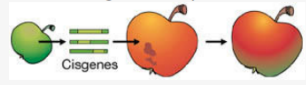
Transgenics in crop improvement:

- Resistance to biotic stresses (insects, viruses, diseses)
- Resistance to abiotic stresses (drought, low temperature, salinity…)
- Herbicide resistance
- Improved food quality
- Ornamental plants

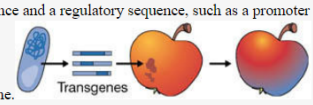
1

Definitions of key terms in relation to plants

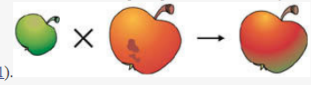
Cisgenesis is the genetic modification of a recipient plant with a natural gene from a crossable—sexually compatible—plant. Such a gene includes its introns and is flanked by its native promoter and terminator in the normalsense orientation.Cisgenic plants can harbour one or more cisgenes, but they do not contain any transgenes.



Transgenesis is the genetic modification of a recipient plant with one or more genes from any non-plant organism, or from a donor plant that is sexually incompatible with the recipient plant. This includes gene sequences of any origin in the anti-sense orientation, any artificial combination of a coding sequence and a regulatory sequence, such as a promoter from another gene, or a synthetic gene.



Traditional breeding encompasses all plant breeding methods that do not fall under current GMO regulations.As the European legal framework defines GMOs and specifies various breeding techniques that are excluded from the GMO regulations,we use this framework as a starting point, particularly the European Directive 2001/18/EC on the deliberate release of GMOs into the environment (European Parliament, 2001). Excluded from this GMO Directive are longstanding cross breeding, in vitro fertilization, polyploidy induction,mutagenesis and fusion of protoplasts from sexually compatible plants (European Parliament, 2001).



Schouten HJ, Krens FA, Jacobsen E. Cisgenic plants are similar to traditionally bred plants: international regulations for genetically modified organisms should be altered to exempt cisgenesis. *EMBO Rep*. 2006;7(8):750-3.

2

Herbicide resistance

Resistance to glyphosate

Example: Roundup Ready maize

Glyphosate works by competitive inhibition of the enzyme EPSP synthase (5-enolpyruvyl-shikimate 3-phosphate). This enzyme catalyzes the synthesis of 5-enolpyruvylshikimate 3-phosphate from phosphoenolpyruvate and shikimate
3-phosphate, a step which is essential for the synthesis of phenylalanine, tyrosine and tryptophane.

EPSPS gene from *Agrobacterium* strain CP_4 was chosen, because of its combination of high catalytic activity and high resistance to the herbicide.

EPSPS is transported in chloroplasts with the help of c*hloroplast transit peptide*, (CTP), which is added to the 5′ end of bacterial EPSPS ORF.

Other enzymes for glyphosate resistance:
- *gox* (glyphosate oxidase), which detoxifies the herbicide, isolated from *Ochrobactrum anthropi*

3

Construct for herbicide resistance development

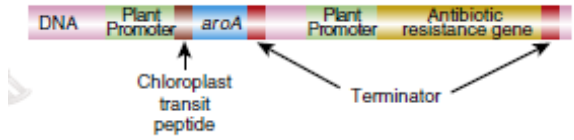


FIGURE 15.18
Expression of the *Agrobacterium aroA* Gene in Plants
The bacterial *aroA* gene must be placed under control of a promoter active in plants. Correct localization of the AroA protein (EPSPS) into the chloroplast requires addition of a chloroplast transit peptide at the N-terminus of the protein.

Clark, D. P., & Pazdernik, N. J. (2015). *Biotechnology*. Elsevier Science.

4

A new generation of glyphosate-resistant crops

- Plants with transgenic EPSPS do not destroy glyphosate, which means that the herbicide can accumulate in the plant tissues.
- Identification of gene in the genus *Bacillus* – glyphosate N-acetyltransferase (GLYAT) – which detoxifies glyphosate by attaching an acetyl group to the herbicide molecule
- A type of directed evolution called multigene shuffling was used that involves taking segments of each member of a multigene family and reassembling the
- segments to create new gene variants – a gene with 10 000-fold the activity of the enzymes present in the original *B. licheniformis* strain was obtained.

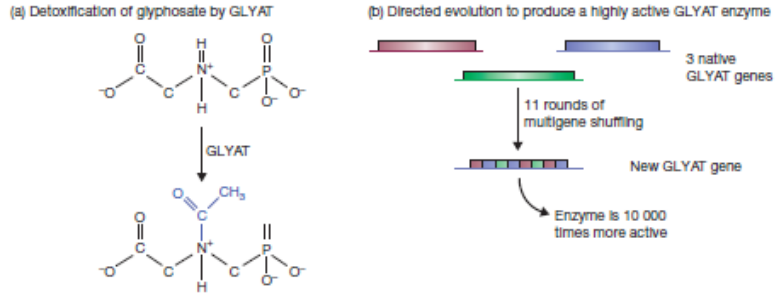


Figure 15.7

Use of glyphosate *N*-acetyltransferase to generate plants that detoxify glyphosate. (a) GLYAT detoxifies glyphosate by adding an acetyl group (shown in blue). (b) Creation of a highly active GLYAT enzyme by multigene shuffling.

