CRISPR/Cas9 FOR IMPROVING CROPS QUALITY TRAITS: A PROMISING APPROACH FOR HUMAN HEALTH

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Abstract

Continued population growth puts increasing pressure on agriculture. Although increasing crop yields remains an important goal of breeding programs, increasing attention is being paid globally to the nutritional quality of crops, which is important for human health. Conventional breeding to achieve this goal is time-consuming, expensive and laborious, but recent advances in genome editing technology offer a valuable alternative to these problems. CRISPR/Cas9 is one of the most successful genome editing tools (awarded the Nobel Prize in Chemistry in 2020), which allows precise and effective manipulation of genes in a living organism. In this paper, we summarize the main advances achieved in agriculture through CRISPR/Cas9, to improve crop quality (elimination of toxic compounds and allergens, enrichment with anthocyanins, extension of shelf life, etc.). We also presented their benefits for human health, as well as their regulation at the global level. In conclusion, CRISPR/Cas9 has immense potential in advancing the nutritional quality of crops and therefore, in promoting human health and food security.

***Key words*: allergens, anthocyanins, CRISPR/Cas9, crop quality, human health,**

INTRODUCTION

Nutrition influences a person's overall health, and poor nutrition can increase the risk of developing certain diseases. Malnutrition, or "hidden hunger," is a serious problem that exacerbates food insecurity. According to Sinha et al. (2019), approximately 800 million people worldwide are malnourished, 98% of whom live in underdeveloped countries.

UNICEF (2021), also estimates that over 340 million people are undernourished, suffering from one or more micronutrient deficiencies, including iron, zinc, iodine, and vitamin A deficiencies.

In addition, population aging and the trend toward a diet rich in protein and fat have led to the expansion of global health problems, and medical solutions are expensive and not widely applicable (Nagamine et al., 2022).

Crops are important food suppliers, meeting the ever-increasing global demand for food, thereby contributing to saving lives (Tilman et al., 2011; Medelete and Panzaru, 2015).

An effective strategy to address these problems is to increase the quantity, quality and nutritional value of food.

Obtaining healthier, more nutritious and tastier food can be achieved through both agnomic practices and traditional breeding methods (hybridization, selection, etc.), but these traditional methods are time-consuming and laborious (Kumar et al., 2022). Therefore, researchers have started to use various genetic techniques.

Thus, since 1996, genetically modified (GMO) crops with interesting traits, including biofortified for desired nutrients, have been developed and marketed through transgenic technology, but unproven concerns regarding their safety for health and the environment as a result of the introduction of foreign genes have led to various controversies among consumers and regulatory restrictions in several countries (Prado et al, 2014; Bonea, 2022; 2023).

The advent of precision genome editing has significantly alleviated public concerns about these GM foods. Genome editing technology produces predictable and heritable mutations in specific regions of the genome, with minimal off-target effects and without the integration of foreign genes (Bhattacharya et al., 2021).

The increasing accessibility and cost-effectiveness of genome editing tools promises numerous benefits for consumers, including improved food safety, improved nutrition, and reduced food waste (Ceasar and Kavas, 2024).

Given the extensive potential and implications that genome editing presents in the agricultural field, we hope that this analysis of genome-edited crops will attract the attention it deserves from a wider audience.

WHAT IS GENOME EDITING?

Genome editing/gene editing is a type of genetic engineering in which DNA is inserted, deleted, modified, or replaced in the genome of a living organism. Unlike early genetic engineering techniques that randomly insert genetic material into a host genome, genome editing targets insertions to specific site locations (Karavolias et al., 2021). There are four main tools for site-specific gene editing, namely: Zinc Finger Nucleases (ZFN), Meganucleases (MN), Transcriptional activator-like effector nucleases (TALEN) and Clustered regularly interspaced short palindromic repeats (CRISPR).

Although the discovery of artificially designed MNs, followed by ZFNs and TALENs, successively increased the efficiency of genome editing, targeting different sites in the genome required the redesign of a new set of proteins.

The difficulty in cloning and engineering ZFNs and TALENs proteins has partly prevented these tools from being widely adopted by the scientific community. In this regard, the CRISPR/Cas9 tool has revolutionized the field, being much simpler to use and more versatile.

