Editorial

Crop genome editing: A way to breeding by design☆

Chuanxiao Xiea,⁎ Yunbi Xub,c, Jianmin Wанд

aNational Engineering Laboratory for Crop Molecular Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China
bInstitute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China
cInternational Maize and Wheat Improvement Center (CIMMYT), El Batan, 56130, Texcoco, Mexico
dChinese Academy of Agricultural Sciences, Beijing 100081, China

Increasing population and consumption in our planet is placing unprecedented challenges on agriculture for meeting food security and sustainability needs [1]. Meanwhile, the adaptation of modern agricultural techniques [2] is central to minimize extensive losses due to abiotic stresses [3] under global climate change. Among these agricultural technology systems, crop breeding is the core node of all technologies and is finally reflected in crops and their products. Crop breeding deals with the creation and selection of the desired variation in target varieties with improved yield, quality and tolerance to abiotic and biotic stresses. Random mutagenesis using physical, chemical, and biological approaches has been limited by the availability of desirable mutant alleles [4].

In 2012, type II cluster regularly interspaced short palindromic repeats (CRISPR)-associated nuclease (Cas) was developed as a system for RNA-programmable genome editing [5]. This study laid a fundamental basis for precise editing, which has been subsequently verified across diverse organisms. The technology of RNA-guided Cas9 activities were quickly in vivo verified by targeted knockout (KO) editing across diverse crops [6,7]. Later on, a number of modified genome editing systems were developed using effector enzymes to generate diverse mutations, including targeted gene activation or repression [8], deletion [9] replacement [9,10] and base editing[11,12]. More heritable mutant systems were optimized using egg cell-specific promoter [13], meristem cell specific Yao promoter [14] and early selection on “Cas9 free” selection [15]. As a worthwhile highlighting area, genome editing has been applied in crop breeding, showing its unparalleled technology advantages over traditional laborious selection methods. The targeted gene KO mediated by CRISPR/Cas9, which generates DNA strand break and then introduces a frame-shift mutation due to endogenous non-homolog end joining repair, is the earliest and easiest targeted mutation tool. As one of the earliest technological tools, CRISPR/Cas9 genome editing has been used to generate a list of diverse cis-regulatory alleles in promoters with beneficial quantitative variation achieved on breeding fruit size, inflorescence branching, and plant architecture in tomato [16]. In addition, crop genome editing technology has also made our dreams in agriculture become true that have no solutions before. Fox example, a triple mutant that consists of chromosome paring, recombination

☆ Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.
⁎ Corresponding author.
E-mail addresses: xiechuanxiao@caas.cn, chxxie@126.com. (C. Xie).

Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.
and sister chromosome separation with an additional asexual reproduction gene null mutation was created, enabling asexual reproduction for the maintenance of hybrids clonally through seed propagation [17,18]. Although it is far from the final application of clonal seeds, its technical feasibility has been initially testified. Recent advances have also provided de novo domestication of wild plant species as a viable solution for redesigning ideal crops [19]. This attempt targets at the development of new cultivated crops from wild species in our future agriculture. Furthermore, the CRISPR/Cas systems has a great potential to enable robust synthetic biology, in which artificial DNA sequences, genetic elements, genes, and chromosome assemblies can be synthesized into recipient genomes by using genome editing tools [20]. To overview the technological advancement in genome editing and its application in crop genetic improvement, we here release a special issue of the Crop Journal. The papers collected in this issue include case studies in genome editing from diverse crops on tool development, optimization, and specific breeding applications. We hope that the presented papers will provide insights and inspirations on both technologies and their application to breeding by design.

For almost all crops, genome-wide single nucleotide polymorphisms (SNPs) substantially contribute to phenotypic diversity. Development of more efficient technologies than former TILLING approach [21] to create targeted SNP variation is highly desirable for both functional genomics and crop improvement. Precise base editing is an important tool for generating sense mutations such as gene correction. Bharat et al. [22] summarized base-editing technologies on the latest developments, as well as their underlying mechanisms, current applications, challenges and perspectives in plants. A number of base editing studies on diverse crops were also reported in this special issue. Besides, this technology can be also used to create new traits such as herbicide resistance. Precise base editing non-allelic ZmALS1 and ZmALS2 was employed using CRISPR/Cas9 nickase-cytidine deaminase fused with uracil DNA glycosylase inhibitor (UGI). The targeted base editing of C-to-T per se along with the phenotype verified in generated mutants demonstrates the power of base editing in maize precise breeding [23].

