

GENOME EDITING TECHNIQUES: APPLICATIONS IN WHEAT BREEDING

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Abstract

Wheat provides the food and nutritional support necessary for human life; therefore the process of wheat breeding is very important to meet the growing demand for cultivars with better agronomic traits. Over time, breeders have tried various breeding techniques to improve desired traits, but these techniques have proven to be time-consuming and labor-intensive. To overcome these problems, scientists have developed new genome editing techniques to accelerate and facilitate crops improvement. The methodology used in this paper focused on processing, analyzing and providing updated information on genome-editing applications for wheat using data from the EU-SAGE platform. To date (January 20, 2024), 43 applications for the CRISPR/Cas technique, 3 for the BE technique and a single application for the TALEN technique have been registered on this platform. The USA is second, after China, in the application of genome editing techniques to wheat. All new wheat genotypes obtained through these applications do not contain foreign DNA, meeting the conditions for regulatory acceptance and ratification of several countries. These include important traits for both farmers and consumers, thereby contributing to increasing global efforts for sustainable agricultural development.

Key words: Base editing, CRISPR/Cas system, grain yield, quality, TALEN

INTRODUCTION

The continued growth of the global population requires an increase in food production. Ensuring sufficient food production is quite difficult due to climate change and other stresses.

Wheat (*Triticum aestivum* L.) is a staple crop for about 35% of the world's population, with over two-thirds of global production being used for human food and one-fifth for animal feed [14].

The area cultivated with wheat in 2021 was 220.7 million hectares, with global production reaching 770.8 million tons [12]. According to [41], to ensure food needs, wheat yield will have to increase by 50% until 2034.

Over time, plant breeders have developed new cultivars through various technologies. The most used approach is breeding through traditional technologies (crossing, selection, etc.), but these technologies are expensive and take many years.

Biotechnologies (transgenesis, genome editing, etc.) offer new opportunities to

improve crops compared to traditional technologies.

Transgenesis is a modern genetic modification technology used in plant breeding by which it is possible to transfer genes from wild relatives into crops, the success of these transgenic plants being mainly linked to herbicide and insecticide tolerance [4, 25].

Despite the advantages of these genetically modified (GM) crops for global food security, their cultivation and use on a large scale has been affected by the opposition of growers and consumers, due to very strict regulations as well as insufficient information [4]. To overcome some objections related to transgenesis, researchers have developed genome editing technology.

Genome editing is a set of innovative molecular techniques aimed at creating targeted mutations by precisely modifying the sequences of an organism's genome using sequence-specific nucleases (SSNs), namely: ZFNs (zinc-finger nucleases), TALENs (transcription activator-like effector nucleases) and the Clustered Regularly

Interspaced Short Palindromic Repeats - associated protein (CRISPR/Cas) [5, 7, 26]. The recent emergence of the CRISPR/Cas technique as a genome editing tool has allowed researchers to precisely and rapidly create new germplasm by manipulating key genes responsible for some specific agronomic traits [33], proving to be a simple tool, efficient, versatile and with the ability to work with several targets simultaneously (multiplexing) [22].

Using CRISPR technique, specific base pair changes as well as small nucleotide deletions can be generated without the addition of foreign DNA [26].

Genome-edited (GE) crops have different genetic properties than traditional GMOs, being similar to crops produced by natural approaches, therefore they are considered to have a low risk to human safety [43].

The induction of precise point mutations in DNA without producing double-strand breaks is possible as a result of the latest advances in the CRISPR/Cas system, namely primary editing and base editing [10].

In this paper, we have provided updated information on genome editing applications in wheat to facilitate a more comprehensive understanding of their importance in ensuring food security.

MATERIALS AND METHODS

This paper was based on the processing and analysis of data collected from the EU-SAGE platform [11] (covering the period 2014 - January 20, 2024) and data provided by other bibliographic sources, the results obtained being synthetically illustrated in the figures. Also, some examples of applications for these new technologies have been summarized and discussed.

RESULTS AND DISCUSSIONS

Distribution of genome editing applications in wheat

Genome editing has been reported in more than 63 crops from 25 countries, with the vast majority in rice, tomato, maize, soybean, and wheat [8].

The first genome-edited crop approved for commercialization in Canada (2014) was an herbicide-tolerant canola variety (Falco™ Canola). In the USA and Canadian markets, besides this variety, the soybean variety with high oleic acid content (Calyno™) is sold. Likewise, in Japan "Sicilian Rouge High GABA" a tomato with a high content of γ -aminobutyric acid (GABA) is marketed, and in the USA, waxy maize (for the chemical industry) with a higher content of amylopectin [25].

According to EU-SAGE Database [11], to date (January 20, 2024), 47 GE applications in wheat have been registered, of which 44 (92%) were developed using the CRISPR/Cas technique, 3 were developed by the BE technique (6%) and 1 by the TALEN technique (2%) (Figure 1). The higher percentage of use of the CRISPR/Cas technique compared to other techniques is due to its versatility and easier customization [8].

