

GENOME EDITING: AN EMERGING TOOL FOR PLANT BREEDERS

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Abstract

Conventional plant breeding has contributed enormously towards feeding the world and has played crucial roles in the development of modern society. The conventional method creates variation by transferring genes between or within the species. In general, these methods are more expensive and takes more time, to overcome these limitations, new technology is required. Genome editing is a powerful tool for biotechnology applications, with the capacity to alter the function of any gene. With the availability of gene information for the majority of the traits, genome editing emerged as a potential to create a new variation with the introduction of any transgene. The important genome editing tools used nowadays are ZFNs, TALEN, Pentatricopeptide repeats protein, adenine base editor, RNA interference, and CRISPR/Cas9. These tools have opened a new era for crop improvement. Due to the complex genetic architecture of most traits, it is challenging to edit genes controlling them. To overcome these challenges, genome editing provides a broader perspective. Among the above-mentioned tools, CRISPR/Cas9 is the most powerful tool for gene editing. These technologies are being used to create abiotic and biotic resistance crop varieties.

Keywords: Conventional method, Cluster regularly interspaced short palindromic sequence, Genome editing, RNA interference, and TALEN.

1. Introduction

Genome editing involves the editing of targeted traits in organisms for the creation of new genetic variation. There are two methods to know the function of the gene namely forward and reverse

genetics. In forward genetics, the main target is to change a particular genotype for obtaining desired phenotypes while in reverse genetics we target the phenotype and then move towards its genotype. With access to map-based cloning and the T-DNA tag, it is possible to detect and screen mutations in the gene of interest to aid in forward genetics (Page and Grossniklaus, 2002). In reverse genetics, we can study a particular phenotype and then try to relate that with particular genotype effect and detect the candidate gene that has been expressed in the population. This approach also reveals some cells genes expressed in multi-environment with the help of bioinformatics tools or gene chip aiding in genetic transform technology involving the knockout gene or overexpression of the gene of interest (Takahashi *et al.*, 1994). The conventional breeding method creates the variation in the population using physical or chemical mutagens in addition to natural variation (Stadler, 1928; Li *et al.*, 2001; Wu *et al.*, 2005; Wang *et al.*, 2008; Talamè *et al.*, 2008; Suzuki *et al.*, 2008). These variations can be exploited in the form of a new variety. These variations involve point mutation, chromosomal mutation, translocation, and deletion in the genome of organism (Kao and Michayluk, 1974) and have been a more widely efficient tool for hybridization. This method enforces the trait to come from both parents that have been utilized in the crossing program. However, this technology can alter the sufficient degree of the genome but still we can't find the target modification easily with this method (Zhang *et al.*, 2017).

2. Why did we need it?

Conventional plant breeding has contributed enormously towards feeding the world and has played crucial roles in the development of any new cultivar for human consumption. Conventional breeding methods depend on using old tools such as natural selection, but it takes much more time to develop a new variety. This method cannot edit any gene in the genome, so it needs new technology to create new variation and for knockout of any unwanted gene.

3. Tools for genome editing

Pre-genomic breeding programs led to the development of stress-tolerant and high-yielding crop varieties. In the past, breeding programs were based on natural, hybridization, and mutant-induced genetic variations to select for favorable genetic combinations. The traditional breeding program

was based on mutagenesis using chemical compounds or irradiation, followed by screening for desired mutations, and has several drawbacks. Mutagenesis methods, intergeneric crosses, and translocation breeding are non-specific; and sometimes large parts of the genome are transferred instead of a single gene, or sometimes thousands of nucleotides are mutated instead of a single nucleotide. To overcome these problems a sophisticated tool is required for the creation of new variations. There are many tools for genome editing as described below.