Brown T.A. 2016. Gene cloning and DNA analysis.

5

Resistance to pests and diseases

Several genes conferring resistance to pests and diseases were identified in animals, bacteria, plants and other organisms were identified.

- **microorganisms**
  - Bt (*Bacillus thuringiensis)* genes – cry proteins (cry toxins, also δ endotoxins, Bt (crystal) proteins)
  - *Ipt* gene – isopentyl transferase from *A. tumefaciens* – codes for a key enzyme in the cytokinin biosynthetic pathway (expression of *ipt* in tobacco has resulted in a decrease in leaf consumption by the tobacco hornworm (*M. sexta*)
  - Gene for cholesterol oxidase from *Streptomyces* gliv (toxic to boll weevil larvae -*Anthonomus grandis*)
  - *Pht* gene from *Photorhabdus luminescens*

- **plants**
  - Proteinase inhibitors (the proteinase inhibitors deprive the insect of nutrients by interfering with digestive enzymes of the insect.)
  - lectins – snowdrop lectin (GNA), pea lectin, rice lectin, etc.

- **animals**
  - Serine proteinase inhibitors from mammals and the tobacco hornworm (*Manduca sexta*)
  - chitinases – enzymes which degrades chitin (major cell wall component of fungi)

6

Bt toxins from *Bacillus thuringiensis*

- Several types of bacteria have evolved defense mechanisms against insect predation, an example being *B. thuringiensis* which, during sporulation, forms intracellular crystalline bodies that contain an insecticidal protein called the δ-endotoxin.
- The δ-endotoxin protein that accumulates in the bacterium is an inactive precursor. After ingestion by the insect, this protoxin is cleaved by proteases, resulting in shorter versions of the protein that display toxic activity.

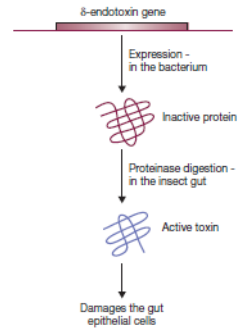


Figure 15.1
Mode of action of a δ-endotoxin.

Brown T.A. 2016. Gene cloning and DNA analysis.

7

Once they are ingested by a susceptible insect, the crystals break down in the alkaline environment of the insect midgut, generally dissolving at pH 8.0 or greater.
At that point, the termini of the Bt pro-toxin proteins are cleaved by specific proteases inside the gut, yielding the toxic protein. The active protein will then bind **to specific protein receptors** on the insect microvillar membrane of the midgut

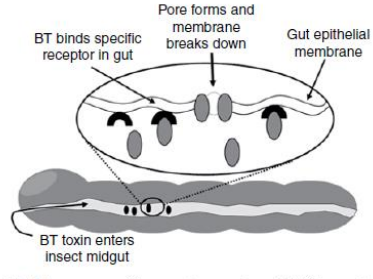


Figure 9.3. The Bt toxin binds to very specific receptors on the epithelial membrane of the insect gut. The toxin then forms channels in the membrane that leads to ion leakage and, ultimately, death of the insect. This mode of action explains the specificity of Bt (from the presence of the necessary receptors) and also shows why the toxin needs to be eaten by the insect to function.

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

8

Only certain species of insects are controlled by particular endotoxins

- the most widely deployed *cry* genes in transgenic plants are members of the cry1A gene family, which are toxic to a broad range of Lepidoptera pests
- some beetle species, such as the Colorado potato beetle (*Leptinotarsa decemlineata*), are targeted by the Cry3A Bt toxin

Table 15.1
The range of insects poisoned by the various types of *B. thuringiensis* δ-endotoxins.

δ-ENDOTOXIN TYPE	EFFECTIVE AGAINST
CryI	Lepidoptera (moth and butterfly) larvae
CryII	Lepidoptera and Diptera (two-winged fly) larvae
CryIII	Coleoptera (beetles)
CryIV	Diptera larvae
CryV	Nematode worms
CryVI	Nematode worms

Fields with Bt crops are required to provide refuge areas to help control resistance!!

http://www.bt.ucsd.edu/crop_refuge.html

9

Resistance to viruses

OPEN

Use of RNAi technology to develop a PRSV-resistant transgenic papaya

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Accepted: 14 September 2017
Published online: 03 October 2017

