Mechanisms and Applications of CRISPR mediated Gene Silencing

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Abstract – *Genetic engineering has fascinated the world with its various techniques to manipulate the genes of organisms. The gene silencing technique, known as CRISPR, has now come to the limelight as it has successfully mitigated the long-standing limitations of the previous gene editing techniques. It is cost-effective, faster, accurate, and highly precise. It can successfully suppress the pathogenic genes, thus reforming the agricultural and healthcare sector. The advanced CRISPR-Cas$_9$ mediated gene editing has become a leading tool in the therapeutics of genetic diseases and disorders. In this article, the mechanism of the CRISPR-Cas$_9$ gene silencing technique and its recent trends in crop improvement and therapeutics have been highlighted along with regulatory, ethical concerns and prospects.*

Key Words**: CRISPR, Cas$_9$ protein, Gene editing, Genetically Modified Crops (GMCs), Gene knockdown, Crop improvement, Therapeutics, Epigenetic modification**

1.INTRODUCTION

Gene silencing, also known as epigenetic modification of gene expression, is a molecular biological process which involves gene silencing and/or gene knockdown and aims to control diseases by eliminating or suppressing the targeted gene from the genome. Since genes can be regulated either at the transcriptional level or at post-transcriptional level, gene silencing can be induced in both levels.

Researchers are studying gene silencing due to its potential to develop genetically modified crops(GMCs) and plants with better yield and higher resistance to target pests and also protect nature [1] (*Zhang Y et al, May 2017*). In the future, it may allow opportunities such as human gene therapy [2] (*Piergentili R et al, April 2021*), programmable RNA targeting, more advancements in agriculture and the health sector, a probable permanent cure for diseases which are incurable to date. It may also produce therapeutics to battle cancer, infectious diseases and neurodegenerative disorders [3] (*Pickar-Oliver A and Gersbach CA., Aug 2019*) and more [4] (*Grand Moursel L et al, April 2021*).

Gene silencing is a highly specific and targeted procedure. With the help of this revolutionary technique, a variety of characteristics of both plants and animals can be altered to a great extent, which will itself eradicate a lot of problems related to pest control, crop yield, food scarcity, human health and well-being. CRISPR-Cas9 mediated gene silencing is a new and intriguing technique which has fascinated the world with its immensity of opportunities.

1.1. HISTORY

CRISPR, which stands for, Clustered Regularly Interspaced Short Palindromic Repeats, was first discovered by Ishino in the year 1987 in Osaka University, Japan, while working with DNA sequences form Escherichia coli bacteria. The exact function and significance were unknown at that time [5] (*Ishino Y et al, March 2018*).

Significant information regarding CRISPR-Cas9 was obtained in 2007 by two French scientists, Rodolphe Barrangou and Philippe Horvath, who had been working with yoghurt cultures of bacteria Streptococcus thermophilus for the Danish company Danisco [6] (*Gostimskaya I, August 2022*).

The first demonstration of the mechanism of CRISPR-Cas9 was made in 2008 by Eugene Koonin and colleagues, National Center for Biotechnology Information, Maryland [7] (*Ratan ZA et al, Feb 2018*). Slowly after that, many more publications were made, and more understanding of this mechanism has been made. In recognition of the importance of CRISPR-Cas9,the Nobel Prize in chemistry was awarded to Jennifer Doudna and Emmanuel Charpentier in 2020 [8] (*Uyhazi KE et al, Jan 2021*).

CRISPR-Cas9 is a revolutionary genome-editing and gene silencing technology. It has high precision, is highly flexible and allows for precise modification of DNA sequences and modification & regulation of genes. It consists of a negative feedback mechanism which knocks down gene expressions and takes part in defence mechanisms. It is better than the previously known genetic engineering techniques due to the fact that it allows removal & introduction of more than one target gene at a time, is much less time-consuming and less costly [9] (*Uddin F et al, Aug 2020*). Also, it is not species-specific and hence, a wide range of organisms which were previously resistant to these genetic engineering techniques can now be explored.

