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Precise Genome editing tools for crop improvement

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Genome or gene editing includes several new techniques to help scientists precisely modify genome sequences. The field of genome editing is experiencing rapid growth as new methods and technologies continue to emerge. Using genome editing to boost agriculture productivity is needed as the world population. This helps us to alter the regulation of gene expression patterns in a determined region and enables novel insights into the functional genomics of an organism. Genetic diversity is a key source for trait improvement in plants. Creating variations in the gene pool is the foremost requirement for developing novel plant varieties. The emergence of genome editing has brought considerable excitement, especially among agricultural scientists because of its easiness, accuracy and effect as it offers new openings to develop improved crop varieties with definite addition or removal of traits of our interest. This is one of the best tools for studying reverse genetics and also to a versatile tool in basic research. Genome edited plants are differentiated from conventional transgenic plants as they may not incorporate foreign DNA. Although genome editing can be used to introduce foreign DNA into the genome, it may simply involve changes of a few base pairs in the plant's own DNA. This distinction makes genome editing a novel and powerful breeding tool that has promising applications in agriculture, especially when genome-edited crops are not regulated as genetically modified crops.

The investigation is in progress to improve crop varieties with higher yields, strengthen both biotic and abiotic stresses, reduction of input costs, and intensification of nutritional value. Genome editing encompasses a wide variety of tools using either a site-specific recombinase (SSR) or a site-specific nuclease (SSN) system. Both systems require recognition of a known sequence. The SSN system generates single or double-stranded DNA breaks and activates endogenous DNA repair pathways. SSR technology, such as Cre/loxP

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and Flp/FRT mediated systems, are able to knock down or knock-in genes in the genome of eukaryotes, depending on the orientation of the specific sites (loxP, FLP, etc.) flanking the target site. There are 4 main classes of SSN developed to cleave genomic sequences, mega-nucleases, zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and the CRISPR / Cas nuclease system (Clustered regularly interspaced short palindromic repeat/CRISPR-associated protein). The recombinase-mediated genome engineering depends on recombinase (sub-) family and target-site and induces high frequencies of homologous recombination.

Improving crops with gene editing provides a range of options: by altering only a few nucleotides from billions found in the genomes of living cells, altering the full allele or by inserting a novel gene in a targeted region of the genome. Due to its precision, gene editing is more specific than either conventional crop breeding methods or standard genetic engineering methods. Thus this technology is a very powerful tool that can be used toward securing the world's food supply. In addition to improving the nutritional value of crops, it is the most effective way to produce crops that can resist pests and thrive in tough climates.

There are 3 types of modifications produced by genome editing; Type I includes altering a few nucleotides, Type II involves replacing an allele with a pre-existing one and Type III allows for the insertion of new gene(s) in predetermined regions in the genome. Because most genome-editing techniques can leave behind traces of DNA alterations evident in a small number of nucleotides, crops created through gene editing could avoid the stringent regulation procedures commonly associated with GM crop development.

Genome editing relies on DNA repair mechanism. DNA damage occurs in all the living cells due to exogenous factors (UV radiation) or endogenous factors (metabolic by-products and free radicals). A most lethal type of DNA damage is the double-strand break (DSB) and must be repaired before DNA replication, which has led to either non-homologous end joining or homology-directed repair. In Non-homologous end-joining (NHEJ), it can quickly and imprecisely, be repaired the break. In one way, several different proteins bind to broken DNA ends and are joined together resulted in the INDELs of nucleotides. In another way, NHEJ, 5′ ends are cut until 3′ overhangs with homology are created. DNA strands then bind at their complementary sequence, and non-homologous DNA are excised and results in

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deletions. NHEJ often leads to frameshift mutations which can result in premature stop codons, resulting in non-functional genes.

The second DNA repair pathway is homology-directed repair (HDR) which relies on template DNA. Homologous recombination is an important process that occurs in somatic cells to repair DSBs and in meiotically dividing cells to exchange genetic material between parental chromosomes. The most common conservative HDR mechanism in plants, which repairs almost all DSBs in somatic cells, is the synthesis-dependent strand annealing (SDSA) pathway.

