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Genome Editing Technologies for Efficient Use of Plant Genetic Resources

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Plant genetic resources (PGRs) are the basis for food and nutritional security, and enhanced utilization of these resources is of paramount importance for genetic improvement of crops. Till date the use of PGRs has been limited in conventional breeding programmes for developing widely adapted crop varieties. In the recent past, genome-wide association studies, genomics and functional genomics approaches using diverse germplasm accessions have facilitated discovery of novel quantitative trait loci (QTLs), genes and alleles associated with useful agronomic traits for use in crop improvement through molecular breeding and genetic engineering. More recently, genome editing has emerged as a new plant breeding technology with great potential for enhancing the use of PGRs, for addressing the challenges of climate change, malnutrition, environmental security, achieving SDGs by 2030, and to sustainably feed 10 billion people by 2050. In this article, an attempt has been made to highlight the use of CRISPR-based genome editing technologies for crop improvement through the use of PGRs.

Introduction

Plant genetic resources (PGRs) have contributed significantly to the development of modern high-yielding cultivars, through the use of conventional breeding methods, which have contributed to the dramatic increase in productivity of major crops since the middle of the 20th century. In recent times, the importance of PGRs has increased manifold for achieving climate resilience and ensuring sustainability in crop production. For instance, the rice landrace FR13A from Odisha, India was identified as a source for introducing *SUB1 (SUBMERGENCE1)* quantitative trait locus (QTL) into mega-varieties for the development of Sub1 rice with submergence tolerance (Bailey-Serres *et al.,* 2010).

While conventional plant breeding methods have played a vital role in developing new crop varieties for increasing food production, advances in genomics, genetic engineering, molecular breeding and the recent development of new plant breeding technologies have enabled breeders to address the challenges of climate change, malnutrition and environmental security. The remarkable progress in plant genomics, sequencing and bioinformatics offers enough opportunities for mining germplasm collections, discovering new genes, elucidating gene function, and identifying superior alleles for use in the new breeding technologies like genome editing (Katiyar *et al.,* 2012, Halewood *et al.,* 2018). Genome editing, which was invented in 2012, is leading to a new revolution by accelerating the pace of genetic improvement of crops, and it is turning out to be indispensable technology for achieving climate resilience and sustainable agricultural development in this 21st century. Genome editing is already revolutionizing crop improvement by introducing desired changes in the plant's native genes with a high level of precision, accuracy and efficiency for developing new crop varieties with improved traits, without the need for introduction of foreign genes.

With the advent of genome editing as the next generation crop breeding technology, plenty of opportunities are now available to develop varieties with increased use efficiency of nutrients, water and radiation, and to create crops with inbuilt resistance to emerging pathogens and environmental stresses on a much faster timescale, not practically feasible with the use of conventional breeding approaches. Further, gene editing technologies could simplify the use of PGRs including crop wild relatives (CWR) and landraces in breeding programmes for expanding specific allelic variations and also eliminating linkage drag of undesirable traits.

Germplasm, Genomics and Pangenomics Facilitate Genome Editing for Crop Improvement

Over 7 million accessions of different plant species are conserved globally in various genebanks. However,

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utilisation of this valuable resource is a major concern today by breeders with only < 1% use reported in crop improvement programmes. Genome editing offers a great promise to enhance the use of PGRs for crop improvement (Fig. 1).