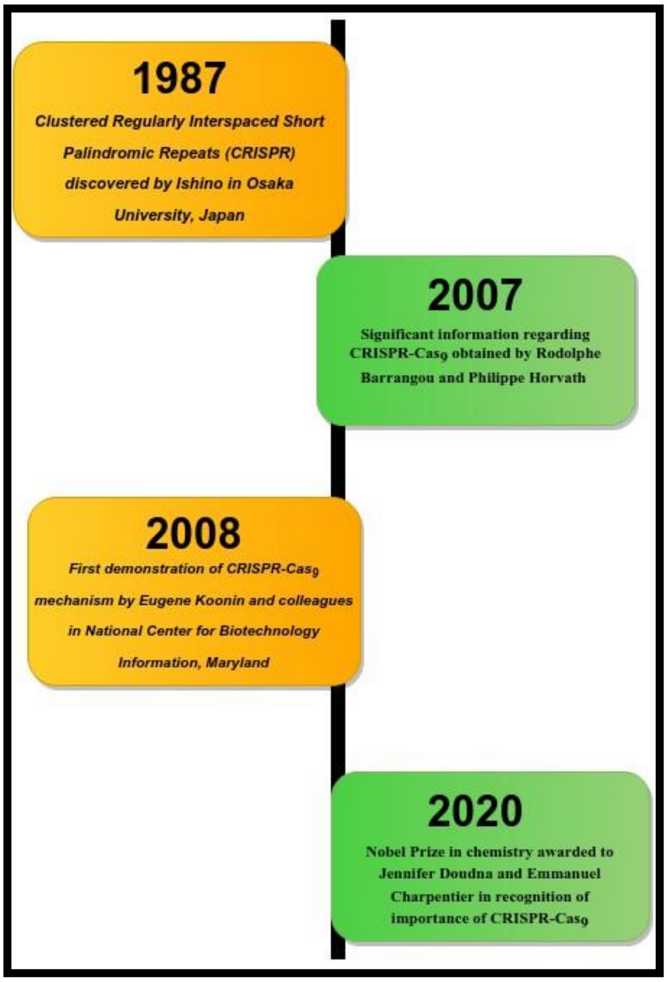

Fig -1: Timeline for CRISPR Gene Silencing Technology

2. MECHANISM

The CRISPR-Cas9 system is used to introduce pre-planned and tightly regulated changes in the genome. It relies on a few key molecules: -

Guide RNA (gRNA) - Approximately 20 bases long, these are pre-designed or synthetic RNA sequences, and can be located within a long RNA scaffold. These scaffolds are produced to be complementary to the target DNA sequences. This aids Cas9 to move to the specific site for gene slicing.

CRISPR scaffold RNAs (*scRNAs*) prove out to be the founding models for synthetic locus-specific transcriptional programming in Yeast [10] (Jesse G Zalatan, 2017).

Protein Cas9 - Protein *Cas9*, is an endonuclease enzyme which act as a pair of '**molecular scissors**'. They have the capability to cut double stranded DNA (dsDNA) at specific locations in the genome guided by gRNA. Cas9 follows gRNA to the exact, precise location in the DNA sequence and cuts this dsDNA across both its strands. This is done so that the required pieces of DNA can be inserted or removed. The mechanism of CRISPR-Cas9 mediated gene silencing is discussed in following section.

The prokaryotes and the eukaryotes contain a series of short, repetitive DNA sequences known as CRISPR. It contains small segments of unique and distinct DNA sequences known as ***spacers*** and are found between the repetitive DNA sequences.

During successive infections, CRISPR sequences are employed to find and remove DNA from related bacteriophages. As a result, the spacers serve as molecular memory. Therefore, these sequences play a crucial part in the organism's antiviral (or anti-phage) defensive system and offer a sort of acquired immunity [11] (Frank Hille et al, March 2018).