Papaya ringspot virus (PRSV) seriously limits papaya (*Carica papaya* L.) production in tropical and subtropical areas throughout the world. Coat protein (CP)- transgenic papaya lines resistant to PRSV isolates in the sequence-homology-dependent manner have been developed in the U.S.A. and Taiwan. A previous investigation revealed that genetic divergence among Hainan isolates of PRSV has allowed the virus to overcome the CP-mediated transgenic resistance. In this study, we designed a comprehensive RNAi strategy targeting the conserved domain of the PRSV CP gene to develop a broader-spectrum transgenic resistance to the Hainan PRSV isolates. We used an optimized particle-bombardment transformation system to produce RNAi-CP-transgenic papaya lines. Southern blot analysis and Droplet Digital PCR revealed that line 474 contained a single transgene insert. Challenging this line with different viruses (PRSV I, II and III subgroup) under greenhouse conditions validated the transgenic resistance of line 474 to the Hainan isolates. Northern blot analysis detected the siRNAs products in virus-free transgenic papaya tissue culture seedlings. The siRNAs also accumulated in PRSV infected transgenic papaya lines. Our results indicated that this transgenic papaya line has a useful application against PRSV in the major growing area of Hainan, China.

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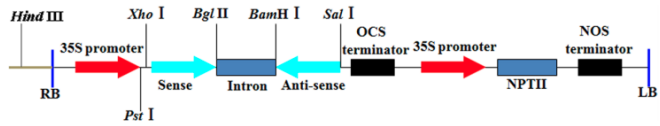

Figure 1. Components of the constructed RNAi vector pCAMBIA2300-35S-OCS. 35 S promoter indicated *Cauliflower mosaic virus* (CaMV) promoter. NOS terminator = nopaline synthase gene terminator, OCS terminator = octopine synthase terminator. NPTII = neomycin phosphotransferase gene. Sense and Anti-sense = conserved 544 bp fragment of CP gene and inverted repeat sequence.

11

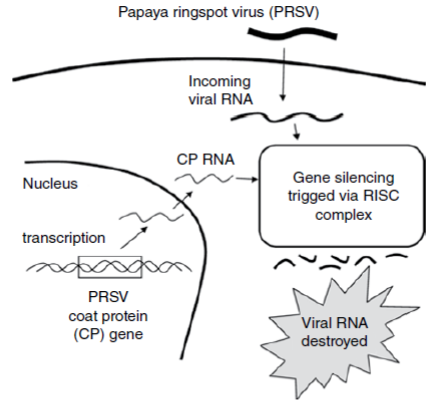

Figure 9.5. Transgenic resistance to papaya ringspot virus (PRSV) is possible because of the process of RNA-mediated gene silencing. To make virus-resistant plants, a portion of the coat protein (CP) gene of PRSV was transferred to and expressed in transgenic papaya plants. Following transcription, the RNA triggers targeted, sequence-specific degradation of similar RNA sequences, such as that found on incoming PRSV viral RNA. The initial degradation of RNA is carried out by an enzyme called *DICER*, and the process is mediated by an enzymatic structure called the *RNA-induced silencing complex* (RISC). Ultimately, this can lead to RNA cleavage, as well as blockage of transcription or translation of the target gene.

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

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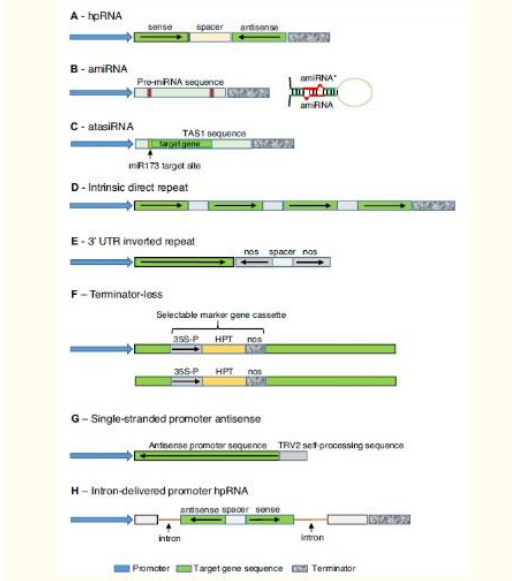

Fig. (1)

Schematic diagrams of the different types of RNA silencing constructs discussed in this review. The target sequence is shown in green, except for the amiRNA construct where the amiRNA and amiRNA* sequences are shown in red. The promoter is shown in blue, and the terminator in textured grey. The predicted amiRNA structure is also shown.

The Basic RNA Silencing Pathway - Hairpin RNA (hpRNA)
Transgene (Transgenes designed to express long self-complementary hairpin RNA (hpRNA)

The miRNA Pathway - Artificial miRNA (amiRNA)
miRNAs are 20-24-nt sRNAs derived from genetic loci known as MIR genes. An amiRNA construct is made by replacing the miRNA and miRNA sequences of a natural miRNA precursor with corresponding target gene sequences.

The Trans-acting siRNA Pathway
The miR173-targeted TAS1 transcript has been modified to express artificial tasiRNAs (atasiRNA).

RNA Silencing in Plants: Mechanisms, Technologies and Applications in Horticultural Crops

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5108043/

13

Pathogen Resistance

Resistance to a particular pathogen can often be conferred by a single plant gene (an R gene, resistance gene), the product of which is active in recognition of the presence or activity of a single virulence factor from the pathogen (encoded by an Avr gene, avirulence gene).

