




Genome editing in plants: a tool for precision breeding and functional genomics

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Abstract

Genome or gene editing (GE) involves a repertoire of innovative molecular techniques that make use of sequence-specific nucleases (SSNs), for the precise modification of an organism's genome sequences. The CRISPR/Cas-based GE system, associated with Clustered Regularly Interspaced Short Palindromic Repeats, has emerged as a potent addition to the expanding genomics toolkit. It enables precise mutagenesis, gene knockouts, multiplex gene editing, and the manipulation of gene expression in plants. Undoubtedly, the application of CRISPR/Cas-based GE in plants has brought about a revolution in basic research, aiding in our understanding of gene functions and significantly advancing applied crop research. This, in turn, underscores its immense potential for crop improvement. Against this backdrop, the current Special Issue on "Genome Editing in Plants: A Tool for Precision Breeding and Functional Genomics" represents a timely effort to assemble a group of leading experts in the field of plant genome editing. This compilation includes a commentary article, two original research papers, and eleven review articles and is expected to bring about substantial progress in the field of plant science, particularly in the domain of genome editing.

Keywords Bioinformatics · CRISPR/Cas · Crop improvement · Genomics · Genome editing

Abbreviations

Acr	Anti-CRISPR
Cas9	CRISPR associated protein 9
CRISPR	Clustered regularly interspaced short palindromic repeats
GE	Genome or gene editing
gRNA	Guide RNA

MNs	Meganucleases
SSNs	Sequence-specific nuclease
TALENs	Transcription activator-like effector nucleases
ZFNs	Zinc-finger nucleases

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Genome or gene editing (GE) encompasses an array of novel molecular techniques that utilize sequence-specific nucleases (SSNs), such as transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and meganucleases (MNs), to precisely modify the genome sequences of an organism. The Clustered Regularly Interspaced Short Palindromic Repeats-associated protein (CRISPR/Cas)-based GE system has been a powerful addition to the growing toolbox of genomics, enabling targeted mutagenesis, gene knockouts, multiplex gene editing, and manipulation of gene expression in plants. It has been a decade since the development of the first gene-edited plant using homing endonucleases and ZFNs. Undoubtedly, the application of CRISPR/Cas-based GE in plants has revolutionized basic research, aiding in understanding gene functions and providing significant momentum to applied crop research, thereby demonstrating its immense potential for crop improvement.

Crop improvement has greatly benefited by adding valuable traits or removing undesirable ones. CRISPR/Cas-based genome editing holds significant promise for plant researchers, due to its simplicity and precision in executing genomic changes. In this backdrop, the current Special Issue on "Genome Editing in Plants: A Tool for Precision Breeding and Functional Genomics" is a timely attempt to bring together a group of elite experts working in the field of plant genome editing to contribute their research outcomes to this compilation. This special issue comprises a commentary article, two original research and eleven review articles. The contributors of the special issue are mostly from national and international laboratories engaged in the research pertaining to genetic modification including GE crops.

Research is underway to utilize powerful GE tools for the development of various crops, including cereals, legumes, oilseeds, fruits, vegetables, flowers, ornamentals, underutilized desert species, vegetatively propagated species and tree crops, including plantation crops. The aim is to enhance crop yield, improve tolerance to both biotic and abiotic stresses, and increase their nutraceutical potential. A glimpse into the scientific articles within this special issue showcases the significant impact of genome editing on crop improvement programs, both at a national and international level (Table 1).

Bioinformatics advances in CRISPR-Cas technology

The rise in popularity of CRISPR/Cas9-mediated genome engineering approaches has spurred the development of numerous *in silico* tools aimed at enhancing gene editing efficiency and minimizing off-target effects. Balakrishnan et al. (2023) shed light on several such online tools available to aid researchers involved in various aspects of genome editing, emphasizing their utility in this special issue. Furthermore, Sharma et al. (2023a, 2023b) review the application of computational tools in gaining a better understanding of CRISPR/Cas9-mediated cellular complexities and their role in crop improvement. In addition, novel machine learning-based models are reported to screen interactions between Anti-CRISPR (Acr) and Cas proteins, aiming to discover natural inhibitors of Cas proteins. This approach seeks to enhance the efficiency of CRISPR-Cas technology by controlling off-target cleavage activities (Murmu et al. 2023).