The CRISPR array is transcribed into a precursor RNA and is processed using the enzyme Cas9. They together are called *CRISPR RNAs* (*crRNAs*). To simplify this complex process, the guide RNA (gRNA) combines with crRNA and hence, they combine their functions in a single RNA molecule. It is now known as the ***Cas9 complex***.

The Cas9 protein consists of 2 key domains - the ***HNH domain*** and the ***RuvC domain***. The HNH and RuvC domains catalyse cleavage of the complementary and non-complementary strands, respectively. These two domains aid in the cleaving process. The ***Protospacer adjacent motif (PAM)*** is a short sequence adjacent to the target DNA and serves as the recognition site [12] (Gleditzsch D et al, April 2019). The Cas9 complex then locates the target DNA and recognizes the PAM. Then the HNH and RuvC domains, cleave the strands and create ***double-strand break (DSB)*** in the DNA.

The CRISPR-Cas9 process now makes use of the cell's natural mechanism of DNA repair. There are two pathways to continue the DNA repair mechanism. ***Non-Homologous End Joining (NHEJ);*** In this pathway, the cell often introduces small insertions or deletions at the DSB sites, thus leading to gene slicing. ***Homology-Directed Repair (HDR);*** This pathway is used to insert specific or target changes into DNA [13] (Xue C et al, July 2021). Here, researchers and scientists prepare a ***repair template,*** which is basically the synthetically prepared piece of DNA with the desired genetic **changes embedded into it, and this template is inserted into** the site of the DSB. Lastly, the DNA repair process leads to target genetic modifications and aids in gene slicing.

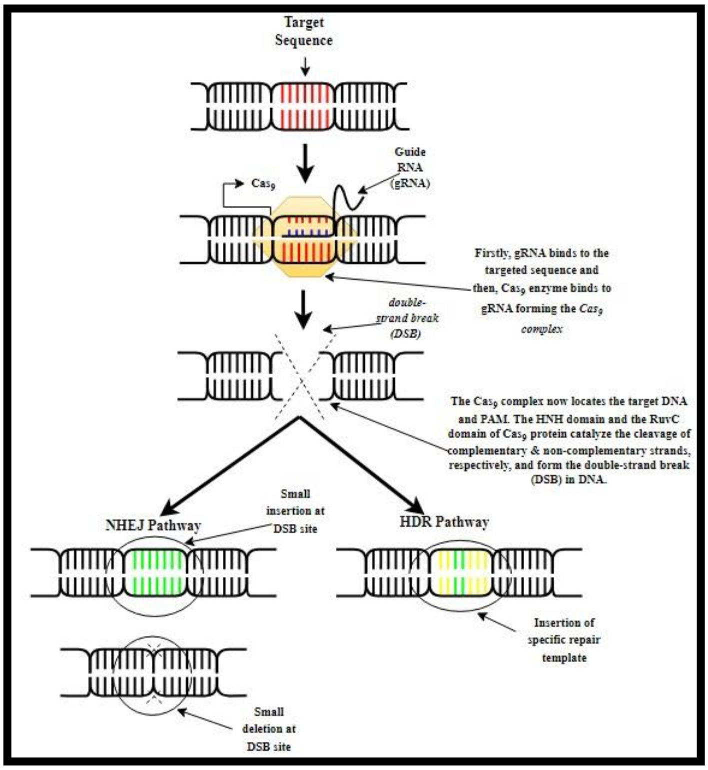

Fig -2: Schematic representation of the mechanism of the CRISPR-Cas$_9$ gene silencing approach

3. RECENT ADVANCEMENTS

3.1. CROP IMPROVEMENT AND AGRICULTURAL SECTOR

Pseudomonas syringae affects tomato plants and the affected plants exhibit dark brown or black lesion encircled by yellow halos in leaves and black lesions in stems and pedicels. CRISPR-Cas$_9$ mediated mutagenesis of a *DMR6* ortholog in tomato plants can be used to create enhanced resistance to bacterial pathogen, *Pseudomonas syringae* [14] (Thomazella DPT et al, July 2021).

