REVIEW

Genome editing and beyond: what does it mean for the future of plant breeding?

Tien Van Vu[1,2] · Swati Das[1] · Goetz Hensel[3,4] · Jae-Yean Kim[1,5]

Abstract
Main conclusion Genome editing offers revolutionized solutions for plant breeding to sustain food production to feed the world by 2050. Therefore, genome-edited products are increasingly recognized via more relaxed legislation and community adoption.

Abstract The world population and food production are disproportionally growing in a manner that would have never matched each other under the current agricultural practices. The emerging crisis is more evident with the subtle changes in climate and the running-off of natural genetic resources that could be easily used in breeding in conventional ways. Under these circumstances, affordable CRISPR-Cas-based gene-editing technologies have brought hope and charged the old plant breeding machine with the most energetic and powerful fuel to address the challenges involved in feeding the world. What makes CRISPR-Cas the most powerful gene-editing technology? What are the differences between it and the other genetic engineering/breeding techniques? Would its products be labeled as "conventional" or "GMO"? There are so many questions to be answered, or that cannot be answered within the limitations of our current understanding. Therefore, we would like to discuss and answer some of the mentioned questions regarding recent progress in technology development. We hope this review will offer another view on the role of CRISPR-Cas technology in future of plant breeding for food production and beyond.

Keywords Genome engineering · New plant breeding techniques · CRISPR-Cas · Crop breeding · Biotechnology regulation

Abbreviations

CRISPR	Clustered regularly interspaced short palindromic repeat
Cas	CRISPR-associated protein
GE	Genome editing
GMO	Genetically modified organism
LMO	Living modified organism
ODM	Oligonucleotide-directed mutagenesis
SDN	Site-directed nuclease
DSB	Double-stranded DNA break
NHEJ	Nonhomologous end-joining
HDR	Homology-directed repair

✉ Goetz Hensel
goetz.hensel@hhu.de

✉ Jae-Yean Kim
kimjy@gnu.ac.kr

1 Division of Applied Life Science (BK21 Four Program), Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea

2 National Key Laboratory for Plant Cell Biotechnology, Agricultural Genetics Institute, km 02, Pham Van Dong Road, Co Nhue 1, Bac Tu Liem, Hanoi 11917, Vietnam

3 Centre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich-Heine-University, Universitätsstraße 1, 40225 Düsseldorf, Germany

4 Centre of Region Haná for Biotechnological and Agricultural Research, Czech Advanced Technology and Research Institute, Palacký University Olomouc, 78371 Olomouc, Czech Republic

5 Division of Life Science, Gyeongsang National University, 501 Jinju-daero, Jinju 52828, Republic of Korea

Introduction

Crop traits are inherited from parent plants through many generations and are encoded by the genetic information contained within the DNA of cells. However, the genetic contents are continuously modified (Vergauwen and De Smet 2017) as a consequence of spontaneous mutations, transcriptional errors, environmentally or artificially induced mutations, transposon activities, meiotic crossing over, and cross-fertilization (allogamy) processes. Plant pests or symbiotic agents, such as *Agrobacterium* spp. (Kyndt et al. 2015) or *Burkholderia* (Pinto-Carbó et al. 2016), integrate their genetic fragments with the hosts, thereby modulating host cells for replication or feeding. Therefore, genetic modifications continuously occur in any genetic containment of living plant cells.

Plant breeding is the process of generating plants containing genetic entities encoding favorable traits that fit our agricultural production, processing, and subsequent consumption. Thus, it includes selection processes among a population of plants with diverse and undesirable traits. Via archeological evidence, plant breeding has been estimated to have been actively carried out by humans a dozen thousand years ago (Vergauwen and De Smet 2017) when seeds of plants with favorable features were saved for the next plantation, a practice known as domestication. The historical milestones (Fig. 1) of plant breeding techniques were achieved in parallel with a more profound understanding of plants and their genetic makeup. With the increases in quantitative and qualitative food consumption, plant breeding has been revolutionized, with key achievements in crossbreeding (hybrid crops) and transgenesis. Hybrid crop backing by heterosis created the first green revolution, starting with semidwarf wheat varieties carrying *reduced height* (*Rht*) alleles developed by Dr. Norman Borlaug in the 1940s–1950s (Swaminathan 2009; Vergauwen and De Smet 2017). Transgenic crops are now widespread globally and are increasingly accepted as food and feed. In the US, one of the leading countries in adopting
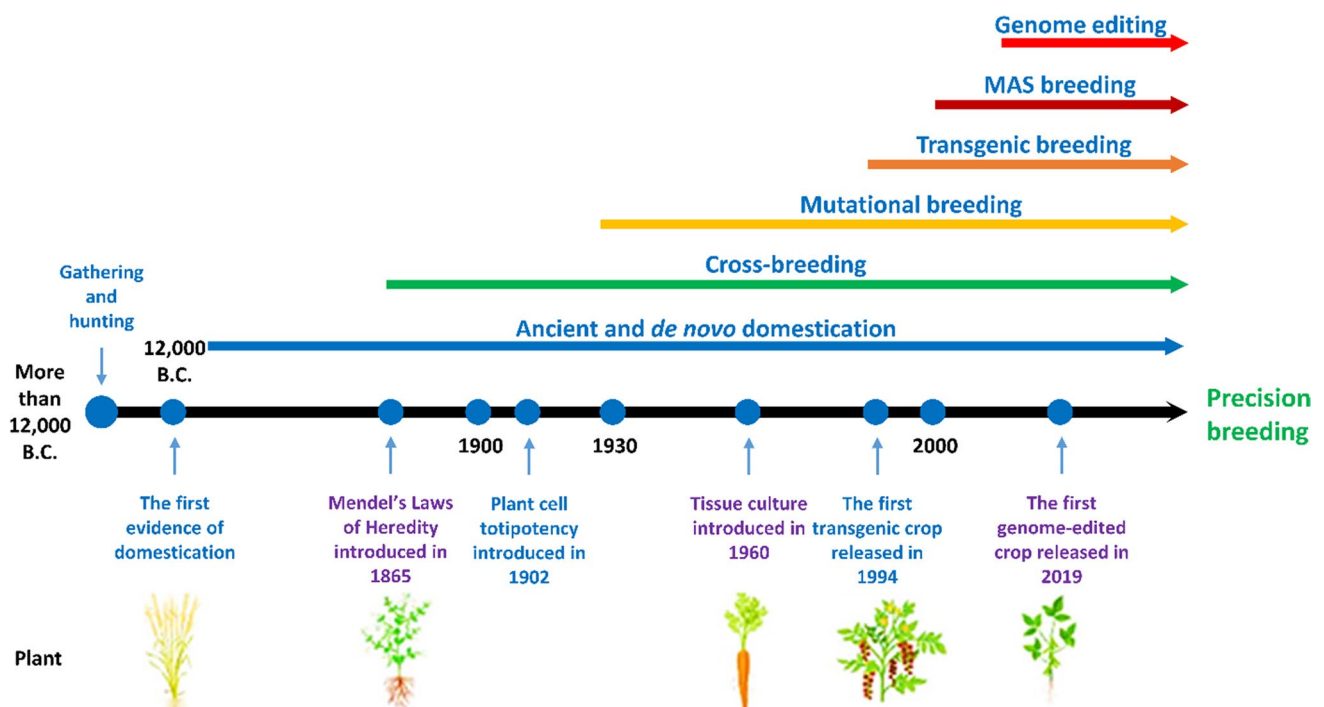
Fig. 1 Plant breeding milestones. The start of domestication and initial plant breeding dates back around 12,000 B.C. when the living style of Human-being changed from gathering and hunting to agriculture. The first-ever domesticated plant was emmer wheat. Since then, ancient domestication and selective breeding were dominant until the discovery of Mendel's laws of genetics. The laws of genetics triggered and enhanced the crossbreeding wave. A milestone in plant breeding that plays an essential role in modern plant breeding was the invention of the totipotency of plant cells in the early 1900s by Gottlieb Haberlandt. As a result, the first in vitro tissue culture was introduced in 1960 with carrot. Plant tissue culture was the critical step for generating the first *Agrobacterium*-mediated transgenic tomato in 1994, known as transgenic breeding. In the meantime, mutational breeding using chemical or physical agents was also introduced in the 1930s and played an important role in generating diverse genetic materials for crop breeding. Biochemical markers further enhanced crossbreeding in marker-assisted selection (MAS) breeding. The recently emerging genome editing approaches have revolutionized plant breeding to precision levels that have never been obtained before. High oleic acid soybean, the first genome-edited crop that was released in 2019, has been opening the wave of genome-edited precision breeding in plants

transgenic crops, nearly all corn, cotton, and soybean areas are covered by transgenic varieties in 2021 (USDA 2020). Transgenesis changes the genetic information of a plant cell, resulting in a so-called genetically modified organism (GMO) or living modified organism (LMO), by adding "foreign," cross-kingdom DNA fragment(s) to achieve beneficial trait(s) that the conventional breeding techniques cannot obtain. While health and biodiversity concerns regarding transgenic approaches are serious, there is very controversially limited evidence to support them. Nevertheless, a long and expensive procedure is required to release GMO/LMO events for cropping or consuming their products as food or feed, limiting the spreading of crops globally.

Recently, genome editing (GE), especially clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein (Cas), has appeared to offer powerful breeding tools without adding exogenous DNAs producing genetic variations, such as natural, spontaneous mutations. The approach has been shown to create many elite traits that conventional breeding either cannot generate or could only generate with a time-consuming and laborious process (Vu et al. 2020). As a result, GE crops, especially those that carry genome modifications mimicking spontaneous mutations, are widely deregulated worldwide except in European countries where stringent GMO rules are applied (Schmidt et al. 2020b). What makes GE products more acceptable than GMOs? GE is usually triggered using "molecular scissors" to cleave the double-stranded DNA genome. The resulting DNA double-stranded breaks (DSBs) prime cellular responses that lead to the repair of broken DNAs primarily by nonhomologous end-joining (NHEJ) or homology-directed repair (HDR) (Puchta 2005; Scully et al. 2019). DSB repair by NHEJ is dominant in somatic cells and usually results in erroneous products containing short base insertions or deletions, thus inducing mutations to the targeted genes (Puchta 2005). These mutations can be produced naturally as spontaneous mutations or in plants created from chemical/radiation-based random mutagenesis. Notably, the introduced molecular scissors can be eliminated from the edited cells, resulting in transgene-free GE plants, thereby eliminating the concerns associated with the derived foods in some countries (Entine et al. 2021; Menz et al. 2020). Moreover, the edited site is controllable and highly specific due to the characteristics of the customized nucleases (Jinek et al. 2012; Gaj et al. 2013). Therefore, GE products are much "cleaner" than random mutagenesis products.

