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Genome editing: a powerful tool for crop improvement

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Introduction

Almost a billion people worldwide suffer from chronic malnourishment, and at the same time, our agricultural systems are declining due to a loss of biodiversity and rising climate change uncertainty. With the growth of the world's population, crop production will need to be doubled by 2050. However, the current annual growth rate of the main crops, wheat, rice, soybean, and maize are 0.9%, 1.0%, 1.3%, and 1.6%, respectively, falling far behind the required 2.4% rate. Other factors, such as reduced arable land and water availability, climate change and increased demand for bio-fuels will further compound the problem in the future. Besides crop yield, there is an increasing demand to develop new varieties with improved traits, such as increased quality, enhanced nutrition, disease resistance, stress tolerance and reduced resource requirements.

The term **"genome editing"** refers to a group of powerful molecular biology methods which allow accurate, effective, and focused changes at specified genomic loci. Genome editing using zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) has been around for two decades, but it has recently come under the spotlight through the development of clustered regularly interspaced short palindromic repeats (CRISPR)/ Cas systems which provide simplicity and ease of targeted gene editing. Though, the benefits of using grafted plants are profuse, not all vegetable species are capable of being grafted, because genetic background, growth characteristics, anatomy, and physiological

The **CRISPR-Cas9 system** has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other genome editing methods. CRISPR-Cas9 was adapted from a naturally occurring genome editing system that bacteria use as an immune defense. When infected with viruses, bacteria capture

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small pieces of the viruses' DNA and insert them into their own DNA in a particular pattern to create segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays that recognize and attach to specific regions of the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus.

Researchers adapted this immune defense system to edit DNA. They create a small piece of RNA with a short "guide" sequence that attaches (binds) to a specific target sequence in a cell's DNA, much like the RNA segments bacteria produce from the CRISPR array. This guide RNA also attaches to the Cas9 enzyme. When introduced into cells, the guide RNA recognizes the intended DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location, mirroring the process in bacteria. Although Cas9 is the enzyme that is used most often, other enzymes (for example Cpf1) can also be used. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of genetic material, or to make changes to the DNA by replacing an existing segment with a customized DNA sequence.

Many gene knockout mutants and some gene replacement and insertion mutants have been produced through the use of genome-editing technologies in a wide variety of plants, and many of these mutants have been shown to be useful for crop improvement. The risks involved in altering genomes through the use of genome-editing technology are significantly lower than those associated with GM crops because most edits alter only a few nucleotides, producing changes that are not unlike those found throughout naturally occurring populations. Once the genomic-editing agents have segregated out, there is no way to distinguish between a ‘naturally occurring’ mutation and a gene edit. Thus, the introduction of genome editing into modern breeding programs should facilitate rapid and precise crop improvement.

Applications of genome editing technologies in crop improvement

In the last several years, genome editing has been used to produce new crop varieties with improved traits, including increased yield, enhanced disease resistance, improved food quality and higher stress tolerance.

A. Improved yield

Grain number, size, and weight—all common quantitative traits—are the key determinants of grain yield, and other genes that impact crop productivity have been identified. In bread wheat, thousand-kernel weight also exhibited an increase after the

three homo-alleles of GASR7, a negative regulator of kernel width and weight, were knocked out using CRISPR/Cas9. It is nevertheless important to remark that increased grain yield per plant and higher thousand-grain weight does not necessarily translate into improved crop yield, because large-scale field trials are necessary to verify the potential agronomic improvements.

B. Disease resistance

Plant diseases are the main cause of crop yield loss, and most importantly, diseases also affect produce quality for fresh consumption and food processing and safety (toxins) in many crops. Genome editing has been applied to increase disease resistance by editing disease-related genes. The rice *OsSWEET14* is a host disease-susceptibility gene that is activated by a type-III effector protein secreted by the bacterial rice pathogen *Xanthomonas oryzae* pv. Oryzae causing bacterial blight. Disruption of the bacterial-derived protein binding sequence in the *OsSWEET14* rice promoter using TALEN resulted in increased resistance to bacterial blight. Virus resistant tobacco and tomato have also been generated by directly targeting viral genomic sequences using CRISPR/Cas9.

C. Herbicide tolerance

Traditionally, herbicide tolerance in crops has been obtained through transgenesis. Genome editing provides a new approach to create herbicide tolerant crops and has been employed to edit endogenous plant genes, such as *EPSPS* and *ALS*, resulting in herbicide tolerant plants. Genome editing-based gene replacement has been used to introduce precise mutations in the ALS gene to produce herbicide tolerant plants, with the first example, tobacco, reported in 2009 using ZFNs and donor templates. Herbicide-resistance maize, soybean, and rice have also been obtained using CRISPR/Cas9 and TALENs to introduce site directed DNA base changes in the *ALS* gene. In plants, EPSPS is a target for glyphosate, a widely used herbicide which binds to EPSPS functional sites to prevent its activity.

D. Healthy food

Genome editing can be used to modify plant components, resulting in healthier foods.

Improved oil composition

A high content of polyunsaturated fatty acids, particularly linolenic acid, in oils results in poor oxidative and frying stability which limits their applications. TALENs were used to simultaneously knock out two soybean FAD2 genes, FAD2-1A and FAD2-1B, resulting in vastly improved oil quality: oleic acid increased from 20% to 80% and linoleic acid decreased from 50% to < 4%. Recently, two independent groups used CRISPR/Cas9 to simultaneously knock out all three FAD2 homeolog genes in the allohexaploid, *camelina*

sativa, producing a significant enhancement in oil composition.

Healthy potatoes

Cold storage of potatoes reduces sprouting and ensures a continuous supply, but it also results in the accumulation of reducing sugars. Mutation of *VINV* in a commercial Ranger Russet potato variety has been achieved using TALENs, with the resulting potatoes having undetectable levels of reducing sugars. Heat processing of the cold-stored potatoes resulted in reduced levels of acrylamide and produced lightly colored chips.

Other examples

CRISPR/Cas9 targeted mutation of the *TMS5* gene in rice cultivars led to the rapid development of temperature-sensitive lines for use in hybrid rice production. The maize *Waxy (Wx)* gene encodes a granule-bound starch synthase (GBSS) responsible for the synthesis of amylose in the kernel. Wild type maize kernels consist of 75% amylopectin and 25% amylose while *wx/wx* lines contain nearly 100% amylopectin which is called *waxy* maize. The economically valuable waxy maize has been produced by CRISPR-mediated *Waxy* gene knockout. High-amylose rice, with potential health advantages, was generated through CRISPR/Cas9-mediated knockout of the starch branching enzymes genes, *SBEI* and *SBEIIb*.

Conclusion

Overall, gene editing technologies, especially CRISPR/ Cas9, have had a revolutionary influence on basic research in plants as well as crop improvement. One of the main advantages of these technologies is that the transgenes initially used to produce the genetic changes can be easily excised from the genome by genetic segregation, and the resulting gene-edited varieties are completely indistinguishable from those generated using conventional breeding methods. The increasing number of researchers using and developing these tools, a revolutionary change is taking place in crop improvement that will help to meet the increasing demand for food and ensure world food security in the future.

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