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A novel plant genome editing CRISPR/Cas9 System: To modify stress tolerance responses in plants

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ABSTRACT

The plant genome modification by Crispr/Cas9 system is gaining ground. In molecular biology, the use of this indispensable tool works on the basis of target recognition due to the existence of a short protospacer adjacent motif (PAM) that is complementary to the desired strand. The components of the genome-editing tool are guide RNA, Cas9 protein. The mechanistic nature of CRISPR/ Cas9 technology i.e. adaptation, expression and interference lead the desirable mutations in the crop plants. According to the need one can use the different types of programmable DNA targeting and editing platform type. The goal of this review is to provide an idea of the production of stress-tolerant crops. The molecular perception from structural and mechanistic research grant a groundwork for rational engineering for the production of plants that can withstand different kinds of stress.

Keywords: Crispr, stress tolerance, growth, abiotic stress, mutation, targeted mutagenesis

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INTRODUCTION

The ground-breaking technology, CRISPR/Cas9 method has brought up a wide range of usage in important practical research of plant biology (Jain 2015). In nature, the CRISPR/Cas9 modern technology is a guard system form the prokaryotic that confer combat to outsider objects such as phages, bacteria, and bacteriophages by stimulating acquired immune comeback. CRISPR is a shortening for "Clustered Regularly Interspaced Short Palindromic Repeats" derived from the "Streptococcus pyogenes", in 1987 (Bortesi and Fischer 2015, Ma, Zhang et al. 2015). CRISPRs are originate in approximately forty percent of bacteria bacterial genomes have been sequenced and ninety percent of archaea genome has been sequenced (Rani, Yadav et al. 2016). The crucial essentials of the CRISPR/Cas9 prearrangement are "Cas9 proteins" and "CRISPR RNAs". Cas9 nucleases, also known as "RNA guided site-specific nucleases (RGNs)", employ single guide RNA (sgRNAs) similar to a precise aimed sequence to lead the Cas9 to begin a double-stranded break (DSB) in a part of DNA or at definite site of curiosity (Stoddard 2011, Voytas 2013, Rani, Yadav et al. 2016).

The catalytic act as a position-specific of the sgRNA/Cas9 complex is definite by an arrangement of only approximately twenty nonstop bases of sgRNA which is slight as to that of ZFNs and TALENs (Kumar and Jain 2015, Wang, Zhang et al. 2015). Pending 2013, the dominant genome editing tools were zinc finger nucleases and "transcription activator-like effector nucleases" (Christian, Cermak et al. 2010, Bortesi and Fischer 2015). ZNF and TALENs together use cleavage area of the Folk1 restriction enzyme and their particular DNA binding domains as the TAL effector and zinc finger protein correspondingly (Gaj, Gersbach et al. 2013, Weeks, Yang et al. 2014).

These nucleases can identify and cut the exact sequence at target loci to generate DSBs, which are then remolded by two primary systems "homologous recombination (HR)" and "non-homologous end joining (NHEJ)". NHEJ repair pathway often causes various modifications of the targeted sequence, such as small deletions or insertions (Belhaj, Chaparro-Garcia et al. 2015, Gao, Wang et al. 2015).

In expectation of the start of genome cutting tools as TALENs, ZFNs, and CRISPR/Cas9, the development and progress of changes have depended on the use of conventional breeding, random mutagenesis, "somatic hybridization" or transgenic methods (Schaeffer and Nakata 2015). The CRISPR/Cas9 method is one such new means of genome editing which connects possible of taking out enduring and required variations in a creature (Endo, Mikami et al. 2014, Rani, Yadav et al. 2016). The comparative acceptance is, swiftness, and living extent of CRISPR/Cas9 for genomic variations are transforming in effect completely zones of molecular biosciences, counting "Functional Genomics", "Genetics", "Applied Biomedical Research", and "Agricultural Biotechnology" (Lowder, Zhang et al. 2015, Qi, Lowder et al. 2015).In plants, three short reports in 2013 designated the first implementation of the CRISPR/Cas9 system to plant genome manufacturing (Li, Norville et al. 2013, Nekrasov, Staskawicz et al. 2013, Endo, Mikami et al. 2014). In order to reduce the off-target questions of CRISPR/Cas9, there is the expansion of double nickase and Cas9-nuclease fusion systems (Schaeffer and Nakata 2015).The ability of CRISPR/Cas9 mains to new walks into RNA innovation, investigation, manipulation and battered have power over to gene expression (Schaeffer and Nakata 2015). It is significant for correct, effective gene targeting for functional genomic checkup of plants and for the production of genetically modified crops and is also helpful in devious of site-specific addition of protein tags, tailored promoters, and other regulatory sequences (Schaeffer and Nakata 2015). Methodologies for exact genome restriction are of great importance

to functional categorization of plant genes and genetic enhancement of agricultural crops (Xie and Yang 2013). Under attack genome editing in plants will not only help functional genomics studies but also help out to discover, enlarge, and create new traits of agricultural importance (Ali, Abul-faraj et al. 2015). A regeneration in plant genome manufacturing began with the progress of a suite of sites precise genome-editing technologies (Schaeffer and Nakata 2015).