Plant Biotechnology Journal

Plant Biotechnology Journal (2018), pp. 1–11 doi: 10.1111/pbi.13042

Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races

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[2]Kachwekano Zonal Agricultural Research and Development Institute, Kabale, Uganda
[3]International Potato Center, Lima, Peru
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Received 18 September 2018; revised 3 November 2018; accepted 9 November 2018.
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Keywords: GM potatoes, transformation, late blight resistance, *Phytophthora infestans*.

Summary

Considered responsible for one million deaths in Ireland and widespread famine in the European continent during the 1840s, late blight, caused by *Phytophthora infestans*, remains the most devastating disease of potato (*Solanum tuberosum* L.) with about 15%–30% annual yield loss in sub-Saharan Africa, affecting mainly smallholder farmers. We show here that the transfer of three resistance (*R*) genes from wild relatives [*RB*, *Rpi-blb2* from *Solanum bulbocastanum* and *Rpi-vnt1.1* from *S. venturii*] into potato provided complete resistance in the field over several seasons. We observed that the stacking of the three *R* genes produced a high frequency of transgenic events with resistance to late blight. In the field, 13 resistant transgenic events with the 3*R*-gene stack from the potato varieties 'Desiree' and 'Victoria' grew normally without showing pathogen damage and without any fungicide spray, whereas their non-transgenic equivalent varieties were rapidly killed. Characteristics of the local pathogen population suggest that the resistance to late blight may be long-lasting because it has low diversity, and essentially consists of the single lineage, 2_A1, which expresses the cognate avirulence effector genes. Yields of two transgenic events from 'Desiree' and 'Victoria' grown without fungicide to reflect small-scale farm holders were estimated to be 29 and 45 t/ha respectively. This represents a three to four-fold increase over the national average. Thus, these late blight resistant potato varieties, which are the farmers' preferred varieties, could be rapidly adopted and bring significant income to smallholder farmers in sub-Saharan Africa.

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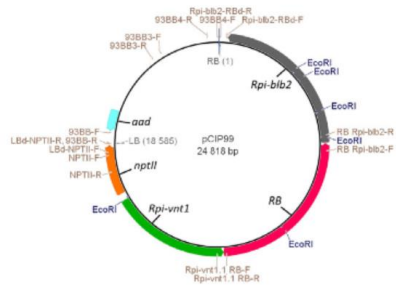

Figure 1 Triple *R* gene stack construct pCIP99 bearing the 18 585 bp T-DNA from right border RB to left border LB with, clockwise, the three *R* genes (*Rpi-blb2*, *RB* and *Rpi-vnt1.1*) and the selectable marker genes (*nptII and aad*) used for transformation of potato and vector selection respectively. Names of the genes and border sequences are indicated inside the circle while primers used to characterize the inserted T-DNA in the potato plants are indicated outside the circle with their sequences provided in Table S1. Positions of the *Eco*RI sites are also indicated on the outside circle.

Figure 4 Confined field trial 60 days after planting during the second season (Oct 14, 2015 – Jan 20, 2016) with a close-up on nontransgenic 'Victoria' plant heavily infected by *Phytophthora infestans*. Each plot is made of three rows of transgenic plants flanked by one row of spreaders on each side as indicated for the front plot. Green potato rows are all transgenic events and 'Cruza 148', whereas the empty rows are the non-transgenic rows of 'Desiree' and 'Victoria'.

15

Traits for Improved Products and Food Quality - Nutritional Improvements

Golden rice – a transgenic plant that produces high levels of beta-carotene or provitamin A in the grain.

Carotenoids are 40-carbon compounds produced from the precursor molecule via a biochemical pathway localized in plastids .
The 40-carbon backbone of beta-carotene is **phytoene,** which is assembled by combination of **two 20-carbon geranylgeranyl diphosphate (GGPP)** molecules by the enzyme **phytoene synthase** (Fig. 9.6). GGPP is naturally produced in rice grains.
Double bonds are then added to phytoene through a series of desaturation steps (**phytoene desaturase from Erwinia spp.)** to produce lycopene, an antioxidant compound found in most plants and that contributes to the red color of tomatoes. Finally, lycopene can be converted to beta-carotene by the enzyme **lycopene cyclase**.

