HS1315

Current Status of Research, Regulations, and Future Challenges for CRISPR Gene Editing in Crop Improvement[1]

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Introduction

Plant breeders and researchers have sought to improve crops since the dawn of agriculture. For hundreds of years, conventional breeding has had a tremendous impact on agricultural productivity. Over the last few decades, researchers have begun to transfer DNA between species in what is known as genetic engineering (transgenic technology). Recently, crop breeding programs started turning to CRISPR gene editing as a means to support commodity development. Considerable efforts have been devoted to applying this gene editing technology in modern agriculture to increase crop yields and improve the quality of food ingredients, especially by many of the major agronomic seed-producing companies. In this article, we outline the recent research updates and regulations on gene editing in crop improvement. The target audience for this report is the general public, including both scientists and nonscientists.

What is CRISPR and how does it work?

CRISPR, or clustered regularly interspaced short palindromic repeats, is a gene editing technology derived from a bacterial defense mechanism. This defense mechanism works in three phases. Phase 1, or Adaptation, occurs when a bacteriophage (or other type of invader) infects bacteria by injecting a small amount of DNA into the cell (Vigouroux & Bikard, 2020). During Adaptation, short fragments of the injected DNA are "saved" by the bacteria within a repetitive region of the genome, referred to as a CRISPR array (Vigouroux & Bikard, 2020). Each array consists of a unique spacer derived from these short fragments, which is interspaced between repeats. During Phase 2, the CRISPR locus is then transcribed and processed, resulting in short RNA sequences, which correspond to the sequences of the invading DNA fragments (Vigouroux & Bikard, 2020). Phase 3, Interference, uses the RNAs processed during Phase 2 as guides for Cas nucleases to create double stranded breaks (DSBs) in the invading DNA, effectively cutting it to pieces (Vigouroux & Bikard, 2020). The native bacterial DNA is protected from DNA damage because the Cas nucleases require a proto-spacer adjacent motif (PAM) site immediately adjacent to the recognized sequence (Doudna & Charpentier, 2014), which is not present in the bacterial DNA.

CRISPR/Cas-mediated gene editing technology takes advantage of this naturally occurring defense mechanism by synthetically adding guide sequences, referred to as guide RNAs (gRNAs), into a CRISPR locus (Figure 1). The lengths of these gRNA sequences vary depending on the Cas nuclease being used and can be designed using a wide range of available software. Cas9 is one of the most common Cas nucleases used in gene editing in plants. Cas9 recognizes a 3 nucleotide (nt) PAM sequence, 5'-NGG-3', where N represents any nucleotide, and most often utilizes gRNA sequences which are 20nt in total length. Variations of Cas nucleases have been engineered to require different PAM sequences, allowing for greater flexibility in selecting targets for gene editing (Hillary and Ceasar, 2023).

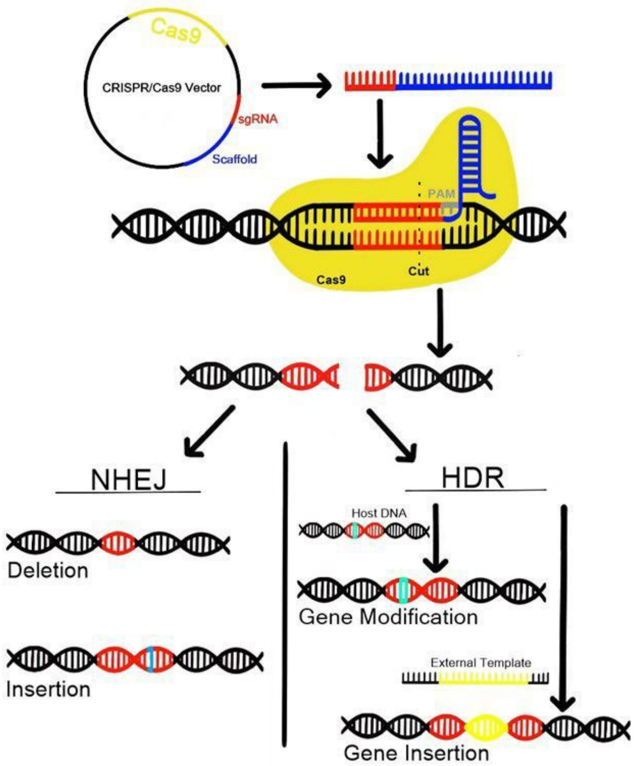

Figure 1. Depiction of CRISPR/Cas9-mediated gene editing.
Credit: Kaitlyn Vondracek