Optimization of genome editing technology has been an important focus in this field. The relevant studies have been largely devoted to transferring the original concept technology into specific species and ensuring its mutant specificity and efficiency. For instance, the fidelity and efficiency are always the two most important technical parameters for gene correction tools. The UGI activity absence or insufficiency of the target-AID or BE3 system usually leads to a high frequency of undesired mutations. As reported in this special issue, plant base-editing systems of BE3 and CDA systems with additional UGI activity combined with the optimized sgRNA expression cassette showed as high as 86.1% and 85.7% clean editing efficiency [24]. The proposed optimization strategy can accelerate the application of precise mutagenesis to fundamental researches and trait improvement in crop plants. Wang et al. [25] reported a multiplex super-assembled adenine base editor (sABE) by combining optimized synergistic core components, including esgRNA, plant codon-optimized Cas9n, rice codon-optimized ecTadA & ecTadA*, and bpNLS. The sABE had higher efficient than previous ABEs version. A multiplex super-assembled CBE system (sCBE) with a rice codon-optimized rAPOBEc1 and dual UGIs was also tested. The optimized sABE and sCBE displayed a greatly improved editing efficiency. Another optimization on an effective multiplex cytosine base editor (SaKHm-pBE) showed more proto-spacer-motif (PAM) recognitions. These attempts can be applied for broaden the targets in recipient plant species [26]. Among CRISPR/Cas systems, CRISPR-Cas12a (formerly called Cpf1) offers a robust multiplex genome editing due to smaller nuclease protein [27]. The CRISPR-associated protein Cas12a has been repurposed due to its possessing two distinct nuclease activities: endonuclease activity for processing its own guide RNAs and RNA-guided DNase activity for target DNA cleavage [28]. Another case study is provided on plant CRISPR-Cas12a optimization in this special issue. A modified tRNA-crRNA array has demonstrated an efficient multiplex genome editing in rice [29]. This modification contributes to CRISPR-Cas12a system especially for genomic loci that have hitherto been difficult to edit in plants.

One of the major challenges for food supply security is to improve yield stability through breeding disease-resistant crops. In this regard, CRISPR/Cas technology has democratized its use in improvement of disease resistance thanks to its ease and robustness [30]. The rice blast caused by Magnaporthe oryzae is one of the most devastating diseases resulting yield loss and bad grain quality in rice. An intron targeted insertion frequency of 3.8% mediated by CRISPR/Cas9 was successfully achieved to create a single amino acid in pi-ta exon2 encoding a rice blast resistant Pi-ta protein [31]. This technology combined with the other R gene pyramiding approaches has provided an effective breeding solution to rice blast resistance.