The next-generation genome sequencing (NGS) technologies are contributing significantly to characterise the genebank accessions at genetic and molecular level, and to identify the genetic variation for enhancing their use in crop breeding. To capture the entire genetic variation in crop germplasm collections including landraces and wild species, advances in sequencing technologies have permitted whole genome sequencing of the diverse accessions of different crop species. In the recent past, efforts have been made to re-sequence 3,000 accessions of rice and over 3,000 accessions of chickpea generating pangenome/gene sequencing data across species with a view to linking genetic variation with traits/phenotypes of agronomic importance (Wang *et al.,* 2018; Varshney *et al.,* 2021). For pangenomics, multiple accessions of a crop species are re-sequenced to determine structural and copy number variations associated with traits such as resistance to biotic and abiotic stresses that are useful for improving crop productivity in variable environments. Since a vast number of genes are not captured in a single reference genome, pangenomics assumes significance (Zanini *et al.,* 2022). Information on pangenomics in major food crops is already being utilised in crop improvement programmes. Efforts are currently in progress in crops like rice, maize, soybean, chickpea, *Brassica* species, tomato and potato. More studies on pangenomics need to be conducted in other crop species, including CWR for identifying relevant genes and alleles associated with stress resistance and other agronomic traits. This will allow application of genome editing for the enhanced use of crop diversity for bringing novel genes/alleles from CWR and landraces to the cultivated gene pool for addressing the issues of climate adaptation of crops, shrinking natural resources, and increasing input use-efficiency, and for improving nutritional quality and architectural features of crops (Gasparini *et al.,* 2021). Additionally, introducing the yield determining attributes from wild species to the cultivated crop genotypes or neo-domestication of wild species is now technically feasible using the CRISPR-based genome editing, base editing, prime editing and/or CRISPR-Combo approaches (Fig. 2).

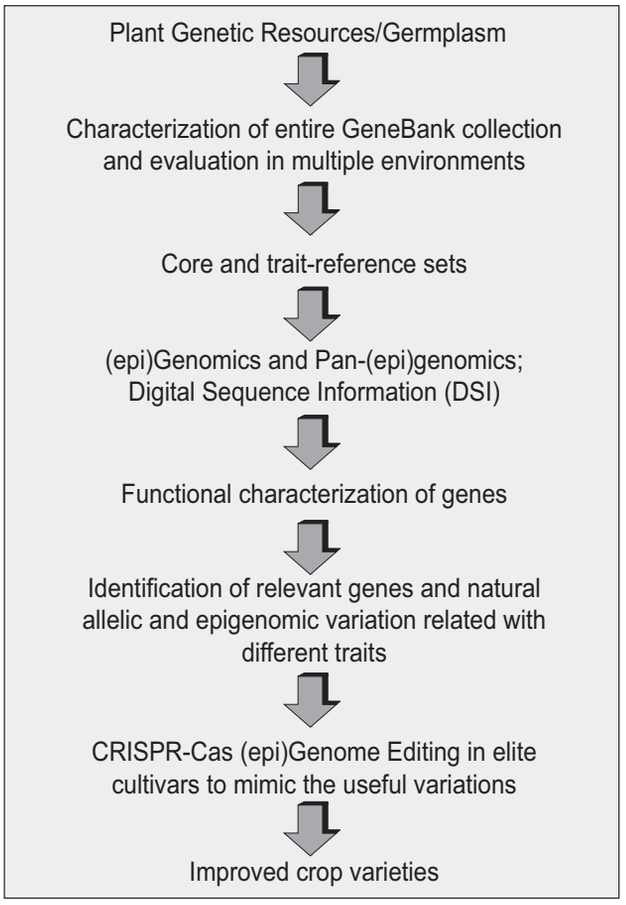

Fig. 1. Plant genetic resources as source of genomic information for crop improvement through (epi)genome editing

Further, during the process of domestication several deleterious alleles have accumulated in the breeding lines and crop cultivars, affecting the fitness of the cultivated crops. Such deleterious alleles, for instance, have been identified by Varshney and his group in a recent study in chickpea including *Cicer* species, landraces, and superior cultivars, which can now be precisely removed through the use of various genome editing approaches (Varshney *et al.,* 2021, Bohra *et al.,* 2021).