Once the guide sequences are cloned into a CRISPR vector, plant tissue is transformed using one of many methods. Agrobacterium-mediated transformation and biolistic transformation via the use of a gene gun are two of the more common transformation methods used in plants, though new methods are continuously being developed for improved editing efficiency as well as for transgene-free applications. Once transformation is complete, the CRISPR/Cas system functions in a similar manner as it does in bacteria. The guide sequences which were cloned into the vector are transcribed and processed into gRNAs which match up to specific locations in the host genome. If the correct PAM site is present adjacent to the matching host sequences, the selected Cas nuclease will be recruited to generate a DSB at that position. If the correct PAM site is not present, the nuclease will not cut the DNA and will move to check the next site which matches the gRNA.

Mutations due to CRISPR gene editing are the product of the DNA repair mechanisms for DSBs. There are two main mechanisms for DSB repair in eukaryotes, non-homologous end-joining (NHEJ) and homology-directed repair (HDR) (Figure 1). HDR utilizes a template, either the sister chromatid of the target chromosome or a template which is externally supplied, to repair the break in the DNA. Using HDR, one can generate a precise insertion or other modification by including the desired sequence as the template. Conversely, NHEJ occurs in the absence of a template and instead relies on an error-prone repair mechanism, which often results in a random insertion or deletion at the position of the break. It is also possible that no error occurs during NHEJ, in which case the host DNA will be indistinguishable from before it was cut.

How does CRISPR gene editing technology improve crops and benefit the public?

Using the CRISPR gene editing technique, researchers can selectively “edit” plant genomes to obtain desired traits. Gene editing is often confused with other transgenic crop improvement approaches (the products of which are colloquially referred to as “genetically modified organisms (GMOs)”) and referred to as the same idea. Schneider et al. (2014) described clearly the differences between genetically modified (GM) foods and GMOs as well as other possible benefits of gene editing for crop improvement. In other EDIS articles (Lee et al. 2016, 2018), we have also described what gene editing techniques are, how they differ from transgenic approaches, and their potential applications for cultivated strawberries.

CRISPR gene editing technology has been applied to numerous crops to modify a wide range of traits (Table 1). For example, to increase yield in tomato, Lippman and colleagues at Cold Spring Harbor Laboratory performed gene editing to develop plants with double the number of branches (Rodríguez-Leal et al. 2017; Rothan et al. 2019). Gene editing has also been applied in tomatoes to enhance nutritional quality and improve resistance to biotic and abiotic stress, traits which would otherwise take decades to improve through traditional breeding methods (Krishna et al. 2019). Gene editing can also help to address disease resistance. Huanglongbing (HLB), or citrus greening, is a devastating disease of citrus worldwide and has reduced Florida citrus production by more than 50% (Graham et al., 2020). There are currently few genetic resistance sources available for HLB-resistance breeding (Dodds et al., 2017; Khachatryan and Choi 2017; Palangasinghe et al., 2024), but Dr. Nian Wang’s group at UF/IFAS has begun working to generate HLB-resistant citrus lines using CRISPR-based gene editing, which could potentially save the Florida citrus industry. Important quality traits in various foods can also be improved using gene editing technology. For example, reduced-gluten wheat, non-browning mushrooms and apples, and soybeans with reduced unhealthy saturated fats can be obtained using CRISPR gene editing tools (Waltz, 2016; Jacobs et al., 2015; Sánchez-León, et al. 2017). Table 2 lists additional crop species in which gene editing has been applied, as well as the current status of the relevant research.

What CRISPR gene edited foods are available in grocery stores?

