The Role of CRISPR/Cas9 for Genetic Advancement of soybean: A Review

ABSTRACT

Soybean is a crucial legume crop that is mainly grown for extracting oil and protein content Which can be used as a food source for human beings as well as livestock. We can also use the protein obtained from soybean for the extraction of biofuel. There is a dire need to increase genetic research on soybean for improvement and enhanced production. One big reason for genetic research on soybean is to make its resilience to the change in the climate. In modern days CRISPR/Cas9 has evolved as an emerging technique that allows us to manipulate the gene of selected traits in most crops including soybean. Advanced tools of biotechnology are widely utilized for the enhancement of crop production, improving quality and yield, introducing disease and insect resistance, and being environmentally friendly. This review gives a glimpse of how the mechanism of CRISPR/Cas9 performs its functions and a brief discussion of CRISPR/Cas9 which has increased the scope of study in the genetic advancement of soybean. It also illustrates some phenomena in which we can use CRISPR/Cas9 for the betterment of soybean.

Keywords*: CRISPR/Cas9, Genetic improvement, soybean, gene editing*

1. INTRODUCTION

Soybean *(Glycine max)* belonging to the Fabaceae family originated in China. About 23,000 different varieties of soybean were first domesticated in Asia, in China, and were then transferred to the USA and Brazil [1]. Due to their high protein content, soybeans are a highly significant legume that is eaten all over the world. They are most frequently utilized in dishes from Japan, Korea, and Indonesia. Typically, soybeans are fermented to create unique ethnic dishes or culinary components or sprouted for use in salads. When soybeans are cooked for around five minutes in Japan, they are frequently eaten as a snack with beer. Tofu is a protein-rich meat alternative that is produced from soybeans in several East Asian nations. Soybean is fermented with a food-grade fungal disease of the Rhizopus organisms in Indonesia to make tempeh, a protein-rich meat alternative [2]. Additionally, soybeans are a valuable commodity in the food, fuel, and fisheries industries due to their high protein content. Soybean is a major commodity crop, used for both food and fuel. Its oil content (18-22%) is second only to peanuts among edible legumes, and its protein content (40-42%) is the highest of any food crop [3]. Pests, diseases, and weeds all negatively impact soybean crop output, with weeds being the primary culprit. Each year, microorganisms including fungi, viruses, bacteria, oomycetes, and nematodes economic loss. Pests that affect soybeans include aphids, beetles, mites, and stinkbugs attack the plant as a whole. The production and quality of soybean crops are both reduced as a result of pathogen infection. Soybean rust, bacterial blight, and soybean mosaic are the most significant diseases, affecting the majority of crop output, on the list of main diseases in soybean caused by numerous pests and pathogens. [4]. Abiotic stressors are the numerous environmental challenges that plants constantly face and can significantly lower their output. Complex and involving many physiological, molecular, and cellular adaptations, plant

responses to these challenges are difficult to comprehend. Different issues severe salinity or drought, excessive heat, and flooding, toxic metals/metalloids, ozone, UV radiation, bright light, etc. are some of the main abiotic stressors. The growth of plants is retarded by the stress due to osmotic pressure in the saline type of soil while the stress of drought which results due to climate change is very dangerous for plant growth. This creates an alarming situation for researchers and farmers as well. Plants are frightened by the harmful consequences of high-temperature stressors as a result of global warming. Some major consequences of high temperature are denaturation of protein, deactivation of enzymes in many reactions, oxygen reactiveness is increased in oxygen species, destruction of membrane structure, and harms in the form of ultra-structural cellular components. Another significant environmental element that frequently impacts plant development and agricultural productivity, again resulting in significant crop losses, is low temperature or cold stress [2]. To combat these stresses and increase the yield advanced technology CRISPER-Cas9 is used. The CRISPR/Cas9 method for gene editing, based on site-specific nucleases, has made strides in the effective targeted alteration of various crops for improved yield and resistance to diseases and other challenges. To create diversity and hasten breeding efforts, CRISPR-Cas9 has proved to be a powerful editing tool for the accurate editing of genome sequences in all types of crops. In this context, the review emphasizes the many functions and uses of the CRISPR/Cas9 system as potent technologies to raise agricultural yields and increase tolerance to environmental stress in agronomically significant soybean, regarding this, the review illustrates the high-efficiency use of CRISPR/Cas9 to boost yield, developing resistance of environmental and biotic stresses [5].