Additionally, PGRs can also serve as a source for exploring epigenomic variation for crop improvement. The epigenome refers to the set of chemical modifications (e.g., DNA methylation, Histone acetylation) in the genome involved in regulating gene expression and holds great potential for crop improvement. It can be a target for the manipulation of regulation of gene expression and, consequently, the phenotype. As pangenome studies on PGRs are conducted to better understand the genetic variation, similar "pan (epi)genome" studies on PGRs can

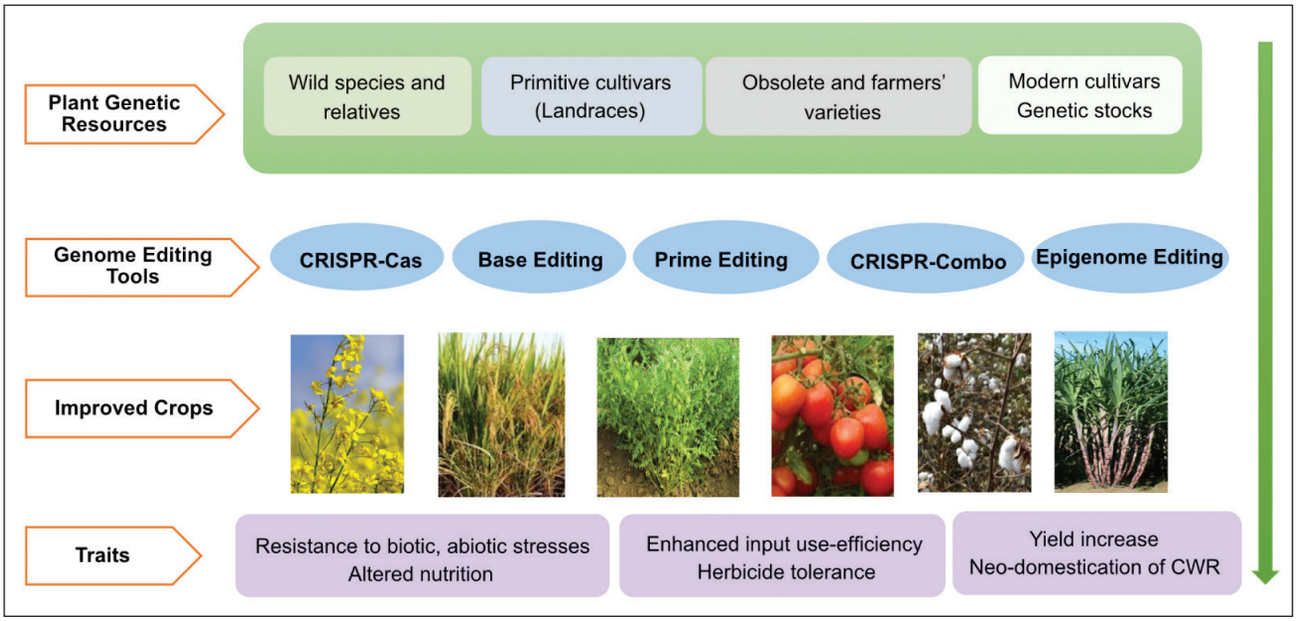

Fig. 2. Utilization of plant genetic resources for crop improvement through genome editing and epigenome editing

be useful to pave the way for modifying the epigenome and exploring the PGRs exhaustively (Fig. 2).

CRISPR-based Approaches for Crop Improvement

The CRISPR-Cas system creates a double-strand DNA break, which is repaired by cell's DNA repair machinery called NHEJ (Non homologous end joining). Through this repair mechanism, deletion or insertion of random nucleotides occurs, knocking out the gene function. The technology also allows precise substitution of nucleotides and insertion (knock-in) of DNA sequences at a predefined position using homologous template sequences through a homology-directed repair (HDR) pathway.

However, HDR-mediated genome editing is less efficient. To circumvent these issues, researchers prefer using base editing to introduce precise point mutations to introduce a trait, as recently shown by Gao and her group to confer resistance to a variety of herbicides in wheat (Zhang *et al.,* 2019). Similarly, a precise single-nucleotide change in *eIF4E1* gene using cytosine base editor rendered *Arabidopsis* plants immune to a disease. In base editing, one single base is converted to another through base editors without the double strand DNA break. Cytosine base editors for transforming C-G to T-A and adenine base editors for converting A-T to G-C are currently in use for crop improvement. This editing process has vast potential for incorporating useful agronomic traits in diverse crop species, thus enhancing the use of PGRs for crop improvement (Molla *et al.,* 2021).