Gene edited soybean oil, cold-storable potatoes, high-fiber and gluten-reduced wheat, and lower-saturated-fat canola have been developed by Calyxt (formerly Cellectis Plant Sciences, Inc., now Cibus). Calyxt’s gene edited soybean oil was cleared by the USDA in 2018 and went on the US market in 2019. A further improved “next generation” was announced in May of 2021, but it's unclear whether products containing the improved trait made it to market ("*Calyxt Announces Next Generation Premium Soybean*", 2021). While Calyxt's high-fiber wheat was cleared by the USDA in 2018, it has not commercialized to date. Starting in 2019, Cibus began cultivating gene edited herbicide resistant canola (SU Canola + Draft herbicide growing system) in North Dakota and Montana. USDA-APHIS cleared herbicide tolerant flax and rice from Cibus in 2020, and waxy corn from Corteva in 2021. Following a merger in 2023, Calyxt became part of Cibus, which now holds more than 1,000 issued and pending patents for gene editing in agriculture and have developed several traits which have been incorporated into several breeding germplasms ("*Cibus Announces Closing of Merger*", 2023).

The EPA will not regulate edited camelina with increased oil content from Yield10 Bioscience, which is now in pre-commercial seed production ("*Yield10 Bioscience Obtains Nonregulated Status*", 2018). Furthermore, the Canadian Food Inspection Agency and United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) additionally cleared new gene edited camelina lines from Yield10 Bioscience in 2024 ("*USDA-APHIS Determines that Yield10 Bioscience's*", 2024; "*Yield10 Bioscience Announces*", 2024). Pairwise released gene edited high-nutrient romaine and low-pungency mustard greens for commercial sale in 2023 (Brown, 2023) and is additionally working on gene editing in strawberry, blackberry, and raspberry. In total, Pairwise holds 21 exemptions from regulation for genome edited crops, with research continuing in a range of crops (Barefoot, 2024).

Gene edited crops are also becoming more widely available in the global market. In 2020, Japan's regulatory agencies approved gene edited tomatoes with increased γ-aminobutyric acid (also known as GABA) content, and Alora (formerly Agrisea), a United Kingdom-based company, secured permission for field trials of salt-tolerant gene edited rice which started in 2023 (Watson, 2023). In 2022, Argentina's Biosafety Commision (Comisión Nacional de Biotecnología Agropecuaria or "CONABIA") cleared Yield10 Bioscience's gene edited camelina ("*Yield10 Bioscience Receives Favorable Ruling*", 2022), and Japan's Ministry of Health, Labour, and Welfare (MHLW) and Ministry of Agriculture, Forestry, and Fisheries (MAFF) cleared Corteva's waxy gene edited corn in 2023 (United States Department of Agriculture, 2023). Biosafety authorities in Kenya are also preparing to release genetically modified corn, cassava, and potato from regulation in the near future, with an additional 42 GM crops in the release pipeline (Gitonga, 2024).

Current Regulations and Policies for CRISPR Gene Edited Crops

The White House Office of Science and Technology Policy issued the Coordinated Framework for Regulation of Biotechnology with the advent of recombinant DNA in the 1980s. In the United States, three Federal agencies—the Environmental Protection Agency (EPA), the United States Department of Agriculture (USDA), and the Food and Drug Administration (FDA)—were charged with the implementation of laws regulating biotechnology products (Food and Drug Administration, 2023). The EPA mainly regulates bioengineered products intended for pesticidal purposes, biofertilizers, bioremediation, and the production of various industrial compounds, including biofuels. As such, the EPA will not regulate CRISPR-edited plants as long as the altered traits are not for synthesis of toxic chemicals such as pesticides or biofuels. Similarly, the FDA focuses on the safe regulation of genetically engineered foods that can be used for dietary supplements, cosmetics, drugs, and medical devices. The FDA ensures food products comply with legal requirements, regardless of their production methods (whether traditional or gene edited). The USDA's Animal and Plant Health Inspection Service (USDA-APHIS) authorizes the importation, interstate movement, and environmental release of plants that may pose a plant pest risk. Regulation of genetically modified and gene edited crops by USDA-APHIS began in 1987 under the Coordinated Framework for Regulation of Biotechnology, 7 CFR part 340 (United States Department of Agriculture, 2022). Further revisions to APHIS's regulatory policy were made in May of 2020 and included several changes to the existing system.

