use CRISPR to knock out MLO1 gene to create powdery mildew pathogen resistant tomato.
Broad applicability: Multiple Plant Species

- Rice blight
- Cabbage black rot
- Wheat streak
- Citrus canker
- Cassava blight
- Banana wilt
- Cotton blight

*Figure modified from the internet*
Gene editing of OsSWEET14 to create Blight (Xanthomonas)-resistant rice

In collaboration with Dr. Deng in Gulf Coast Rec, SWEET gene has been edited for testing citrus canker disease resistance.

Similar strategy can be employed to other plant species.
Broad applicability: polyploidy plants

Simultaneous targeting of multiple copies of same genes in polyploidy crops

WT and tadep1-aabbdd mutant (high yield)
High Gene Editing Efficiency in Allotetraploid Tobacco and Octoploid Strawberry

Allotetraploid Tobacco

Octoploid Strawberry

In collaboration with UF/IFAS Gulf Coast Rec
Non-GMO: NO Foreign DNA

GMO approach

Genome editing (e.g. CRISPR/Cas) (non-GMO)

The Transgene (e.g. R-gene) insertion makes the product

Non-GMO approach

Final product with the edit but no transgene

Accept

Discard
Breeding Non-GMO Lettuce with CRISPR-Genome Editing

Use your eyes to find Non-GMO plants

NPT-GFP::pCassava Mosaic Virus
pUBI::HypaCas9
ProAtU6::crRNA-2 x Bsal-crRNA

Control Gene Edited

Lettuce Seeds are inhibited by Florida high temperature, but gene edited lettuce can germinate well

F2 Segregated Seeds

UF IFAS UNIVERSITY OF FLORIDA
Complimentary To Traditional Breeding: Solution to Linkage Drag

Never has progenies with good flavor and better postharvest quality, disease resistant tomato.
Complimentary To Traditional Breeding: Solution to Linkage Drag

Gene editing can remove linkage drag to create better plants

- Good Flavor
- Disease Susceptibility
- Poor postharvest quality
- High Yield

CIRSPR Editing
How Different Is Between Conventional Breeding And CRISPR Genome Editing?

<table>
<thead>
<tr>
<th></th>
<th>Radiation mutagenesis</th>
<th>Conventional Breeding</th>
<th>GMO</th>
<th>Genome Editing</th>
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<tbody>
<tr>
<td>genetic modification</td>
<td>YES, Artificial (e.g. radiation)</td>
<td>Yes, by nature (e.g. reshuffling of DNA fragment or recombination</td>
<td>Yes, Transgene</td>
<td>Yes, transgene</td>
</tr>
<tr>
<td>Turn gene off</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Turn gene on</td>
<td>No (extremely rare)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Foreign DNA</td>
<td>No</td>
<td>No</td>
<td>yes</td>
<td>No (case by case)</td>
</tr>
<tr>
<td>Distinguished from conventional breeding</td>
<td>no</td>
<td>-</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Efficiency</td>
<td>low</td>
<td>low</td>
<td>high</td>
<td>Very high</td>
</tr>
<tr>
<td>Precision</td>
<td>random</td>
<td>random</td>
<td>random</td>
<td>precise</td>
</tr>
<tr>
<td>Cost</td>
<td>high</td>
<td>high</td>
<td>High (mainly for dealing with regulation)</td>
<td>Low</td>
</tr>
<tr>
<td>Difficulties of procedures</td>
<td>easy</td>
<td>easy</td>
<td>complicated</td>
<td>complicated</td>
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</tbody>
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### Rise of Private Sector in Application of Genome Editing Techniques for Crop Improvement

<table>
<thead>
<tr>
<th>Company</th>
<th>Location</th>
<th>Year Established</th>
<th>Selected Tools/Services</th>
<th>Focus Crops</th>
</tr>
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<tbody>
<tr>
<td>Benson Hill Biosystems</td>
<td>St. Louis, MO</td>
<td>2012</td>
<td>CROP-OS software; gene editing using CRISPR-Cpf1 and -Cms1</td>
<td>Row crops edited for higher yield, stress resistance, and herbicide tolerance</td>
</tr>
<tr>
<td>Corteva (DowDuPont)</td>
<td>Wilmington, DE</td>
<td>2018</td>
<td>CRISPR-Cas9</td>
<td>Waxy corn modified for altered starch composition</td>
</tr>
<tr>
<td>Pairwise</td>
<td>Durham, NC</td>
<td>2018</td>
<td>CRISPR-Cas9 with base editing</td>
<td>Row crops such as corn and soybeans with increased productivity, disease resistance; more-convenient fruits and vegetables</td>
</tr>
<tr>
<td>Syngenta</td>
<td>Basel, Switzerland</td>
<td>2000</td>
<td>CRISPR-Cas9</td>
<td>Corn, soy, wheat, tomato, sunflower, modified to increase yield</td>
</tr>
<tr>
<td>Tropic Biosciences</td>
<td>Norwich, UK</td>
<td>2016</td>
<td>CRISPR and other techniques</td>
<td>Disease-resistant bananas, decaffeinated coffee</td>
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<tr>
<td>Yield10 Bioscience</td>
<td>Woburn, MA</td>
<td>2015</td>
<td>CRISPR-Cas9</td>
<td>Camelina engineered for higher oil content</td>
</tr>
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Rise of Public Sector in Application of Genome Editing Techniques for Crop Improvement

