DOI 10.18551/rjoas.2021-05.15

GENOME EDITING TECHNOLOGY IN PLANT BREEDING: A REVIEW

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ABSTRACT

Global population growth is demanding for more food production in the future. The selection of the plant for cropping has led to the loss of valuable genotypes. Genome-editing technology is the recent advances in plant breeding which allows the insertion, deletion or substitution of the specific loci in the target host cell. Among the homologous recombination (HR) technology, transcription activation-like effector nuclease (TALEN), zinc finger nucleases (ZFN) and clustered regularly interspaced short palindromic repeats (CRISPR); the most popular these days is CRISPR/Cas. Here, we review the methods of genome-editing, their applications, potentials, and the regulatory issues related to genetically modified organisms.

KEY WORDS

Genome-editing, homologous recombination, CRISPR/Cas, indels, crop improvements.

Plant breeding is the art and science of changing the genetics of plants for the benefit of humankind. The work in plant breeding can be accomplished by the different techniques; from simply selecting plants having desirable characteristics as a propagative material, to more complex molecular techniques.

The practice of plant breeding has started near the beginning of human civilization. Since then it is practiced worldwide. Nowadays, plant breeders engaged in different institutions, governments, organizations, universities, and industries associated with various crops or research centers are practicing breeding. Classical breeding techniques use interbreeding of closely related individuals for producing new varieties of crop or crop lines having desirable characteristics. These techniques are labor-intensive and time-consuming. To address this problem, there is need of trails for molecular breeding for safety and traits evaluation.

Conventional plant breeding begins with genetic engineering through extracellular manipulated DNA for constructing plasmid vector to incorporate gene of interest to the host cell. Gene of interest is the specific sequence of DNA which is transferred to the selected organism. The entire plasmid or the fragments of DNA can be delivered to the host cell by Agrobacterium-mediated gene transfer or by particle bombardment method [1]. X-rays-induced random mutagenesis has also been a tool for conventional plant breeding. All these breeding as; traditional breeding, intergeneric crosses and conventional breeding are non-specific as they transfer a large part of genome instead of a single desired gene. Genetic modification technology has been developed since the mid-1990s [2], used for the transfer of specific genes to the elite varieties/genotypes for developing the high value crops. These transgenic crops were mainly developed by introducing resistance gene against pests and disease as example: Bt-cotton and Bt-maize, or for herbicide tolerance e.g. Bt-cotton and for the modification of plant products enriching nutrients e.g. Golden Rice. However, many

concerns about the genetically modified crops regarding health and environment are there and for the endorsement of deregulation to be commercialized, it requires noteworthy time and costs [3]. The Cartagena Protocol on Biosafety in 2000, came into force since 2003 and it covers the discharge of GMOs in the environment and also for its movement across the different terrain worldwide for food production [4].

New plant breeding technologies have emerged for alleviating fears which are associated with genetically modified crops. One of the recent advances in plant breeding is genome editing which ensures the manipulation of the target genome for achieving a therapeutic effect [5]. It can be used to modify a gene directly in the genome of selected plant [6-8]. Observance of more precise breeding; genetic engineering technology using transcription activator-like effector nucleases (TALENs), site-directed mutagenesis (SDNs), targeted gene inactivation/modification, zinc-finger nucleases (ZFNs), clustered regularly interspaced short palindromic repeats associated Cas nucleases (CRISPR-Cas), have developed for accelerating breeding by attaining desirable traits [9]. Among these methods the most popular method these days is CRISPR-Cas system with Cas9 or Cas12a associated proteins. CRISPR Cas genome editing technology is becoming the technique of choice among people because of its various advantages over other methods as easy to use, flexibility in editing genome, and its facility to cleave methylated loci [10; 11].

METHODS OF GENOME EDITING

Homologous recombination. When the DNA is damaged producing gaps in DNA, Double stranded breaks (DSBs) and interstrand crosslink in DNA (ICLs); they need to be repaired for providing high-fidelity in all forms of life. This repairing process of DNA is known as homologous recombination. During homologous recombination there is the exchange of genetic material between the two strands of DNA containing similar base sequences [12] (Figure 1).

Figure 1 – Homologous recombination, Source: National Human Genome Research Institute

This occurs naturally in eukaryotic organisms, certain viruses and bacteria and it is a potent tool for genetic engineering. When the double stranded breaks were introduced by meganucleases which have a recognition site of 18 bp, a paradigm shift was there in 1900 in the field of breeding [13]. Homologous recombination (HR) helps in preserving the genome by duplication and support for DNA replication [14]. Double stranded breaks can be a major threat to stability of the genome as its failure to repair can result in loss of chromosome, apoptosis, carcinogenesis and chromosomal rearrangement [15]. By using meganucleases, position for the homologous recombination was predicted exactly [16].

Homologous recombination can be presynapsis, synapsis and postsynapsis. The paired chromosomes from both parents have the opportunity to flip flop or cross-over one another which results in the shuffling of genetic material. This crossing over of genetic material produces homologous recombination.