Research on the CRISPR-Cas9 system for gene editing and crop improvement is being performed worldwide, but there is still no internationally agreed-upon regulatory policy for gene edited products. In the United States, the FDA, EPA, and USDA-APHIS have taken different steps toward regulating agricultural products produced by new plant-breeding technologies, including CRISPR gene editing. The FDA has no additional requirement for the food safety assessment of gene edited crops. In 2024, guidelines for companies to voluntarily engage with the FDA prior to marketing gene edited foods were released. These voluntary guidelines will allow companies to outline food safety precautions taken and may help to ease transition of new gene edited plant products to the commercial market (Bickell, 2024). The FDA will continue to apply a risk-based approach to gene edited foods with a focus on the objective characteristics and intended use(s). However, the FDA will still regulate gene editing used in animals as "new animal drugs."

On May 24, 2023, the EPA announced a final rule for an exemption proposed in October of 2020 regarding regulation of plant-incorporated protectants (PIPs) (Environment Protection Agency, 2023; Mendelsohn et al, 2023; Stockstad, 2023). PIPs are pesticidal substances which are produced in and used in and used by plant as pesticide (Mendelsohn et al., 2023). The 2023 final rule reflects advancements made in biotechnology since 2001, when PIPs derived from conventional breeding were exempted from regulation while maintaining regulatory requirements for PIPs produced through biotechnology (Environmental Protection Agency, 2023). Under the final rule, gene edited plants will be exempt from the in-depth review process if the introduced trait already exists within the plant's gene pool and if data is submitted indicating the gene edited plant won't harm the plant's ecosystem or cause harm to consumers (Stokstad, 2023). This regulatory change comes as a result of significant advancements in biotechnology which enable the creation of PIPs through gene editing and genetic engineering that are virtually indistinguishable from those produced using traditional breeding methods (Environmental Protection Agency, 2023). On March 28, 2018, USDA Secretary of Agriculture, Sonny Perdue, issued a formal statement on innovative plant-breeding techniques (including CRISPR gene editing), explaining that the USDA does not have plans to evaluate gene edited plants for health and environmental safety if they could otherwise have been developed through traditional breeding, unless these gene edited plants are potential plant pests or developed using plant pests (United States Department of Agriculture, 2018). The 2020 revisions to 7 CFR 340 provide a more in-depth definition for plants eligible for non-regulated status (United States Department of Agriculture, 2020(b)). Under these updates, regulations do not apply to organisms which contain one of the following modifications: cellular repair of targeted double-stranded break in absence of externally provided template (7 CFR 340.1(b)(1)), targeted single base pair substitution (7 CFR 340.1(b)(2)), or introduction of a gene known to occur within the plant's gene pool, change of target sequence to match a known allele within the plant's gene pool, or targeted changes to correspond to a known structural variant in the plant's gene pool (7 CFR 340.1(b)(3)). A plant can additionally be considered non-regulated following submission of exemption proposals by either USDA-APHIS or an external party if the resulting modification(s) could be achieved through conventional breeding (7 CFR 340.1(b)(4)(i) and 7 CFR 340.1 (b)(4)(ii)). On May 14, 2020, USDA Secretary of Agriculture, Sonny Perdue, announced a press release revealing a final rule to update USDA regulations of biotechnology under the plant protection act (United States Department of Agriculture, 2020(a)). This final rule, titled the Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient (SECURE) rule, will enable more efficient and effective regulatory oversight by focusing on risks posed by traits introduced during gene editing rather than whether the gene edited plant was developed using a plant pest (Hoffman, 2021; United States Department of Agriculture, 2020(a)). The SECURE rule additionally includes a mechanism for rapid initial review to identify and separate plants which do not plausibly increase plant pest risk from those that do (Hoffman, 2021). Plants which are identified to plausibly increase plant pest risk will be subject to further regulation.

In contrast to the United States, the European Court of Justice has ruled that gene editing techniques fall within the European Union's 2001 GMO directive, meaning that gene- edited products should be treated like traditional transgenic products (Faure and Napier 2018) and be subjected to a mandatory risk assessment (Callaway 2018). According to Faure and Napier, CRISPR gene editing technology will not be profitable in the European Union and is unlikely to be implemented soon. However, loosened restrictions of gene edited plants have been considered recently, and the European Parliament voted to ease regulation of gene edited crops in early 2024 (Stokstad, 2023,2024; "EU rethinks genome editing", 2023; Voigt, 2023).

