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Revolutions of CRISPR/Cas9 in modern era as genome editing tool

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ABSTRACT

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) is a latest genome engineering technology that has created a buzz in the scientific world as it has enabled scientists and geneticists to efficiently edit genomes of not only prokaryotic but eukaryotic organisms as well including Human beings with a greater level of precision leading to advancements in medical field that possesses genetic components. Technically, CRISPR works by slicing out chunks of DNA and substituting that removed chunk with a new, altered and updated sequence of DNA. This enables the modifications in mutant genes such that the target of curbing the transmission of genetic diseases in descendants could be achieved. CRISPR/Cas9 genome engineering technology is being practiced routinely now a day as it is a faster, cheaper and a more precise technique of DNA editing with a versatile range of potential applications and future perspectives. The readers will get a precise overview of CRISPR/Cas9 genome engineering technology, its mechanism, applications and role in procuring advanced, modified and disease resistant traits in plants and humans with a brief account of ethical considerations after overviewing this review article.

Key word: Genome; tool; population; biotechnology; DNA

INTRODUCTION: CRISPR technology, an innovative and worth considering tool for editing genomes to modify gene function for acquiring required traits and enhance the quality of gaining products i.e. (improving crops) CRISPR stands for "Clusters of Regularly Interspaced Short Palindromic Repeats" (Zhang *et al.*, 2016). CRISPR/ Cas9 is a distinctively exclusive genome editing technique, it enables genetic researchers to edit parts of genome by adding, deleting, substituting or modifying sections of the sequences of DNA (Savić and Schwank, 2016). It is a specifically specialized region of DNA comprised of two distinct features: The presence of nucleotide repeating sequences, Interspersed spacers (Liu *et al.*, 2017). The potential to regulate genomes in a site-specific way has the functionality to convert plant natural research and biotechnology. Particular genome alternate can provide control over genetic statistics and can be used to deal with basic natural questions and to accelerate biotechnology (Voytas, 2013). For this reason, genome-enhancing device will facilitate sensible genomic studies to cope with essential questions regarding plant boom, development and responses to the surroundings. Linking genotype to phenotype remains a number one assignment in useful genomics. Information about gene characteristic and regulation may have big programs ranging, as an example, from engineering flowers for superior inclinations to address genetic diseases (Liu *et al.*, 2017). With the super advancements in sequencing technologies, the era of series records is incredibly tractable; however information gene characteristic remains a challenge.

Various medical conditions that involve genetic components, i.e. high level of cholesterol in the body, Tumors, hepatitis B or cancer can be treated and further risks could be eradicated by utilizing CRISPR/Cas9 as a genome editing tool. Its applications usually involve editing of the genomes of somatic cells that are not related to reproduction, interests are shown by various genetic engineers to apply this genome engineering technology to germ line cells that are involved in the process of reproduction so that required trait could be transferred to further generations but it obviously has certain vital ethical implications.

Molecular biologists have attempted to have a have a look at how a selected genotype influences a phenotype by means of developing opposite genetics processes which incorporates focused gene inactivation with the aid of harnessing homology directed repair processes (Adams and Sekelsky, 2002). The gene focused on technique pioneered with the aid of Mario Capecci, Martin Evans and Oliver Smithies became very inexperienced in mouse embryonic stem cells (ESC) proficient in the homology directed repair (HDR) (Capecchi, 2005). The software of this method in other cellular types and eukaryotic organisms has not been a success because of the inefficiencies of HDR mechanisms in most extraordinary organisms, which include plant life. For this reason, studies recognition has been to booming the efficiency of HDR. Several opinions located that the technology of double-strand breaks (DSBs) stimulates the cellular repair manner and could grow the performance of HDR through using several orders of magnitude (Townsend *et al.*, 2009). Therefore, attempts had been made to apply internet site online-particular nucleases (SSNs) to generate internet site-particular DSBs and harness the cell restore gadget to regulate the collection of the gene of interest (GOI). Era of net page-precise DSBs calls for a protein that may be reprogrammed to bind to any series of interest and cleave double-stranded DNA simultaneously. Such proteins do now not exist in nature, but may be made as chimeric synthetic versions amongst a customizable or programmable DNA-binding module and a nonspecific nuclease catalytic area (Townsend *et al.*, 2009).