Salinity has adverse effects on rice plants, including death and loss in quality of rice. Critical salinity levels can lead to huge loss in rice yield. Research shows how CRISPR-Cas$_9$ targeted mutagenesis of *OsRR22 gene* enhances rice salinity tolerance, thus increases the plant's adaptability to saline conditions [15] (Zhang, A. et al, March 2019).

Drought stress typically leads to leaf rolling, stunting, yellowing and, in extreme cases, permanent wilting and stomatal loss in *Arabidopsis*. CRISPR-Cas$_9$ mediated targeting of *protein AREB1* leads to drought stress tolerance [16] (Roca Paixão J.F. et al, May 2019).

Grain width and size are crucial traits while determining the plant yield production. It also influences the appearance and marketability of the grain. In reply, research indicates that rice grain nutritional quality and the aleurone layer are enhanced by a CRISPR-Cas9-mediated mutation in the GRAIN WIDTH and WEIGHT2 (GW2) locus [17] (Achary V.M.M. et al, Nov 2021).

Soyabean seeds contain approximately 12-15% of fatty acid components. India consumes different oils, including soybean oil, and vegetarians and vegans rely heavily on soy & soy products. It may lead to obesity, coronary heart disease, certain types of cancer, cardiovascular diseases, and early death. Thus, this makes it a bad choice in food applications. Improved oil content in soybeans originated from a significant decrease in the concentration of saturated fatty acid in soybean seeds induced by the CRISPR-Cas9-mediated deletion of *GmFATB1* [18] (Ma J. et al, April 2021).

Cauliflower Mosaic Virus (CaMV) is a virus which leads to chlorosis, mosaic, vein clearing and stunting of cauliflower. It impacts the agricultural sector and economical sector. Research on CRISPR-Cas$_9$ mediated mutation of *CaMV CP gene* could lead to successful resistance to cauliflower mosaic virus(CaMV) [19] (Liu H et al, March 2018).

Fragrance is one of the most important qualitative traits for rice breeding. It moulds consumer preferences and thus it affects market value and plays a vital role in global trade and identity. Adding onto this, research conducted on *Oryza sativa* explains how CRISPR-Cas$_9$ -mediated editing of the *BADH2 gene* triggers the revolution of rice fragrance [20] (Imran M et al, Feb 2023).

Fusarium oxysporum is a soil borne fungal disease commonly observed in watermelon. It leads to stunting, wilting and early systemic yellowing of the watermelon, leading to the early death of the plants. Its known for its destructive characteristics and significant loss in the yield and individual weight of watermelons. To prevent this, gene editing of the *Clpsk1* gene using the CRISPR-Cas$_9$ mechanism, makes the watermelons more resistant to *Fusarium oxysporum f.sp. niveum* [21] (Zhang M et al, March 2020).

Papaya ringspot virus (PRSV) is a common & destructive disease seen in Papayas. It leads to yellowing and stunted growth of the plant and circular spots on the fruits & leaves. Economically, it has drastic effects and is one of the major hurdles in Papaya cultivation. Research conducted on this, shows how the mutagenesis of *melon Prv gene* with the help of CRISPR-Cas$_9$ gene editing mechanism, helps the plant to develop a resistance towards this disease [22] (Nizan S et al, Aug 2023).

Fire Blight disease is caused by the bacterium *Erwinia amylovora* is responsible for death of apple and pear fruits, twigs, roots, shoots, blossoms, leading to death of the entire plants. This has become a major limiting factor for apple and pear cultivation, ultimately leading to heavy economic losses. CRISPR-Cas$_9$ gene knockout of *MdDIPM4* can minimize the adverse effects of this disease [23] (Pompili V et al, Sept 2021).

Pathogenic fungus, *Ganoderma boninense*, is significantly affecting the cultivation of the Oil palm plants. It leads to the rotting of different regions and an early death of the plants. Its been observed that within a span of half a year, it can cause about 43% of economic losses in South East Asia. CRISPR-Cas_9 mediated gene knockout of the *EgEMLP gene* has been performed to control this adverse situation [24] (Budiani et al, Jan 2020).