Several scientists in Europe together with the European Commission's top scientific advisory panel have sharply rebuked both the European Court decision on gene editing and Europe's entire framework for regulating genetically modified organisms (Conrow, 2018; Pothering, 2019). Gene editing is also currently considered GM in New Zealand and controlled by the Environmental Protection Authority (EPA). While gene edited crops are still heavily regulated globally, several countries have started loosening restrictions on edited crops, including India (Government of India Ministry of Science & Technology Department of Biotechnology, 2022), Argentina, Brazil, Chile, Colombia, Paraguay, Israel, Japan, and Australia (Menz et al., 2020). While the EU and New Zealand have yet to loosen restrictions for gene edited crops, there are calls for updates to the regulatory systems based on decades of regulatory experience and consumer studies (Dayé et al., 2023; Van Der Meer et al., 2023; Voigt, 2023).

Conclusion

CRISPR gene editing techniques are fundamental breakthroughs in plant genetic improvement that can adjust desired traits more quickly and precisely than traditional breeding. In recent years, gene editing has been applied in many crop systems to improve important agronomic traits such as yield, nutritional value, and disease resistance. However, there remains some public concern and regulatory uncertainty over food ingredients from gene edited crops. Nevertheless, such foods will soon be available on supermarket shelves, and therefore it is important that the development and regulatory status of gene edited foods is clarified and that consumers are adequately informed about new technologies.

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Table 1. A selection of companies using CRISPR gene editing for crop improvement.

Company	Gene-Edited Crops
Alora	Rice
Arcadia Biosciences	Wheat
Bayer (through various collaborations)	Canola, Corn, Cotton, Pennycress, Soybean, Tomato
Benson Hill Biosystems	Row crops
Cibus	Canola, Corn, Rice, Soybean, Wheat
Corteva	Canola, Corn, Sorghum, Soybean
GreenVenus	Avocado, Grape, Lettuce
Inari Agriculture	Corn, Soybean, Wheat
J.R. Simplot	Avocado, Potato, Strawberry
Nexgen	Tomato
Pairwise	Blackberry, Cherry, Corn, Lettuce, Mustard greens, Raspberry, Soybean, Strawberry
PLANTeDIT	Soybean
Sanatech Seed	Tomato
Syngenta	Corn, Rice, Soybean, Sunflower, Tomato, Wheat
Tropic Biosciences	Banana, Coffee, Rice
Yield10 Bioscience	Camelina, Canola, Soybean

Table 2. Development status of various CRISPR-modified crops.

Crop	Purpose of Gene-Editing	Country	Current stage
Alfalfa	Improve quality	United States	Commercially available
Apple	Non-browning	Canada, United States	Commercially available
Avocado	Reduced browning	United States	Research in progress
Banana	Reduced browning	Philippines	Commercially available
Cacao	*Phytophthora tropicalis* disease resistance	United States	Research in progress
Camelina	Increased oil content, improved oil quality	Argentina, Canada, Chile, United States	Commercially available
Citrus	Citrus greening disease resistance	United States	Research in progress
Corn	Waxy corn, drought resistance, shorter growth height	Argentina, Brazil, Canada, Chile, United States	Commercially available
Grape	Powdery Mildew disease resistance	United States	Research in progress
Groundcherry tomato	Improve productivity	United States	Research complete, not commercially released
Lettuce	Increased nutrient quantity	United States	Commercially available
	Non-browning	United States	Commercially available
Mustard greens	Reduced pungency	United States	Commercially available
Mushroom	Longer shelf-life	United States	Commercially available

Crop	Purpose of Gene-Editing	Country	Current stage
Potato	Cold storage, reduced acrylamide	United States	Commercially available
	Reduced browning	United States	Commercially available
Rice	Improve yield	China	Research continuing on additional varieties
	Salt tolerance	Canada, Kenya, United Kingdom, Vietnam,	Field-trial stage
	Drought resistance	India	Research in progress
Soybean	High oleic acid, less saturated fat	United States	Commercially available
Strawberry	Extend shelf life	United States	Research in progress
Tomato	Increase fruit size, yield, and lycopene accumulation	European Union	Field-trial stage
	Increased antioxidant content	United States	Commercially available
	Improved nutrient content	Japan, Philippines	Commercially available

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