Under the current conditions of plant breeding and cropping technologies, it is impossible to produce sufficient foods to feed the world by 2050. Additionally, cropping for foods faces many hurdles due to climate change and the shortage of arable lands as a result of urbanization. Therefore, adopting a revolutionized plant breeding technique such as GE is needed to generate new traits/cultivars that can enhance yield or sustain food production under unfavorable conditions. In this review, we discuss the foundation of CRISPR-Cas technology regarding crop genetic improvement and the potential uses of its derived foods for humans compared with other genetic engineering technologies. Future perspectives of GE are also provided to suggest another view of the applied legislation.

In summary, CRISPR-Cas technology enables rapid and precise modification of breeding material, with targets, such as product ingredients, plant architecture, improved nutrient uptake or distribution in the crop, contributing to agricultural sustainability and the UN Sustainable Development Goals (SDGs). Increased yields using less fertilizer, pesticide, or water are further goals of CRISPR-Cas technology.

Food production in the CRISPR era

According to an analysis performed by van Dijk and coworkers, under the impacts of climate change, global food production needs to be increased from +30 to +62% between 2010 and 2050 (van Dijk et al. 2021). If we also consider nutritional needs, then food production may be sufficient in 2050 following radical changes in food types, such as consuming more plant-based alternatives of meat and dairy, and societal adaptation (Berners-Lee et al. 2018), which is likely impossible. Earlier, in the middle of the twentieth century, novel semidwarf wheat and rice varieties played a vital role during the first green revolution (Van Vu et al. 2019), which reversed the catastrophic prediction of hunger due to the food crisis. Food production was dramatically increased in Mexico, India, and other countries following the importation of new wheat and rice varieties (Khush 2001). The breeding technology behind the success of the green revolution was crossbreeding that pyramided elite genes into elite plant varieties, which were selected under various geographical conditions (Swaminathan 2009; Vergauwen and De Smet 2017). Recently, it was predicted that crop-based food production may not be sufficient to feed the world by 2050 (Ray et al. 2013). To reverse the prediction, we must improve crop productivity with high-yield and/or climate-resilient varieties. Hence, novel varieties are expected to play the same role in solving the next food crisis.

GE technology has paved the way for breeding crop plants to enhance growth and production under challenging environmental conditions. It can also help improve crop plants' qualitative traits to sustain human health and other needs. Theoretically, any modification of a known genome site can be made at high efficiency and precision with the various GE tools currently available (Chen et al. 2019; Van Vu et al. 2019; Marzec and Hensel 2019). Early in the development of GE tools, rice varieties that conferred resistance to bacterial

blight disease (Li et al. 2012) and fungal blast (Wang et al. 2016) were developed (Table 1). A similar GE tool was also used to create powdery mildew-resistant wheat (Wang et al. 2014). By enhancing yield and improving the starch components in maize, superior corn was introduced in 2020 (Gao et al. 2020). Recently, more attention has been given to the improvement of nutritional and health-functional traits of crop plants, such as oil contents of soybean (Haun et al. 2014), the first commercialized GE product, starch quality in potato (Andersson et al. 2018) and gluten-free wheat (Sánchez-León et al. 2018). Japan was the first country to commercialize a genome-edited tomato product that contains high γ-aminobutyric acid (GABA) contents (Nonaka et al. 2017) in September 2021. Increasingly, GE-derived foods and products enhance global food production and security with levels of speed, efficacy, and precision that no other plant breeding technologies can offer. Likewise, with the huge potential of GE technology, global food production can match the projected demands in 2050 in the CRISPR era if the technology is widely accepted in a similar manner to random mutagenesis.

What would new plant breeding technology such as CRISPR-Cas-based gene editing offer?

New breeding methods, such as CRISPR-Cas technology, allow rapid and precise manipulation of a target genome for the first time in human history. All previous mutation techniques either involve introducing many unwanted changes (e.g., chemical and radiation mutagenesis) or are purely random and unpredictable (e.g., natural mutations).

In crossbreeding, which is most commonly used for combining positive traits, the genome of the elite background must then be enriched again through several backcrossing steps. Crossbreeding is time-consuming and costly. In addition, this technology has the disadvantage that certain genomic regions cannot be separated from each other due to a lack of recombination. This phenomenon is called linkage drag.

CRISPR-Cas technology can also increase breeding diversity within a species. Here, a reduction in genetic diversity has occurred in the course of domestication due to the processes described above (Voss-Fels et al. 2019). Enhanced genetic diversity can now be achieved through the targeted induction of double-strand breaks between previously inseparable loci (for review, see Rönspies et al. 2021) (Rönspies et al. 2021). Several approaches have described heritable inversions and translocations in this way. Schmidt and colleagues (2020) used the *Staphylococcus aureus* Cas9

Table 1 Several representative CRISPR-Cas-based food crops suggested in this minireview

No.	Crop	Modification	Added trait	Targeted gene(s)	Targeted site	Regulation status	Release status	References
1	Rice	Indel mutation	Bacterial blight resistance	*Os11N3* (*OsS-WEET14*)	Cis-element	NA	NA	Li et al. (2012)
2		Indel mutation	Rice blast Resistance	*OsERF922*	Coding sequence	NA	NA	Wang et al. (2016)
3	Wheat	Indel mutation	Powdery mildew resistance	*TaMLO*	Coding sequence	NA	NA	Wang et al. (2014)
4		Indel mutation	Reduced gluten content	*α-gliadin* genes	Coding sequence	NA	NA	Sánchez-León et al. (2018)
5	Maize	Indel mutation	High-yield waxy corn	*ZmWx1*	Coding sequence removal	NA	NA	Gao et al. (2020)
6	Soybean	Indel mutation	High oleic acid oil	*GmFAD2-1A and GmFAD2-1B*	Coding sequence	Passed in the US	February 2019	Haun et al. (2014)
7	Potato	Indel mutation	Altered starch contents	*StGBSS*	Coding sequence	NA	NA	Andersson et al. (2018)
8	Tomato	Indel mutation	Highly accumulated GABA	*SlGAD2* and *SlGAD3*	Coding sequence, autoinhibitory domain removal	Passed in Japan	September 2021	Nonaka et al. (2017)

nuclease and the ovule-specific promoter in *Arabidopsis* to invert an 18-kb fragment. Subsequently, the same group succeeded in changing the difference between the two *Arabidopsis* ecotypes *Ler*-1 and *Col*-O, known as a heterochromatic knob (*hk4S*) inversion on chromosome 4 (Schmidt et al. 2020a). This inversion made a meiotic crossover in this region on chromosome 4 possible for the first time. Recently, a translocation of 0.5- to 1-Mbp chromosomal segments was reported (Beying et al. 2020). Fragments could be exchanged between *Arabidopsis* chromosomes 1 and 2 and between chromosomes 1 and 5. These changes were also detectable in subsequent generations. Even though similar evidence for crop plants has been lacking, it is now possible to exchange positively correlated alleles between accessions of a species in a targeted manner using the methods described above.

Another advantage of CRISPR-Cas technology is the acceleration of the breeding process. Knowing the molecular basis for domestication genes, such as fruit color, fruit size, or the number of fruits, makes it possible to precisely convert a wild species into a cultivated plant within one generation (for review, see Fernie and Yan 2019) (Fernie and Yan 2019). This process is also referred to in the literature as de novo domestication, and several examples already exist. For example, by altering six loci in the precursor of our present-day tomato *Solanum pimpinellifolium*, plants were generated that had a threefold increase in fruit size, a tenfold increase in the number of fruits, and a 500-fold increase in the accumulation of lycopene (Zsögön et al. 2018). Parallel attempts were made in the same species to alter daylength sensitivity, shoot architecture, flower and fruit production, and nutrient content by simultaneous gene editing (Li et al. 2018). These authors also reported successful domestication in only one step. Similar approaches in *Physalis pruinosa* (Lemmon et al. 2018) and allotetraploid wild rice (Yu et al. 2021) further demonstrated the potential of this technology for the breeding process.

Although many agronomically relevant traits are not based on single modifications, some examples illustrate that this is still possible. The domestication-related barley genes for brittleness and row-type *Btr1*, *Btr2* and *Vrs1* (Komatsuda et al. 2004, 2007) demonstrate that few modifications dramatically change the phenotype. Either the deletion of 1 or 11 nucleotides allows the barley grains to stay in the spike until harvest, which allowed ancient farmers easier harvest (Pourkheirandish et al. 2015). For the switch from two- to six-rowed barley, a mutation in the *Vrs1* gene is necessary. It has been illustrated using RNAi technology that the causal gene has been identified (Sakuma et al. 2013). Using CRISPR/Cas-mediated knockout, full development of the lateral grains was observed (Hensel et al., unpublished). Another example of fungal resistance in wheat further supports the idea that slight modifications have a severe impact. The *Lr34* gene decides whether a wheat accession is susceptible or tolerant to fungal infections. As barley (Chauhan et al. 2015) shows, just one amino acid can alter the resistance of a cultivar. Using CRISPR-Cas technology as prime editing can achieve such allele exchange.

All targeted mutagenesis platforms rely on the knowledge of sequence information. With the tremendous improvements in sequencing technologies, discovering new genomes is possible within hours and for an affordable amount of money. This development allows the direct comparison of multiple accessions of the same species (known as pangenome, (Golicz et al. 2016)) and identifies the beforementioned necessary sequence polymorphisms.