Improvising of CRISPR/Cas9 technology: CRISPR technology was improvised from the natural defense mechanism (of

archaea and bacteria Cas proteins, accompanied with Cas9, to shield the attack of viruses, and other foreign bodies. The process is brought into regulation by chopping up and destructing the genetic material (DNA) of a foreign invading body (Adams and Sekelsky, 2002). These components are further utilized for the manipulation and editing of genes when introduced in more complex organisms (Pan *et al.*, 2016).

Introduction of DSB (Double Strand Break): The site specific genome engineering is made possible by Cas9 that is CRISPR associated enzyme. Cell repair Double strand breaks (DSB) are introduced at a specific site of interest that is RNA specified loci of chromosomes (Cong *et al.*, 2013). The Double Stranded Breaks (DSBs) are repaired by the cell utilizing two pathways: (1) NHEJ (Non Homologues End Joining) Pathways. (2) HDR (Homology Directed Repair) Pathways (Mao *et al.*, 2013).

Mechanism of CRISPR/Cas9 system: CRISPR/Cas9 mediated genome engineering mechanism involves the presence of a chimeric single-guide RNA that mediates the recruitment of Cas9 to the specifically targeted DNA. The targeted sequence of DNA is recognized via protospacer followed by PAM (Protospacer Adjacent Motif) DSBs induced via Cas9 are repaired either via NHEJ or via HDR. Indel mutations arise in case of repairment by NHEJ whereas; Repair of DSBs via HDR enables desired alterations in the sequence (Anders *et al.*, 2014).

Progression of type 2 CRISPR system: CRISPR/Cas9 gene editing system is carried by utilizing TYPE 2 CRISPR system where the required genes are inserted into the host/target cells via vectors (plasmids) or by inoculating the naked DNA into the cell. Type 2 CRISPR SYSTEM progresses by utilizing single gRNA which is formed by genetically engineering technique taking into consideration various tracer RNA and crRNAs (Song *et al.*, 2016).

Cas9 with customizable specificities: The process of editing of genome utilizing genetic engineering as a tool has nowadays become a major emergence in the latest field of genetics in order to make various biological researches successful. UTILIZING CRISPR Cas9 system we can assemble RNA guided Nucleases with customizable specificities. This technology has emerged to be very successful in handling and manipulating the bio-medically significant cell types and organisms that have been a question to manipulate in the long run. CRISPR Cas9 System has the bravura to perform specifically targeted and highly beneficial editing and transformation in the genome sequence and will be initiating the development of unique and latest tools to curb the human diseases (Mir *et al.*, 2018).

Basis of supremacy of CRISPR/Cas9 technology: If we bring under consideration the comparison of CRISPR/Cas9 technology with other nuclease technologies, then the bases of supremacy of CRISPR/Cas9 technology are acknowledged which are elaborated as under: Editing of genome via HDR or NHEJ is stimulated in plants or animal's genome at specific loci via CRISPR/Cas9 genome engineering technology (Briner and Barrangou, 2016; Gong *et al.*, 2018).

Lower unit cost: In order to retarget the Cas9 to a new sequence of DNA we just need to purchase a pair of (20-nt guide sequence encoding) oligos, (Mao *et al.*, 2013) which is much customizable as compared to the construction of two new TALEN genes (Zhang *et al.*, 2016).

Specific cleavage site: Enzyme is transformed into DNA nicking enzyme by either mutating the residues of catalysis of HNH or RuvC nuclease domains of Cas9 there is a cleavage at the targeted sequence between 17th and 18th bases (Nishimasu and Nureki, 2017) whereas; there is nonspecific cleavage in other nuclease technologies (TALENs and ZFNs) (Zhang *et al.*, 2016).