3. 1 TALEN

Transcription activator-like effectors nuclease is an emerged tool for genome editing in crop plants (Ma *et al.*, 2015). TALEN uses a double-strand break (DSB) in a similar type to the use of zinc finger nucleases (ZFNs). Both TALEN and ZFNs contain nonspecific FOKI endonuclease. However, the FokI domains of ZFNs fuse with specific DNA-binding domains that are highly conserved repeats derived from transcription activator-like effectors [Ma *et al.*, 2015]. Approximately 50 genes have been targeted to induce mutation by TALEN in different crops, for example, Arabidopsis, soybean, barley, tomato, wheat, rice, tobacco, and maize, etc. (Ray *et al.*, 2013). TALEN scaffolds were optimized for high activity in plants [Zhang *et al.* 2013]. TALEN scaffolds are demonstrated by the mutagenesis for targeting a trait in Arabidopsis (Christian *et al.*, 2013), brachy podium (Shan *et al.*, 2013), tomato (Lor *et al.*, 2014) and wheat (Wang *et al.*, 2014). In recent times, TALEN created a heritable mutation in rice (Zhang *et al.*, 2014) and which will ultimately aid in plant breeding. In another study, TALEN has been used to engineer disease resistance for *Xanthomonas oryzae pv oryzae* by destroying the target sequence TALE effectors in rice (Li *et al.*, 2012). The FAD2 gene has been used to improve quality in soybean (Haun *et al.*, 2014). In wheat 3 homologous of MLO genes have been successfully knockout simultaneously for conferring heritable disease resistance for powdery mildew (Wang *et al.*, 2014). TALEN has been used to improve the fragrance (Shan *et al.*, 2015) and storage tolerance (Ma *et al.*, 2015) in rice. In potatoes, cold tolerance and processing traits are engineered by the TALEN. These studies are based on protein coding genes for mutagenesis but also have studied for non-coding genes and regulatory elements (Li *et al.*, 2012). There are some studies that have been conducted to generate chromosomal deletion of any crops plant (Christian *et al.*, 2013; Malzahn *et al.*, 2017).

3. 2 Zinc finger nuclease

Zinc finger nuclease (ZFNs) is a genome-editing tool involving the cleavage of target DNA that is designed to cut at specific sites of DNA sequence (Carroll 2011). ZFNs create a DSBs through the replacement of homologous recombination genes. Each ZFN consists of a DNA binding domain that is having two finger chain modules. The DNA cleaving domain has a FokI DNA nuclease domain, that recognized a unique pattern of 6-bp hexamer in the DNA sequence (Carlson *et al.*, 2012; Gupta *et al.*, 2012). These domains are joined together to form a Zinc finger protein. DNA cleaving and DNA binding domains were fused together, to make one unit work together as specific genomic scissors (Carlson *et al.*, 2012; Gupta *et al.*, 2012). ZFN originates the site-specific cut in double-stranded and breaks the DNA permanently to edit the genome (Carlson *et al.*, 2012; Gupta *et al.*, 2012). ZFN is only dependent on ZFP that can target a specific DNA sequence in the genome. The best possible structure provided by the Cys2His2 ZFP to develop suitable ZFNs that have a required sequence specificity (Pabo *et al.*, 2001). ZFP stabilizes the chelation of zinc ions to conserve the Cys2His2 amino acids that are having approximately 30 amino acids (Thakore and Gersbach 2015; Pavletich and Pabo 1991). The ZF motifs cover and bind the DNA sequence in its genome by incorporating a major groove in the double helix DNA (Pavletich and Pabo 1991). The ZF interacts with ZFN that have specific sequence interaction with specific the DNA sequence (Pavletich and Pabo 1991; Sahi and Berg 1995). In this triplet, the sequence can be bound by each finger. Linking multiples zinc finger motifs to form ZFPs is possible to bind longer DNA strands (Liu *et al.*, 1997; Kim and Pabo 1998). The methylase domain (M), FokI-cleavage domain (N), transcription activator domain (A), and transcription repressor domain (R) are fused with ZFP to form a ZFN [Kim and Cha 1998; Alwin *et al.*, 2005].

3. 3 Pentatricopeptide repeat proteins

The organellar genome has a low number of promoters. The organellar RNA number is more than that of nuclear RNA. However, the regulation of RNA cleavage, RNA splicing, and RNA editing is insufficient to control the expression of the organellar genome. RNA binding protein is developed by the organelles to regulate the gene at the post-transcriptional level (Small *et al.*, 2013; Schmitz-Linneweber and small 2008). The organellar genome has tetratricopeptide repeats protein