The fundamental prerequisite of the aforementioned accelerated evolution is the simultaneous targeting of multiple target sites in a genome. Various methods have been developed and applied for this purpose. Either several gRNA arrays can be united in one T-DNA using the methods based on type-II restriction enzymes (Hahn et al. 2020), or tRNA-based techniques are used (Xie et al. 2015). Reducing allergens in food is highlighted here from the endless array of applications. First, gluten-free wheat should be mentioned (Sánchez-León et al. 2018). Simultaneously, targeting 35 different *α-gliadin* genes in bread wheat reduced the coeliac disease-triggering gluten content by 85%. This reduction makes the production of gluten-reduced foods possible. Recently, CRISPR-Cas was used to remove the major allergen, 2S albumin class gene *Bra j* I in brown mustard (*Brassica juncea*), and mutant plants with reduced allergenicity were generated (Assou et al. 2021).

The CRISPR-Cas technology application can go far beyond inducing a double-strand break by fusing functional domains to Cas proteins. For example, genes could be activated in rice (Xiong et al. 2021), repressed in *Arabidopsis* (Lowder et al. 2015), their position in the *Nicotiana tabacum* genome visualized by reporter genes (Dreissig et al. 2017), or modified using base editors. Review articles on how these approaches can be used for breeding salt-tolerant rice (Ganie et al. 2021) or tomato (Vu et al. 2020) have recently been published.

A variety of prime editing (PE) and base editing (BE) variants have been developed over the past years and have been successfully tested in plants (for review, see Molla et al., 2021) (Molla et al. 2021). Prime editing (Anzalone et al. 2019) and base editing (Gaudelli et al. 2017; Ren et al. 2018) now allow modification of the target sequence, which does not require induction of a double-strand break. With these, applications, such as regulation of cis-elements, modification of RNA splice sites, integration of synthetic miRNAs, or adaptation of miRNA-binding sites, are possible. This mechanism could also alter the binding sites of effectors released by fungal pathogens to target plant susceptibility genes. In this way, heritable resistance might be conferred.

Using CRISPR-Cas technology, generating resistance to herbicides is also possible. Here, a wealth of herbicide resistance can be produced in agronomically essential plants through the targeted selection of target genes. While most plants for commercial use constitute a GMO through the transfer of bacterial genes, the adaptation of plant targets, such as *EPSPS*, *HPPD*, and *ALS* genes from *Zea mays*, *Avena sativa*, and *N. tabacum*, can be made using CRISPR-Cas technology. This review provides a detailed overview (Han and Kim 2019).

What makes CRISPR-Cas the most powerful gene-editing technology?

CRISPR-Cas technology, unlike zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), is an RNA-based gene modification system (Jinek et al. 2012). ZFNs and TALENs, in contrast, are based on the attachment of polypeptides to DNA. In ZFN, a finger-like peptide binds a DNA triplet (Urnov et al. 2010), whereas, in TALEN, approximately 33 amino acid-length peptides bind specifically to a DNA nucleotide (Boch et al. 2009). In ZFN and TALEN, these DNA-binding domains are coupled to the nonspecific FokI endonuclease. However, this is only active as a dimer (Vanamee et al. 2001), which means that ZFN and TALEN approaches require two target molecules on the respective complementary strand. This mode of action makes cloning more difficult because larger and repetitive DNA fragments have to be assembled. The target sequence area is thus larger, which increases the specificity and reduces the probability of an off-target mutation. The basis for this is that a DNA region of approximately 40 nucleotides per target genome is more likely to be unique than a region only 20 nucleotides long, as with CRISPR-Cas. However, this potential negative feature of the CRISPR system can be reduced by carefully selecting the target sequence. Various online tools, such as CRISPOR (Concordet and Haeussler 2018) and CRISPR-Plant (Xie et al. 2014), will only be mentioned here as examples. A review on this has been published previously (Cui et al. 2018; Naim et al. 2020).

Proof of the development of revolutionary technology is its broad application. Apart from the introduction of next-generation sequencing technology, there has been no other technology in the life sciences in recent years that has been used so widely and successfully in such a short time. The reason for this is its universal use in every organism tested to date, but above all, its simplicity in creating the necessary reaction reagents. For the most common usage, the transformation of a simple vector carrying expression cassettes encoding a CRISPR-Cas protein and a guide RNA (gRNA) is sufficient in many cases. Additionally, the creation of complex constructs is straightforward through the implementation of methods based on type-II restriction enzymes (Engler et al. 2008). Furthermore, in recent years, commercial suppliers have CRISPR products in their portfolios at affordable prices, further fueling the use of CRISPR-Cas technology.

As CRISPR-Cas technology uses a relatively short sequence (on average, 20 nucleotides) to navigate the double-strand inducing Cas protein, special attention must be paid to gRNA selection. Multiple online platforms allow the identification of target genome-specific sequences (Liu et al. 2020). It has to be mentioned that not just sequence similarities of the gRNA are essential. The necessity of being adjacent to a PAM sequence further reduce putative off-targets (Jinek et al. 2012). Several studies have shown that mismatches in the twelve nucleotides in front of the PAM abolish the functionality of the RNP complex (Zheng et al. 2017). Another possibility to reduce potential off-targets is using preassembled RNP complexes (Park and Choe 2019). As these complexes do not contain DNA that becomes integrated into the host genome and is transmitted during cell divisions, inducing off-targets is reduced.

The biotechnological use of the CRISPR-Cas system involves two components. These are target sequence-specific gRNAs and double-strand break-inducing Cas enzymes (Jinek et al. 2012). The Cas enzyme to bind to the target DNA requires a so-called protospacer adjacent motif (PAM) sequence. This PAM sequence is specific for each Cas protein. For the most commonly used SpCas9, NGG somewhat limits the choice of a target sequence. Although there are sufficient NGG positions in each genome, if one needs to cut at a specific place due to a functional amino acid or protein domain being present, this can lead to limitations. This disadvantage has been overcome by identifying Cas proteins from other organisms or the synthetic evolution of existing Cas proteins (for review, see Liu et al. 2021) (Liu et al. 2021).

Another advantage of the CRISPR-Cas system is its DNA-binding properties. By inactivating the catalytic domains, activity-killed Cas protein can target specific areas of a target genome. This approach can be used for gene activation (Xiong et al. 2021), repression of gene function (Lowder et al. 2015), visualization of specific genomic regions (Dreissig et al. 2017), or setting epigenetic marks (Nakamura et al. 2021). This diversity of applications demonstrates the broad and universal use of a modified bacterial immune system (Huang and Puchta 2021).

In summary, CRISPR-Cas technology has revolutionized the life sciences as a whole, not only basic biology or agriculture. Many applications use the technology in medicine as exemplified by diagnostics (Hajian et al. 2019) or altering cancer treatments (Stadtmauer et al. 2020). It is one of the most powerful technologies discovered recently.

What are the differences between CRISPR-Cas and other genetic engineering techniques?

The era of conventional plant breeding methods

Since the Neolithic age, plant breeding has been a paramount part of an evolving human civilization. It is based on the introduction of genetic variability, yield increment, and improved nutritional value in crops by crossing wild species and landraces (Fig. 1) (Podevin et al. 2013). However, in this contemporary age of agriculture, conventional breeding techniques, such as crossbreeding and mutagenesis breeding, using irradiation or chemical mutagenesis are unlikely to be adapted to mass production to feed approximately 10 billion people by 2050 (FAO 2017; Gao 2021). In the late 1950s, the first Green Revolution, introducing "dwarfing" gene mutations, was bred into major staple crops, such as wheat (*Triticum aestivum*) and rice (*Oryza sativa*), to obtain high-yield crops (Khush 2001). In chemical and radiation-induced mutation breeding, genome-wide random mutagenesis was observed, which led to genetic variation (Holme et al. 2019). However, both the crossbreeding and mutation breeding techniques encountered limitations, such as introducing undesirable traits, high labor requirements, and the amount of time needed to choose a rare variety that harbored the desired feature (Gao 2021).

The long history of plant breeding pivoted its focus from crossing, random mutagenesis, and transgenic breeding to genome editing (Hickey et al. 2019; Chen et al. 2019). Modern plant breeding techniques can be one of the critical solutions for securing food production to feed the world under emerging biotic and abiotic risks, such as environmental and climatic changes and disease and pest management (Podevin et al. 2013).

Transgenic breeding and new plant breeding techniques

Transgenic breeding opened a new era, but its products contain foreign DNA fragments with random insertions in the genome that may cause potential unintended effects. New plant breeding techniques were introduced to overcome some of these transgenic products. These technologies include cisgenesis and intragenesis, grafting, agroinfiltration, reverse breeding, RNA-directed DNA methylation, genome elimination, oligonucleotide-directed mutagenesis (ODM), and site-directed nucleases (SDNs) (Enfissi et al. 2021). However, most of them could not overcome the regulatory limitations in many countries

(Podevin et al. 2013). Cisgenesis and intragenesis can be categorized under a traditional genetic transformation technology of plant breeding, as they involve T-DNA integration into a plant genome. Intragenesis creates new genes with desired traits by isolating functional genetic elements, such as promoters, coding regions, or terminators of existing genes, rearranging them in vitro, and inserting the 'intragenic' DNA combination back into the plant (Rommens et al. 2007). In contrast, a 'cisgenic plant' is a crop plant that has been genetically modified with one or more genes (containing conserved introns and flanking regions, such as native promoter and terminator regions in a sense orientation) isolated from a crossable donor plant (Schouten et al. 2006). Cis/intragenic lines should be free from foreign sequences (i.e., selectable markers and reporter genes) (Liu et al. 2013).

The use of *Agrobacterium tumefaciens*-derived T-DNA borders is a particular concern for public acceptance in GMO regulation; thus, it can be preferentially replaced by plant-derived P-DNA borders (Schouten and Jacobsen 2008). The debate for using P-DNA borders for *Agrobacterium*-mediated transformation over T-DNA borders is that DNA sequences integrated into the recipient plant should be derived from the sexually compatible DNA pool (Rommens et al. 2004). However, in the case of T-DNA sequences, it can be identified within different plant species, as sometimes it integrates into the genome without integration of T-DNA border sequence, thus making an alternative way to identify and select transformants (Zhu et al. 2013).