Abiotic stress is a multifaceted attribute, which is directed by numerous genes. There is a considerable collaboration between technologies of several collaborative, directing and metabolic machines, that cause root abiotic stress reaction/variation (Jain 2015). The main constraints to agricultural making are owing to a range of abiotic stresses, such as hydration, salt stress, temperature, excess of water, ion toxicity and emission (Hussain, Lyra et al. 2016). By taking the advantage of CRISPR/Cas9 genome editing technology, we can produce the stress-tolerant crop plants by targeting those genes which are involved in stress-related segments of DNA communicating network, signal transduction and metabolite creation(Zhang, Zhang et al. 2014, Osakabe, Watanabe et al. 2016).Subsequently 2013, CRISPR/Cas9 has been effectively supportive by brief-expression and persistent lines of transgene in numerous kinds of plant, such as Arabidopsis, Nicotiana benthamiana, rice, wheat, maize, and tomato (Jiang, Zhou et al. 2013, Liang, Zhang et al. 2014, Zhou, Liu et al. 2014, Qi, Lowder et al. 2015, Wang, Zhang et al. 2015,

Schedel, Pencs et al. 2016) including monocots rice, sorghum, maize, wheat and dicots: tomato (Fan, Liu et al. 2015). Cas9 system is revealed to be active in soybean by kicking-out a “green fluorescent protein (GFP)” transgene and changing nine endogenous positions on the chromosome (Jacobs, LaFayette et al. 2015).Mutations could be induced in firm transgenic potato via this structure. Through this process, we can get monoallelic and balletic homozygous mutant organisms in the T1 generation. It is an efficient tool to uphold the practical lessons of unsystematic fragments of DNA in potato (Wang, Zhang et al. 2015).

This review is focused on the stress-tolerant crop plants by applying CRISPR/CAS9 with special emphasis. We can produce new stress resistance varieties by targeting those genes which are involved in stress-related gene regulatory network, also discussed the known mechanisms of CRISPR/CAS9 and finally, the current insights into research on CRISPR/CAS9 followed by prospects of developing new stress-tolerant crop varieties.

ORGANIZATION/ STRUCTURE OF CRISPR

This systematic tool consists of different components that pay a great part in the editing and designing modifications in the genetic makeup and revolutionizing the genome editing era by rational engineering with the aid of Crispr the Disrupter tool.

REPEATS

An arrangement of small, repetitive DNA sequences (~20–40 bp) in length, categorized as repeats (Ratner, Sampson, & Weiss, 2016). Each CRISPR-Cas9 locus contains several regions. These repeats are necessary to regulate the CRISPR-Cas9 function and are present in the center of the system. Hence the CRISPR repeats act as a regulatory guide (Alkhnbashi et al., 2016).

LEADER

The region upstream of the main recurrence of the CRISPR collection comprises a signal, the so-called leader sequence, which grasps regulatory elements that are essential for edition. The leader region differs in their size, ranging from 47 bp in certain bacteria to a few hundred base pairs in numerous hyperthermophiles Archaea, and they are accountable to reveal extended sections of less complicated sequence, with partial conservation of sequence (Alkhnbashi et al., 2016).

CAS9 GENES

A fundamental set of Cas9 genes is linked with CRISPR array. A lot of the Cas9 genes are similar to helicase and endonuclease families in sequence or those genes which encode for nucleic acid binding proteins (Haft, Selengut, Mongodin, & Nelson, 2005; Ratner et al., 2016). Cas9 complex is an enzyme that snips DNA, and CRISPR is a pool of DNA sequences that informs Cas9 precisely where to leave. Scientists can modify or inserts DNA sequences within the cell in order to repair the damage caused by this cutting. The required particular of Cas9 enzyme with the target DNA is influenced by not only the gRNA-DNA base pairing but also a protospacer-adjacent motif (PAM, sequencing) nearly 3' end of the target site. Cas9 consists of two nuclease domains (HNH and RuvC-like), which chop one part of double-stranded DNA at the identical position (three base pairs from the PAM), leading to a DSB (Wiedenheft, Sternberg, & Doudna, 2012; Xing et al., 2014).

SPACER

Repeats that are interspaced with non-repetitive sequences labeled as spacers (Ratner et al., 2016). CRISPR duplication sequence is intervened by foreign DNA segments, composed of hundreds of repeat-spacer elements (Alkhnbashi et al., 2016). As the virus DNA incorporates into the CRISPR region, it turns into a molecular most-hunted arcade, demonstrating the enemies about the entry of the microbe in model organisms(V. Kumar & Jain, 2015).