(PPR), that regulates post-transcriptional regulation. Pentatricopeptide repeat protein (PPR) is characterized by the tandem repeats motifs that consist of 35 amino acids (Schmitz-Linneweber and small 2008). PPRs have classes such as P-class (35 amino acids), L-class (35–36 amino acids), S-class (short, approximately 31 amino acids), and E-class (extended domain) [Barken and Small 2014; Shikanai 2006]. DYW tripeptide motif (Aspartate-Tyrosine-Tryptophan) is highly conserved by the PLS type PPRs at the C terminal ends that is the most likely show behavior of the editing domain (Lurin *et al.*, 2004). The DYW domain is having most of the zinc-binding affinity that is necessary to catalytic activity for the editing of any genome (Boussardou *et al.*, 2014). The I-class PPRs have amino acid residue that is involved in RNA binding for the targeted nucleotides. But the E domain is not having proper catalytic activity hence it helps to interact with protein to protein with editing enzymes (Okuda *et al.*, 2007). The helices form a solenoid antiparallel helix-turn-helix structure that provides the basis for a specific sequence for RNA binding. Position 6 and 1 are responsible for the recognition of nucleotide and for RNA binding, as reported by Barkan *et al.*, (2012). PPRs recognize the transcription in a modular fashion at positions 1 and 6. The amino acid position at 1 and 6 recognize each PPRs to bind each nucleotide. PPRs binds the 5' ends of RNA in a parallel fashion. The interaction between nucleotide and PPRs motifs occurred by the Van der Waals interaction and combination with asparagine position 1 and threonine position 6 to recognize adenine nucleotide, whereas asparagine position at 6 and aspartic acid position at 1 recognize uracil nucleotides (Barken *et al.*, 2012; Yin *et al.*, 2013; Yagi *et al.*, 2013). PPRs binds the RNA sequence as a monomeric by employing the amino acid residues at position 6 and 1 position. The interaction of PPRs with tRNA helped by the amino acid at position 3. This position was created by the hydrophobic amino acid (Fujii *et al.*, 2011). PPRs motifs provide greater versatility for editing due to presence of higher diversity in amino acid composition. PPRs are having the LAGLIDADG that is involved in splicing events (Toole *et al.*, 2008; De Longevialle *et al.*, 2008).

3. 4 Adenine base editor

Spontaneous Conversion of 5-methylcytosine and cytosine to thymine and uracil respectively originate by the hydrolytic deamination in cells and resulting in the mutation of C-G and T-A (Nishida *et al.*, 2016; Zong *et al.*, 2017). This event occurs 100- 500 times per cell in 24 hours

(Gaudelli *et al.*, 2017). Therefore, these editing processes are restricted to the conversion of C-G to T-A. Hence the conversion of A-T base pair into G-C base pair in target gene loci is based on SNPs (small nucleotide polymorphism). This hurdle is overcome by the Adenine base editors (ABEs). The ABEs convert the A-T to G-C in bacteria and humans, but nowadays it is also involved in plants (Gaudelli *et al.*, 2017). ABEs are high conversion accuracy of A-T to G-C targeted genomic, and purity (Gaudelli *et al.*, 2017). Adenine base editors generate point mutation than the CRISPR/Cas9 nuclease-based genome editing that is having low rates of INDELS (0.1%) and also having high product purity (99.9%) with less off-target mutations (Gaudelli *et al.*, 2017). In this process, the deoxyadenosine deaminase TadA is fused with catalytically impaired Cas9 nickase with a corresponding single guide RNA (sgRNA) to perform the task [Gaudelli *et al.*, 2017]. Thus, the targeted DNA sequence is bound by the fused TDA and CAS9 that is guided by the program of RNA, thus exhibiting a small single-stranded DNA bubble. The conversion of adenine to inosine within the bubble is with the help of TadA Deoxyadenosine deaminase. The C terminal end of Cas9-nickase fused with TadA instead of the N- terminal ends and starts the editing activity (Gaudelli *et al.*, 2017). Doubling the length of the linker between TadA and Cas9 nickase by using the 32-amino-acid-long (SGGS)2-STEN-(SGGS)2 linker leads to higher editing efficiencies [Gaudelli *et al.*, 2017]. TadA is a homodimer, so one monomer catalyzes the activity of deamination process and other acts as a docking station of tRNA substrate (Gaudelli *et al.*, 2017). But this technology exhibits the limited editing of targeted sequences when any genome has multiple adenine nucleotides. But these limitations have been overcome by the use of Tad Cas9 as it targeted two separate sites in which one is TAT and other is TAA in the kanamycin resistance genes.