Over 50 genes have been targeted for mutations using TALEN in plants, including *Arabidopsis*, Barley, *Brachypodium*, maize, tobacco, rice, soybean, tomato and wheat. TALEN scaffolds were optimized for high activity in plants. The optimized TALEN scaffold was then demonstrated by targeted mutagenesis in *Arabidopsis*, tomato, *Brachypodium* and wheat. More recently, TALEN was shown to induce a variety of heritable mutations in rice, demonstrating its usefulness in plant genome editing. As an effective genome editing tool, TALEN has been applied to generate useful traits in crops. In an elegant study, TALEN was used to engineer disease resistance in *Xanthomonas oryzae pv. oryzae* by destroying the target sequence of TALE effectors in rice. In soybean, the FAD2 gene was targeted for improved oil quality. In wheat, three homologs of MLO were successfully targeted for simultaneous knockout, conferring heritable disease resistance to powdery mildew. Improved rice seeds have been engineered with TALEN, creating traits such as fragrance and storage tolerance. Improved cold storage and processing traits have also been engineered in potato. Most of these studies targeted protein coding genes for mutagenesis. Other types of NHEJ based editing can also be achieved by TALEN, such as targeted mutagenesis of non-protein coding genes and regulatory elements, and generating large chromosomal deletions.

Another important tool on genome editing is CRISPR/Cas9 which is a unique technique that enables geneticists and medical scientists to edit a particular sequence of the genome by addition or deletion or by altering the sections of the DNA sequence. This is currently a precise and versatile technique in the manipulation of gene. This system contains two molecules which are necessary for the introduction of mutation in the DNA sequence. One molecule is the enzyme, Cas9. This is the molecular scissor that can cut the double helical stand of the DNA at a particular site/location and that portion can be easily removed

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or substituted or added to the genome. Another molecule is a small segment of RNA called as guide RNA which is predesigned with 20 bases long and is present inside the RNA scaffold. This part binds to the DNA the pre-designed sequence 'guides' Cas9 to the right part of the genome. This makes sure that the Cas9 enzyme cuts at the right point in the genome.

The guide RNA is designed to find and bind to a specific sequence in the DNA. The guide RNA has the basic unit of our genetic instructions containing adenine (A), cytosine (C), guanine (G) and uracil (U) in RNA that are preferentially binding to the bases A to U and C to G to those of the target DNA sequence in the genome. This means that, at least in theory, the guide RNA will only bind to the target sequence and no other regions of the genome. The enzyme Cas9 follows the guide RNA to DNA sequence location and make the cut in both the DNA double-helical strands.

CRISPR-PLANT is a newly designed web portal supported by PennState and Arizona Genomics Institute (AGI) established to help researchers to use the CRISPR-Cas9 system for genome editing. It estimates the highly specific sgRNA. After the target site confirmation, target specific oligonucleotides (20nt) are designed which further fuses with tracrRNA sequence to form sgRNA. sgRNA is further placed in a vector either along with Cas9 sequence (a binary vector) or individually under a suitable promoter for an optimal expression. The constructs are then transformed using a suitable method. The delivery systems vary based on plant species, research purpose, and requirements. F gRNA-Cas9 mediated editing can be detected by a restriction enzyme digestion suppressed PCR (RE-PCR) method, which investigates the NHEJ-introduced mutations. RE-qPCR can also be performed for a more accurate estimation of genome-editing efficiency.

CRISPR/Cas9 sequence specific nuclease editing is an effective approach to combat rice blast disease. OsERF922 gene in rice was targeted and 21 CRISPR-ERF922 induced mutants were identified from 50 T0 transgenic plants. Furthermore, the high throughput can be obtained by coalescence of cytidine deaminase enzyme with Cas9, which permits high-efficiency emendation of target codons in rice. dCas9 fusion with cytidine deaminase allows direct conversion of cytidine to uridine leading to a point mutation from C/G bp to T/A bp during replication in one of the daughter cells.

For this reason many scientists believe plants improved with the more precise gene editing techniques will be more acceptable to the public than transgenic plants. With genome

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editing comes the promise of new crops being developed more rapidly with a very low risk of off-target effects. It can be performed in any laboratory with any crop, even those that have complex genomes and are not easily bred using conventional methods.