PAM

PAM (protospacer adjacent motif) consists of a conserved sequence motif (NGG) (Rani et al., 2016) present at 3' end of the desired sequence that is vital designed for RNA-guided Cas9 to distinguish and divide the target site (Gasiunas, Barrangou, Horvath, & Siksnys, 2012). It comprehends the strong profit of absorbent an essential PAM acknowledgment arrangement of just two nucleotides (GG) (Jiang, Yang, & Weeks, 2014). The amount of PAMs is positively associated with genome size of eight typical plant species as the study of nuclear genome sequences such as "*Arabidopsis thaliana, Medicago truncatula, Glycine max, Solanum Lycopersicum, Brachpodium distachyon, Oryza sativa, Sorghum bicolor,* and *Zea mays*" have shown that there is a frequency of 5-12 times of presence of PAM (NGG/NAG) site after every 100bp (V. Kumar & Jain, 2015). CRISPR/Cas9 technology is an analog of RNA-directed interference (Rani et al., 2016). It could be distinguished into three different key phases adaptation, expression, and interference (V. Kumar & Jain, 2015).

MECHANISM OF CRISPR

CRISPR system contains three genes, with one which codes Cas9 nuclease and two RNA genes: transactivating crRNA (tracrRNA) and progenitor crRNA (pre-crRNA) which are the template. The programmable pre-crRNA, which has nuclease guide sequences called as spacers interspaced by similar direct repeats, is administered to mature crRNA in association with tracrRNA (Sander & Joung, 2014).The transformed single guide RNA consists of two RNA genes which are hairpin that imitates the crRNA–tracrRNA compound. By directing CAS9 with the correct guided RNA, scientists can edit different parts in DNA sequence, up to 20bp long, inside the genome at any desired place.

ADAPTATION

It is the slightest understood stage in the CRISPR/Cas9 system where a distant DNA part from attacking genetic material is incorporated into the locus of CRISPR for future identification of that foreign DNA(Alkhnbashi et al., 2016).In the spacer acquisition phase, DNA of invader is recognized and managed into small, spacer-sized sequences that are made known to into the CRISPR array, to be lined by a couple of repeat sequences (Ratner et al., 2016). The presence of reworking signs in the leader area is also strengthened by evolutionary knowledge (Alkhnbashi et al., 2016).

LEADERLESS

The manifestation of adaptation indicators in the section of the leader is sustained by the presence of leaderless CRISPR-groups in some crenarchaea, which do not accomplish novel spacers (Alkhnbashi et al., 2016). From the results, it can be concluded that 13% of 980 archaeal CRISPR loci, and 24% of 2852 bacterial loci, were believed to be leaderless (Alkhnbashi et al., 2016). Even though it is recognized that adaptation is affected by signals existing in the area upstream of the CRISPR array, the so-called leader sequence, no bioinformatics software is present that can inevitably explain these leader sequences to date. That is the reality that the identified leader sequences disclose only restricted sequence conservation (Alkhnbashi et al., 2016).

EXPRESSION

It implicates the production of guide RNA units (crRNA) and their incorporation into huge RNA–protein effector complexes (Alkhnbashi et al., 2016).In this phase, crRNA is matured because of the preparation of functional crRNAs from the transcript of the repeat–spacer array where the tracrRNA combines at repeat region of the pre-crRNA with the aid of Cas9 (Rani et al., 2016).CrRNAs combine with Cas9 enzyme to produce ribonucleotide complex that identified nucleic acids of foreign origin. It shows no inclination between the coding and non-coding strands, which is suggestive of an RNA-guided DNA-targeting scheme(Semenova et al., 2011).

INTERFERENCE

In this phase effector complexes carefully examination for and cut down occupying genetic material previously identified by and united into the CRISPR-Cas9 system (Alkhnbashi et al., 2016). It means here crRNA: tracrRNA duplex directed cleavage of invading foreign genetic elements by Cas9 nucleases occurs. These Cas9 nucleases induce a double-strand break to destroy the invading viral genome. The Cas9 recognizes only those viral sequences that are complementary to the guide crRNA. If the PAM(NGG) sequences are not present at the CRISPR locus, it prevents the RGN from cleaving the CRISPR locus itself (Rani et al., 2016). The specificity of CRISPR. The specificity of the CRISPR system is governed by the gRNA, as it directs the Cas9 to a specific target site to cut. Due to this capability, we can construct gRNA having specific target sequences to knock out, knock-in and knock down the expression of a particular gene.