Xanthomonas oryzae pv. Oryzae is a fungal pathogen which causes *Bacterial blight*. It leads to stunted growth of young/new plants, yellow/brown spots on the leaves leading to drying & early death of the leaves and plants. CRISPR-Cas_9 gene silencing technique is used to understand how the sucrose transporter gene *OsSWEET13*, which is identified as the disease-susceptibility gene for *PthXo2*, can be exploited to develop a resistance towards this disease [25] (Zhou J et al, May 2015).

Pathogens such as *Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ringspot Virus (PRSV), and Watermelon Mosaic Virus (WMV)* belong to the *Potyviridae family*. They are responsible for yellowing of leaves, leaf mosaic patterns, stunted growth, systemic mottling, and necrosis of the plants. To mitigate this hurdle, gene knockout of the *elF4E gene* has been done using the CRISPR-Cas9 mechanism and the results show a profound resistance of the disease in cucumbers [26] (Fidan Hakan et al, March 2023).

Table -1: Recent Advancements in Crop Improvement and in the Agricultural Sector

CROP/PLANT	TARGET SEQUENCE	TRAIT	REFERENCE
Tomato (*Solanum lycopersicum L.*)	*DMR6 ortholog gene*	*Pseudomonas syringae* exhibits dark brown or black lesion encircled by yellow halos in leaves and black lesions in stems & pedicels	Thomazella DPT et al, July 2021
Rice (*Oryza sativa*)	*OsRR22 gene*	Death & quality loss of *Oryza sativa* due to salinity	Zhang, A. et al, March 2019
Thale cress *(Arabidopsis)*	*protein AREB1*	Drought stress leads to leaf rolling, stunting, yellowing, permanent wilting and stomatal loss	Roca Paixão J.F. et al, May 2019
Rice (*Oryza sativa*)	*GRAIN WIDTH and WEIGHT2 (GW2) gene*	Accentuation of nutritional quality, grain width and grain size of rice to improve marketability	Achary V.M.M. et al, Nov 2021
Soyabean (*Glycine max*)	*GmFATB1 gene*	Consumption of soyabeans (containing 12-15% of fatty acids) may lead to obesity, coronary heart disease, etc	Ma J. et al, April 2021
Cauliflower (*Brassica oleracea var. botrytis*)	*CaMV CP gene*	*Cauliflower Mosaic Virus* (CaMV) leads to chlorosis, mosaic, vein clearing and/or stunting	Liu H et al, March 2018
Rice (*Oryza sativa L*)	*BADH2 gene*	Improving the fragrance aspect impacts consumer preference and global marketability	Imran M et al, Feb 2023
Watermelon (*Citrullus lanatus*)	*Clpsk1* gene	*Fusarium oxysporum f.sp. niveum* is known for its destructive characteristics and significant loss in the yield and individual weight	Zhang M et al, March 2020
Papaya (*Carica papaya L.*)	*melon Prv gene*	*Papaya ringspot virus (PRSV)* leads to yellowing and stunted growth of the plant and circular spots on the fruits & leaves	Nizan S et al, Aug 2023
Apple (*Malus domestica*)	*MdDIPM4 gene*	*Fire Blight disease* caused by bacterium *Erwinia amylovora* is responsible for death of apple and pear fruits, twigs, roots, shoots, blossoms, leading to death of the entire plant/tree.	Pompili V et al, Sept 2021
Oil palm (*Elaeis guineensis*)	*EgEMLP gene*	*Ganoderma boninense* causes the rotting of different regions and an early death leading to heavy economic losses	Budiani et al, Jan 2020
Rice (*Oryza sativa*	*OsSWEET13*	*Bacterial blight* caused by *Xanthomonas oryzae pv. Oryzae*	Zhou J et al, May