Genome editing stands as an invaluable tool to uncover the functions of novel genes, alleles, and haplotypes, which can be strategically employed in crop improvement programs. In the realm of functional genomics, the utilization of gene editing techniques and the array of technological advancements available for exploring the gene-function

Table 1 List of articles in this special issue

S. No.	Title of Manuscript	Authors	Article type
<i>Bioinformatics advances in CRISPR-Cas technology</i>			
1	Computational tools and scientometrics for CRISPR-based genome editing	Balakrishnan et al.,	Review article
2	Prediction of protein–protein interactions between anti-CRISPR and CRISPR-Cas using machine learning technique	Murmu et al.,	Original article
3	A review on bioinformatics advances in CRISPR-Cas technology	Sharma et al.,	Review article
4	Precision genetic technologies for cereal functional genomics	Biswal et al.,	Review article
5	Strategies to improve genome editing efficiency in crop plants	Mohannath et al.,	Review article
<i>Genome editing in crop improvement</i>			
6	Accelerating crop domestication through genome editing for sustainable agriculture	Pattnaik et al.,	Review article
7	Improvement of floricultural traits in ornamental crops using genome editing tools	Jadhav et al.,	Review article
8	Genetic manipulation of microRNAs: approaches and limitations	Sharma et al.,	Review article
9	Applications and challenges of harnessing genome editing in oilseed crops	Vaikuntapu and Kumar	Review article
10	Genome editing for vegetatively propagated crops improvement: a new horizon of possibilities	Lakhani et al.,	Review article
11	Genome editing advancements in potato (<i>Solanum tuberosum L.</i>): operational challenges and solutions	Mali et al.,	Review article
<i>Original articles and case studies</i>			
12	Low phytate soybean: Next generation metabolic engineering using CRISPR-Cas 9 genome editing technology	Krishnan et al.,	Original article
13	CRISPR/Cas9 mediated editing of phytoene desaturase gene in squash	Thakur et al.,	Original article
14	Nutraceutical and flavor profiles in underutilized desert legumes of India: gene editing strategies towards sustainable food development	Joshi et al.,	Commentary
<i>Regulatory framework</i>			
15	The evolving landscape of global regulations on genome edited crops	Vaikuntapu et al.,	Review article

relationship in cereal crops are enumerated by Biswal et al., (2023). In addition to the benefits of simplicity, high specificity, and cost-effectiveness, enhancing the efficiency of the CRISPR-Cas9 system has emerged as a significant area of research focus as described by Mohannath et al. (2023) in this issue.

Genome editing in crop improvement

This special edition features review articles that outline the progress made in genome editing across various species, including ornamental plants, oil seeds, vegetatively propagated crops, and potato. Accelerating crop domestication through the genome editing route is a compelling application of this approach, as highlighted by Pattnaik et al. (2023). The article discusses the potential of genome editing in overcoming the limited genetic diversity present in cultivated varieties. By doing so, crop wild relatives can be harnessed for their invaluable agronomic traits, facilitating the accelerated improvement of cultivated crops and making it a reality. Similarly, CRISPR-Cas9-based genome editing is anticipated to have a significant influence on the breeding and genetic manipulation of ornamental crops. Jadhav et al. (2023) have outlined numerous applications of genome editing in altering floricultural traits, including flower color, size, shape, scent, agronomic traits such as disease resistance, and post-harvest traits like vase life, among others. Despite the availability of over 200 whole genome sequences of ornamental crops, only a few have been extensively researched using genome editing, emphasizing the vast potential for the application of modern breeding tools in these crops.