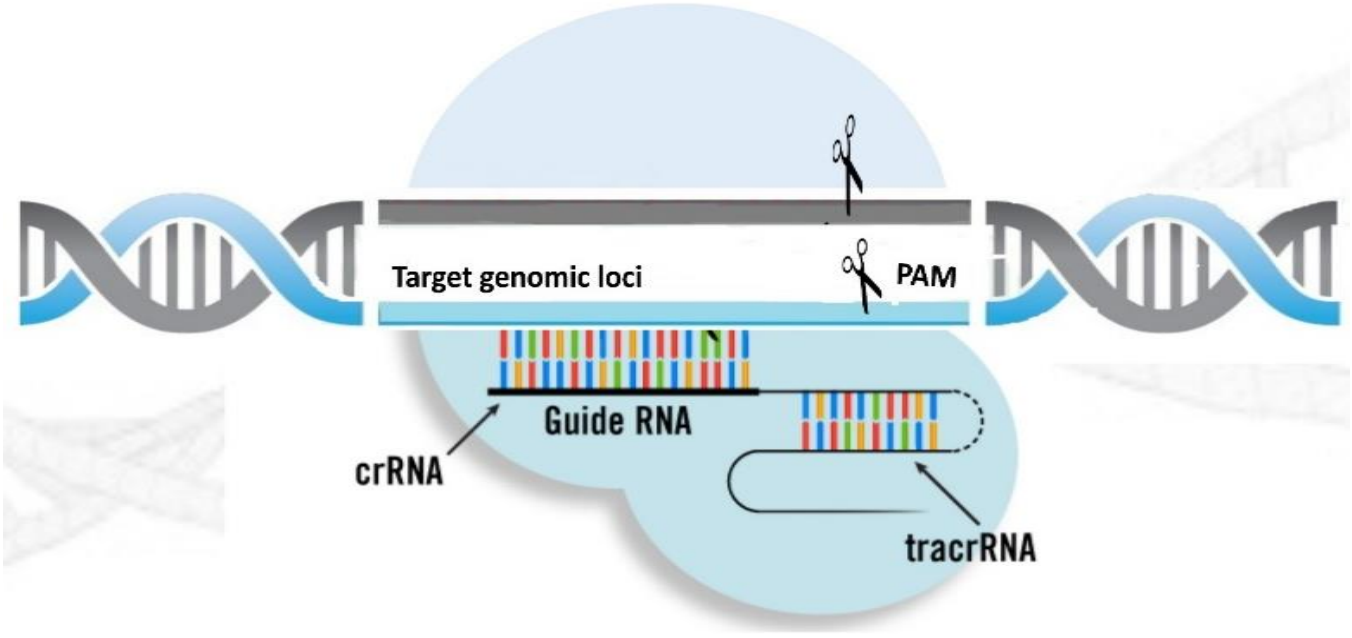

Figure 1.1 Interference of CRISPR- CAS9 system,

CLASSIFICATION OF CRISPR

This is classified consisting of different parameters either Cas9 protein or some other factors into type and subtypes. Here is the basic classification of the CRISPR

TYPE1

The ability of CRISPR/Cas9 system as a DNA cutting tool is described by Charpentier and Doudna (2012), they classify the CRISPR into class I, II or III, on the origin of occurrence of unique Cas9 proteins(Cas6,Cas9) that is determined adjacent to the CRISPR array (Rani et al., 2016; Ratner et al., 2016). All variants of CRISPR/Cas9 system must contain two proteins i.e. Cas1 and Cas2 proteins. Cas1 is a metal-dependent DNase with no sequence specificity and has a function of integrating CRISPR locus with spacer DNA. Cas2 is also a metal-dependent protein with endonuclease activity, but the exact function of this protein is still unveiled. HD domain is present in Cas3 protein, and involve in the metal-dependent nuclease activity on double helix of oligo-nucleotides (V. Kumar & Jain, 2015). Cas4 has RecB-like nuclease activity and involved in acquisition of spacer. Cas5 and Cas6 are known as repeat-association mysterious proteins (RAMPs) because these two Cas9 proteins are distantly related to each other. both these proteins contain minimum one RNA recognition motif and a characteristic glycine-rich loop (V. Kumar & Jain, 2015). Different Cas9 variants, like native Cas9, dCas9 (nuclease-deficient Cas9), and Cas9 nickase, have different applications. To generate gene knockouts in mammalian cells, the wild-type humanized Cas9 (hCas9) has been used (V. Kumar & Jain, 2015). It has been proved through editing of various loci at a time that CRISPR–hCas9 system can be used for various high-throughput applications (Cho et al., 2013). However, due to some deleterious and un-wanted side effects, the application of hCas9 for the purpose of rearrangement of genome is restricted (V. Kumar & Jain, 2015).

TYPE 2

The type II system is the very commonly used tool for genome editing. Type II system is the first successfully employed and patented CRISPR/Cas9 system for the eukaryotic genome editing (Rani et al., 2016). In type II system Cas9 protein has important role. Type II systems use RNase III for the purpose of cleavage due to the lack of the Cas6 gene. Extra small RNA is encoded by functional type II systems that have a sequence complementary to the repeat sequence and is known as tracrRNA (Dugar et al., 2013).