Zinc-finger nucleases (ZFNs). Zinc finger nuclease is an endonuclease which consists of artificially designed zinc finger protein fused to cleave the domain of FokI restriction enzyme [17]. Zinc finger nucleases is replaced by the site-directed nucleases (SDN) and the mode of action of transcription activator like effector nucleases (TALENs) and meganucleases is similar to the mode of action of ZFNs. These TALENs, ZFNs and meganucleases constitute SDN [18]. The DNA binding and restriction proteins which are designed for recognizing specific DNA is known as site-directed nucleases (SDNs) [2].

Figure 2 – Design of Zinc finger nucleases [19]

Figure 3 – An overview of DNA double-stranded break and the consequent repair mechanism [20]

ZFN bound to its target and each contains the FokI domain linked to an array of 3-6 ZFNs which are specially designed to recognize the sequences and locate cleavage site. The optimum spacing between these half sites is 5-7bp.

Transcription activator-like effector nucleases (TALENs). It is the non-specific DNA cleaving nuclease which is fused to a DNA binding region and can easily be engineered such that it can target any sequence essentially [7]. It provides the insertion, deletion or substitution of a gene in the target loci precisely to alter the sequence.

In figure 3 shown that FokI dimer is fused by a single polypeptide. The double stranded breaks generated by TALENs can be repaired by homologous recombination which gives a way for desired genome editing. The non-homologous end joining leads to the generation of small interstitial deletion/addition or substitution (INDELs). HR with double-stranded repair template -DNA leads to precise gene editing and gene replacement.

Clustered regularly interspaced short palindromic repeats (CRISPR). The CRISPR/Cas system is an RNA-mediated defense mechanism of bacteria and archaea for the defense against the viral phages. It consists of CRISPR repeat spacer arrays and Cas9-a protein. It is also known as RNA guided DNA interference [21]. The two components of CRISPR/Cas9 systems are: Cas9 nuclease and single guide RNA (sgRNA). Single guide RNA after recognizing the target DNA sequence which is located by proto-spacer adjacent motif (PAM); it generates double-strand breaks (DSB) in a specific site. One of the two DNA repair pathways is non-homologous end joining (NHEJ) and homology-directed repair (HDR) [22]. The double-stranded breaks can be repaired by producing nucleotide insertions/deletions (indels) or substitutions by non-homologous end joining (NHEJ). Another way to repair double-stranded break include homology-directed repair (HDR) at the time of DSB formation when the homologous donor templates are present (Figure 4).

Figure 4 – CRISPR/Cas for genome editing:

a) Two system of CRISPR/Cas as: Cas9 and Cpf1. b) depending on the double stranded breaks there are multiple outcomes of CRISPR/Cas: I, II and III in the NHEJ repair pathway which is dominant and IV and V in HDR pathway. c) Various applications of dCas9 based genome manipulation. dCas9 is fused with other proteins, transcriptional activators/repressors, fluorescent proteins and epigenetic effectors used for gene activation, gene repression, epigenome editing and genome labelling. d) Cas13a is used for RNA targeting and dCas13b is fused with adenosine deaminase acting on RNA (ADAR) is used for RNA editing [23].

Application and potential of genome editing in present plant breeding. The genome-editing by non-homologous end joining (NHEJ) mainly leads to loss of functional mutation causing frameshift and protein truncation [13]. The amino acid located in the functional domain may also cause the possibility of substitutions which may lead to elusive functional changes or to complete loss of function. However, after the development of CRISPR/Cas, site-directed mutagenesis has been developing at a faster rate. For cultivating crop in a wide topographical area, the important factors are time to flowering and sensitivity to day length. QTL sequencing approach in tomato for detecting flowering time between wild type and cultivated tomato using SNPs showed that plant overexpressing SELF PRUNING 5 G (SP5G) to be very late flowering as compared to those of two mutants edited with CRISPR/Cas [24]. Also for tomato, poor microsporogenesis and low yield under moderately high or low temperatures prevented year-round cropping. The CRISPR/Cas technique for editing EMS mutated AGAMOUS-LIKE 6 gene (SIAGL6) lead facultative parthenocarpy and fruiting in tomato under high temperature [25]. Similarly, in a commercial breeding program, double haploid (DH) production for inbred line development [26], haploid production in corn [27], temperature-sensitive genic male sterility (TGMS) and photoperiod sensitive genic male sterility (PGMS) for hybrid production in China [28], and single-strand oligonucleotide and CRISPR/Cas for developing herbicide tolerance in flax [29], etc. are very important traits in agriculture brought by genome editing technology. The applications of genome editing technology for precision breeding are:

1. Knockout-mediated crop trait improvement: Increasing yield; Improving quality; Biotic and abiotic stress resistance;
2. Speeding hybrid breeding;
3. Crop trait improvement through Knock-in and replacement;
4. Application of base editors in plants;
5. Fine tuning gene regulation in plants;
6. Antiviral plant breeding strategies;
7. High throughput plant mutant libraries.