Nevertheless, this technique randomly integrated foreign DNA into plant genomes and was strictly subjected to government regulations for introducing foreign genes; the products were called GMOs (genetically modified organisms). To date, public and government opinions remain largely undecided regarding the safe usage of the final products created by cisgenesis and intragenesis approaches (Raman 2017; Gao 2021). Apart from transgenic breeding that maintains inserted genes, another class of NPBTs temporally introduces recombinant genes that change the expression of one or more endogenous genes, resulting in reverse breeding or early flowering. Another extended method is RNA-directed DNA methylation for gene silencing induced by transient expression via agroinfiltration or grafting to a GM stock (Bally et al. 2018; Enfissi et al. 2021). In the absence of an introduced recombinant gene or removal by segregation, these techniques result in no change in their native genome in the final products (Schaart et al. 2016).

Plant genome editing

The era of gene editing in NPBT makes precise manipulation of the genome possible with SDNs (Table 2), which are primarily executed as molecular scissors of the genome

Table 2 Examples of SDN-1, SDN-2, and SDN3 in plant genome editing

Product type	SDN tool	Application	Plant	Target gene	References
SDN-1	TALEN	Bacterial leaf blight resistance caused by *Xanthomonas oryzae* pathovar *oryzae* in Rice	*Oryza sativa*	Partial deletion (5 to 10 bp) of a specific region in the *Os11N3* gene	Li et al. (2012)
	TALEN	Resistance against *Blumeria graminis* f.s.p. *tritici* (*Bgt*) in hexaploid Bread wheat	*Triticum aestivum*	S-gene disruption of *Mildew resistance locus o* (*Mlo*) gene; six *Mlo* alleles	Wang et al. (2014)
	TALEN	Improving food quality by converting oleic acid to linoleic acid in Soybean (*Glycine max*)	*Glycine max*	*FAD2-1A* and *FAD2-1B*	Haun et al. (2014)
	TALEN	Accumulation of reducing sugars during cold storage in tetraploid commercial potato variety	*Solanum tuberosum*	Four alleles of *Vacuolar invertase* (*Vinv*)	Clasen et al. (2016)
	CRISPR-Cas	Increased Brassinosteroid signaling by targeting a negative regulator in BR signaling pathway of Lettuce (*Lactuca sativa*)	*Lactuca sativa*	*Brassinosteroid insensitive 2* (*BIN2*)	Woo et al. (2015)
	ZFN	*Arabidopsis* stress-response regulator *ABA-INSENSITIVE* (*ABI4*) gene, by customed designed ZFN	*Arabidopsis thaliana*	*ABA-INSENSITIVE* (*ABI4*)	Osakabe et al. (2010)
	ZFN	High frequency targeted mutagenesis in *Arabidopsis thaliana* using zinc finger nucleases	*Arabidopsis thaliana*	indel mutation in *ADH1* and *TT4*	Zhang et al. (2010)
	TALEN	Custom TALEN design for *Arabidopsis* ADH1 target gene using functional analog of *AvrHah1*	*Arabidopsis thaliana*	indel mutation in *ADH1*	Cermak et al. (2011)
	CRISPR-Cas	Bacterial blight susceptible genes in Rice	*Oryza sativa*	*OsSWEET14* and *OsSWEET11*	Jiang et al. (2013)
	CRISPR-Cas	*AtPDS3* and *AtFLS2* targeted for indel mutation	*Arabidopsis thaliana*	*AtPDS3* and *AtFLS2*	Li et al. (2013)
	CRISPR-Cas	i*Inositol oxygenase* (*inox*) and *phytoene desaturase* (*PDS*) gene using cell suspension culture of wheat	*Triticum aestivum*	*inox* and *pds* indel mutation	Upadhyay et al. (2013)
	TALEN and CRISPR-Cas	*ZmPDS*, *ZmIPK1A*, *ZmIPK*, *ZmMRP4* using TALEN and *ZmIPK* gene using CRISPR-Cas in maize	*Zea mays*	*ZmPDS*, *ZmIPK1A*, *ZmIPK*, *ZmMRP4*	Liang et al. (2014)
	TALEN	*Vacuolar Invertase* (*VInv*) breaks down sucrose to glucose and fructose in Potato	*Solanum tuberosum*	*VInv*	Clasen et al. (2016)
SDN-2	ODM	Tolerance to imidazolinone herbicides in Maize	*Zea mays*	Acetohydroxyacid synthase gene (*AHAS*)	Zhu et al. (2000)
	ZFN	The *gfp* coding region was replaced by the *hpt* coding region and the *ppt* coding region, in *Arabidopsis*	*Arabidopsis thaliana*	*gfp*- coding region	de Pater et al. (2009)
SDN-3	ZFN	Maize with reduced phytate content and herbicide tolerance	*Zea mays*	*Inositol-1,3,4,5,6-pentakisphosphate 2-kinase* (*IPK*)	Shukla et al. (2009)
	TALEN	Targeted replacement of 322-bp donor molecule differing by 6 bp from *ALS* coding region in *N. benthamiana*	*Nicotiana benthamiana*	gene target replacement in *ALS*	Zhang et al. (2013)
	CRISPR-Cas	*NbPDS* locus designed with donor template containing AvrII site flanked by a 533-bp left homology arm and 114-bp right homology arm	*Nicotiana benthamiana*	HDR in *NbPDS*	Li et al. (2013)

by programmable DNA-binding proteins that recognize a specific DNA sequence of a DNA target and induce DSBs (Gaj et al. 2013; Shrivastav et al. 2008; Enfissi et al. 2021). Although uncertainties remain over the future applications of plant genome editing, this technique has the potential to surpass the time-consumption and regulatory limitations associated with conventional (crossing and random mutagenesis) and transgenic breeding techniques, respectively (Lusser and Rodríguez-Cerezo 2012). The advantage of gene editing over genetic engineering is that the end product acquires no foreign genes (Schaart et al. 2016). The associated plants produced via the SDN1 method are designed to introduce random mutations (substitutions, insertions, and deletions) using double DSBs to a specific gene, followed by a NHEJ repair pathway (Roth et al. 2012; Waterworth et al. 2011). Deletions of regulatory regions, exons, introns, or large chromosomal fragments by introducing one or two DSBs at different sites using either one or two SDNs lead to frameshifts, duplication, inversion, and translocation events (Petolino et al. 2010; Şöllü et al. 2010; Lee et al. 2012; Fauser et al. 2012). SDN2-edited products are developed in the presence of the donor DNA repair template containing targeted mutations by single-base substitution or short indels and have high sequence identity to the endogenous gene by exploiting HDR pathway (Podevin et al. 2013; Schaart et al. 2016). The final edited products from SDN3 can be maneuvered for targeted gene correction or to introduce gene/allele replacement for creating new phenotypes. SDN3 defines an insertion of new DNA fragments at a predefined locus using sequence-specific donor DNA templates with flanking DNAs showing homology to the target locus by both NHEJ and HDR, which would otherwise integrate randomly in the genome at naturally occurring DSBs (Naegeli et al. 2020; Podevin et al. 2013). The ODM approach does not rely on exogenous nucleases but uses oligonucleotides to introduce targeted mutations in the genome, usually of one or a few adjacent nucleotides, an approach that is distinct from SDN-based techniques (Zhu et al. 2000).

The products of NPBT developed as SDN-1, SDN-2, and SDN-3 (Table 2) are implemented using molecular scissors, such as protein-guided zinc finger nucleases (ZFNs, the first generation of editing tools) (Bitinaite et al. 1998; Laity et al. 2001; Urnov et al. 2005; Kim et al. 1996) and transcription activator-like effector nucleases (TALENs, the second generation) (Miller et al. 2011; Li et al. 2011). The third generation of molecular scissors is an RNA-guided protein complex, CRISPR-Cas systems. The most widely used CRISPR-Cas systems are Cas9 and Cas12a (Cpf1) (Jinek et al. 2012; Zetsche et al. 2015; Kim et al. 2016).

The GE tools ZFN, TALENs, and the CRISPR-Cas system can be used as SDN-1 (for random mutagenesis), SDN-2 (predicted mutagenesis of a targeted genomic locus), and SDN-3 (precision insertion of a DNA sequence). However,

the ODM technique resembles the SDN-2 type. Recent GE findings of base editing and prime editing (Komor et al. 2016; Anzalone et al. 2019; Lin et al. 2020) generate specific base changes in the target sequence without inducing DSBs or positioning any template DNA in the targeted locus. SDN tool delivery can be executed in plant genome editing by stable DNA integration, transient expression, or 'DNA-free' methods. The SDN modules integrated into the genome can be removed by segregation in edited progeny events in sexually propagated crops. In asexually (vegetatively) propagated crops, transient expression or DNA-free delivery of the SDN tool is required (Ma et al. 2017). For both ODM chemically synthesized oligonucleotide and 'DNA-free' delivery, RNA expressing the SDN module or the protein (TALEN, ZFN) or ribonucleoprotein complex (in case of CRISPR-Cas) itself can be delivered directly into plant cells (Metje-Sprink et al. 2018; Okuzaki and Toriyama 2004). In summary, CRISPR-Cas technology provides the most efficient tools to modify endogenous gene sequences precisely, resulting in foreign gene-free GE products.

Would its products be labeled as "conventional" or "GMO" or something else?

There is a continuous debate regarding the acceptance of GMO/LMO products as foods or feeds (Teferra 2021). The tendency is to assure no potential harm can be caused to consumers' health or no environmental threat. One of the primary reasons for the regulation of GMOs is that cross-kingdom genetic materials are integrated into plant genomes and overexpressed during plant growth and development (Teferra 2021). However, it is different in the case of GE plants, when in some cases, indel mutations are indistinguishable from wild plants since there are no foreign genetic materials integrated into edited plant genomes (Wolt et al. 2016). Therefore, GE plants and derived products are as readily accepted as foods and feed as those obtained from conventional breeding if they do not contain exogenous genetic materials (Entine et al. 2021; Menz et al. 2020). Rapid applications and relaxed regulations have accelerated the release of the first (high oleic soybean) and second (GABA tomato) GE products in less than eight years from the first publication of TALEN and CRISPR-Cas9 techniques, respectively. Significantly, the commercialization of the GE products is much less expensive than that of GMO products thanks to the omission of the costly regulatory process that applied to GMO products. Thus, for the first time, small enterprises, such as Calyxt or Sanatech Seed Co., Ltd, have released new biotech products. Many requests for the deregulation of GE events were from small or medium enterprises (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regul

ated/regulated_article_letters_of_inquiry/regulated_artic
le_letters_of_inquiry, accessed on 30.11.2021). These facts
encourage other small-scale labs and companies to develop
new GE crops, thereby bringing hope to the idea that global
food production can feed the world by 2050. This section
analyzes the two GE cases to understand how they were
deregulated by the US and Japan.