L)	*gene*	leads to stunted growth of young/new plants, yellow/brown spots on the leaves leading to drying	2015
Cucumber (*Cucumus sativus L.*)	*elF4E gene*	*The Potyviridae family* is responsible for yellowing of leaves, leaf mosaic patterns, stunted growth, systemic mottling, and necrosis	Fidan Hakan et al, March 2023

3.2. THERAPEUTICS AND HEALTHCARE SECTOR

Porcine Reproductive and Respiratory Syndrome (PRRS), also known as Blue ear disease, is a contagious viral disease and causes diminishing immune responses, respiratory problems, reproductive failure or infertility as well as death in animals, especially in pigs. CRISPR-CAS9 gene editing of the *CD163 gene* in pigs makes them resistant to this highly pathogenic disease [27] (Chen J et al, Jan 2019).

Dehorning of dairy animals is a practice done on dairy farms to prevent injury of animals or others, including herd members or the caretakers. It involves removal of horns from the skull using chemicals, hot iron or blades and is an excruciatingly painful and traumatic experience. Research produced hornless dairy cattle from genome-edited cell lines, to relieve the dairy cattle [28] (Carlson DF et al, May 2016).

Malaria is a disease caused by plasmodium parasite and spread by female *Anopheles stephensi* mosquitoes. It mostly causes recurring fever & chills, nausea, vomiting, diarrhea, anaemia and jaundice in humans. CRISPR-Cas9 technology is being implemented to modify this mosquito population to prevent the spread of this disease in the future [29] (Gantz VM et al, Dec 2015).

Avian leukosis virus (ALV) causes *lymphoid leukosis* disease in poultry animals, especially chickens which can cause tumour formation in liver, spleen and/or in kidneys or sex organs. It leads to heavy economic losses to the poultry sector, locally and globally. CRISPR-Cas9 mediated gene editing of the *NHE1 gene has led to r*esistance of J subgroup of ALV in chickens [30] (Koslová A et al, Jan 2020).

Lung carcinoma, commonly known as Lung cancer, is a malignant tumor which develops in the lungs. It commonly leads to breathing troubles and is a fatal disease. Smoking may lead to lung cancer. According to WHO, lung cancer held the 2nd position (2.21 million cases) globally, in cancer related deaths in 2020, just after breast cancer. While there are medicines and other clinical approaches to cure this, it has led to very poor results and cases of drug resistance have also been observed. Since we know briefly that genetic mutations lead to activation of the oncogenes, gene modification technologies, especially CRISPR-Cas9 technology is trying to target the tumor-suppressor genes and the oncogenes which are commonly the sites for mutation and tumor growth, to modify and regulate their expressions in cells. This could prove to be more effective and a persistent cure when compared to conventional methods [31] (Akbari Kordkheyli V et al, April 2021).

Cancer is a deadly disease where a malignant tumor formation takes place. It causes pain, fatigue, weight loss, changes in the brain and nervous system and, in extreme cases, death. Various clinical approaches have been devised to fight off cancer. One of them is *immunotherapy*. In immunotherapy, body's immune system works to find and eliminate cancer cells. This process is often applied alongside chemotherapy or radiation therapy. CRISPR-Cas9 technology can further boost the capacity and tend to the limitations of immunotherapy. With this, CRISPR-Cas9 based genome-wide screening of the target oncogenes might be possible [32] (Liu Z, Shi M et al, Feb 2023).

Sickle cell disease is an autosomal recessive blood disease caused by single point mutation of nitrogenous base A instead of T in the sixth(6th) codon of the β-globin gene. This leads to a change in amino acids from glutamic acid to valine(V) and further leads to formation of a distorted sickle-like red blood cell. This affects the longevity of RBCs and thus they die prematurely. Patients suffering from sickle cell disease, hence, often acquire anaemia, suffer from obstructed blood flow, which may in turn lead to stroke, cardiovascular abnormalities, and early demise. CRISPR-Cas9 is used in target disruption of the *HBG1 and HBG2 gene* promoters in an approach to treat Sickle Cell Disease [33] (Sharma A et al, Aug 2023).