TYPE 3

Cas6 is utilized by type III systems, however, stem-loops are not produced by their repeats. the process of cleavage is occurred by the longer transcript twisting around the Cas6 and cleavage happens upstream the repeat sequence (Niewoehner, Jinek, & Doudna, 2014).

APPLICATIONS

The CRISPR/Cas9 system has a broad circle of applications in different kinds of organisms, such as the editing of genome, regulation of genome, screening of genome, and many other applications that are ecologically important (J.-F. Li et al., 2013; Shan et al., 2013). Gene expression could also be regulated by employing CRISPR/Cas9 system in addition to genome editing. For gene silencing, a CRISPR interference (CRISPRi) platform” has been formulated recently, and this approach is complementary to RNA interference process (Larson et al., 2013; Qi et al., 2013). Moreover, effects of these genes are influenced by some other different genes (hereditary background, epistatic involvement), by salinity scale and conformation and on the ecological situations (“genotype-3-environment interaction”). Due to this complication, discrepancy in the genetic makeup of the wild or parent type germplasm for quantitative-agronomic characters like resistance to the traumas of environment remains mostly untouched (Cuartero, Bolarin,

Asins, & Moreno, 2006). There are limited reports on plant genome engineering mediated by the application of this editing tool, CRISPR/Cas9 system up till now. In 2013, targeted modifications in plant genome are reported simultaneously by three different research groups (V. Kumar & Jain, 2015). For the exploration of economically important traits of plants and for molecular breeding assistance in agriculture, the creation of genetic variation at allelic levels in plants is essential (Rani et al., 2016). By using CRISPR/Cas9 system, faulty gene can be repaired by cutting out and normal gene is inserted into the cell. By pairing with other techniques and tools of molecular biology CRISPR could develop a major strength in ecology and protection. Particular genome engineering has the tendency to alter not only us but the whole world and all other its ecosystems (Zaidi, Mansoor, Ali, Tashkandi, & Mahfouz, 2016).

Organism Name	Common Name	Genes targeted	Modification Made	When	References
Populustomentosa Carr	Chinese White Poplar		Editing and targeted gene mutation of the genome in a woody species	2015	(Fan et al., 2015)
Arabidopsis thaliana	Mouser Cress		Multiplexed editing of gene And transcriptional initiation or suppression of plant endogenous genes	2015	(Lowder et al., 2015)
			targeted gene modification	2013	(Jiang et al., 2013)
			Gene Editing and Modified Genes inherited in the T2 and T3 Generations	2014	(Jiang et al., 2014)
Oryza sativa	Rice		Multigene Knockout Using Off-Target mutations	2014	(Endo, Mikami, & Toki, 2014)
			A large number of deletions in chromosome and heritable small genetic changes	2014	(Zhou, Liu, Weeks, Spalding, & Yang, 2014)
			Targeted gene modification	2013	(Jiang et al., 2013)
Glycine max	Soya bean		Targeted genome modifications	2015	(Jacobs, LaFayette, Schmitz, & Parrott, 2015)
Solanumtuberosum	Irish Potato		Efficient targeted mutagenesis	2015	(S. Wang et al., 2015)
Nicotianatabacum	Tobacco		targeted mutagenesis	2014	(Gao et al., 2015)
			targeted gene modification		(Jiang et al., 2013)
Lactuca sativa	Garden Lettuce		DNA-free genome editing	2015	(Woo et al., 2015)
Sorghum bicolor	*Sorghum*		targeted gene modification	2013	(Jiang et al., 2013)

Biological stresses, like infectious particles, weeds, fungi and bacteria, can easily be combat by molecular piling of numerous herbicide-resistant, chitinase, defending genes into the plant species to lessen the use of chemical pesticide and thereby, crop plants yield is improved (Rani et al., 2016; Xu et al., 2015). The reports on abiotic pressure tolerance in crops have not been available yet. Any order in the genome can be manipulated by using the CRISPR/Cas9 system and it is partial only by the accessibility of a PAM position. The genomes of bacteria, animals and plants have been edited successfully and efficiently by employing CRISPR/Cas9 system (Feng et al., 2013; Jiang et al., 2013; Shan et al., 2013). Based on the acumen of CRISPR/Cas9 system, desired changes can be introduced by using this system such as targeted single and many gene knock-outs of harmful genes in plants and for

enlightening economic traits SNPs are introduced into a gene of interest. CRISPR/Cas9 system can also be considered for improving quality development program of oilseed plants (Rani et al., 2016).