Case #1: GABA tomato

Gamma-aminobutyric acid (GABA) is an amino acid that
is not used to make proteins. Consumption of GABA may
help to reduce blood pressure and may have several other
sound health effects. GABA is an intermediate product of a
biochemical pathway called the GABA shunt that converts
glutamate to succinate (Bown and Shelp 1997). In plants,
GABA accumulation was enhanced under stresses that stim-
ulate the Ca^{2+}/calmodulin-dependent activities of glutamate
decarboxylase (GAD, EC 4.1.1.15). Suppressing *GAD* genes
by RNAi reduced GABA accumulation in plants (Takayama
et al. 2015). GAD's C-terminal calmodulin-binding domain
(CaMBD) plays an autoinhibitory function in switch-
ing GAD activities between normal and stress conditions.
Thus, removing it enhanced the GABA contents in toma-
toes (Takayama et al. 2017). CRISPR-Cas-based targeted
mutagenesis of *GAD2*, *GAD3*, and *GABA transaminase*-
encoding genes (*GABA-T*) resulted in hyperaccumulation
of GABA in tomato (Sicilian Rouge CF cultivar) (Nonaka
et al. 2017). The line containing a mutation that eliminated
the CaMBD of GAD2 was the first commercial GE tomato
globally.

Sanatech Seed Co., Ltd., the owner of the high GABA
line, sent a letter to the Animal and Plant Health Inspec-
tion Service (APHIS, USA) to request the exclusion of
the high GABA tomato from the APHIS oversight under 7
C.F.R. Part 340 article. It is a T2 segregated line validated as
T-DNA-free by conventional PCR covering the entire binary
plasmids used for *Agrobacterium*-mediated transformation.
The edited allele contains a single-base insertion resulting
in a premature stop codon just before the CaMBD domain,
eliminating it from the GAD2 protein. Therefore, the line
accumulated GABA at levels 4- to fivefold higher than in its
parents, and the traits were stable for at least three genera-
tions (T0, T1, and T2) (https://www.aphis.usda.gov/biote
chnology/downloads/reg_loi/20-140-01_air_CBIdel_a2.
pdf, accessed on 29.11.2021). More importantly, the high
GABA content does not affect the growth and development
of the line. A steroidal glycoalkaloid, tomatine, and its con-
tents in ripened fruits were not altered, indicating that toxin
or allergen production does not increase. Ultimately, the
high GABA tomato line was confirmed to be deregulated
from APHIS oversight under 7 C.F.R. Part 340 in a process
called "Am I Regulated (AIR)" (https://www.aphis.usda.gov/

biotechnology/downloads/reg_loi/20-140-01_air_response_
signed.pdf, accessed on 29.11.2021).

Case #2: high oleic acid soybean

The GE event of soybean that increased oleic acid content
was the first GE soybean to be released to the US market by
Calyxt (previously known as Cellectis Plant Sciences). On
May 5, 2015, APHIS issued a letter to Cellectis Plant Sci-
ences for deregulating a TALEN-based FAD2KO soybean
that accumulated high levels of oleic acid from the 7 C.F.R.
Part 340 (https://www.aphis.usda.gov/biotechnology/downl
oads/reg_loi/brs_response_cellectis_air_fad2k0_soy_cbidel.
pdf, accessed on 30.11.2021). The FAD2KO event contains
knockout alleles of the *Fatty acid desaturase 2-1a* (*FAD2-
1a*, a 63-bp KO allele) and *FAD2-1b* (a 23-bp KO allele)
genes, which play roles in converting oleic acid to linoleic
acid (Haun et al. 2014). Seeds collected from plants carrying
homozygous knockout alleles of the two genes increased the
oleic acid content up to 80% compared to 20% in its parental
lines. They reduced the linoleic acid content to under 4%
(Haun et al. 2014). In the US, the cropping area of FAD2KO
has dramatically increased since 2018 (Menz et al. 2020).
The event was confirmed not to contain T-DNA by PCRs
that amplified three distinct regions of the T-DNA, including
the TALEN gene, the selection marker, and the right border
(Haun et al. 2014).

Would production be sufficient with gene-edited varieties?

It is difficult to answer this question since better future tech-
nologies can replace the present. Moreover, if the world pop-
ulation continues to increase at the current rate, we will need
more improved crop cultivars created by diverse technolo-
gies than just GE plants. However, at present, GE varieties
are bringing hope to sustain and secure global food produc-
tion to meet the demand by 2050 since the technology can
quickly generate or domesticate desirable alleles into elite
cultivars at low costs and with no linkage drag. Neverthe-
less, GMO/LMO varieties still play essential roles, featur-
ing crucial traits that GE has not been able to successfully
generate, such as those that require a strong expression of
genes, such as herbicide- or insect-resistant alleles. Usually,
genome editing is sufficient to introduce herbicide-resistant
alleles into a plant. However, the expression levels, driven
by endogenous promoters, might not be adequate to support
durable herbicide resistance at high doses while maintaining
enzymatic activities (Li et al. 2016; Jin et al. 2021; Hum-
mel et al. 2018). Alternatively, the endogenous promoter can
also be engineered by the CRISPR-Cas system to increase
its strength (Rodríguez-Leal et al. 2017), which may further

advance the technology for those traits. For insect resistance, it seems that only cross-kingdom genes have been successful at this point; hence, insect-resistant GMO/LMO varieties are still needed.

Future perspectives

Several significant threats endanger global food security, especially in developing countries (Khush 2001; Ray et al. 2013). The first is the decrease in arable lands due to urbanization, which is greatly extended in developing countries. Second is the shifting of environmental conditions caused by climate changes that can affect the sustainability of plant-based food production because the growth and development of plants are sensitive to subtle changes in environmental conditions. The third is the yield barrier limiting conventional breeding techniques from achieving higher productivity. More importantly, the three significant threats become more serious when combined. In the meantime, the global population in developing countries is increasing at speeds much higher than that of food production, threatening developing countries with hunger and undernourishment. Therefore, the UN's Sustainable Development Goals to "end hunger by 2030" may not be reached in time.

All plant breeding techniques have significantly contributed to global food production regardless of the legislation applied to them or the products derived from performing these techniques. There is a clear trend of technological advances in plant breeding and food productivity, indicating their importance. However, when technologies are advanced, new components added to the traditional system cause concerns about potential risks to consumers' health. Therefore, even when proven to be safe for humans, GMO/LMO products are still subjected to costly and lengthy regulation procedures (Teferra 2021). However, transgene-free GE products cannot be more "traditional" and much "cleaner" than products obtained by random mutagenesis. There are even difficulties distinguishing a GE plant from its parents because legislators cannot find any convenient detection tools (Grohmann et al. 2019). Then, the question is, do we have to consider GE plants as riskier than their parents? The answer is no from the leading countries in biotechnological innovation, such as the US and Japan, where the products of the first GE crops were commercialized like traditional ones. Many countries have now deregulated the GE crops listed in the SDN-1 category (Table 2) (Van Vu et al. 2019; Menz et al. 2020). Surprisingly, GE plants have been listed along with GMOs/LMOs in Europe, where cropping of GMOs/LMOs is not allowed, even though these regions are major importers of GMO/LMO products. Fortunately, scientists are raising voices to defend GE plants and their products; for example, the EU-based petition called "give CRISPR a chance" (Vangheluwe et al. 2020) has successfully changed the minds of legislators to reconsider regulatory bills for GE plants (https://www.reuters.com/world/europe/eu-calls-rethink-gmo-rules-gene-edited-crops-2021-04-29/, accessed on 03.12.2021). Moreover, after Brexit, the UK considered genome editing as a science innovation and opened the door for its use (https://www.nature.com/articles/d41586-021-01572-0. Accessed on 03.12.2021).

Although the first concern about a new biotech product is always consumer safety, overestimating their risks in a non-scientific manner has been preventing innovations in plant breeding and food production (Jorasch 2020; Whelan et al. 2020). It may not be a significant issue to wealthy countries like those in the European zone but may affect food security in developing countries. Therefore, giving CRISPR a chance allows developing countries to ensure adequate food production, which will help reach the UN's Sustainable Development Goals to "end hunger by 2030" and further overcome the tragic prediction of food crises in 2050.

Concluding remarks

Plant breeding has played a critical role in securing food production to feed the world. Regardless of the breeding technique applied to generate a new variety, safety assessments based on the product and not the technology used should be used to determine whether the plant should be released into the environment. Many crop cultivars obtained by random mutagenesis were released into the environment without any restriction simply because they have an extended historical profile of safe use as foods and feeds (Holme et al. 2019). If everything is decided in a science-based manner, then most GE plants should also be considered at least as safe as those obtained from random mutagenesis (Entine et al. 2021; Jorasch 2020; Whelan et al. 2020). In other aspects, compared with GMO/LMO plants, many GE traits can be generated without the incorporation of transgenes. GE plants are likewise very similar to those that evolved in nature but require only months to obtain compared to multiyear selection on multiple generations of ancient domesticated crops. Therefore, it is natural not to regulate GE plants, as shown by Sanatech's high GABA content and Calyxt's FAD2KO events. GE technology is continuously improving, and we are expecting more exciting applications in crop breeding and the global acceptance of GE plants and foods for ending hunger and poverty in every country in the world.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

Andersson M, Turesson H, Olsson N, Fält AS, Ohlsson P, Gonzalez MN, Samuelsson M, Hofvander P (2018) Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. Physiol Plant 164(4):378–384. https://doi.org/10.1111/ppl.12731

Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A, Liu DR (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576(7785):149–157. https://doi.org/10.1038/s41586-019-1711-4

Assou J, Zhang D, Roth KDR, Steinke S, Hust M, Reinard T, Winkelmann T, Boch J (2021) Removing the major allergen Bra j I from brown mustard (Brassica juncea) by CRISPR/Cas9. Plant J. https://doi.org/10.1111/tpj.15584