Improvement of agricultural product quality is an important aspect in crop breeding. In this special issue, gene editing has been used to improve product quality for three major crops. In soybean, its grains contain three lipoxigenase isozymes, LOX1, LOX2, and LOX3, that are involved in the formation of beany flavor [32]. A pooled CRISPR/Cas9 strategy targeting three GmLox genes, as reported in this special issue, was employed to generate gmlox1gmlox2gmlox3 triple mutants and binary mutants with better beany flavor [33]. In rice, as a staple food for huge populations in world, improvement of eating and cooking quality has been one of major objectives in breeding. The OsaAAP6 and OsaAAP10 KO mutants were generated in three japonica varieties and one japonica line by using CRISPR/Cas9 system. The data showed that OsaAAP6 and OsaAAP10, especially the latter, could be manipulated to rapidly reduce grain protein content and significantly improve eating and cooking quality in rice, providing a new strategy for breeding cultivars with desirable eating and cooking quality [34]. In maize, waxy property is an important grain trait associated with starch quality. An efficient quality breeding using in vivo CRISPR/Cas9 machinery was demonstrated with triple selections on target mutation, background recovery, and “transgene-free” [35]. This research provides a practical solution to commercial hybrid breeding on a stable transformation recalcitrant species.
In addition to breeding applications, genome editing tools are also of great value in fundamental researches such as gene function analysis. For example, gene knockout (KO) is an earliest targeted mutation tool in genome editing. It is a frequently used and effective strategy for gene function study since the null mutants can provide direct morphology or physiology phenotypes. Using CRISPR/Cas9 systems, large-scale mutant libraries [36], multiplex null mutants [37,38], and surely single gene KO mutant [39,40] have been generated across diverse plant species. In this special issue, we include three case studies on gene functional analysis using CRISPR/Cas9 tool. Pollen fertility is an important trait that not only strongly influences rice yield but also holds a great prospect on seed industrial application. Luo et al. [41] presented an example of generating loss-of-function mutants of LSP1/OsABCG3 using CRISPR/Cas9 for gene function study. Their findings suggest that LSP1/OsABCG3 is critical for pollen fertility and involved in the molecular mechanisms underlying rice pollen wall development. This example demonstrates that genome editing is also an important tool for basic studies in plants. The second case study is about phytohormone abscisic acid (ABA), which plays an important role in plant growth and development [42]. The performance of OsABA8ox2 KO mutation mediated by the CRISPR/Cas9 system verifies that the mutation conferred drought tolerance in plants due to the drought-induced ABA and indole-3-acetic acid accumulation in roots [43]. The third case study involves two mitochondrial phosphate transporter genes, OsMPT3;1 and OsMPT3;2, from which double KO mutants were generated using CRISPR/Cas9 to analyze their osmotic regulatory factor in rice [44]. The results suggest that the OsMPT3s are crucial regulatory factors affecting the transport of phosphate and providing energy for ATP synthesis encoding in rice. The last but not the least, there are many genome editing tools that can be used to generate target sense mutations on target genes, holding a great potential in basic studies.

In summary, the reports presented in this special issue provide a wealth of information on the technical implications of gene editing as a molecular design tool in crops. The unprecedented ability of crop genome editing has led to exciting and tremendous advances in both basic research and crop breeding. Due to its simplicity, versatility, and robustness, genome editing has made it a powerful tool for precise genetic improvement via gene knockout, replacement, point mutations, knock-in, targeted gene regulation, and other modifications at desire gene loci in crops [45]. Each of the targeted mutation types mentioned here holds great potential for crop genetic improvements and biotechnological applications. Genome editing is also useful for construction of high-throughput mutant library [36]. A number of technical hurdles with genome editing, involving knock-in efficiency, delivery systems and mutant specificities, still remain to be overcome for a wider application of genome editing technologies across crop species. Synthetic biology, systems biology and functional genomics, coupled with the development of genome editing technologies, next-generation sequencing and many other related technologies, will enable the engineering of advanced crops by design. Besides, a worldwide CRISPR patent landscape shows strong geographical biases, where China is now one of the most important players by surpassing the United States as the biggest holder in plant CRISPR intellectual properties in the world [46]. However, ambiguities regarding the regulatory status of genome editing techniques across countries have confused regulators and product developers using genome editing tools for crop improvement [47]. China’s conservatism and hesitation in application policy on crop genome editing have obviously limited its own technological advantages, stopping the leading efforts in crop genome editing from transforming into crop productivity. Genome editing differs from transgene technology due to its significantly different technical characteristics. For ribonucleoprotein based genome editing, no any DNA participates during the whole process of generating the target mutation. In this regard, a classified regulatory framework should be proposed and established for genome-editing based genetic improvement.

Acknowledgments

The editors of this special issue thank all authors for their contribution and reviewers for their comments and suggestions for improving manuscripts. We thank the Editorial Office of The Crop Journal for their efficient handling and processing of the manuscripts selected for this special issue. We also acknowledge the support to this special issue from National Engineering Laboratory for Crop Molecular Breeding.

REFERENCES


Y. Zhang, X. Wang, Y. Luo, L. Zhang, Y. Yao, L. Han, Z. Chen, L. Wang, Y. Li, OsABA8ox1, an ABA catabolic gene, suppresses root elongation of rice seedlings and contributes to drought response, Crop J. 8 (3) (2020) 480–491.

S. Huang, S. Xin, C. Xie, J. Han, Z. Liu, B. Wang, S. Zhang, Q. Wu, X. Cheng, Mutagenesis reveals that the rice OsMPT3 gene...
is an important osmotic regulatory factor, Crop J. 8 (3) (2020) 465–479.