2. Genome Editing Using CRISPR/Cas9:

CRISPR/Cas9 consists of the immune system of prokaryotes (those organisms which don't have prominent Nuclei). It works upon the guidelines of RNA while genome editing on a specific site. This system is widely used to cure human genetic diseases. This is a very effective system but at the back end, it has some drawbacks like off-target effects [6]. Long before the discovery of CRISPR/Cas9, it was very difficult to edit the genes. The two most important techniques (i) Zinc finger nucleases (ZFN) and (ii) Transcription activator-like effector nucleases (TALENs) was very popular for editing target DNA. But their drawback was high cost and they were more time-consuming than CRISPR/Cas9 [7]. As there are many types of stresses in the plant they can be biotic or abiotic. Researchers always want to edit the genome of plants for the genetic improvement of different traits. CRISPR/Cas system is used for improvement in nutrition, biotic and abiotic stress resistance, metabolic engineering, etc. It has a wide range of applications [8].CRISPR/Cas9 is a simple system that is very easy to use for the fast and efficient editing of genes [9]. It is most recent technique is genome engineering [10]. CRISPR/Cas9 system is categorized into 2 classes according to the function of the Cas protein. Class 1 has Cas protein complexes and includes type1,3and 4 while Class 2 is simple and has single proteins which include types 2,5 and 6. Cas protein and guide RNA are the most important in CRISPR/Cas9 system [7]. Efficient genome editing through this system needs a vector system, target sites, and an appropriate way of transformation. Special vectors are designed to achieve highly efficient results with accurate editing [2]. So CRISPR/Cas9 is a potential tool for genome editing. The complete process of CRISPR/Cas9 is consist of just 3 steps. First of all (i) Recognition, then (ii) Cleavage, and at the last (iii) Repair. Single-guided RNA must be present for the working of Cas protein. sgRNA signals the Cas protein to recognize the targeted DNA sequence. It then cut the sequence at specific sites.Then there are two mechanism of repairs for Double-Stranded break for homologous and non-homologous ends [7].There are two setbacks of this efficient systems, one is that there is specific PAM sequence for ortholog of each Cas9 while other is there is danger of mutation which is not needed at all. [11].

3. IMPLEMENTATION OF CRISPR/CAS9 IN SOYABEAN

In 2015, CRISPR/Cas9 was used first time [12]. After the successful execution of initial attempts, many researchers started to use this technique for the manipulation of genes of soybean for research and development and made remarkable progress. This technique has great potential to improve the soybean by targeting mutagenesis and its qualitative and quantitative traits also.

3.1 Steps involved in the application

First of all selection of a plant and the selection of the targeted site for mutagenesis and PAM sequence is recognized. In the second step, sgRNA was designed and then CAS9 and sgRNA were assembled in a suitable vector. In the next step, the vector is transformed into the selected soybean plant and transformants are identified. Finally detection of mutation by sequencing or PCR/Re assay. By following the above mention procedure CRISPR/CAS9 system was utilized for genome editing in soybean for improving its various traits by targeting its genes. By knockout of genes (SPL9a, SPL9b, SPL9c, SPL9d) and(GmLHY1a, GmLHY1b, GmLHY2a, GmLHY2b) plant architecture features influences and by targeting (GmAP1, Gmprr37, GmE1, GmFT2a) flowering period is affected which increases the crop yield. In the same way by knocking out different genes various traits in soybean such as crop nutrition and quality, and nodulation were improved, and tolerance to biotic and abiotic stresses is achieved.