Table 1 – Applications of genome-editing technology [30]

Plant species	Application perspectives
BIOTIC STRESS TOLERANCE	
Virus stress tolerance	
<i>Arabidopsis thaliana</i>	Potyvirus resistance (TuMV)
<i>Arabidopsis thaliana</i> and <i>Nicotiana benthamiana</i>	Beet severe curly top virus (BSCTV) tolerance
<i>Nicotiana benthamiana</i>	Tomato yellow leaf curl virus (TYLCV) resistance
	Virus tolerance
<i>Cucumis sativus</i>	Ipomovirus immunity, tolerance to the Zucchini yellow mosaic virus and Papaya ring spot mosaic virus
Fungus stress tolerance	
<i>Oryza sativa</i>	Blast (caused by <i>Magnaporthe oryzae</i>) tolerance
<i>Solanum lycopersicum</i>	Powdery mildew resistance
<i>Triticum aestivum</i>	Powdery mildew (<i>Blumeria graminis</i> f. sp. <i>Tritici</i>) resistance
Bacteria stress tolerance	
<i>Citrus paradise</i>	Citrus canker (caused by <i>Xanthomonas citri</i> subspecies <i>citri</i>) tolerance
<i>Citrus sinensis</i> Osbeck	Canker resistance
<i>Oryza sativa</i>	Bacterial blight (caused by <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>) tolerance
ABIOTIC STRESS TOLERANCE	
Herbicide tolerance	
<i>Arabidopsis thaliana</i>	Cold, salt and drought stress tolerance
	Glufosinate resistance and reduced trichomes formation
<i>Lotus japonica</i>	Bioavailability of soil organic nitrogen and capability to accommodate nitrogen-fixing bacteria intracellularly to fix its own nitrogen
<i>Linum usitatissimum</i>	Glyphosate tolerance
<i>Oryza sativa</i>	Herbicide resistance
	Herbicide tolerance
	Glyphosate tolerance
<i>Solanum tuberosum</i>	Reduced susceptibility to ALS-inhibiting herbicides
Drought stress tolerance	
<i>Zea mays</i>	Improved grain yield under field drought stress conditions

Potentials of genome editing. The potentials and future prospects of genome-editing can be the improvement of traditional crops for the development of novel crops [31]. Plants are the primary source of food for all living creatures and the diverse source of raw materials for medicines and industries. The major limiting factor for the growth of plants is nitrogen and the nitrogen content in plants is mainly located in nitrogen fixation (nif) genes [32]. The genome-editing mechanism can allow the transfer of genes from legumes to cereals, making them able to fix nitrogen by themselves and this will increase crop productivity [33].

Selective breeding practiced nowadays is creating a loss of valuable genotypes and this affects their fitness under assured environmental conditions [34]. The intensive breeding has also affected many crop cultivars as tomato and the stress due to biotic and abiotic factors can be managed by increasing tolerance in them. Their growth habit, nutritional characteristics, flowering and fruiting time and production can be accelerated without losing the original character of wild genotype [35]. Besides, improved delivery of genome-editing reagent in the target plant cells [36], improved specificity of CRISPR/Cas system in enabling the exclusion of off-target effects during seed multiplication, commercial crop production through the cleavage of off-target by Cas9 or Cpf1 in rice, cotton [37], and increased efficiency of precise gene editing by homology-directed repair and controlling invasive species with CRISPR/Cas-Based gene, etc. are the major potentials of genome editing in the present days.

Figure 5 – Comparison of breeding used in modern agriculture [23]

In genome editing, the improvement of a trait by precisely modifying the target gene or rearranging chromosomes in elite varieties requires less time than that of cross breeding, mutation breeding, and transgenic breeding.

Regulatory issue. Besides all the potentialities of genome editing techniques, their inappropriate use may cause societal problems and problems in the field of agriculture and environmental applications. With genome-edited crops, the occurrence of off-target mutations is one of the important issues in agriculture. Some off-target mutations might result in loss of function, immunogenicity or toxicity in food products, although no such evidence to date has been documented of any adverse effect of foods produced from genetically modified crops [38]. If such immunogenicity or toxicity is seen in agricultural products, the consequence of their consumption will be problematic. Some organisms modified with genome editing might seem to be identical with naturally occurring organisms, and this may lead to difficulty in characterizing them. Such organisms need to require scientific scrutiny before being released into the environment. If these organisms are unintentionally released

in the environment without risk assessments, they may affect local biome by seriously threatening native species [39].

CONCLUSION

The extraordinary capacity of genome editing has enabled us to generate diversity in plants and this diversity has led to broad application. In the changing world, the development of such technologies has created a powerful tool in precise breeding. Their ability for inserting a desired genome into the target host cell has enabled us to improve crops according to our needs and interests. Although having certain regulatory issue in agriculture and environment, the concept of synthetic biology advances in functional genomics and this in combination with genome editing techniques will allow advancement in crop breeding with the development of improved traits.

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