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

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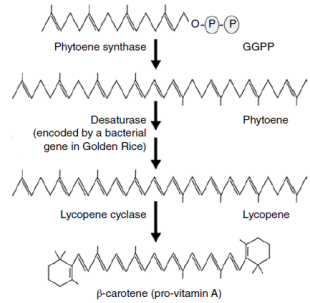
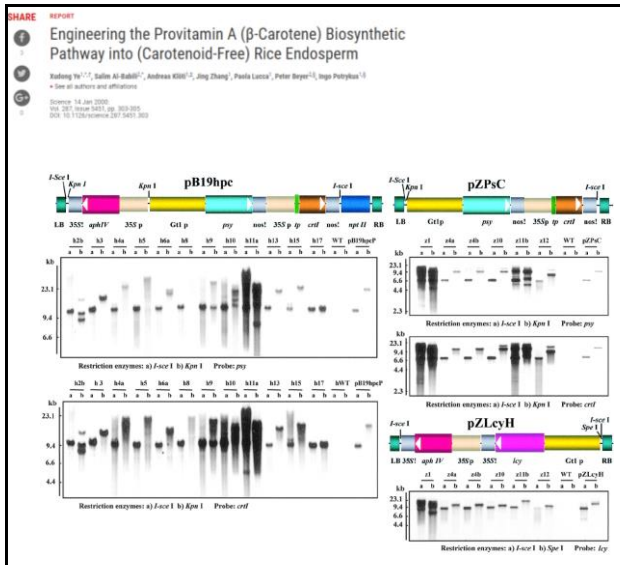


Figure 9.6. The production of β-carotene in Golden Rice was made possible by high-level, tissue-specific expression of the necessary enzymes in rice. Rice grains normally produce geranylgeranyl–diphosphate (GGPP). A gene-encoding phytoene synthase was transferred to rice from daffodil (for the original Golden Rice) or maize (in Golden Rice 2), and this led to production of phytoene in rice grains. A desaturase enzyme necessary to add double bonds to the structure was provided by transfer of a bacterial gene to rice (the two arrows at this step represent the multiple reactions that are necessary to add all double bonds). Finally, lycopene was converted in rice grains by an endogenous lycopene cyclase activity to the yellow-orange endproduct, β-carotene.

An improved version of transgenic rice referred to as Golden Rice 2, using a phytoene synthase gene from corn rather than daffodil, was subsequently produced that accumulated levels of carotenoids over 20 times higher than in the original Golden Rice (Paine et al. 2005). It is estimated that by eating modest amounts of Golden Rice 2, enough beta-carotene can be provided to overcome vitamin A deficiency.

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

17



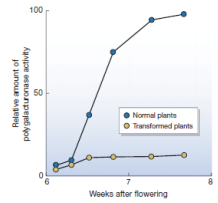
Structures of the T-DNA regions of three vectors: pB19hpc, pZPsC and pZLcyH used in co-transformations.

Representative Southern blots of independent transgenic T_0-plants are given below the respective *Agrobacterium* vectors. LB, left border; RB, right border; "!", polyadenylation signals; p, promoters; *psy*, phytoene synthase; *crtI*, bacterial phytoene desaturase;*lcy*, lycopene β-cyclase; *tp*, transit peptide.

18

FlavrSavr, Calgene, USA, 1994
Antisense RNA to inactivate polygalacturonase gene - This enzyme slowly breaks down the polygalacturonic acid (pectic acid) component of the cell walls in the fruit pericarp, resulting in a gradual softening.

Figure 15.10
The differences in polygalacturonase activity in normal tomato fruits and in fruits expressing the antisense polygalacturonase gene.



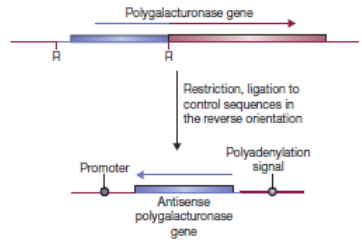


Figure 15.9
Construction of an antisense polygalacturonase gene.

Brown T.A. 2016. Gene cloning and DNA analysis.

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Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8805–8809, December 1988
Biochemistry

Reduction of polygalacturonase activity in tomato fruit by antisense RNA

(plant transformation/cauliflower mosaic virus 35S promoter/lycopene accumulation)