Discovered in 2012 and awarded the 2020 Nobel Prize in Chemistry, this tool acts like a pair of scissors, cutting DNA at a specific point to allow very precise changes in the traits of a crop or animal. Currently, different CRISPR/Cas systems are used for genome targeting, but the most widely used is the CRISPR-Cas9 type II system from *Streptococcus pyogenes* (Adli, 2018).

The CRISPR/Cas9 tool involves two essential components: a guide RNA and the Cas9 endonuclease (protein). The guide RNA directs the Cas9 protein to the desired location in the genome for editing, and the Cas9 enzyme acts as a molecular scissor, making a precise cut in the DNA and causing a double-strand break (DSB) at the designated location (Figure 1). Normal cellular processes then occur to repair the break through non-homologous end joining (NHEJ) or through homology-directed repair (HDR), resulting in different types of modifications, depending on the presence or absence of a DNA template.

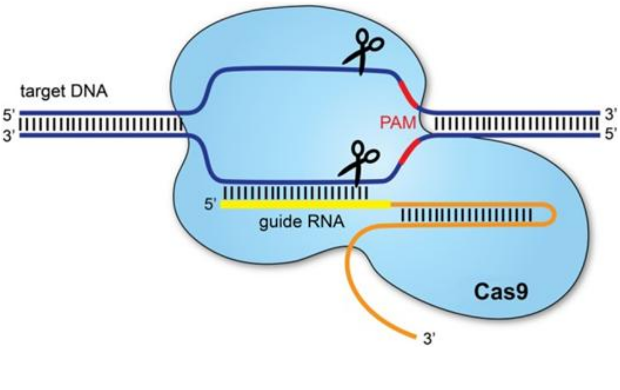

Figure 1. CRISPR/Cas9 tool diagram
Source: Redman et al. (2016)

GENOME-EDITED (GE) CROPS AND THEIR BENEFITS

The emergence of genome-editing tools, especially CRISPR/Cas9, has attracted the attention of researchers because they remove the limitations of traditional breeding methods. These techniques can create precise mutations in targeted genes without the introduction of foreign DNA. The development of this GE technology promises a wide range of benefits for consumers through improved nutrition, increased food safety, and reduced food waste (Zhang and Zhu, 2024).

Gene-edited crops have already reached the market, but many other innovations are in development, awaiting commercial approval. Most genome-edited crops are approved and sold in the United States, Japan, and Canada (Table 1).

Table 1. Examples of genome-edited (GE) crops via CRISPR/Cas9 and their benefits for human health

Crop/improved trait	Description	Company (country)	Year of approval or testing	Patent or reference
GE crops found on the market				
Tomato (*Lycopersicum esculentum)* Purple tomatoes with high anthocyanin content	*Purple tomato* contain two genes from the snapdragon flower that provided the purple pigment and increased the concentration of anthocyanins. Anthocyanins are associated with a number of health benefits (reducing inflammation and promoting heart health)	Norfolk Healthy Produce, Ltd., (UK)	2022-available in the US	https://www.lens.org/lens/patent/172-128-558-724-105/frontpage?l=en NPS (2023)
Tomato (*Lycopersicum esculentum*) GABA tomato with higher levels of gamma-aminobutyric acid	*Sicilian Rouge was obtained* by inactivating the gene encoding the *calmodulin binding domain* (*CaMBD*), thereby allowing increased activity of the enzyme decarboxylase, which catalyzes the decarboxylation of glutamate to *GABA*. High levels of γ-aminobutyric acid (*GABA*) are thought to help lower blood pressure and promote relaxation.	Sanatech Seed Co, Ltd. (Japan)	2021-available in the Japan	https://patents.google.com/patent/JP6058872B2/en USDA (2020)
Lettuce (*Lactuca sativa*) Non-browning romaine lettuce	*GreenVenus lettuce* contains combinations of polyphenol oxidase (*PPO*) gene mutations that reduce browning, tip burning, extending shelf life and improving nutrition.	GreenVenusLLC./Intrexon(California, US)	2024-available in the US	https://www.lens.org/lens/patent/185-656-263-620-844/fulltext?l=en IFT (2019)
Mustard (*Brassica juncea*) Mustard greens with decreased bitterness	*Conscious Greens* has functional modifications of the multigene *myrosinase* type I family, which removes the pungency from fresh leaves, doubling the nutritional value compared to romaine lettuce and extending its shelf life	Pairwise Plants Services Inc (US)	2023 - available in the US and Canada	https://www.lens.org/lens/patent/143-473-546-918-944/frontpage ISAAA (2022)
Banana (*Musa* spp.) Reduced browning	*Non-browning bananas* were obtained by eliminating the function of the polyphenol oxidase enzyme, which is why they can remain yellow for longer, thus reducing food waste and CO2 emissions (the equivalent of removing 2 million cars from the roads per year).	Tropic Biosciences, Ltd (UK)	2023 - available in Philippines	https://www.lens.org/lens/patent/089-116-471-909-450/fulltext?l=en Tropic Biosciences