The *Human Immunodeficiency Virus*, abbreviated as HIV, is a retrovirus which attacks the immune system of the host organism and renders it highly vulnerable. It has 3 phases viz. acute HIV, chronic HIV infection and AIDS (*acquired immunodeficiency syndrome*). HIV greatly affects the longevity of the organism. Many treatment techniques are in the market but none of them can provide a complete cure for this disease or improve the health status of the target individual. *Antiretroviral Technology* (ART) has been effective in suppressing the HIV virus up to a great extent but has severe drug toxicity as one its limitations. Thus, researchers are now looking out for novel approaches which will have high precision, accuracy and less toxic effects on the host organism. The CRISPR-Cas9 technique has already been used to target the HIV-1 genome in both *in-vivo & in-vitro* studies involving non-human primates. CRISPR has the potential to be launched into the market as the primary genome editing tool to eradicate the HIV virus [34] (Bhowmik R and Chaubey B., Dec 2022).

Table -2: Recent Advancements in Therapeutics and Healthcare Sector

TARGET POPULATION	TARGET SEQUENCE	DISEASE/TRAIT	EFFECT	REFERENCE
Pig (Sus domesticus)	CD163 gene	Porcine Reproductive and Respiratory Syndrome (PRRS)	It causes diminishing immune responses, respiratory problems, reproductive failure or infertility as well as death	Chen J et al, Jan 2019
Dairy Cattle		Dehorning is done to prevent injury of animals.	It involves removal of horns from the skull blades, chemicals and other painful methods	Carlson DF et al, May 2016
Female Anopheles stephensi mosquitoes		Malaria is a disease caused by plasmodium parasite	It mostly causes recurring fever & chills, nausea, vomiting, diarrhea, anaemia and jaundice in humans	Gantz VM et al, Dec 2015
Chicken (Gallus gallus)	NHE1 gene	Avian leukosis virus (ALV) causes lymphoid leukosis disease	It can cause tumour formation in the liver, spleen and/or in the kidneys or sex organs	Koslová A et al, Jan 2020
Homo sapiens		Lung carcinoma	It commonly leads to breathing troubles and is a fatal disease	Akbari Kordkheyli V et al, April 2021
Homo sapiens		Cancer	It causes pain, fatigue, weight loss, changes in the brain and nervous system and, in extreme cases, death	Liu Z, Shi M et al, Feb 2023
Homo sapiens	HBG1 and HBG2 genes	Sickle cell disease	It causes distortion of the RBC, leading to reduced lifespan of RBC, can lead to obstructed blood flow, which in turn leads to stroke, cardiovascular abnormalities and early demise	Sharma A et al, Aug 2023
Homo sapiens		Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS)	It causes fatigue, weight loss, muscle and joint pain, shingles, swelling of lymph nodes. It renders the immune system highly vulnerable, leading to early death.	Bhowmik R and Chaubey B., Dec 2022

4. ADVANTAGES & DISADVANTAGES

In recent years, scientists have been utilizing the gene silencing technique in drug development. The pharmaceutical research and development team are always in steady search of potential new molecules for drug development and therapeutics [35] (Grand Moursel L et al, April 2021). Gene silencing is growing to become a powerful tool in genetic engineering. Just like every coin has two sides, gene silencing techniques also come with their pros and cons.

4.1. ADVANTAGES

Gene silencing is a technique which regulates gene expression in a cell and prevents expression of a target gene. Once this target gene is silenced using various techniques such as RNAi, CRISPR-Cas9, TGS or PTGS, its expression gets significantly reduced. Hence, gene silencing can be used for epigenetic modification where the gene expression is regulated and altered without changing the DNA sequence in the target plant/crop or animal.

CRISPR-Cas9 mediated gene silencing is being employed to provide resistance to infectious as well as fatal diseases [36] (Borrelli VMG et al, Aug 2018). Gene silencing can also be employed in suppression of viral genes. It can thus become a potent tool in battling with viral diseases such a HIV/AIDS, in the future [37] (Shahriar SA et al, June 2021).