RAYMOND E. SHEEHY, MATTHEW KRAMER, AND WILLIAM R. HIATT*

Calgene, Inc., 1920 Fifth Street, Davis, CA 95616

Communicated by E. Peter Geiduschek, August 4, 1988

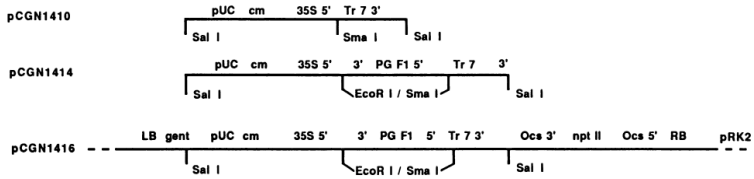
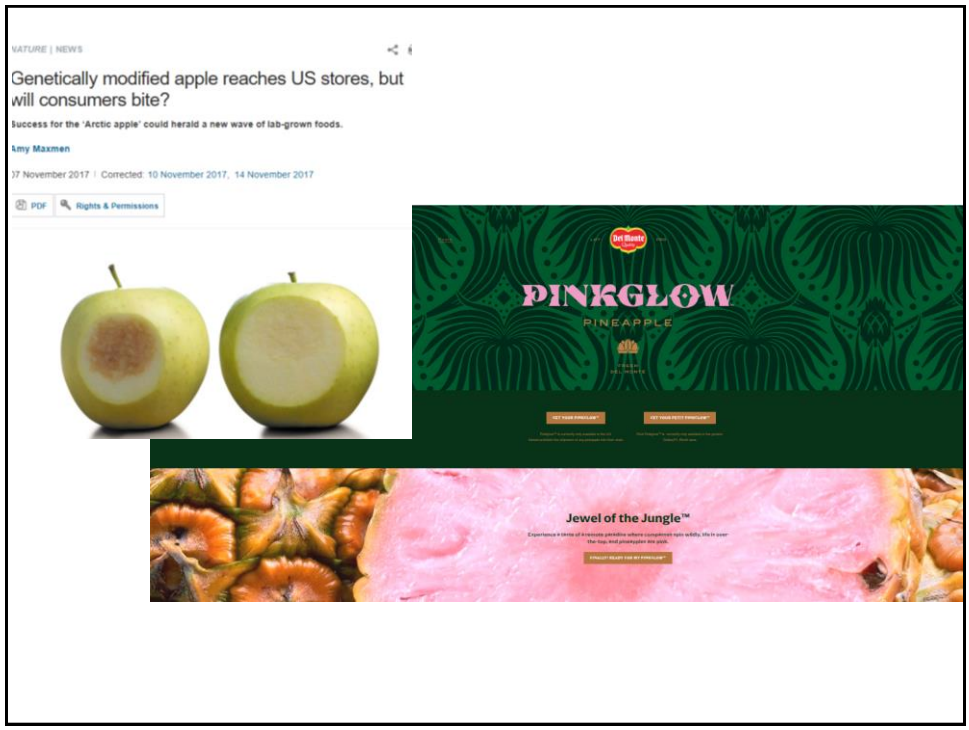


FIG. 1. Construction of the binary plasmid pCGN1416. LB, left border of the transferred DNA (T-DNA) region of the Ti plasmid of *Agrobacterium tumefaciens;* gent, gentamycin-resistance gene; pUC, pUC plasmid origin of replication; cm, chloramphenicol-resistance gene; 35S 5′, cauliflower mosaic virus 35S promoter; PG F1, full-length PG cDNA (base pairs 1624 to 1) in the antisense orientation; Tr 7 3′, 3′ region of transcript 7 of the Ti plasmid; Ocs 3′, octopine synthase 3′ region; npt II, kanamycin-resistance gene; Ocs 5′, octopine synthase 5′ region; RB, right border of the T-DNA region of the Ti plasmid; pRK2, pRK290 broad host-range replicon.

20



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Genome edited plants

22

Plant Biotechnology Journal

Plant Biotechnology Journal (2017) **15**, pp. 207–216 doi: 10.1111/pbi.12603

ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions

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Keywords: maize, ARGOS, CRISPR-Cas9, genome editing, drought tolerance, grain yield.

Summary

Maize *ARGOS8* is a negative regulator of ethylene responses. A previous study has shown that transgenic plants constitutively overexpressing *ARGOS8* have reduced ethylene sensitivity and improved grain yield under drought stress conditions. To explore the targeted use of *ARGOS8* native expression variation in drought-tolerant breeding, a diverse set of over 400 maize inbreds was examined for *ARGOS8* mRNA expression, but the expression levels in all lines were less than that created in the original *ARGOS8* transgenic events. We then employed a CRISPR-Cas-enabled advanced breeding technology to generate novel variants of *ARGOS8*. The native maize GOS2 promoter, which confers a moderate level of constitutive expression, was inserted into the 5′-untranslated region of the native *ARGOS8* gene or was used to replace the native promoter of *ARGOS8*. Precise genomic DNA modification at the *ARGOS8* locus was verified by PCR and sequencing. The *ARGOS8* variants had elevated levels of *ARGOS8* transcripts relative to the native allele and these transcripts were detectable in all the tissues tested, which was the expected results using the GOS2 promoter. A field study showed that compared to the WT, the *ARGOS8* variants increased grain yield by five bushels per acre under flowering stress conditions and had no yield loss under well-watered conditions. These results demonstrate the utility of the CRISPR-Cas9 system in generating novel allelic variation for breeding drought-tolerant crops.

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BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency's regulatory process — making it the first CRISPR-edited organism to receive a green light from the US government.

"The research community will be very happy with the news," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yinong Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (*Agaricus bisporus*) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a handful of base pairs in the mushroom's genome, Yang knocked out one of six PPO genes — reducing the enzyme's activity by 30%.

STUART MCCALL/GETTY

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

AGENCY RULES

The mushroom is one of about 30 genetically modified organisms (GMOs) to sidestep the USDA's regulatory system in the past 5 years. official. "They were very excited," Yang says: "There was certainly interest and a positive feeling" at the meetings. He followed up with an official letter of enquiry to the agency later that month.