				LTD. (2023).
Corn (*Zea mays*) Corn with high starch content	*Waxy corn* was obtained by targeted deletion of the waxy gene (*Wx1*), which increased the amylopectin content of the starch (by almost 100%). It offers potential benefits especially for athletes, due to its rapid digestion and ability to quickly replenish muscle glycogen stores, aiding recovery.	Pioneer Hi-Bred International Inc	2024 - available in Japan	https://www.lens.org/lens/patent/066-043-105-670-954/frontpage?l=en USDA and GAIN (2023).
Successfully tested GE crops awaiting approval				
Camelina (*Camelina sativa*) Camelina with enhanced omega-3 oil	Camelina modified to accumulate *EPA* and *DHA* - fatty acids that are beneficial for health and are normally only found in omega-3 fish oils.	Rothamsted Research (UK)	2022 - Field testing (UK)	Halleron (2022)
Tomato *(Lycopersicum esculentum)* Tomatoes biofortified with vitamin D	To biofortify tomatoes with provitamin D3, the activity of the *Sl7-DR2* gene was blocked. Globally, approximately one billion people suffer from vitamin D deficiency, and their number is increasing due to inadequate food availability	John Innes Centre, Norwich Research Park (UK)	2022- Field testing (UK)	Li et al. (2022)
Wheat (*Triticum aestivum*) Low asparagine wheat	Wheat improved by deletion of asparagine synthase (*TaASN2*) to reduce the concentration of free asparagine in the grain. Free asparagine is known to be converted to acrylamide (a carcinogenic contaminant) during high-temperature processing, baking, and roasting.	Rothamsted Research (UK)	2021 - Field testing (UK)	Raffan et al. (2021)
Cruciferous vegetable (*Brassica oleracea*) Cabbage, broccoli and kale with increased levels of glucosinolates	Cruciferous vegetables improved by eliminating the *MYB28* gene, to increase the level of glucosinolates, organic compounds that have beneficial effects on human health (improved blood sugar control, reduced risk of cardiovascular disease, anticancer effect)	John Innes Centre (UK)	2021 Field testing (UK)	Galvin (2021)

Regulation of genome-edited products at the international level

Most countries where genome-edited products are regulated consider products obtained through the SDN-1 technique to be non-GMOs, and those obtained through the SDN-3 technique to be improved GMOs. For products obtained through the SDN-2 technique, a case-by-case approach is preferred (Podevin et al., 2013).

SDN1, SDN2 and SDN3 refer to modifications obtained through genome editing (including CRISPR/Cas9), which are classified according to the way in which the double-strand break (DSB) is repaired (Figure 2).

SDN-1 applications initiate a double-strand break in DNA, without the addition of foreign substances, the repair of this break (by NHEJ) can lead to the insertion or deletion of nucleotides, causing the elimination, silencing or modification of the activity of a gene. SDN-2 applications initiate a double-strand break and, while the break is repaired by the cell (by HDR), a small template of nucleotides complementary to the target region is provided, which will be used in the natural repair process. This template contains one or more small sequence changes in the genomic code that are copied into the genetic material of the plant, resulting in a desired change in the target gene. SDN-3 applications initiate a double-strand break in DNA, but is accompanied by a template that contains a gene or other sequence of genetic material. Subsequently, this template is used in the cell's natural repair process (by HDR), resulting in the introduction of new genetic material into the genome (Islam and Ahuya, 2024).