3. 5 RNA interference

This is the post-transcriptional mechanism that regulates the gene expression in a different direction (Zong *et al.*, 2017; Gaudelli *et al.*, 2017; Small 2007). This mechanism can be originated due to siRNA (small interfering RNA) (Axtell *et al.*, 2006; Agrawal *et al.*, 2003), piRNA (PIWI-interacting RNA) (Seto *et al.* 2004; Klattenhoff and Theurkauf 2008), miRNA (microRNAs) (Carrington and Ambros 2008). siRNA is produced by the double-stranded RNA that encompasses approximately 20-30 bp noncoding single-stranded nucleotides and its guides to

DNA cleavage and for translation repression. siRNA is exhibiting a complementary sequence and leads to the knockdown effects of gene function (Jackson *et al.*, 2006; Birmingham *et al.*, 2007). RNAi was discovered by Andrew Fire and Craig Mello; they received the Nobel prize in 2006 and reveals the importance of RNAi. The siRNA acts as a form of RISC (RNA induced silencing complex) that acts as a silencing or degradation target gene in mRNA (Pratt and Macrae 2009; Kawamata and Tomari 2010). In siRNA methylation of RNA binding sites can alter the chromatin configuration (Zhou *et al.*, 2014; Kawasaki *et al.*, 2004). miRNA is directly derived from the genome whereas siRNA is derived from exogenous sources. Mostly it has been observed that miRNA exhibits the mismatched pair due to the presence of extended terminal D-loop, whereas siRNA exhibits complete and correct base pairing (Zhu *et al* 2013; Lau *et al.*, 2001). The origin of siRNA and miRNA is different but both require RISC. A few siRNA is produced from the plant cells while the majority of siRNA are from viruses. But the presence of cell walls in plant cells makes it difficult to transfer siRNA into cells. Transfer of siRNA into plant cells is achieved by the hairpin loop hairpin RNA that folds back to generate a double-stranded region. DCL/DICER like enzymes recognized by double-stranded the region which later makes complexes with Argonaute for the regulation of the silencing process (Kurzynska-Kokorniak *et al.*, 2015; Kurihara and Watanabe 2004). Most of the modified virus is used to induce the RNAi and knockdown genes this process called the virus-induced gene silencing (VIGS) (Lu *et al* 2003; Burch-Smith *et al* 2006). VIGS is very helpful to transfer transgene into the target gene. This process is very helpful to those plants that are recalcitrant to genetic transformation. While miRNA and siRNA are produced from the endogenous genome that is exhibiting a natural origin. Dicer enzymes cleave the pre-miRNA that is having a hairpin precursor with specificity (Kurihara and Watanabe 2004). The expression of several genes is controlled by the miRNA that is conserved evolutionary small RNA (Warnefors *et al.*, 2014; Li and Mao 2007). The target transcript is cleaved by generating artificial miRNA (Tiwari *et al.*, 2014; Gasparis *et al.*, 2017). Inhibit expression of genes with the help of a small 7-nucleotide complementarity of the siRNA. However, forecasting off targeted effects using siRNA is more difficult. Limitation of sequence specificity of siRNA, difficult to separate screening of expressed genes. Mutation in gene changes the stable effects and functional effects of mutation can be predicted easily. However, RNA interference shows the effects on genes. Hence, two plants that have been interfered with by RNA to show different effects.

3. 6 Cisgenesis and Intragenesis

This is the main technique used to induce genome editing, target transgene generation, and genetic mutation. Recent time's somatic hybridization used to transfer genes from two different species separated by the genetic barrier. The development of new technology led to the isolation of genes from cross-species, this gene called the cisgenesis. Cisgenesis or intragenesis refers to the transfer of genes from between the same species or genetically cross-compatible species without linkage drag, this may be helped by the DNA sequencing (Hartung *et al.*, 2014; Schouten *et al.*, 2006). Genes remain unchanged in cisgenesis, but in intragenesis only a part of a gene such as a promoter or a regulator is transferred. Cisgenesis may result in a new organism that might not be distinguishable by one obtained from conventional crossbreeding, whereas intragenesis results in an organism that cannot be obtained by conventional crossbreeding [Hou *et al.*, 2014]. A large no of cisgenesis gene has been isolated with the help of DNA sequencing technology; these genes do not have any marker genes with them. It leads to genome modification while remaining the gene pool. Cisgenesis plants are highly demanded by the public than transgenic plants. Cisgenes has been successfully utilized in cereal plants where the phytase gene was introduced to confer bioavailability of phosphate (Holme *et al.*, 2014). In wheat durum species The 1D \times 5 and 1Dy10 glutenin subunits with native endosperm promoter and terminator are using a transformation method. The bread quality is enhanced by the using transformation method in wheat (162). In poplar for gibberellin signaled five cisgenesis genes such as PtGA20ox7, PtGA2ox2, Pt RGL11, PtRGL12, and PtGAI1 were transferred to Populustremula x alba (Han *et al.*, 2011). PtGA20ox7 is responsible for the early growth and rate of shoot generation (Han *et al.*, 2011). PtRGL12 is responsible for longer xylem fibers while PtGAI1 cisgenesis is responsible for increasing regeneration (Han *et al.*, 2011). Cisgenesis and transgenesis is the same approach except that in cisgenesis transfer of genes from the same plants or crossable species by breeding technique as well. (Mohanta *et al.*, 2017).