CRISPR-Cas9 helps in improving productive traits and in producing biomedical models for research and development [38] (Torres-Ruiz R et al, Jan 2017).

In the agricultural sector, gene silencing is used extensively utilized to generate pest resistant crops which target and kill only the targeted insects or pests, making it non-lethal for others. It is also used to design commercially important crops having higher nutritional content, higher yield, size, aroma, taste, texture, and other desirable traits.

CRISPR-Cas9 also takes part in animal welfare and reduces the number of the animals that are constantly being used for scientific experiments. Gene silencing is also being used to target oncogenes to inhibit tumour growth. It is used to design effective cancer treatments [39] (Balon K et al, Jan 2022).

Gene silencing can reform the health and pharmaceutical industry with effective medicines having reduced side effects. It could also prove to effectively suppress disease causing genes. Gene silencing can target genes with high precision, leading to almost zero percent chances of off-target effects.

4.2. DISADVANTAGES

Even though the chances are close to nil, gene silencing might unintentionally affect non-target genes which might cause unexpected and drastic consequences [40] (Guo C et al, March 2023).

Gene silencing might not be a permanent cure, thus leading to multiple sessions of therapy. It is also a highly challenging task to efficiently deliver gene silencing molecules to the target cells or tissues.

Gene silencing may trigger immune responses, which in turn might lead to unexpected and adverse reactions in the body.

It also raises ethical concerns as it leads to silencing of genes and even knockdown of genes. The use of gene silencing on the human genome is yet to be completely legalized by the government [41] (Shinwari ZK et al, Sept 2017).

It requires complex machinery & specialized expertise. Long-term safety of gene silencing techniques, limited applicability and regulatory challenges are still under tight investigation which also needs to be addressed.

5. REGULATORY AND ETHICAL CONCERNS

The CRISPR-Cas9 system might affect a non-target region which might be similar in structure or function, leading to unintended genetic modifications. This might pose unknown threats to the crop or organism subjected to the above technology [42] (Naeem M et al, July 2020).

The ethical, social, and biosafety aspects need to be thoroughly studied and understood before introduction of genetically modified crops [43] (Shinwari ZK et al, Sept 2017). The long-term effects of consumption of crops modified using the CRISPR-Cas9 mechanism are yet to be understood in depth.

Extensive research and development is being conducted to understand how editing the germ line by bringing alterations in the sequences of the ovum or the sperm, can affect the future progenies.

The CRISPR-Cas9 gene silencing technique has the capability for precise gene slicing and gene knockout, making it a powerful tool. While the CRISPR-Cas9 gene editing has been implemented in various crops, leading to increased tolerance to biotic as well as abiotic stress, their impact in the ecosystem is yet to be analysed and quantified. CRISPR-Cas9 is a recent gene editing mechanism, it further needs well-defined regulatory framework and guidelines which necessitates heavy restrictions and thoughtful ethical implications before releasing the products into the market.

6. CONCLUSIVE REMARKS

Gene silencing has revolutionized genetic engineering in recent years. It is a precise and powerful technique which regulates the expression of a targeted gene and inhibits its expression. It could be utilized in the plant & crop industry to enhance their yield, nutritional content, taste, size, aroma, reduce allergic properties, and other characteristics. CRISPR technology has applications to enhance livestock production, taking into consideration the global food demand, reducing the impact of farming on the environment, enhancing the overall health of humans and livestock.

Few of the applications of CRISPR are in animal welfare, providing pest resistance for plants by suppressing them or altering plants such that they only target pests get eradicated, providing resistance to infectious as well as fatal diseases, improving productive traits, producing biomedical models for research, among others [44] (Liu Z et al, June 2022).

It has a huge capability in medical research and therapeutics [45] (Grand Moursel L et al, April 2021). Gene silencing in human genome is associated with ethical concerns. However, efficient and accurate use of gene silencing techniques has the potential to treat those diseases, including cancers. With the judicious and ethical use of this powerful technique, the world will witness a new era of advancement and prosperity.

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