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Editorial

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Genome Editing: New Approaches to Create Disease-Resistant Crops

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Editorial

Genome editing achieves a greater precision in genetic modification of living organisms while minimizing the unintended consequences and opposition to products developed through Genetically Modified Organism (GMO) technologies [1]. These technologies are powerful and versatile tools and have revolutionized methods of modifying living organisms for many intended purposes. Targeted genome editing using specialized nucleases offers methods with increased accuracy by introducing deletions, insertions, and replacement to site-specific genomic locations. Examples include the use of Zinc Finger Nuleases, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), Oligonucleotide-directed mutagenesis, RNA-dependent DNA methylation, and precision breeding for crop plant improvement [2,3]. The CRISPR/Cas9 was first discovered in the mid-1980s, in bacteria as a part of their immune system against viruses. The Cas9 nuclease target specific genomic sites with the help of a single guide RNA (sgRNA). Each sgRNA (targeting molecule) is composed of a 20-nucleotide spacer immediately upstream of a Proto-spacer Adjacent Motif (PAMs). The sequence of spacer and PAM must be complementary to a specific genomic location, allowing targeted mutagenesis of genes.

Genome editing has been effectively utilized in direct genetic improvement of different crops for several agriculturally important traits. Techniques are also important for asexually/vegetative propagated crops and species with a long juvenile life cycle. This is important particularly in crop species with limited genetic diversity, which limits the number of disease resistant traits that can be introgressed. Genome editing systems have been used to control diseases caused by the three main types of pathogens: fungi, bacteria, and viruses. In general, two approaches are used to control plant diseases using CRISPR, targeting either pathogen genes or plant host genes required for infection. Genome editing technologies have been successful in controlling the powdery mildew fungi through the *mutation in* the host *susceptibility* factor, mildew-resistance locus (MLO) in wheat and tomato [4,5]. developing resistance against rice blast disease (*Magnaporthe oryzae*), rice bacterial blight (*Xanthomonas oryzae* pv. oryzae), and tomato bacterial speck [6,7]. Additionally, the system has also found utility in controlling plant RNA and DNA viruses [8-12]. A sgRNA targeting the origin of replication conserved in the geminiviruses (Family *Geminiviridae*) conferred to resistance different viruses [8-11]. Introducing a mutation in a eukaryotic translation initiation factor4E (eIF4E) led to broad host resistance against five RNA viruses under the greenhouse conditions [12]. In addition, the CRISPR-Cas9 system can be used to elucidate the molecular and cellular mechanisms of plant defense responses against plant pathogens by generating non-transgenic plant and pathogen mutants.

Although the CRISPR/Cas9 system described in the publications is straightforward, it has several limitations. It is difficult to implement in crops that are recalcitrant to gene insertion and *in vitro* regeneration. The success of genome editing system is dependent on several factors such as implementing proper bioinformatics-specific pipelines, setting up workflows and transformation efficiency. Mutating plant genes can result in unexpected changes in the plant and may intervene with the cellular and development functions, which may pose a risk to crop yields and performance. There exists an urgent need to utilize bioinformatic pipelines that could accurately predict/design sgRNA sequences in conjunction with complete genome sequences to pinpoint the precise location of target genes. This information minimizes editing of unintended genomic target regions and subsequently reduces off-target genes.

In addition, genome editing systems for improved disease resistance depend on several factors. The availability of genome sequence information of both plant host and pathogen would facilitate the implementation of genome editing on different pathosystem**s.** Currently, only a limited amount of information has been generated on genes involved in host/pathogen interactions that can be used as targets for the CRISPR/Cas9 system. However, genome editing will open new avenues of research for studying detailed host-pathogen interactions and targeting specific genes for enhancing disease resistance. On the other hand, targeting individual pathogens might be ineffective due to genetic variation in populations, recombination, and the emergence of new variants with altered host ranges. The solution could be targeting different pathogen genes through the integration of different sgRNAs in the plant host genome, which would be highly laborious and time-consuming. This might result in unexpected changes in cellular and development functions such as plants host genome instability, and reduce transformation efficiency, thereby reducing crop yield and performance.

The development of new genome editing technologies for crop improvement has raised questions about whether the products developed through such techniques would be subject to the regulations currently in place for transgenic/GMO crops. From the biosafety point of view and risk associated with the genome-edited crops, the regulatory issues play a significant role. However, the speed in the development of regulatory protocols for plants developed through new techniques has not kept pace with the actual development of new technologies. Recently, the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) stated that they will not regulate crops/new cultivars developed with genome editing as such crops are developed using techniques that are similar to conventional breeding procedure (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/brs-news-and-information/2018_brsnews/pbi-details). However, European regulators have determined that crops developed using genome editing techniques would be regulated

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and tested just like their transgenic/GMO counterparts [13]. It remains to be seen how these rules would be modified as a greater number of crop cultivars with improved traits are developed using genome editing technologies. There appears to be a greater consensus among the scientific community for developing a set of updated regulatory approval procedures that are consistent, harmonized, evidence-based, and enable synchronous development of technology and trade [14]. Thus, future regulatory protocols for biosafety issues and risk associated with the genome-edited crops will be crucial in determining how crops developed through genome editing are tested and commercialized.

In summary, the development and application of genome editing for crop improvement have its own unique strengths and limitations. Genome editing can play a significant role in understanding the cellular and molecular basis of plant-pathogen interactions, and help to construct the signaling networks underlying plant-pathogen interactions. Still, there is a huge demand for optimization and development of protocols for plant genome editing. These include a set of vector systems that are compatible with the plant species, highly efficient plant transformation methods and delivery systems, high throughput screening of transformation events, which can be streamlined to enable rapid product development. Several research teams worldwide are currently optimizing and applying genome editing technologies, and we expect to see more research published rapidly in the near future.

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