Table 1. NIH Funding for CRISPR-Related Research, FY2011-FY2018

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Projects</th>
<th>Total Funding</th>
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<tbody>
<tr>
<td>2011</td>
<td>7</td>
<td>$5,070,129</td>
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<tr>
<td>2012</td>
<td>9</td>
<td>$7,432,520</td>
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<tr>
<td>2013</td>
<td>30</td>
<td>$12,505,507</td>
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<tr>
<td>2014</td>
<td>161</td>
<td>$85,298,742</td>
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<td>2015</td>
<td>551</td>
<td>$267,055,410</td>
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<td>2016</td>
<td>1,245</td>
<td>$603,205,999</td>
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<tr>
<td>2017</td>
<td>2,031</td>
<td>$947,465,783</td>
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<tr>
<td>2018</td>
<td>2,651</td>
<td>$1,155,385,840</td>
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<tr>
<td>Total</td>
<td>6,685</td>
<td>$3,083,419,930</td>
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</table>

<table>
<thead>
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<th>Year</th>
<th>Publications</th>
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<td>87</td>
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<tr>
<td>2012</td>
<td>137</td>
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<tr>
<td>2013</td>
<td>300</td>
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<td>2014</td>
<td>670</td>
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<tr>
<td>2015</td>
<td>1,457</td>
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<tr>
<td>2016</td>
<td>2,594</td>
</tr>
<tr>
<td>2017</td>
<td>3,738</td>
</tr>
<tr>
<td>2018</td>
<td>3,917</td>
</tr>
<tr>
<td>Total</td>
<td>12,900</td>
</tr>
</tbody>
</table>

November 20, 2018.
USDA Will Not Regulate CRISPR-Edited Crops
Restrictions will remain on transgenic plants, which contain artificially inserted genes from other species.

The USDA says Crispr-edited foods are just as safe as ones bred the old-fashioned way
By Katherine Ellen Foley • April 2, 2016

The USDA Just Gave the Green Light to CRISPR’d Food
Kristen V. Brown
3/30/18 2:38 pm • Filed to: CRISPR

USDA confirms it won't regulate CRISPR gene-edited plants like it does GMOs
Rich Hardy • April 3rd, 2018

USDA slams EU's decision on regulating gene-edited products
Chris Koger
July 30, 2016 06:08 PM

Trending in The Packer
USDA-Regulated?

Not regulated as long as
❖ they are not plant pests or developed using plant pests.
❖ Noxious weed

This would include plant varieties with the following changes:
• Deletions
• Single base pair substitutions
• Insertions from compatible plant relatives (foreign DNA from bacteria, insects, virus etc. will be still regulated)
• Off-springs of a genetically engineered plant that does not retain the change of its parent
### USDA Authorizations: Products Of Genome Editing

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Permits and Notifications</th>
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<tbody>
<tr>
<td>2013</td>
<td>1</td>
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<tr>
<td>2014</td>
<td>21</td>
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<tr>
<td>2015</td>
<td>56</td>
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<tr>
<td>2016</td>
<td>90</td>
</tr>
<tr>
<td>2017</td>
<td>124</td>
</tr>
<tr>
<td>June/2018</td>
<td>62</td>
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<tr>
<td>Total</td>
<td>354</td>
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</table>

Dupont/Pioneer aim to launch the first CRISPR-enabled **waxy corn** around 2020.
Food And Drug Administration (FDA) - Regulated?

- All food is regulated, regardless of how plant varieties are bred
- No unique requirements exist for food developed with biotechnology
- All food must meet universal regulatory requirements
  - All food must be safe
  - GE food labelling? Yes
  - Genome-Edited food labeling? probably

GE Soybean with increased levels of oleic acid

New name: “High oleic soybean oil”

GE soybean oil
Global Regulatory Status

- **Canada**: Not regulated unless product is novel
- **United States**: ‘Am I Regulated?’ inquiries
- **Argentina, Brazil, Chile**: Case by case approaches; foreign DNA insertions generally regulated
- **Israel**: Foreign DNA insertions regulated
- **Norway**: Proposed; foreign DNA insertion regulated; otherwise case-by-case
- **Europe**: YES
- **China, Japan, Korea**: Issue still being debated; no formal guidance
- **Australia**: Under review; likely regulated whenever foreign DNA involved; otherwise case-by-case
Challenges

• Availability of Genomic Sequence
• Efficient plant transformation pipe line
• DNA-free genetic transformation method for perennial plant species
IP Licensing Agreements Landscape

Free for academic researchers
Social Acceptance And Ethical Concerns

• Consumer acceptance

• Ecological concern

• Health Concern

• Ethical concern
Summary

• Genome editing requires similar procedures used for Genetic Engineering (GMOs), yet creates precise mutation in plant genomes containing non-foreign DNA
• Resulting products are indistinguishable from products of natural variability or mutagenesis, yet genome edited plants are regulated by USDA in a manner of case-by-case
• Limitation of genome editing application are plant transformation pipeline and genome availability
• Both public and private sector have significant opportunities to apply genome editing in their breeding programs
Thank you!