Bally J, Jung H, Mortimer C, Naim F, Philips JG, Hellens R, Bombarely A, Goodin MM, Waterhouse PM (2018) The rise and rise of Nicotiana benthamiana: a plant for all reasons. Annu Rev Phytopathol 56:405–426

Berners-Lee M, Kennelly C, Watson R, Hewitt CN (2018) Current global food production is sufficient to meet human nutritional needs in 2050 provided there is radical societal adaptation. Elementa: Sci Anthropocene. https://doi.org/10.1525/elementa.310

Beying N, Schmidt C, Pacher M, Houben A, Puchta H (2020) CRISPR-Cas9-mediated induction of heritable chromosomal translocations in Arabidopsis. Nat Plants 6(6):638–645. https://doi.org/10.1038/s41477-020-0663-x

Bitinaite J, Wah DA, Aggarwal AK, Schildkraut I (1998) FokI dimerization is required for DNA cleavage. Proc Natl Acad Sci 95(18):10570–10575

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. Science (new York, NY) 326(5959):1509–1512. https://doi.org/10.1126/science.1178811

Bown AW, Shelp BJ (1997) The Metabolism and Functions of [gamma]-Aminobutyric Acid. Plant Physiol 115(1):1–5. https://doi.org/10.1104/pp.115.1.1

Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res 39(12):e82. https://doi.org/10.1093/nar/gkr218

Chauhan H, Boni R, Bucher R, Kuhn B, Buchmann G, Sucher J, Selter LL, Hensel G, Kumlehn J, Bigler L, Glauser G, Wicker T, Krattinger SG, Keller B (2015) The wheat resistance gene Lr34 results in the constitutive induction of multiple defense pathways in transgenic barley. Plant J 84(1):202–215. https://doi.org/10.1111/tpj.13001

Chen K, Wang Y, Zhang R, Zhang H, Gao C (2019) CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol 70:667–697. https://doi.org/10.1146/annurev-arplant-050718-100049

Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebu R, Davison S, Ray EE, Daulhac A, Coffman A, Yabandith A, Retterath A, Haun W, Baltes NJ, Mathis L, Voytas DF, Zhang F (2016) Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnol J 14(1):169–176. https://doi.org/10.1111/pbi.12370

Concordet JP, Haeussler M (2018) CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. Nucleic Acids Res 46(W1):W242-w245. https://doi.org/10.1093/nar/gky354

Cui Y, Xu J, Cheng M, Liao X, Peng S (2018) Review of CRISPR/Cas9 sgRNA design tools. Interdiscip Sci Comput Life Sci 10(2):455–465. https://doi.org/10.1007/s12539-018-0298-z

de Pater S, Neuteboom LW, Pinas JE, Hooykaas PJ, van der Zaal BJ (2009) ZFN-induced mutagenesis and gene-targeting in Arabidopsis through Agrobacterium-mediated floral dip transformation. Plant Biotechnol J 7(8):821–835. https://doi.org/10.1111/j.1467-7652.2009.00446.x

Dreissig S, Schiml S, Schindele P, Weiss O, Rutten T, Schubert V, Gladilin E, Mette MF, Puchta H, Houben A (2017) Live-cell CRISPR imaging in plants reveals dynamic telomere movements. Plant J 91(4):565–573. https://doi.org/10.1111/tpj.13601

Enfissi EM, Drapal M, Perez-Fons L, Nogueira M, Berry HM, Almeida J, Fraser PD (2021) New plant breeding techniques and their regulatory implications: An opportunity to advance metabolomics approaches. J Plant Physiol 258:153378

Engler C, Kandzia R, Marillonnet S (2008) A one pot, one step, precision cloning method with high throughput capability. PLoS ONE 3(11):e3647. https://doi.org/10.1371/journal.pone.0003647

Entine J, Felipe MSS, Groenewald J-H, Kershen DL, Lema M, McHughen A, Nepomuceno AL, Ohsawa R, Ordonio RL, Parrott WA, Quemada H, Ramage C, Slamet-Loedin I, Smyth SJ, Wray-Cahen D (2021) Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world. Transgenic Res 30(4):551–584. https://doi.org/10.1007/s11248-021-00257-8

FAO F (2017) The future of food and agriculture–Trends and challenges. Annual Report 296

Fauser F, Roth N, Pacher M, Ilg G, Sánchez-Fernández R, Biesgen C, Puchta H (2012) In planta gene targeting. Proc Natl Acad Sci 109(19):7535–7540

Fernie AR, Yan J (2019) De novo domestication: an alternative route toward new crops for the future. Mol Plant 12(5):615–631. https://doi.org/10.1016/j.molp.2019.03.016

Gaj T, Gersbach CA, Barbas CF III (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31(7):397–405

Ganie SA, Wani SH, Henry R, Hensel G (2021) Improving rice salt tolerance by precision breeding in a new era. Curr Opin Plant Biol 60:101996. https://doi.org/10.1016/j.pbi.2020.101996

Gao C (2021) Genome engineering for crop improvement and future agriculture. Cell 184(6):1621–1635. https://doi.org/10.1016/j.cell.2021.01.005

Gao H, Gadlage MJ, Lafitte HR, Lenderts B, Yang M, Schroder M, Farrell J, Snopek K, Peterson D, Feigenbutz L, Jones S, St Clair G, Rahe M, Sanyour-Doyel N, Peng C, Wang L, Young JK, Beatty M, Dahlke B, Hazebroek J, Greene TW, Cigan AM, Chilcoat ND, Meeley RB (2020) Superior field performance of waxy corn engineered using CRISPR–Cas9. Nat Biotechnol 38(5):579–581. https://doi.org/10.1038/s41587-020-0444-0

Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR (2017) Programmable base editing of A●T to G●C in genomic DNA without DNA cleavage. Nature 551(7681):464–471. https://doi.org/10.1038/nature24644

Golicz AA, Bayer PE, Barker GC, Edger PP, Kim H, Martinez PA, Chan CK, Severn-Ellis A, McCombie WR, Parkin IA, Paterson AH, Pires JC, Sharpe AG, Tang H, Teakle GR, Town CD, Batley J, Edwards D (2016) The pangenome of an agronomically important crop plant Brassica oleracea. Nat Commun 7:13390. https://doi.org/10.1038/ncomms13390

Grohmann L, Keilwagen J, Duensing N, Dagand E, Hartung F, Wilhelm R, Bendiek J, Sprink T (2019) Detection and identification of genome editing in plants: challenges and opportunities. Front Plant Sci 10:236. https://doi.org/10.3389/fpls.2019.00236

Hahn F, Korolev A, Sanjurjo Loures L, Nekrasov V (2020) A modular cloning toolkit for genome editing in plants. BMC Plant Biol 20(1):179. https://doi.org/10.1186/s12870-020-02388-2

Hajian R, Balderston S, Tran T, deBoer T, Etienne J, Sandhu M, Wauford NA, Chung J-Y, Nokes J, Athaiya M, Paredes J, Peytavi R, Goldsmith B, Murthy N, Conboy IM, Aran K (2019) Detection of unamplified target genes via CRISPR–Cas9 immobilized on a graphene field-effect transistor. Nat Biomed Eng 3(6):427–437. https://doi.org/10.1038/s41551-019-0371-x

Han Y-J, Kim J-I (2019) Application of CRISPR/Cas9-mediated gene editing for the development of herbicide-resistant plants. Plant Biotechnol Rep 13(5):447–457. https://doi.org/10.1007/s11816-019-00575-8

Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, Retterath A, Stoddard T, Juillerat A, Cedrone F, Mathis L, Voytas DF, Zhang F (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnol J 12(7):934–940. https://doi.org/10.1111/pbi.12201

Hickey LT, Hafeez AN, Robinson H, Jackson SA, Leal-Bertioli SCM, Tester M, Gao C, Godwin ID, Hayes BJ, Wulff BBH (2019) Breeding crops to feed 10 billion. Nat Biotechnol 37(7):744–754. https://doi.org/10.1038/s41587-019-0152-9

Holme IB, Gregersen PL, Brinch-Pedersen H (2019) Induced genetic variation in crop plants by random or targeted mutagenesis: convergence and differences. Front Plant Sci 10:1468

Huang T-K, Puchta H (2021) Novel CRISPR/Cas applications in plants: from prime editing to chromosome engineering. Transgenic Res 30(4):529–549. https://doi.org/10.1007/s11248-021-00238-x

Hummel AW, Chauhan RD, Cermak T, Mutka AM, Vijayaraghavan A, Boyher A, Starker CG, Bart R, Voytas DF, Taylor NJ (2018) Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava. Plant Biotechnol J 16(7):1275–1282. https://doi.org/10.1111/pbi.12868

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res 41(20):e188. https://doi.org/10.1093/nar/gkt780

Jin M, Chen L, Deng XW, Tang X (2021) Development of herbicide resistance genes and their application in rice. Crop J. https://doi.org/10.1016/j.cj.2021.05.007

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816–821. https://doi.org/10.1126/science.1225829

Jorasch P (2020) Will the EU stay out of step with science and the rest of the world on plant breeding innovation? Plant Cell Rep 39(1):163–167. https://doi.org/10.1007/s00299-019-02482-2

Khush GS (2001) Green revolution: the way forward. Nat Rev Genet 2(10):815–822. https://doi.org/10.1038/35093585

Kim D, Kim J, Hur JK, Been KW, Yoon SH, Kim JS (2016) Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. Nat Biotechnol 34(8):863–868. https://doi.org/10.1038/nbt.3609

Kim Y-G, Cha J, Chandrasegaran S (1996) Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci 93(3):1156–1160

Komatsuda T, Maxim P, Senthil N, Mano Y (2004) High-density AFLP map of nonbrittle rachis 1 (btr1) and 2 (btr2) genes in barley (*Hordeum vulgare* L.). Theor Appl Genet 109(5):986–995. https://doi.org/10.1007/s00122-004-1710-0