3. 7 CRISPR/CAS9

In the era of high throughput genotyping, precise and effective genome editing technologies are needed to implement reverse genetics. Genome editing is of great importance for functional

characterization and genetic improvement of agricultural plants. There are various technologies that are being available including transcription activator-like effectors nucleases (TALENs), zinc fingers, and homing meganucleases for target genome modifications (Stoddard, 2005; Doyon *et al.*, 2011; Xie *et al.*, 2011). Although with the availability of such tools, there is a need for a new technology for genome editing which could be affordable, robust, scalable, and easy to engineer. This has led to the emergence of type II clustered regularly interspaced short palindromic sequences repeat (CRISPR) technology using RNA-guided nucleases (Cas9) for precise insertions, deletions, and sequence changes in a broader range of cell types and organisms. CRISPR allows precise genome editing with being comparatively easy, inexpensive, user-friendly, and rapidly adopted editing technology (Kim *et al.*, 2011). Traditionally, crops were being improved by using conventional breeding but with the constrained of ever declining existing genetic variation in plants, hampering the production for future feeding. The development of genetically modified crops with CRISPR is similar to those by mutation or conventional breeding, but CRISPR is an extremely versatile tool for providing sustainable agriculture for the rapidly increasing population. In the last decade, CRISPR has been successfully performed for targeted mutagenesis and regulation of transcriptional control in the model and agriculturally important crop plants, demonstrating the revolutionary aspect of this novel system. CRISPR has been extensively used for gene replacement, multiplex editing, gene knockouts, and targeted gene editing for improvement or designing a new crop plant.

The functioning of CRISPR-Cas9 contains combinations of two Cas9 genes. The sequence from the non-coding RNA element is known as crRNA while the other sequence is from trans-encoded CRISPR RNA termed as trans acting crRNA (tracrRNA). Guide RNA is formed by complex of these two RNA sequences (crRNA + tracrRNA) ultimately determining the specificity of the target sequence in the genome along with the Protospacer Adjacent Motif (PAM) (Jinek *et al.*, 2014). The cleavage of DNA took place within this protospacer boundaries when targeted by guide RNA. CAS9 protein is an endonuclease responsible for double-strand break (DSB) when targeted by guide RNA and facilitates endogenous DNA repair mechanism resulting in edited DNA (Mali *et al.*, 2013). There are various variants available for Cas9 nucleases, guide RNA, and their combination for genome editing (Doudna and Charpentier, 2014). The desired combination or type depends upon the crop species you are working and the gene of interest.

Till now, genome editing in plants is restricted to changes in enzymatic functions for a single purpose. CRISPR-Cas9 provides the most robust and reliable system for genome editing for multipurpose plant systems. There is an ongoing study at John Innes center for characterizing the pathways for nitrogen fixation in plants using bacteria (Xie *et al.*, 2012). Some studies are working to incorporate these pathways into wheat (*Triticum aestivum*). This will ultimately result in a reduction in dependency on inorganic fertilizer as plants will be able to fix atmospheric nitrogen (Cook Martin and Bastow, 2014). The other major focus in the present world is to convert C3 rice (*Oryza sativa*) to C4 using CRISPR, as engineered C4 rice has scope for increasing yield. This conversion requires the conversion of a single cell C3 cycle to a two-celled C4 cycle (Baltes *et al.*, 2014). There are various successful studies using CRISPR to remove unwanted cells from different plants, but there is scope for new genic regulatory sequences insertion to plant genome. The transfer of new DNA segments into plants is of special interest for conferring new biological functions. With CRISPR-Cas9 there is tremendous potential for multiple gene stacking with very little variability in gene expression. The gene stacking approach has been successfully demonstrated in maize (*Zea mays*) where 5 % of transgenic progeny witnessed proper integration (Alagoz *et al.*, 2016). Similar gene stacking was performed in cotton (*Gossypium hirsutum*) (D'Halluin *et al.*, 2013). There is no doubt that plant breeders are working hard to engineer disease resistance, high yielding, drought tolerance and nutritional varieties taking genes from other varieties or species. But this effort, the introgression of desired traits, takes several years in conventional breeding approaches. CRISPR-Cas9 is now widely used to improve agricultural important crop plants. A wheat line is recently developed resistant to powdery mildew using a gene-editing approach. Jointly with other gene-editing tools, CRISPR-Cas 9 is emerging as a game-changer for transformation in plants.