The CRISPR/Cas9 system has extensive range of applications in different organisms, like introduction of point alterations alike to ordinary single nucleotide polymorphisms, alteration in the function of gene , and incorporation of external genes for the purpose of gene knockouts, gene pyramiding, transfer of protein to Genetic loci, initiation and repression of gene expression, and modifications of epi-genome (Jain, 2015; V. Kumar & Jain, 2015). Another vital application of the CRISPR/Cas9 system is the generation of conditional alleles in direction to study the functionality of the fatal gene by giving three-dimensional and temporal control upon gene expression (Gayatonde & Vennela; V. Kumar & Jain, 2015). CRISPR technology is the advancement of plant breeding techniques. For the production of mutant plants non-transgenic approaches are also present to deliver such nucleases to plants. Consequently, varieties of crops generated through

these techniques may be suitable as non-GM and may have tremendous influence on plant breeding and biotechnology (Jain, 2015; Marton et al., 2010).

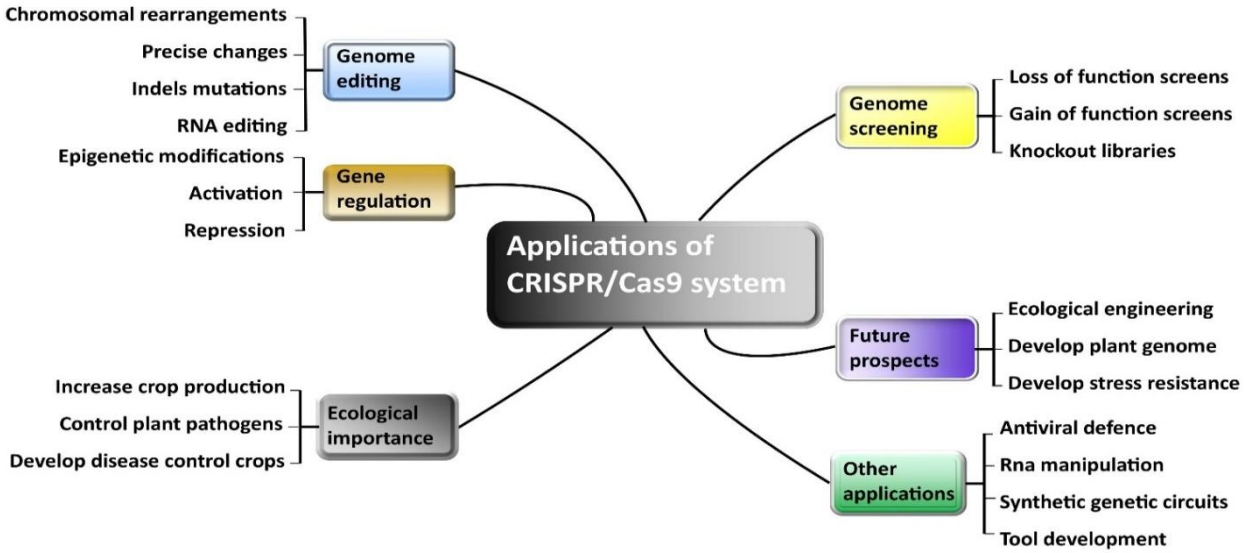

Figure 1.2 The broad biological application of CRIPPR-CAS9 system and future prospects

SPECIFIC GENES INVOLVED IN DROUGHT STRAIN RESPONSE AND ACCEPTANCE

Mostly in crops, only 20% of the attainable yields are basically the actual yields (Todaka, Nakashima, Shinozaki, & Yamaguchi-Shinozaki, 2012). Abiotic stresses are the major dominating factors in reducing crop yields for instance excess or deficiency of water, temperature changes i.e. low or high, and high level of salinity. Therefore, actual yields tremendously increased by improving abiotic stress tolerance in most crops (Kasuga, Liu, Miura, Yamaguchi-Shinozaki, & Shinozaki, 1999; K. Kumar, Kumar, Kim, Ryu, & Cho, 2013). Cellular gene expression changes are responsible for the physiologic response to such abiotic stresses. As plants are naturally non-motile so adaptive mechanisms have been developed by them against abiotic stress conditions. Thousands of genes are involved in the activation and integration of these mechanisms are activated and integrated (Kant, Kant, Gordon, Shaked, & Barak, 2007; W. Wang, Vinocur, & Altman, 2003). It is demonstrated that these stress conditions induce the expression of various genes. These genetic factor products can be categorized into two groups: the first group of

gene products contains proteins that are involved in the function of protecting cells from water loss or dehydration. These proteins include the enzymes vital for the biosynthesis of various osmoprotectants, antifreeze proteins, chaperones, late-embryogenesis-abundant (LEA) proteins, and detoxification enzymes. The second group contains transcription factors (Huang & Liu, 2006).