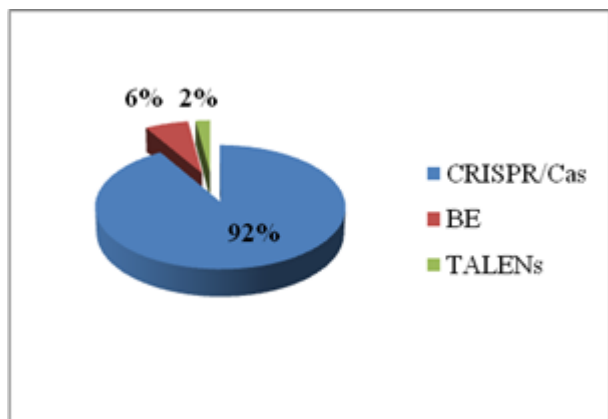


Fig. 1. Distribution of GE applications for wheat
Legend: GE - genome-edited; TALEN - Transcription activator-like effector; BE - Base editing; CRISPR/Cas - clustered regularly interspaced short palindromic repeats-associated protein (Cas);
Source: Own design and processing based on the data from [11].

According to [10], the development of genome-edited plants through the CRISPR/Cas9 technique (the basic flow) is carried out in five steps shown in Figure 2.

CRISPR-Cas systems are classified into two classes and six major types. The CRISPR/Cas9 system is part of class 2, type II CRISPR systems and has as its main components a single-guide RNA and an RNA-guided Cas9 endonuclease [19].

Among the 47 genome editing applications in wheat, 44 (94%) are SDN-1 genetic modifications, and according to the European Commission these changes are called "targeted mutagenesis"[8].

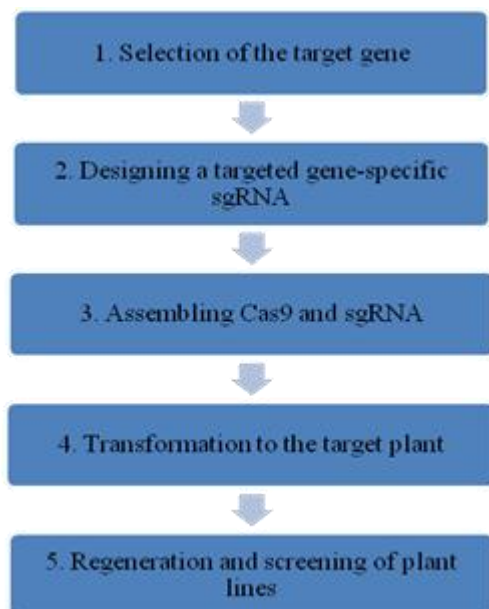


Fig. 2. Steps (basic flow) of the development of genome-edited plants by the CRISPR/Cas9 technique. Source: Own design adapted from [10].

These genome editing applications in wheat relate to the improvement of the following traits: plant yield and growth, food/feed quality, biotic and abiotic stress, industrial use and herbicide tolerance. The distribution by traits category was illustrated in Figure 3.

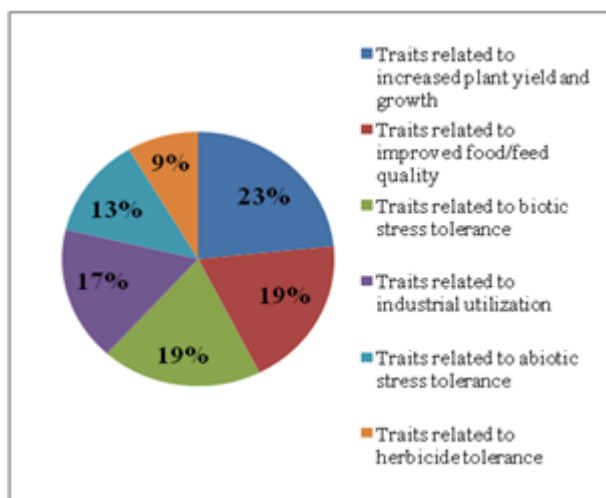


Fig. 3. Distribution of GE applications for wheat by trait category. Source: Own design and processing based on the data from [11].

In terms of the distribution of applications by country, the results showed that China ranks first with 31 applications, USA is far behind with only 8 applications, the UK with 5 applications and Pakistan with 3 applications in wheat breeding (Figure 4).

Research on genome-edited (GE) crops, their trade, cultivation and use as food and feed depend on the regulations of each country. These regulations should take into account the fact that the genome-edited plants contain only the mutations in the target gene(s) (no foreign DNA) and are phenotypically identical to those resulting from natural or induced mutations [25].