Since the last decade, genome editing has been adopted from mammals to plants to achieve a wide range of modifications from single nucleotide alterations to deletions of megabases in the DNA. Plant genome editing has great potential for the ambiguous project for the plant improvements. Most of the CRISPR-Cas9 information is obtained from mammals and it is assessed that most of these findings are universal which could be executed in diverse crop species. Future research will focus on optimizing the strategies for designing the guide RNA variants, as it is critical for

inducing breaks in the DNA. There is a huge scope for designing guide RNA for polyploid species like wheat, potato (*Solanum tuberosum*), and sugarcane (*Saccharum officinarum*) as it requires information from variation in diverse allelic forms. Another major challenge in gene editing is the lack of a high throughput platform for identifying edited gene events. Keeping in consideration for all the upcoming challenges and the number of researchers involved in CRISPR-Cas9 there are potentials for swift improvements with a new batch of genome editing tools.

4. Applications of genome editing

Genome editing technology has several advantages resulting in a wide application in crop improvement. This is one of the biggest tools for modern biotechnology, crop varieties are continuously improving with the help of genome editing, such as biotic, and abiotic resistances and also improved nutritional values in crops (Osakabe *et al.*, 2016; Xiong *et al.*, 2015; Kissoudis *et al.*, 2014; Chantrenongpiur *et al.*, 2016). Genome editing tools are used in plant breeding for the improvement of new desirable characters to create the point mutation that is similar to natural SNPs (Jacobs *et al.*, 2015; Xu *et al.*, 2017). There have been many modifications with the help of genome editing such as alteration of genes, knockout of genes, gene pyramiding, repression or activation of genes expression, epigenetic changes, etc. For instance, in *Arabidopsis thaliana* and *Zea mays* has been edited with the help of ZFN tools for herbicide resistance (Osakabe *et al.*, 2010; Townsend *et al.*, 2009; Shukla *et al.*, 2009). In the last few years, genome editing is used to produce new varieties for insect resistance, disease resistance, increased yield, and improved quality, etc.

4.1 Improved yield

Yields of the plant mainly depend on grain number, grain weight and grain size, these characters are contributed by the quantitative trait loci (Xing and Zhang, 2010; Bai *et al.*, 2012). For improvement of yield, need knockout of genes those who have negative effects such as DEP1, GW2, TGW6, GS5, Gn1a, and GS3 in rice, this is the direct way to the improvement of crops. Gn1a, Gs3 and DEP1 have been utilized to improve traits through the use of CRISPR/Cas9 (Li *et al.*, 2016b; Shen *et al.*, 2016). TGW6, GW2 and GW5 have been knocked out, resulting in

approximately 29.8% increase in thousand-grain weight in the triple mutant (Xu *et al.*, 2016). Thousand kernel weight may be increased if these genes knockouts, effects of three homo-alleles of GASR7 who have the negative effects of kernel width and weight in bread wheat are improved with the help of CRISPR/Cas9 (Zhang *et al.*, 2016b). It is nevertheless important to remark that increased grain yield per plant and higher the thousand-grain weight does not necessarily translate into improved crop yield because large-scale field trials are necessary to verify the potential agronomic improvements.