Multi Genomic loci targeted simultaneously : The editing of genome is accomplished much efficiently by utilizing CRISPR/Cas9 genome engineering technology as a mass/combination of sgRNAs could be delivered to the sites of interest (targeted sites) simultaneously (Globyte *et al.*, 2019).

Mechanistically, CRISPR Cas9 system functions in 3 distinct stages:

(1) Adaptation, acquiring of new spacers from invaders simply for immunization (Brooks *et al.*, 2014).

(2) crRNA Biogenesis, small interfering crRNAs are transcribed (Schiml *et al.*, 2014).

(3) Interference, crRNAs guide the Cas machinery to cleave the nucleic acids of invaders (Gao *et al.*, 2015).

CRISPR loci encoded adaptive immune system: In order to acquire immunity against the infection caused by bacteriophages, many bacteria have developed a specific Adaptive immune system that is typically RNA guided and Cas genes play key role in ensuring safe plasmid transfer. New spacers which are actually the short fragments of foreign DNA are incorporated into the host chromosome during the process of immunization (Gao *et al.*, 2015; Hirano *et al.*, 2016). This immunization process helps the host to stay protected in future if invaded by the same invader as the genetic record of previous infection remains in the memory without being altered (Svitashev *et al.*, 2015).

CRISPR system (Grouping into six types): According to the latest classification, CRISPR Cas9 System has been grouped into six different types on the basis of specific and unique sets of CRISPR associated (CAS proteins). If our aim is to target sequence degradation or crRNA BINDING then TYPE-1 and TYPE-3 CRISPR Systems are considered which have a large complex of multi cas9 proteins. Each DNA strand is cleaved with a specific domain of nuclease and for the maturation of crRNA, the basic requirement is tracer RNA (Esvelt *et al.*, 2013; Kleinstiver *et al.*, 2016).

Role of Cas9 enzyme: Cas9 is basically a DNA endonuclease that is multi-tasking Cas9 has two different nuclease domains: (1) HNH-like nuclease domain. (2) RuvC-like nuclease domain HNH-like nuclease domain is specified for cleaving the DNA strand that is complementary to the sequence of targeted strand i.e. guide RNA. RuvC-like nuclease domain is specified for cleaving the strand of DNA that is opposite to the sequence of non-targeted strand (Lim *et al.*, 2016; Barrangou and Horvath, 2017).

Contrast between RuvC and HNH nuclease domains: In order to cleave the non-targeted DNA strand a catalytic mechanism utilizing two metal ions is practiced by the RuvC Nuclease Domains. Mechanism of one metal ion is utilized when cleavage of targeted DNA is required and this sort of cleavage is carried out by HNH Nuclease Domains (Jinek *et al.*, 2012; Braff *et al.*, 2016). The RuvC nuclease domain resembles the structure of retroviral integrases on the other hand HNH nuclease domain comprises beta-beta-alpha metal fold structure. There is a conserved of base histidine in cleaving

enzymes of one metal ion dependent nucleic acid and aspartate residue in the cleavage enzymes of two metal ions dependent nucleic acids (Leenay *et al.*, 2016). If the aim is mutagenesis of either HNH or RuvC Nuclease domain, then cas9 is converted into nickase (Anders *et al.*, 2014). If we take into account the mutagenesis of both HNH and RuvC nuclease domains of cas9 ultimately resulting into dcas9 which is also known as (dead cas9), then endonuclease activity of cas9 remains no more functional, but DNA binding ability guided by RNA remains functional in dcas9 (dead cas9) (Fauser *et al.*, 2014).

Apo state bi-lobed structure of cas9: If we take into account the apo state structure of cas9, so it consists of two lobes; (Nishimasu and Nureki, 2017)**,** The alpha-helical recognition (REC) lobe**,** The nuclease (NUC) lobe**:** The nuclease (NUC) lobe further consists of two domains: The conserved HNH nuclease domain; The split RuvC nuclease domain: In addition, more variable C-Terminal Domain (CTD) is also present. Initially, when the apo-cas9 enzyme is not bound to the guide-RNA, it is not capable of recognizing the target DNA in other words apo-cas9 enzyme is in an inactive configuration prior to binding with the g-RNA (Fauser *et al.*, 2014).