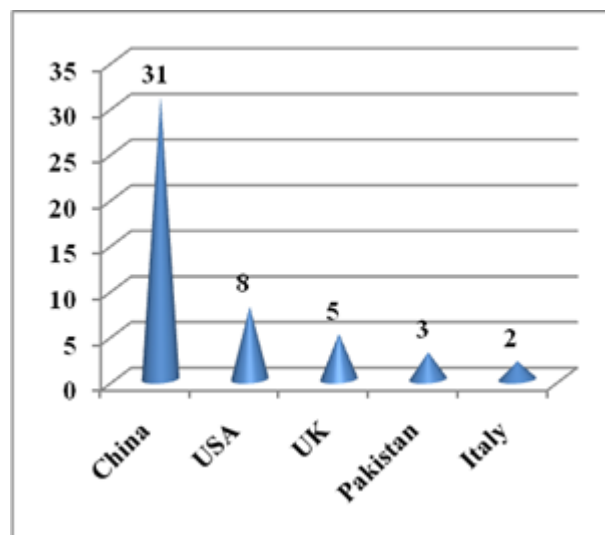


Fig. 4. Distribution of GE applications for wheat by country. Source: Own design and processing based on the data from [11].

Plant yield and growth

Grain yield is the most important objective of wheat breeding, and it depends primarily on the number and weight of grains in the spike. By applying CRISPR/Cas9, scientists removed some negative regulatory genes, which led to improved yield. For example, [40], using CRISPR/Cas9 developed knockout mutants of *TaGW2* that had significantly increased thousand-grain weight values relative to those of the wild-type control.

Another strategy for increasing grain yield is increasing the efficiency of nutrient use. Thus, by using CRISPR/Cas9 for the targeted mutagenesis of *TaARE1* genes, mutant lines were obtained that showed high nitrogen use

efficiency, increased yield, and delayed senescence [44].

Modern wheat cultivars that have a semi-dwarf architecture contain the *Rht-B1b* and *Rht-D1b* alleles, alleles that reduce height but negatively affect nitrogen use efficiency and grain filling. By simultaneously eliminating the *Rht-B1* and *ZnF-B* genes (encoding a RING-type E3 ligase), [32] obtained semi-dwarf wheat plants with a more compact architecture and improved yield.

Food/feed quality

For some consumers the quality of the wheat is very important. For example, the proteins in wheat gluten (gliadin and glutenin) can cause several diseases in susceptible people, including celiac disease. As a result, through the CRISPR/Cas system, researchers [31] were able to obtain wheat mutant lines with reduced α -gliadin content. Also, [24] by knockout mutants of two γ -gliadin genes (*Gli- γ 1-1D* and *Gli- γ 2-1B*) reduced the gliadin content of wheat grains.

Chronic non-infectious diseases (coronary diseases, diabetes, colon cancer, etc.) affect millions of people. It is believed that the consumption of cereals rich in resistant starch would reduce the risk of these diseases, and cereal grains with high amylose content are important sources of resistant starch. Therefore, [20] reported obtaining high-amylose wheat mutant lines by targeted mutagenesis of *TaSBEIIa* genes using CRISPR/Cas9.

Free asparagine is considered the precursor to acrylamide, a contaminant that forms during high-temperature processing of wheat foods. According to the International Agency for Research on Cancer [16], acrylamide is a carcinogen. Using CRISPR/Cas9 to reduce asparagine synthetase (*TaASN2*) gene expression in the grain, [30] reported obtaining plants whose grains have a reduced content of free asparagine.

In general, cereal grains contain phytic acid which is considered an antinutrient agent because it reduces the bioavailability of iron and zinc in the human body, which can lead to malnutrition. Therefore, [17] reported the development of biofortified wheat with high

iron and zinc content by disrupting the *TaIPK1* gene using CRISPR/Cas9.

Abiotic stress tolerance

Drought is a big challenge for sustainable agriculture, especially because of the consequences of climate change. Abiotic factors (drought, excess moisture, salinization, etc.) can influence and modify not only crops yield [34], but also soil quality, with a negative impact on agriculture [29].

Recent researches have demonstrated that genome editing is an effective technology in increasing drought tolerance in wheat. For example, wheat plants with loss-of-function mutations of the *12-OXOPHYTODIENOATE REDUCTASE SUBFAMILY III (OPRIII)* genes were obtained, which showed longer seminal roots and improved drought tolerance [13]. Using the sgRNA-CRISPR/Cas9 multiplex genome editing system, [2] created *TaSall* mutant lines with improved growth under drought conditions, and [38] created a mutant *TaPP2C158* gene that can serve as a direct target for genome editing while its favorable haplotype (alleles) can be useful for improving drought tolerance.