4. 2 Disease Resistance

Severe loss in yield due to infection by the disease. The disease affects consumption, freshness, and food processing safety in many crop plants (Savary *et al.*, 2012). Disease resistance may be increased through the use of genome editing of targeted traits in any crop. Bacterial pathogen *Xanthomonas oryzae* pv. *Oryzae* that is causing bacterial blight, this secret type II effector protein activated to OsSWEET14 is a host disease- susceptibility gene (Li *et al.*, 2012b). One more gene, CsLOB1 host susceptibility plays a pivotal role in both activities such as enhance the eruptive pustule formation and for pathogen growth ((Hu *et al.*, 2014). CsLOB1 promoter has been modified with the help of CRISPR/Cas9 in which two groups have been generated in citrus canker (Jia *et al.*, 2017; Peng *et al.*, 2017). In rice, ERF transcription factor OsERF922, a negative regulator of blast susceptible has been knockout, resulting in an enhancement in yield (Wang *et al.*, 2016a). Another one more example in wheat TaMLO that is a contribution to disease susceptibility has been edited, resulting in disease resistance (Wang *et al.*, 2014). MLO allele has been knocked out in Arabidopsis, tomato and barley that causes the powdery mildew disease to produce durable resistance and broad-spectrum *Blumeria graminis* f. sp. *Tritici* (Bgt)s (Wang *et al.*, 2014). This gene creates the mutation in all three TaMLO homologous genes induced by the TALEN, these genes produced heritable variation for powdery mildew resistance in bread wheat (Wang *et al.*, 2014). In tomato, gene SIMLO1 produces the powdery mildew has been disrupted with the help of using CRISPR/Cas9 (Nekrasov *et al.*, 2017). There are many bacteria, eukaryotic and other viruses that have been edited through using the genome editing approach. Tobacco and tomato are most affected by the virus, so these plants have been generated for targeted viral genome sequences through the use of the CRISPR/Cas9 editing approach (Ali *et al.*, 2015; Baltes

et al., 2015; Ji *et al.*, 2015). The eukaryotic translation initiation factor eIF4E is a host factor required by plant RNA viruses to maintain their life cycle and mutations in this gene have produced broad virus resistance in T3 non-transgenic cucumbers (Chandrasekaran *et al.*, 2016) (Ali *et al.*, 2015; Baltes *et al.*, 2015; Ji *et al.*, 2015).

4. 3 Herbicide tolerance

In the past, the herbicide resistance crops were produced by using transgenic technology (Lombardo *et al.*, 2016). Nowadays genomic editing approach has created a new approach for herbicide resistance plants to edit endogenous plant genes such as ALS and EPSPS, resulting in herbicide tolerance (Lombardo *et al.*, 2016). The acetolactate synthase enzymes encoded by the ALS gene, that is being participated in the biosynthesis of branched-chain amino acids likes isoleucine, valine, and leucine (Lee *et al.*, 1988; Chipman *et al.*, 1998). Inhibitors of ALS are used as herbicides that slowly starve affected plants of these amino acids, eventually leading to inhibition of DNA synthesis, but specific point mutations within the conserved region of ALS can confer resistance to these herbicides (Lee *et al.*, 1988; Chipman *et al.*, 1998; Svitashv *et al.*, 2015). Precise mutation in the ALS gene, there are many plants that have been developed through the replacement of genes with the help of genome editing approach, the first example of tobacco, use of donor template and ZNF has been reported in 2009 (Townsend *et al.*, 2009). Site-directed DNA base changes in the ALS gene have been created with the help of CRISPR/Cas9 for herbicide resistance in tomato, soybean, and maize (Li *et al.*, 2015; Svitashv *et al.*, 2015; Li *et al.*, 2016c). A 5-enolpyruvylshikimate-3- phosphate synthase is encoded by the EPSPS that is necessary for biosynthetic aromatic amino acid that is essential for plant survival (Kishore and Shah, 1988). EPSPS has widely used herbicide in plants that is responsible for glyphosate that binds to EPSPS functional sites and prevents its activity (Kishore and Shah, 1988). The usual method to introduce glyphosate tolerance in plants is to modify the EPSPS protein structure in order to disrupt herbicide binding site while maintaining its catalytic activity (Sammons and Gaines, 2014). In *Linum Usitatissimum* (Flax) substitution of two nucleotides through HDR-based genomes editing that is responsible for EPSPS binding sites that have been used with the help of single-stranded DNA repair templates and CRISPR/Cas9 (Sauer *et al.*, 2016). The EPSPS modified genes show a high level of herbicide tolerance than control. There is one more similar approach that has

been used a modification of EPSPS gene in rice in which substitution of two nucleotides has been edited for glyphosate resistance (Li *et al.*, 2016a).