Engineered nucleases enabling genome editing:

A DSB is stimulated at a targeted genomic loci and genome editing is promoted via cas9. Once the cleavage is done by cas9, the DNA damage repair of targeted loci is achieved by utilizing any one of the two pathways as enlisted below (Karvelis et al., 2019):

- The error-prone NHEJ pathway
- The high fidelity HDR pathway

Both of these pathways can be utilized in order to achieve the required genomic editing. In order to nullify the effect of larger genome deletions, a bulk of DSBs might be utilized. If we consider HDR, then it is a major pathway for repairing DNA (Amrani *et al.*, 2018). Alterations or modifications at the targeted loci may be achieved by HDR but the presence of repair template is must that is introduced via an external source. If we talk about repair template so, it either may be in the form of double stranded DNA or single stranded DNA oligonucleotides (ssODNS) (Chen *et al.*, 2017) Introduction of single nucleotide mutation is a comparatively minor genome editing and it can be made successful by utilizing single stranded DNA oligonucleotides (ssODNS) (Jinek *et al.*, 2012). The proficiency of HDR pathway can be different according to different scenarios, typically depending upon the state of cell , type of cell as well as the presence or absence of repair template. The HDR pathway is most probably suitable for those cells that are in the process of cell division (Koonin *et al.*, 2017).

Role of CRISPR/Cas9 genome engineering technology in humans: CRISPR/Cas9 Genome Engineering Technology plays a vital role in HUMANS via editing and modifications in germline and pleuripotent cells. Genes possessing resistance traits against various diseases are administered in the pleuripotent stem cells as a result of which descendants possess all beneficial disease resistant trait possessing genes (Vassena *et al.*, 2016).

Ethical contemplations: If we take into account the applications of CRISPR Technology in the field of Reproduction, it enhances our level of perception regarding gene functioning which may ameliorate medical skills and know-hows. Gene editing may boost the reproductive sovereignty of Humans by amending the defects that cause sterility in future parents and rectification of diseases in progeny (Gong *et al.*, 2018) but their lie some ethical contraventions regarding the genome editing in human germ-line cells as according to one point of view there would be modifications in Human species (Vassena *et al.*, 2016). An intently linked objection against genome editing associates the peril to human dignity and the concern for rejuvenation of eugenics. (UNESCO International Bioethics Committee, 2015)

CRISPR/Cas9 technology: Genetically edited crops: Recent advancements in genome editing skills have stirred substantial exhilaration among the agricultural scientists (Ahmed *et al.*, 2017; Ahmed *et al.*, 2019). These techniques propose new prospects for developing upgraded plant lines with the introduction of beneficial traits or excision of detrimental traits. Enhanced espousal of genome editing has been boosted by precipitously developing clustered regularly interspaced short palindromic repeats (CRISPR) (Noman *et al.*, 2016)

CONCLUSION: CRISPR/Cas9 has been declared as a unique genome engineering technique that has facilitated most efficient homologous recombination. The massive success of CRISPR/Cas9 genome engineering technology has been empowered by highly specific and efficient nature of DNA successful modulation of disease causing alleles in-vivo in animals and ex-vivo in somatic as well as pluripotent stem cells is a strongly convincing advancement in the future perspectives of Clinical Therapeutic genome editing, specifically for treating Human Cancer. We suggest, making efficient use of CRISPR/Cas9 of fruit plants, along with efforts to reduce diseases susceptibility, alternate plant structure or flower morphology, enhance fruit developments, and maximum fruit yield. Moreover, thorough characterization of cotton genes and beneficial modification in the genes via CRISPR/Cas9 to enhance cotton yield could be a possible perspective as well. Latest development in genome modifying affords a remarkable possibility for the genetic improvement of those agronomically essential fruit plants.

CONFLECT OF INTERST: Authors declare that they have no conflict of Interest

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