Also, soil contamination with arsenic due to mining, irrigation of As-tainted groundwater or the use of As-based pesticides, negatively influences the growth of plants and contributes to increasing the content of toxic substances in their organs, with a serious risk to human health. In this context, [39] generated CRISPR-edited *TaPHT1;9* wheat mutants with increased tolerance to AsV (arsenate) and low As (arsenic) concentrations.

Biotic stress resistance

Plants possess both resistance genes and disease susceptibility genes, and the modification of susceptibility genes through genome editing techniques has been shown to be promising for increasing disease resistance [46]. For example, through TALEN-mediated genome editing, [36] created a wheat MLO mutant, *tamlo1-aabbdd* with broad-spectrum resistance to powdery mildew but with accelerated senescence.

Subsequently, by CRISPR/Cas9-mediated precision editing, [23] created a *Tamlo-R32* mutant with a 304 kb deletion in the wheat

MLO-B1 locus, balancing powdery mildew resistance with a high efficiency.

Pandemic stripe rust caused by *Puccinia striiformis* f. sp. tritici frequently affects wheat crops. CRISPR/Cas9 inactivation of *TaPsIPK1* (*Puccinia striiformis*-Induced Protein Kinase 1) conferred rust resistance to wheat [37].

The fungus *Fusarium graminearum* causes the disease called Fusarium head blight in wheat, which causes great damage to farmers. By CRISPR-mediated targeting of the *TaNFXL1* gene, [6] obtained wheat mutants with enhanced resistance.

Herbicide tolerance

Weeds compete with agricultural crops for growing space, food and sunlight, directly or indirectly spreading diseases and pests. Furthermore, their seeds or pollen may contain toxins. An effective method of weed control is the use of chemical herbicides, but their use has many disadvantages, namely, the development of weeds resistant to herbicides [9], degradation of soil structure [3], and environmental pollution due to the noxious produced by chemical factories [28], etc. To overcome these problems, the development of herbicide-resistant crops is needed. After researchers [18] published (in 2012) the complete mechanism of the "gene scissors" of the CRISPR/Cas system, techniques based on this genome editing system were used to improve plants for desired traits, including herbicide resistance. For example, by base editing of the acetolactate synthase (ALS) and acetyl-coenzyme A carboxylase genes, [42] obtained wheat mutations that confer tolerance to imidazolinone, sulfonylurea and aryloxyphenoxy propionate herbicides. Also, using A-to-G base editing with *WhiteABE*, [15] obtained a Met-to-Thr mutation in wheat tubulin alleles with increased resistance to the herbicide dinitroaniline.

Industrial utilization

Male sterility has an important role in obtaining hybrid wheat; therefore, CRISPR technique has recently been used to obtain male-sterile lines.

For example, with this technique, [27] generated *Ms1* knockout wheat lines that exhibited male sterility in the first generation.

Also, by targeting all three homeologs of *TaNPI* genes, [21] created complete male sterile mutants, and [45] by individually targeting genes such as Dicer-like 4 (*TaDCL4*), Dicer-like 5 (*TaDCL5*) or RNA polymerase 6 (*RDR6*), using CRISPR/Cas9, generated mutants with male sterility.

Pre-harvest sprouting (PHS) that occurs due to prolonged high humidity before harvest leads to reduced wheat yield and quality [35]. Thus, [47] converted a white-grained cultivar into a red-grained cultivar improving its tolerance to PHS and at the same time the antioxidant capacity of the grains, due to the functional restoration of the *Tamyb10-B1a* allele. Also, through genome editing of homeologous *Qsd1* loci, the researchers [1] obtained a triple-knockout wheat mutant with longer dormancy, thereby reducing the risk of PHS.

CONCLUSIONS

Current climate change and continued population growth require rapid crop improvement so that new cultivars with better adaptability to a changing environment are available to farmers. In this context, scientists have proven that genome editing techniques have an important role in accelerating crop breeding.

Genome-edited crops promote sustainable and ecological farming systems, reducing the environmental impact associated with conventional systems by improving resource use efficiency, reducing chemical inputs and increasing stress tolerance.

The importance of wheat in ensuring global food security is indisputable, which is why genome editing has been used efficiently to improve some agronomic traits in wheat.

The potential of wheat genome editing is illustrated by the 47 applications registered in the EU-SAGE platform, of which 43 (92%) use CRISPR/Cas technique. Most applications were aimed at improving plant yield and growth (23%). The USA is second, after China, in the application of genome editing techniques to wheat.

All new wheat genotypes obtained through these applications do not contain foreign

DNA, meeting the conditions for regulatory acceptance and ratification of some countries. The applications presented in this paper represent successful attempts in genome editing for the development of new improved wheat cultivars, cultivars that promise numerous benefits for consumers (improvement of nutritional quality), farmers (higher grain yield, tolerance to biotic and abiotic stress, tolerance to herbicides), as well as for biodiversity, offering solutions to malnutrition and food insecurity.

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