Komatsuda T, Pourkheirandish M, He C, Azhaguvel P, Kanamori H, Perovic D, Stein N, Graner A, Wicker T, Tagiri A, Lundqvist U, Fujimura T, Matsuoka M, Matsumoto T, Yano M (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc Natl Acad Sci U S A 104(4):1424–1429. https://doi.org/10.1073/pnas.0608580104

Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533(7603):420–424. https://doi.org/10.1038/nature17946

Kyndt T, Quispe D, Zhai H, Jarret R, Ghislain M, Liu Q, Gheysen G, Kreuze JF (2015) The genome of cultivated sweet potato contains Agrobacterium T-DNAs with expressed genes: an example of a naturally transgenic food crop. Proc Natl Acad Sci U S A 112(18):5844–5849. https://doi.org/10.1073/pnas.1419685112

Laity JH, Lee BM, Wright PE (2001) Zinc finger proteins: new insights into structural and functional diversity. Curr Opin Struct Biol 11(1):39–46

Lee HJ, Kweon J, Kim E, Kim S, Kim J-S (2012) Targeted chromosomal duplications and inversions in the human genome using zinc finger nucleases. Genome Res 22(3):539–548

Lemmon ZH, Reem NT, Dalrymple J, Soyk S, Swartwood KE, Rodriguez-Leal D, Van Eck J, Lippman ZB (2018) Rapid improvement of domestication traits in an orphan crop by genome editing. Nature Plants 4(10):766–770. https://doi.org/10.1038/s41477-018-0259-x

Li C, Hao M, Wang W, Wang H, Chen F, Chu W, Zhang B, Mei D, Cheng H, Hu Q (2018) An efficient CRISPR/Cas9 platform for rapidly generating simultaneous mutagenesis of multiple gene homoeologs in allotetraploid oilseed rape. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00442

Li J, Meng X, Zong Y, Chen K, Zhang H, Liu J, Li J, Gao C (2016) Gene replacements and insertions in rice by intron targeting using CRISPR–Cas9. Nat Plants 2(10):16139. https://doi.org/10.1038/nplants.2016.139

Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013) Multiplex and homologous

recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. Nat Biotechnol 31(8):688–691. https://doi.org/10.1038/nbt.2654

Li T, Huang S, Jiang WZ, Wright D, Spalding MH, Weeks DP, Yang B (2011) TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. Nucleic Acids Res 39(1):359–372. https://doi.org/10.1093/nar/gkq704

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30(5):390–392. https://doi.org/10.1038/nbt.2199

Liang Z, Zhang K, Chen K, Gao C (2014) Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. J Genet Genomics 41(2):63–68. https://doi.org/10.1016/j.jgg.2013.12.001

Lin Q, Zong Y, Xue C, Wang S, Jin S, Zhu Z, Wang Y, Anzalone AV, Raguram A, Doman JL, Liu DR, Gao C (2020) Prime genome editing in rice and wheat. Nat Biotechnol 38(5):582–585. https://doi.org/10.1038/s41587-020-0455-x

Liu G, Zhang Y, Zhang T (2020) Computational approaches for effective CRISPR guide RNA design and evaluation. Comput Struct Biotechnol J 18:35–44. https://doi.org/10.1016/j.csbj.2019.11.006

Liu J, Gerken H, Huang J, Chen F (2013) Engineering of an endogenous phytoene desaturase gene as a dominant selectable marker for Chlamydomonas reinhardtii transformation and enhanced biosynthesis of carotenoids. Process Biochem 48(5–6):788–795

Liu R, Liang L, Freed EF, Gill RT (2021) Directed evolution of CRISPR/Cas systems for precise gene editing. Trends Biotechnol 39(3):262–273. https://doi.org/10.1016/j.tibtech.2020.07.005

Lowder LG, Zhang D, Baltes NJ, Paul JW 3rd, Tang X, Zheng X, Voytas DF, Hsieh TF, Zhang Y, Qi Y (2015) A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. Plant Physiol 169(2):971–985. https://doi.org/10.1104/pp.15.00636

Lusser M, Rodríguez-Cerezo E (2012) Comparative regulatory approaches for new plant breeding techniques. In: Workshop proceedings. European Commission. JRC Technical Report EUR

Ma J, Xiang H, Donnelly DJ, Meng F-R, Xu H, Durnford D, Li X-Q (2017) Genome editing in potato plants by agrobacterium-mediated transient expression of transcription activator-like effector nucleases. Plant Biotechnol Rep 11(5):249–258. https://doi.org/10.1007/s11816-017-0448-5

Marzec M, Hensel G (2019) More precise, more universal and more specific - the next generation of RNA-guided endonucleases for genome editing. FEBS J 286(23):4657–4660. https://doi.org/10.1111/febs.15079

Menz J, Modrzejewski D, Hartung F, Wilhelm R, Sprink T (2020) Genome edited crops touch the market: a view on the global development and regulatory environment. Front Plant Sci. https://doi.org/10.3389/fpls.2020.586027

Metje-Sprink J, Menz J, Modrzejewski D, Sprink T (2018) DNA-Free genome editing: past, present and future. Front Plant Sci 9:1957. https://doi.org/10.3389/fpls.2018.01957

Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ, Dulay GP, Hua KL, Ankoudinova I, Cost GJ, Urnov FD, Zhang HS, Holmes MC, Zhang L, Gregory PD, Rebar EJ (2011) A TALE nuclease architecture for efficient genome editing. Nat Biotechnol 29(2):143–148. https://doi.org/10.1038/nbt.1755

Molla KA, Sretenovic S, Bansal KC, Qi Y (2021) Precise plant genome editing using base editors and prime editors. Nat Plants 7(9):1166–1187. https://doi.org/10.1038/s41477-021-00991-1

Naim F, Shand K, Hayashi S, O'Brien M, McGree J, Johnson AAT, Dugdale B, Waterhouse PM (2020) Are the current gRNA ranking prediction algorithms useful for genome editing in plants?

PLoS ONE 15(1):e0227994–e0227994. https://doi.org/10.1371/journal.pone.0227994

Nakamura M, Gao Y, Dominguez AA, Qi LS (2021) CRISPR technologies for precise epigenome editing. Nat Cell Biol 23(1):11–22. https://doi.org/10.1038/s41556-020-00620-7

Nonaka S, Arai C, Takayama M, Matsukura C, Ezura H (2017) Efficient increase of γ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. Sci Rep 7(1):7057. https://doi.org/10.1038/s41598-017-06400-y

Okuzaki A, Toriyama K (2004) Chimeric RNA/DNA oligonucleotide-directed gene targeting in rice. Plant Cell Rep 22(7):509–512

Organisms EPoGM, Naegeli H, Bresson JL, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ (2020) Applicability of the EFSA Opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. EFSA J 18(11):e06299

Osakabe K, Osakabe Y, Toki S (2010) Site-directed mutagenesis in Arabidopsis using custom-designed zinc finger nucleases. Proc Natl Acad Sci U S A 107(26):12034–12039. https://doi.org/10.1073/pnas.1000234107

Park J, Choe S (2019) DNA-free genome editing with preassembled CRISPR/Cas9 ribonucleoproteins in plants. Transgenic Res 28(Suppl 2):61–64. https://doi.org/10.1007/s11248-019-00136-3

Petolino JF, Worden A, Curlee K, Connell J, Moynahan TLS, Larsen C, Russell S (2010) Zinc finger nuclease-mediated transgene deletion. Plant Mol Biol 73(6):617–628

Pinto-Carbó M, Sieber S, Dessein S, Wicker T, Verstraete B, Gademann K, Eberl L, Carlier A (2016) Evidence of horizontal gene transfer between obligate leaf nodule symbionts. ISME J 10(9):2092–2105. https://doi.org/10.1038/ismej.2016.27

Podevin N, Davies HV, Hartung F, Nogue F, Casacuberta JM (2013) Site-directed nucleases: a paradigm shift in predictable, knowledge-based plant breeding. Trends Biotechnol 31(6):375–383. https://doi.org/10.1016/j.tibtech.2013.03.004

Pourkheirandish M, Hensel G, Kilian B, Senthil N, Chen G, Sameri M, Azhaguvel P, Sakuma S, Dhanagond S, Sharma R, Mascher M, Himmelbach A, Gottwald S, Nair SK, Tagiri A, Yukuhiro F, Nagamura Y, Kanamori H, Matsumoto T, Willcox G, Middleton CP, Wicker T, Walther A, Waugh R, Fincher GB, Stein N, Kumlehn J, Sato K, Komatsuda T (2015) Evolution of the grain dispersal system in barley. Cell 162(3):527–539. https://doi.org/10.1016/j.cell.2015.07.002

Puchta H (2005) The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. J Exp Bot 56(409):1–14. https://doi.org/10.1093/jxb/eri025

Raman R (2017) The impact of Genetically Modified (GM) crops in modern agriculture: a review. GM Crops Food 8(4):195–208. https://doi.org/10.1080/21645698.2017.1413522

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS ONE 8(6):e66428. https://doi.org/10.1371/journal.pone.0066428

Ren B, Yan F, Kuang Y, Li N, Zhang D, Zhou X, Lin H, Zhou H (2018) Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9-Guided hyperactive hAID mutant. Mol Plant 11(4):623–626. https://doi.org/10.1016/j.molp.2018.01.005

Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB (2017) Engineering quantitative trait variation for crop improvement by genome editing. Cell 171(2):470–480.e8. https://doi.org/10.1016/j.cell.2017.08.030

Rommens CM, Haring MA, Swords K, Davies HV, Belknap WR (2007) The intragenic approach as a new extension to traditional plant breeding. Trends Plant Sci 12(9):397–403

Rommens CM, Humara JM, Ye J, Yan H, Richael C, Zhang L, Perry R, Swords K (2004) Crop improvement through modification of

the plant's own genome. Plant Physiol 135(1):421–431. https://doi.org/10.1104/pp.104.040949

Rönspies M, Schindele P, Puchta H (2021) CRISPR/Cas-mediated chromosome engineering: opening up a new avenue for plant breeding. J Exp Bot 72(2):177–183. https://doi.org/10.1093/jxb/eraa463

Roth N, Klimesch J, Dukowic-Schulze S, Pacher M, Mannuss A, Puchta H (2012) The requirement for recombination factors differs considerably between different pathways of homologous double-strand break repair in somatic plant cells. Plant J 72(5):781–790