In the European Union, genome-edited products are still subject to the regulation of genetically modified organisms (GMOs). In July 2023, the European Commission proposed a relaxation of the GMO rules for plants in the NGT1 category, a proposal that the European Parliament approved in February 2024, thus paving the way for negotiations with the Council (Bonea, 2024).

Subsequently, in March 2025, the Council agreed on its position for negotiations with the European Parliament on new genomic techniques. The new provisions thus provide for: the option for EU Member States to prohibit the cultivation of plants in the NGT2 category; the transmission of information on patents filed for plants in the NGT1 category to a public database; mandatory labelling of plants in the NGT2 category; and the prohibition of the herbicide tolerance trait for plants in the NGT1 category (Dionglay, 2025).

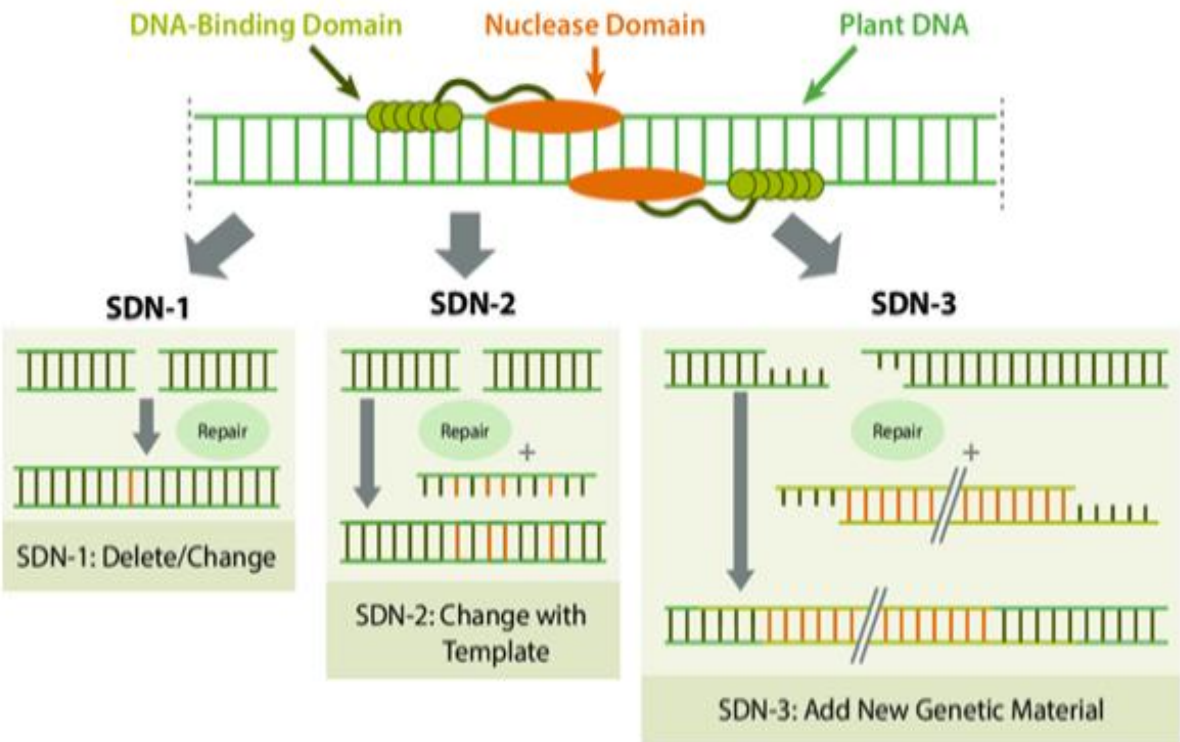

Figure 2. SDN-1, SDN-2, and SDN-3 techniques
(Source: Islam and Ahuja (2024)

CONCLUSIONS

Malnutrition and undernutrition worldwide are driving researchers to develop crops with high yields and improved quality.

Genome editing is a revolutionary, precise and efficient technology that has the potential to contribute to solving these goals. Through its tools, especially CRISPR/Cas9, crops without foreign DNA have been developed, which are already commercialized in some countries.

The results obtained with the CRISPR/Cas9 tool analysed above are examples of how genome editing technology can be applied to crops to improve quality traits important for human health.

Consumer interest in these GE foods can be improved by providing relevant information on their benefits, and their wide expansion can be achieved through a harmonization of international regulations.

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