4.4 Improved oil composition

In polyunsaturated fatty acids particularly, linoleic acid has poor availability, poor frying and oxidative nature, these criteria put restrictions on oil use. Improvement of quality and fatty acid composition requirement to change the FAD genes (fatty acid desaturase). Conversion of oleic acid into linoleic acid is responsible with the help of the family FAD2 genes, while the FAD3 genes family encodes enzymes to catalyze the production of linoleic acid into linolenic acid (Demorest *et al.*, 2016). Two soybean FAD2 genes such as FAD2-1A and FAD2-1B has been used for the improvement of oil quality by the TALEN editing approach, in which linoleic acid reduced from 50% to <4% but oleic acid increased from 20% to 80% (Haun *et al.*, 2014). TALENs were used for the improvement of oil in soybean in which FAD3A introduced into fad2-1a/fad2-1b in soybean plants, resulting in a decrease of linolenic acid and increased linoleic acid (Demorest *et al.*, 2016). Three FAD2 homoeologous genes in the allohexaploid, camelina sativa have been simultaneously knockout using two independent groups CRISPR/Cas9, resulting in producing a composition oil (Morineau *et al.*, 2016; Jiang *et al.*, 2017).

4.5 Healthy potatoes

Potato is a good source of carbohydrates, minerals, vitamins, etc. The requirement for protection of potatoes from infection, diseases, and pests. We need to conserve potatoes in cold storage. Cold storage provides a sufficient quantity of potato, saves from sprouting and also gives the continuous supply to the public domain but there is one more problem created with a potato that reduces the accumulation of sugars. This sugar produces free amino acid that originates during high temperatures and produces browning, bitter test and increases the level of acrylamides, to overcome this problem, the requirement of new technology for human safety (Clasen *et al.*, 2015). Conversion of sucrose into glucose and fructose is catalyzed by the vacuolar invertase that is encoded by the VINV, VINV plays an indispensable role to reduce sucrose in storage potato tubers. TALEN used to mutate VINV in a russet potato variety that nowadays is used for

commercial use in which potato having undetectable levels of reducing sugars. The heating of potatoes in the cold storage is responsible for reduced levels of acrylamide and producing high levels of lightly colored chips (Clasen *et al.*, 2015).

4.6 Other examples

Mutate of TMS gene in rice with the help of CRISPR/Cas9 leads to the development of a temperature-sensitive line for use of hybrid seed production (Zhou *et al.*, 2016). Granule-bound starch synthase is encoded by the maize waxy gene (Wx) that is responsible for the synthesis of amylose in the kernels (Nelson and Rines, 1962). Wild type maize kernels contain 25% amylose and 75% amylopectin whereas wx/wx lines contain approximate 100% amylopectin so-called the waxy maize. Waxy gene knockout from maize to use of CRISPR/Cas that is having more economic value (Chilcoat *et al.*, 2017). Knockout of the starch branching enzymes genes such as SBEI and SBEIIb that creates high amylose rice with potential health advantages has been generated to the use of CRISPR/CAS9 (Sun *et al.*, 2017; Zhang *et al.*, 2017).

4.7 For functional genomics

Nowadays, there are many tools used for genome editing such as ZFN, TALEN, ODM, and CRISPR/Cas9. Such types of new tools create the point mutation, deletion and insertion of new nucleotide or substitution of a nucleotide in the targeted genome [Gaj *et al.*, 2013; Kumar and Jian 2015; Chain *et al.*, 2016; Zheng *et al.*, 2016; Gao *et al.*, 2015; Lowder *et al.*, 2016; Perez-Pinera *et al.*, 2012]. Introducing a new gene into the genome is to detect the function of foreign genes. Researchers have proved which one individual gene or cell has a function. For example, the unique ability of the CRISPR/Cas9 system to selectively bind to specific DNA sites helps to regulate gene activity [Cong *et al.* 2011; Zhang *et al.* 2011; Lowder *et al.*, 2016]. Promoter activity for protein activation or repressing that is controlled by the function of a gene can be attached to the catalytic inactive Cas9 protein. It has been proved that the binding of targeted DNA at binding sites can be stimulated or inhibited the function of target trait (Lowder *et al.*, 2016). In addition, targeted to different genome sites has been simultaneously introduced in the cells with the help of CRISPR/Cas9 system [Li *et al.*, 2016; Cong *et al.*, 2013; Wang *et al.*, 2013]. In crop domestication,

there are many genes that have been identified using this CRISPR/Cas9 technology. CRISPR/Cas9 helps to decide the intergenic interaction of any genes (Lowder *et al.*, 2016).

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