The USDA's answer came this week. "APHIS does not consider CRISPR/Cas9-edited white

The United States is revamping its rules for regulating GMOs, which collectively are known as the Coordinated Framework for Regulation of Biotechnology. To that end, the US National Academies of Sciences, Engineering and Medicine have convened a committee that is charged with predicting what advances

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DUPONT PIONEER'S NEXT GENERATION OF WAXY CORN SHOWS THE GREEN SIDE OF CRISPR-CAS9

Written by LVS on Thursday 16 February 2017 in the category Insights with the tags food, agriculture, crispr.

CRISPR-Cas9 is making its way into the agricultural sector. Agricultural heavyweight DuPont Pioneer is clearly taking the lead in bringing the technology into plant breeding: about a year ago, the company announced that it would develop a new and improved waxy corn variety with CRISPR-Cas9. Is CRISPR the technology that will make genetic engineering for crops available globally?

Waxy corn: first in the CRISPR line

Last year, DuPont Pioneer announced its first product that would be developed with CRISPR technology: a new and improved waxy corn variety. Waxy corn has been around since the early twentieth century. While normal corn kernels contain 75% amylopectin and 25% amylose, a deletion in the waxy gene results in waxy corn kernels that contain over 97% amylopectin, essentially eliminating amylose from the kernel.

Vir: https://biovox.eu/insights/detail/dupont-pioneer-rsquo-s-next-generation-of-waxy-corn-shows-the-green-side-of-crispr-cas9

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Food Ingredients About Us Growers Food Manufacturing

February 26, 2019

First Commercial Sale of Calyxt High Oleic Soybean Oil on the U.S. Market

Calyxt successfully markets Calyno™ High Oleic Soybean Oil as a premium, high-quality food ingredient

First commercial sale of High Oleic Soybean Meal as a premium non-GMO feed ingredient for livestock

Minneapolis-St. Paul, Minn. – February 26, 2019 – Calyxt, Inc. (NASDAQ: CLXT) a consumer-centric, food- and agriculture-focused Company, announced today the successful commercial launch of its highly anticipated Calyno™ High Oleic Soybean Oil, which is the Company's first product to be sold on the U.S. market. This first commercial sale of Calyno oil is to the foodservice industry for frying and salad dressing, as well as sauce applications.

Calyno oil contains approximately 80 percent oleic acid and up to 20 percent less saturated fatty acids compared to commodity soybean oil as well as zero grams of trans fat per serving. One of the exciting potential sustainability benefits of Calyno oil is that it has up to three times the fry life and extended shelf life compared to commodity oils, providing a more sustainable product.

https://calyxt.com/first-commercial-sale-of-calyxt-high-oleic-soybean-oil-on-the-u-s-market/

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Research article

CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*☆

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ABSTRACT

The CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9-mediated genome editing system has been widely applied as a powerful tool for modifying preferable endogenous genes. This system is highly expected to be further applied for the breeding of various agronomically important plant species. Here we report the modification of a fatty acid desaturase 2 gene (*FAD2*), which encodes an enzyme that catalyzes the desaturation of oleic acid, in *Brassica napus* cv. Westar using the CRISPR/Cas9 system. Two guide RNAs were designed for *BnaA.FAD2.a* (*FAD2.Aa*). Of 22 regenerated shoots with *FAD2.Aa* editing vectors, three contained mutant alleles. Further analysis revealed that two of three mature plants (Aa1#13 and Aa2#2) contained the mutant alleles. The mutant *fad2.Aa* allele had a 4-bp deletion, which was inherited by backcross progenies (BC_1) in the Aa1#13 line. Furthermore, plants with the *fad2.Aa* allele without transgenes were selected from the BC_1 progenies and plants homozygous for *fad2.Aa* were then produced by self-crossing these BC_1 progenies (BC_1S_1). Fatty acid composition analysis of their seeds revealed a statistically significant increase in the content of oleic acid compared with that in wild-type seeds. These results showed that the application of the CRISPR/Cas9 system is useful to produce desirable mutant plants with an agronomically suitable phenotype by modifying the metabolic pathway in *B. napus*.

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Gen

REVIEW

Applications and potential of genome editing in crop improvement

Yi Zhang[1], Karen Massel[2], Ian D. Godwin[2] and Caixia Gao[3,4*]

Abstract

Genome-editing tools provide advanced biotechnological techniques that enable the precise and efficient targeted modification of an organism's genome. Genome-editing systems have been utilized in a wide variety of plant species to characterize gene functions and improve agricultural traits. We describe the current applications of genome editing in plants, focusing on its potential for crop improvement in terms of adaptation, resilience, and end-use. In addition, we review novel breakthroughs that are extending the potential of genome-edited crops and the possibilities of their commercialization. Future prospects for integrating this revolutionary technology with conventional and new-age crop breeding strategies are also discussed.