Sakuma S, Pourkheirandish M, Hensel G, Kumlehn J, Stein N, Tagiri A, Yamaji N, Ma JF, Sassa H, Koba T, Komatsuda T (2013) Divergence of expression pattern contributed to neofunctionalization of duplicated HD-Zip I transcription factor in barley. New Phytol 197(3):939–948. https://doi.org/10.1111/nph.12068

Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, Barro F (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J 16(4):902–910. https://doi.org/10.1111/pbi.12837

Schaart JG, van de Wiel CCM, Lotz LAP, Smulders MJM (2016) Opportunities for products of new plant breeding techniques. Trends Plant Sci 21(5):438–449. https://doi.org/10.1016/j.tplants.2015.11.006

Schmidt C, Fransz P, Rönspies M, Dreissig S, Fuchs J, Heckmann S, Houben A, Puchta H (2020a) Changing local recombination patterns in Arabidopsis by CRISPR/Cas mediated chromosome engineering. Nat Commun 11(1):4418. https://doi.org/10.1038/s41467-020-18277-z

Schmidt SM, Belisle M, Frommer WB (2020b) The evolving landscape around genome editing in agriculture. EMBO Rep 21(6):e50680

Schouten HJ, Jacobsen E (2008) Cisgenesis and intragenesis, sisters in innovative plant breeding. Trends Plant Sci 13(6):260–261 (**author reply 261–263. doi:10.1016/j.tplants.2008.04.005**)

Schouten HJ, Krens FA, Jacobsen E (2006) Do cisgenic plants warrant less stringent oversight? Nat Biotechnol 24(7):753–753

Scully R, Panday A, Elango R, Willis NA (2019) DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat Rev Mol Cell Biol 20(11):698–714. https://doi.org/10.1038/s41580-019-0152-0

Shrivastav M, De Haro LP, Nickoloff JA (2008) Regulation of DNA double-strand break repair pathway choice. Cell Res 18(1):134–147

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, Rebar EJ, Gregory PD, Urnov FD (2009) Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature 459(7245):437–441. https://doi.org/10.1038/nature07992

Şöllü C, Pars K, Cornu TI, Thibodeau-Beganny S, Maeder ML, Joung JK, Heilbronn R, Cathomen T (2010) Autonomous zinc-finger nuclease pairs for targeted chromosomal deletion. Nucleic Acids Res 38(22):8269–8276

Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, Mangan PA, Kulikovskaya I, Gupta M, Chen F, Tian L, Gonzalez VE, Xu J, Jung IY, Melenhorst JJ, Plesa G, Shea J, Matlawski T, Cervini A, Gaymon AL, Desjardins S, Lamontagne A, Salas-Mckee J, Fesnak A, Siegel DL, Levine BL, Jadlowsky JK, Young RM, Chew A, Hwang WT, Hexner EO, Carreno BM, Nobles CL, Bushman FD, Parker KR, Qi Y, Satpathy AT, Chang HY, Zhao Y, Lacey SF, June CH (2020) CRISPR-engineered T cells in patients with refractory cancer. Science (new York, NY). https://doi.org/10.1126/science.aba7365

Swaminathan MS (2009) Obituary: Norman E. Borlaug (1914–2009). Nature 461(7266):894. https://doi.org/10.1038/461894a

Takayama M, Koike S, Kusano M, Matsukura C, Saito K, Ariizumi T, Ezura H (2015) Tomato glutamate decarboxylase genes SlGAD2 and SlGAD3 play key roles in regulating gamma-aminobutyric acid levels in tomato (*Solanum lycopersicum*). Plant Cell Physiol 56(8):1533–1545. https://doi.org/10.1093/pcp/pcv075

Takayama M, Matsukura C, Ariizumi T, Ezura H (2017) Activating glutamate decarboxylase activity by removing the autoinhibitory domain leads to hyper γ-aminobutyric acid (GABA) accumulation in tomato fruit. Plant Cell Rep 36(1):103–116. https://doi.org/10.1007/s00299-016-2061-4

Teferra TF (2021) Should we still worry about the safety of GMO foods? Why and why not? A review. Food Sci Nutr 9(9):5324–5331. https://doi.org/10.1002/fsn3.2499

Upadhyay SK, Kumar J, Alok A, Tuli R (2013) RNA-guided genome editing for target gene mutations in wheat. G3 (bethesda) 3(12):2233–2238. https://doi.org/10.1534/g3.113.008847

Urnov FD, Miller JC, Lee Y-L, Beausejour CM, Rock JM, Augustus S, Jamieson AC, Porteus MH, Gregory PD, Holmes MC (2005) Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435(7042):646–651

Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD (2010) Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11(9):636–646. https://doi.org/10.1038/nrg2842

USDA (2020) Genetically engineered soybean, cotton, and corn seeds have become widely adopted

van Dijk M, Morley T, Rau ML, Saghai Y (2021) A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food 2(7):494–501. https://doi.org/10.1038/s43016-021-00322-9

Van Vu T, Sung YW, Kim J, Doan DTH, Tran MT, Kim JY (2019) Challenges and perspectives in homology-directed gene targeting in monocot plants. Rice (n y) 12(1):95. https://doi.org/10.1186/s12284-019-0355-1

Vanamee ES, Santagata S, Aggarwal AK (2001) FokI requires two specific DNA sites for cleavage. J Mol Biol 309(1):69–78. https://doi.org/10.1006/jmbi.2001.4635

Vangheluwe N, Swinnen G, de Koning R, Meyer P, Houben M, Huybrechts M, Sajeev N, Rienstra J, Boer D (2020) Give CRISPR a chance: the GeneSprout initiative. Trends Plant Sci 25(7):624–627. https://doi.org/10.1016/j.tplants.2020.04.011

Vergauwen D, De Smet I (2017) From early farmers to Norman Borlaug - the making of modern wheat. Curr Biol 27(17):R858–R862. https://doi.org/10.1016/j.cub.2017.06.061

Voss-Fels KP, Stahl A, Hickey LT (2019) Q&A: modern crop breeding for future food security. BMC Biol 17(1):18. https://doi.org/10.1186/s12915-019-0638-4

Vu TV, Sivankalyani V, Kim EJ, Doan DTH, Tran MT, Kim J, Sung YW, Park M, Kang YJ, Kim JY (2020) Highly efficient homology-directed repair using CRISPR/Cpf1-geminiviral replicon in tomato. Plant Biotechnol J. https://doi.org/10.1111/pbi.13373

Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Liu YG, Zhao K (2016) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS ONE 11(4):e0154027. https://doi.org/10.1371/journal.pone.0154027

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32(9):947–951. https://doi.org/10.1038/nbt.2969

Waterworth WM, Drury GE, Bray CM, West CE (2011) Repairing breaks in the plant genome: the importance of keeping it together. New Phytol 192(4):805–822

Whelan AI, Gutti P, Lema MA (2020) Gene editing regulation and innovation economics. Front Bioeng Biotechnol. https://doi.org/10.3389/fbioe.2020.00303

Wolt JD, Wang K, Yang B (2016) The regulatory status of genome-edited crops. Plant Biotechnol J 14(2):510–518. https://doi.org/10.1111/pbi.12444

Woo JW, Kim J, Kwon SI, Corvalán C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS (2015) DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol 33(11):1162–1164. https://doi.org/10.1038/nbt.3389

Xie K, Minkenberg B, Yang Y (2015) Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. Proc Natl Acad Sci 112(11):3570. https://doi.org/10.1073/pnas.1420294112

Xie K, Zhang J, Yang Y (2014) Genome-wide prediction of highly specific guide RNA spacers for CRISPR-Cas9-mediated genome editing in model plants and major crops. Mol Plant 7(5):923–926. https://doi.org/10.1093/mp/ssu009

Xiong X, Liang J, Li Z, Gong BQ, Li JF (2021) Multiplex and optimization of dCas9-TV-mediated gene activation in plants. J Integr Plant Biol 63(4):634–645. https://doi.org/10.1111/jipb.13023

Yu H, Lin T, Meng X, Du H, Zhang J, Liu G, Chen M, Jing Y, Kou L, Li X, Gao Q, Liang Y, Liu X, Fan Z, Liang Y, Cheng Z, Chen M, Tian Z, Wang Y, Chu C, Zuo J, Wan J, Qian Q, Han B, Zuccolo A, Wing RA, Gao C, Liang C, Li J (2021) A route to de novo domestication of wild allotetraploid rice. Cell 184(5):1156-1170.e1114. https://doi.org/10.1016/j.cell.2021.01.013

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163(3):759–771. https://doi.org/10.1016/j.cell.2015.09.038

Zhang F, Maeder ML, Unger-Wallace E, Hoshaw JP, Reyon D, Christian M, Li X, Pierick CJ, Dobbs D, Peterson T, Joung JK, Voytas DF (2010) High frequency targeted mutagenesis in Arabidopsis thaliana using zinc finger nucleases. Proc Natl Acad Sci U S A 107(26):12028–12033. https://doi.org/10.1073/pnas.0914991107

Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ, Voytas DF (2013) Transcription activator-like effector nucleases enable efficient plant genome engineering. Plant Physiol 161(1):20–27. https://doi.org/10.1104/pp.112.205179

Zheng T, Hou Y, Zhang P, Zhang Z, Xu Y, Zhang L, Niu L, Yang Y, Liang D, Yi F, Peng W, Feng W, Yang Y, Chen J, Zhu YY, Zhang LH, Du Q (2017) Profiling single-guide RNA specificity reveals a mismatch sensitive core sequence. Sci Rep 7:40638. https://doi.org/10.1038/srep40638

Zhu S, Duwal A, Su Q, Vossen JH, Visser RGF, Jacobsen E (2013) Vector integration in triple R gene transformants and the clustered inheritance of resistance against potato late blight. Transgenic Res 22(2):315–325. https://doi.org/10.1007/s11248-012-9644-9

Zhu T, Mettenburg K, Peterson DJ, Tagliani L, Baszczynski CL (2000) Engineering herbicide-resistant maize using chimeric RNA/DNA oligonucleotides. Nat Biotechnol 18(5):555–558

Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas DF, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. Nat Biotechnol 36(12):1211–1216. https://doi.org/10.1038/nbt.4272