EXAMPLE

Naturally, dehydration approachable element-binding factors (DREB) are activated in plants in response to abiotic stress (i.e., water deficiency, salinity, and freezing). The DREB is classified into different types including DREB1A, DREB1B, and DREB2A. Their products bind to the cis-acting elements CRT/DRE that are located on the promoter region of genes involve in stress tolerance in order to suppress their effects (Hsieh et al., 2002; Yamaguchi-Shinozaki & Shinozaki, 2005). For dehydration responsive gene regulation such cis-regulatory elements are very important, and these cis-elements are located in the promoter regions of genes that are involved in dehydration and cold stress tolerance. To increase the efficiency of this mechanism, there is a need to enhance the binding ability of DREB factors with promoter region and stress tolerance should be improved. In previous experiments, some of the approaches used by the scientists to enhance the promoter binding ability of DREB by introducing different promoters for constitutive expression of the genes involved in stress tolerance i.e. 35S cauliflower mosaic virus (CaMV) promoter and rd29A for gene transfer. They introduced a gene that is responsible for encoding a stress-inducible transcription factor that played important roles in stress-tolerant genes regulation in *Arabidopsis thaliana*.

The functionality and capability of stress-tolerant genes are improved with the use of 35S cauliflower mosaic virus (CaMV) advocate in *Arabidopsis thaliana*, but growth retardation also occurred under normal conditions. The expression investigation of dehydration-inducible genetic factors in *Arabidopsis* exposed that there are minimum of four independent signal pathways involved in the induction of stress-inducible genes in response to dehydration. Among these pathways two are abscisic acid (ABA) dependent and other two are ABA independent. Among the two ABA-independent pathways, one of those pathways is involved in cold-response (Yamaguchi-Shinozaki & Shinozaki, 2005). There are various stress-inducible genes, for example *rd29A (Kasuga, Miura, Shinozaki, & Yamaguchi-Shinozaki, 2004)*, and ABA independent pathway is used for their induction. Within the *rd29A* gene promoter region, cis-regulatory elements i.e. dehydration-responsive element (DRE) (Hsieh et al., 2002) with a sequence TACCGACAT are discovered, and these are involved to respond in cold and dehydration stress conditions. So, we can add stress-induced *rd29A* promoter by using CRISPR/CAS9 technology for the expression of *DREB1A.* This promoter is very efficient in stress tolerance having reduced growth retardation effects. It is observed that transgenic plants have better stress tolerance ability and under non-stress conditions these plants show better growth (Kasuga et al., 1999; Kasuga et al., 2004).

FREEZING

cDNA analysis of *Arabidopsis* plants revealed that there are cis-regulatory elements named CRT/DRE CRT/DRE within the DREB genes promoter region. The freezing tolerance to the plants is provided when the CRT/DRE binding protein (CBP1) binds with such cis-acting elements to express DREB genes (Kasuga, Liu et al. 1999). For enhancing freezing tolerance, we can use CRISPR/Cas9 technology to get the overexpress of the gene. A gene named *SabNAC* isolated from *Spartina alterniflora* is introduced into the *Arabidopsis thaliana* and its overexpression made it resistant to drought and salinity. So we can improve salinity and drought tolerance through this modification (Karan,

DeLeon, Biradar, & Subudhi, 2012) A gene named *GhRAV1* is isolated from *Gossypium hirsutum* is introduced in *Arabidopsis thaliana* and it's overexpression increased the sensitivity to drought and salt stress. In contrast to wild type the plant modified with *GhRAV1* gene has decreased seed germination, seedling growth, chlorophyll content and maximum photochemical efficiency (Zhang et al., 2015).

RICE

Oryza sativa L. is one of the vital crops and more than half of the world's population used this crop as their staple diet. It is the model plant for monocotyledonous plants. However, rice is very sensitive to salt stress and is one of the most salt-sensitive cereal crop having a threshold of 3 dSm-1 for most cultured varieties (Hoang et al., 2016; Liang et al., 2015)

SALINITY

Elevated levels of different salts in water and soil are generally termed as salinity. These salts include magnesium sulfates, sodium chloride, and calcium sulfates, magnesium bicarbonates, and calcium bicarbonates (Hoang, Williams, Khanna, Dale, & Mundree, 2014). Depending upon the light intensity, climate conditions, plant species, and soil conditions there are variations in the harmful effects of salinity (Acosta-Motos et al., 2017). The fundamental knowledge about molecular mechanisms related to stress acceptance in plants is significant to boost the yield of plants under salt stress conditions. Like other plant species, various mechanisms have been developed by rice to manage with salinity stress consisting (i) biosynthesis and growth of osmolytes for Osmo defense; (ii) ion homeostasis and compartmentation; (iii) antioxidants—ROS detoxification and (iv) planned cell death (Acosta-Motos et al., 2017).