Table 1 Crop traits that have been improved by genome-editing techniques

Crop species	Gene editor	Target gene	DNA repair type	Target trait	Reference
Maize	ZFNs	ZmPK1	HR	Herbicide tolerant and phytate reduced maize	[14]
Maize	ZFNs	ZmTLP	HR	Trait stacking	[15]
Rice	ZFNs	OsQQR	HR	Trait stacking	[16]
Rice	TALENs	OsSWEET14	NHEJ	Bacterial blight resistance	[18]
Wheat	TALENs	TaMLO	NHEJ	Powdery mildew resistance	[19]
Maize	TALENs	ZmGL2	NHEJ	Reduced epicuticular wax in leaves	[20]
Sugarcane	TALENs	COMT	NHEJ	Improved cell wall composition	[21]
Sugarcane	TALENs	COMT	NHEJ	Improved saccharification efficiency	[22]
Soybean	TALENs	FAD2-1A, FAD2-1B	NHEJ	High oleic acid contents	[23]
Soybean	TALENs	FAD2-1A, FAD2-1B, FAD3A	NHEJ	High oleic, low linoleic contents	[24]
Potato	TALENs	Vinv	NHEJ	Minimizing reducing sugars	[25]
Rice	TALENs	OsBADH2	NHEJ	Fragrant rice	[26]
Maize	TALENs	ZmMTL	NHEJ	Induction of haploid plants	[27]
Brassica oleracea	TALENs	FRIGIDA	NHEJ	Flowering earlier	[28]
Tomato	TALENs	ANT1	HR	Purple tomatoes with high anthocyanin	[29]
Rice	CRISPR/Cas9	LAZY1	NHEJ	Tiller-spreading	[39]
Rice	CRISPR/Cas9	Gn1a, GS3, DEP1	NHEJ	Enhanced grain number, larger grain size and dense erect panicles	[40]
Wheat	CRISPR/Cas9	GW2	NHEJ	Increased grain weight and protein content	[41]
Camelina sativa	CRISPR/Cas9	FAD2	NHEJ	Decreased polyunsaturated fatty acids	[42]
Rice	CRISPR/Cas9	SBEIIb	NHEJ	High amylose content	[43]
Maize	CRISPR/Cas9	Wx1	NHEJ	High amylopectin content	[44]
Potato	CRISPR/Cas9	Wx1	NHEJ	High amylopectin content	[45]
Wheat	CRISPR/Cas9	EDR1	NHEJ	Powdery mildew resistance	[46]
Rice	CRISPR/Cas9	OsBRP922	NHEJ	Enhanced rice blast resistance	[47]
Rice	CRISPR/Cas9	OsSWEET13	NHEJ	Bacterial blight resistance	[48]
Tomato	CRISPR/Cas9	SlMLO1	NHEJ	Powdery mildew resistance	[49]
Tomato	CRISPR/Cas9	SlAZ2	NHEJ	Bacterial speck resistance	[50]
Grapefruit	CRISPR/Cas9	CsLOB1 promoter	NHEJ	Alleviated citrus canker	[51]
Orange	CRISPR/Cas9	CsLOB1 promoter	NHEJ	Citrus canker resistance	[52]
Grapefruit	CRISPR/Cas9	CsLOB1	NHEJ	Citrus canker resistance	[53]
Cucumber	CRISPR/Cas9	eIF4E	NHEJ	Virus resistance	[54]
Mushroom	CRISPR/Cas9	PPO	NHEJ	Anti-browning phenotype	[55]
Tomato	CRISPR/Cas9	SP5G	NHEJ	Earlier harvest time	[56]
Tomato	CRISPR/Cas9	SlAGL6	NHEJ	Parthenocarpy	[57]
Maize	CRISPR/Cas9	TMS5	NHEJ	Thermosensitive male-sterile	[58]
Rice	CRISPR/Cas9	OsMATL	NHEJ	Induction of haploid plants	[59]
Tomato	CRISPR/Cas9	SP, SP5G, CLV3, WUS, GGP1	NHEJ	Tomato domestication	[60]
Rice	CRISPR/Cas9	ALS	HR	Herbicide resistance	[61]
Rice	CRISPR/Cas9	ALS	HR	Herbicide resistance	[62]
Rice	CRISPR/Cas9	EPSPS	NHEJ	Herbicide resistance	[63]
Rice	CRISPR/Cas9	ALS	HR	Herbicide resistance	[64]
Soybean	CRISPR/Cas9	ALS	HR	Herbicide resistance	[65]
Maize	CRISPR/Cas9	ALS	HR	Herbicide resistance	[66]
Potato	CRISPR/Cas9	ALS	HR	Herbicide resistance	[67]
Flax	CRISPR/Cas9	EPSPS	HR	Herbicide resistance	[68]
Cassava	CRISPR/Cas9	EPSPS	HR	Herbicide resistance	[69]
Maize	CRISPR/Cas9	ARGOS8	HR	Drought stress tolerance	[70]

CRISPR clustered regularly interspaced short palindromic repeats, *HR* homologous recombination, *NHEJ* non-homologous end joining, *TALEN* transcription activator-like effector nuclease, *ZFN* zinc-finger nuclease

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