Another approach called prime editing has been developed with ability to perform sequence deletion, addition and substitution. This method requires no donor DNA as template or double-strand breaks. It employs catalytically impaired Cas9 endonuclease (nCas9), that nicks only one DNA strand, fused to reverse transcriptase and the prime editing guide RNA (pegRNA), which carries specified edits and the target site information. This allows direct transfer of new genetic information as desired edits from the pegRNA into a specified genomic site (Molla *et al.,* 2021). To overcome the low efficiency of this system, a new version of prime editing, enpPE2 has been recently developed and tested in rice (Li *et al.,* 2022).

Further, to overcome limitation of the classical CRISPR-mediated genome editing system of deletion or insertion confined to single genes, a novel breeding approach has been developed more recently by Yiping Qi and his group and called it CRISPR-Combo for multiple gene editing and simultaneously altering gene expression of other native genes without any deletion/insertion (Pan *et al.,* 2022). This system is also useful for enhancing *in-vitro* regeneration efficiency of recalcitrant crops varieties, in addition to simultaneously editing multiple genes, as demonstrated in poplar cells (Pan *et al.,* 2022).

Besides the above-mentioned approaches, epigenome editing is another novel approach for crop improvement (Gardiner *et al.,* 2022). Targeted manipulation of epigenetic marks is called epigenome editing and is achieved by combining sequence-specific DNA binding modules with effectors that can add or remove these marks from the genome (Gardiner *et al.,* 2022). Several epigenome editing tools have been developed using CRISPR-Cas9 systems to activate or repress gene expression by modifying the epigenetic marks in plants (Gallego-Bartolome *et al.,* 2018; Ghoshal *et al.,* 2021; Lee *et al.,* 2019; Papikian *et al.,* 2019). For example, inactive catalytic versions of Cas9 (dCas9) were combined with catalytic domains of DNA methyl transferases to silence gene expression by adding repressive DNA cytosine methylation marks (Ghoshal *et al.,* 2021; Papikian *et al.,* 2019). In a similar approach, the catalytic domain of human TEN-ELEVEN TRANSLOCATION 1 enzymes is combined with dCas9 to develop tools for activating gene expression by removing DNA cytosine methylation (Gallego-Bartolome *et al.,* 2018). Initially, these tools were tested and generated in the model plant *Arabidopsis thaliana*; currently, research is underway to expand their use to crops.

Conclusions and Future Perspective

In conclusion, it may be highlighted that genome editing has great potential for the enhanced use of crop diversity for bringing novel resistance genes/alleles from CWR and landraces to the cultivated gene pool for addressing the issues of climate adaptation of crops, shrinking natural resources, and increasing input use-efficiency. Direct genome editing in elite cultivars is likely to replace backcross breeding for altering alleles and creating optimal genetic variation required for desired traits.

However, to harness the full potential of these technologies, urgent attention will be needed for QTL/gene discovery through functional genomics using the diverse accessions of major crops for key traits (Das *et al.,* 2016). This requires that enough emphasis is given for characterisation of entire germplasm collections followed by genomics and pangenomics, and functional characterisation of genes for establishing precise links between phenotype and the genomic regions/genes or genetic elements in a given crop (Archak *et al.,* 2016; Kumar *et al.,* 2017; Phogat *et al.,* 2020).

Further, it is desired that simplified, reproducible *in-vitro* regeneration and genetic transformation systems are developed in a range of agriculturally important crops and elite cultivars for efficiently generating genome-edited crop events. Similarly, successful genetic transformation of wild crop species and innovations in developing simplified methods for introducing genetic material will prove useful for accelerating crop domestication with improved traits related to climate resilience. These novel breeding technologies in due course of time could pave the way for enhanced utilisation of germplasm accessions conserved globally in different genebanks for sustainable food and nutritional security.

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