The role of small RNAs, especially miRNAs, in the endogenous genetic regulation of various biological processes and pathways has gained recognition. Understanding miRNA function and fine-tuning miRNAs for the desired phenotype necessitate the use of genetic engineering approaches. Sharma et al. (2023) have highlighted the editing of miRNAs using ZNF proteins, meganucleases, TALENs, and CRISPR/Cas9, and its implications for crop improvement.

The utilization of genetic engineering for enhancing oilseed crops has faced limited success due to their *in vitro* recalcitrance nature. This challenge is particularly more pronounced in oilseed crops like castor, sesame, jatropha, which hold significant importance in the Indian context. Therefore, this special issue presents a comprehensive compilation of potential applications and gene targets for genome editing in oilseeds (Vaikuntapu and Kumar 2023). In a review by Lakhani et al. (2023), the role of CRISPR/Cas-based genome editing in augmenting the nutrition, agronomic performance, and industrial value of clonally

propagated crops is discussed. The article also sheds light on various barriers, such as polyploidy, low transformation efficiency, and the absence of high-throughput screening methodologies, that hinder the adoption of GE technologies in these crops. The authors also propose potential ways to overcome these obstacles.

In another significant food and commercial crop, potato, Mali et al. (2023) summarize recent advancements achieved through the adoption of the CRISPR/Cas9 system. The article highlights various traits, such as the development of amylase-free and steroid glycoalkaloids-free, starch-resistant potatoes, among others. Additionally, the article describes operational challenges and potential bottlenecks encountered in genetic manipulation of potatoes, which are applicable to other vegetatively propagated crops as well.

Case studies

Krishnan et al. (2023) report on a CRISPR/Cas9 modification system using a highly effective chimeric gRNA, showcasing the development of low phytate soybean with immense potential for the food and feed industry. The adoption of gene editing tools to explore underutilized or neglected legumes grown in the Indian desert regions for the supply of nutrients and health-benefitting nutraceuticals is notably demonstrated by Joshi et al. (2023). The article also underscores potential areas of research and identifies gaps that warrant the attention of the research and academic communities in utilizing the underutilized legumes of semi-arid tropics. CRISPR/Cas9-based gene editing of phytoene desaturase gene in squash provides a valuable platform for rapid genetic modification of cucurbitaceae species (Thakur et al. 2023).

Regulatory framework

Compared to conventional transgenic crops, genome editing has the advantage of potentially bypassing stringent regulatory procedures due to its non-transgenic approach. Many countries seem to share a consensus that non-transgenic edited plant lines may be regarded similarly to conventionally bred varieties, potentially requiring no additional regulatory framework. Consequently, many country-specific legislations advocate for the exemption of genome-edited crops from GMO regulations governing crop plants). In this context, a review by Vaikuntapu et al. (2023) provides an overview of the current legal and regulatory framework worldwide for genome-edited plant varieties.

Concluding remarks

This special issue serves as a comprehensive compilation, encompassing selected review articles, commentary, original research articles that include wet lab experiments and computational approaches, and incisive opinions. It is anticipated that this compilation will significantly advance the field of plant science, especially in the realm of genome editing. We, the editors, extend our sincere gratitude to all the authors for their valuable contributions to this special issue. Additionally, we acknowledge the indispensable support from the peer-reviewers who diligently assessed the suitability of the manuscripts submitted to this special issue and provided valuable suggestions to authors for enhancing the quality of their work. This special issue owes its existence and success to the unwavering support and encouragement of the Editor-in-Chief, Dr. Ramamurthy Srinivasan. The Editors of this special issue express their sincere gratitude for his invaluable guidance and encouragement. Additionally, the Editors acknowledge and appreciate the indispensable support received from Dr. Mamta Kapila (Executive Editor), Dr. Karen Grace Corpuz (Journal Editorial Office), Ms. Jeyapradha Saravanan (Production Editor), and their dedicated teams at Springer. Their assistance and expertise have played a vital role in bringing this special issue to fruition.

Declaration

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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