UNDER STRESS CONDITIONS

Many events in the cell are induced whenever plants encounter salt stress. Sensory responses are initiated firstly and then response mechanisms are activated. Ion concentration is changed such as increased level of Na^+ ions, less availability of water. During the stress response, phospholipids including DAG, stands for diacylglycerol, IP3 stands for inositol triphosphate, and PA stands for phosphatidic acid have essential structural roles in inducing cytosolic Ca^{2+} spiking (L. Li et al., 2017). Phospholipase C is involved in their activation (L. Li et al., 2017), alterations in the cytosolic homeostasis of Ca^+ and K^+ ions, ROS (Reactive oxygen species) accumulation and disturbance of cell wall membrane geometry. It is reported that ROS levels have been increased, and salt stress caused significant injury and eventual death in plants. Such events result in the activation of mechanosensitive chemicals like CNGCs, osmosensors, purinoceptors, annexins and histidine kinases by membrane-bound ion transporters. This activation of mechanoreceptors results in the increase in the concentration of second messengers (Ca^+, cGMP, ROS). Rice varieties are reported to show defense mechanisms in contrast to enhanced radical construction during salt stress by preserving the antioxidant enzymes action, biosynthesis of ABA and reduction in root membrane potential. These sensory events result in the activation of response mechanisms. These response mechanisms include ABA independent and dependent pathways, MAPKs, SnRK2s, SOS pathways. Such mechanisms result in the reduction of driving force for Na^+ ions uptake and stress producible genes along with transcription factors are activated (Conde, Chaves, & Gerós, 2011; Munns & Tester, 2008).

MODIFICATIONS

Multiple genes are controlled the salt stress tolerance along with its quantitative trait. During the past few years, various genes involved in salt tolerance in plants had been isolated and these genes have role in sign transduction and transcriptional maintenance, transporters of ions and metabolic paths. In rice, genes involved in antioxidant and ROS detoxification are CAT1 and GST, the products of these genes are catalases and Glutathione S-transferase. These enzymes have antioxidant activities. So

CRISPR/CAS9 technology could be utilized to target these all genetic factors to enhance the activities of GST, CAT, and SOD and reduce H_2O_2 for improving the salinity tolerance (Hoang et al., 2016; Zhao & Zhang, 2006).

CRISPR/Cas9 genome editing t technology is used to introduce another gene into the rice named *GlyII,* for the purpose of enhancing salinity tolerance. This gene produces a protein called Glyoxalase II (Singla-Pareek, Yadav, Pareek, Reddy, & Sopory, 2008). Some genes like *OsKAT1, OsNHX1* have a function of ion homeostasis and compartmentation. We can also utilize these genes for rice genome editing to make it salinity tolerant. *OsNHX1*, *OsKAT1* gene overexpression*,* obtained from *Oryza sativa* by using CRISPR/CAS9 technology to prevent off-target effects has enhanced salt tolerance. Transgenic plants had improved growth, osmotic potential reduction under NaCl stress by making protein vacuolar (Na^+, K^+)/H^+ antiporter (Biswas et al., 2015; Fukuda et al., 2004).

Fourteen DREB-type genes are present in the genome of rice, including *OsDREB1F, OsDREB1A*, and *OsDREB2A* are brought by the stress of salt and their overexpression proved very efficient to combat with abiotic stress. By using CRISPR/Cas9 technology we can make transgenic plant *Arabidopsis* or crop like rice for overexpressing *OsDREB1A*, *OsDREB1F,* and *OsDREB2A* to improve the plant's tolerance to stress*.* OsDREB1A and OsDREB1D activate the promoter region of *OsDhn1* containing the DRE sequence. Hence, we can say that the DREB/CBF TFs are preserved in rice and *Arabidopsis* and DREB-type genetic factor have crucial role in enhancing the resistant to salt pressure in transgenic rice. ABA-independent kinases also have roles in salinity stress tolerance. In transgenic rice overexpression of CDPK, OsCDPK7 improved resistant to stress of salt and the *OsCDPK7* appearance level is associated with the extent of salt resistant. NF-YC gene isolated from bermudagrass can be introduced into rice through CRISPR/Cas9 technology and its overexpression make it resistant to salinity and drought by lower levels of ion leakage and higher levels of RWC and higher level of chlorophyll contents.

CONCLUSION

We expect that this literature review will help as a valuable preliminary point for groups seeing to learn further about CRISPR/Cas9 and approve the skills for their species of the target. In this paper, we tried to employ the CRISPR/Cas9 scheme for genetic manufacturing in plants to harvest resistance in them. Targeted changes by CRISPR/Cas9 can offer an invention in producing mutants in earlier difficult-to-approach genes, to alter multiple loci and to produce large deletions. Its capacity for detailed nucleotide mutations using intended sgRNA arrangements could be used to change the regulatory hereditary elements located upstream of genetic factor and addition of large sequences at definite loci to augment transcription of genes defining agricultural performance (Rani et al., 2016).

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