3.2 Improvement of yield

The prime objective of many researchers is to bounce the yield. Yield is the result of different factors like gene and their interaction with the environment and many abiotic and biotic factors which affects the crop

in vegetative and developmental growth stages. It is the most crucial trait which is measured by different aspects like the number of pods, the number of grains, seed weight some other factors which affect yield indirectly such as plant height, node number, leaf length, width, and shape, as well as branch count, are all intermodal [13]. The four crucial genes, "GmSPL9a, GmSPL9b, GmSPL9c, and GmSPL9d, from the SPL9 family," were swapped out using CRISPR/Cas9. Soybean plant architecture was studied after mutations were induced in four genes [13]. It was concluded from research that the mutant spl9a/spl9b from T2 double homozygous has a small plastochoron value. From the experiment, it was found that the T2 double homozygous mutant spl9a/spl9b had a shorter plastochron length. This led to the uplift of trifoliate leaves, nodes, and branches of the soybean plant.[14]. These characteristics like more branches, more node number, and an increase in dry weight show a positive response in correlation with yield [13]. Height is also a yield determinant factor as a long plant means longer inter-nodal distance and stem which results in lodging. Thus yield is ultimately decreased due to grain loss [15, 16]. Another effect that was observed was due to lodging is the reduction of seed, seed loss, and unhealthy and less vigorous seed.[17]. Many steps are practiced with time for the reduction of plant height in numerous staple crops [18]. In Soybeans, the CRISPR/Cas9 was used to target four major genes which are responsible for the Late Elongated Hypocotyl (LHY) family of genes to explain their potential regarding plant height and inter-nodal distance. There is a significant role of quadruple mutants in soybean for the reduction of plant height along with inter-nodal distance for improving crop yield in comparison to wild-type plants. It is also an observation that the experimental mutant soybean has less amount of gibberellic acid while the wild type has a great quantity. Gibberellic acid acts as a role in developmental stages and improves plant height, elongation, and leaf expansion also. So gibberellic acid content also altered through this.

3.3 Altering plant architecture

Exemplarily Plant structure is very necessary for better production Ideotype is a necessary character for good yielding and its character is under the control of the growth habitat of soybean cultivar. So in this way genes such as GmLHY linked by gibberellic acid are altered through editing [13].

3.4 Improving Quality

CRISPR has an inevitable role in case of quality improvement in soybean which is discussed as under

3.4.1 Improving Seed Sugar Content

Soluble sugar contents, amino acids, and mineral nutrition are very important for the quality of seed. GmSWEET10a and GmSWEET10b assess the size of the seed, quality of oil, and protein contents at the same time in soybean. These two genes also check the allocation of sugar for the seed coat and embryo [19]. Soybean has a much larger seed as compared to Arabidopsis and it requires much more sugar. Therefore, minor changes in their sugar content will create a strong effect on seed development [20].

3.4.2 Decreasing the unpleasant seed beany taste

Soybean possesses a huge quantity of protein content. It also has a characteristic of special taste like soy flavor which inhibits the acceptance of soybean and its products for humans on the big canvas. There are three Lipoxygenases in soybean which are under the supervision or control of the *Lox1*, *Lox2*, and *Lox3* genes. This lipoxygenase plays a vital role in the oxidation of linoleic acid and linolenic acid. As a result, they produce a grassy and beany taste in soybean and its byproducts. For getting Lipoxygenase-free soybean lines, the CRISPR/Cas9 system was applied to cause a mutation in *GmLox1*, *GmLox2*, and *GmLox3* genes. They could be used for the reduction of beany flavor in soybean. [21].

3.4.3 Production of the hypoallergenic soybean crop

As an immunodominant protein, Gly m Bd 30 K (P34) has already been identified. To develop hypoallergenic soybeans for human consumption was also attempted to replicate low-P34 soybean germplasm [22]. Fifteen different proteins have been isolated from the serum of people who are allergic to soybeans. Important allergenic proteins in soybeans were identified as Gly m Bd 60 K, Gly m Bd 30 K, and Gly m Bd 28 K. CRISPR/Cas9 technology was utilized to alter the Gly m Bd 30 K and Gly m Bd 28 K genes in soybean plants to make them hypoallergenic. Mutant soybean plants did not store high levels of either allergen in their seeds. Identified immunodominant protein in mutant soybean seeds [21].

3.5 Regulation of Flowering time

Soybeans' flowering time is a key agronomic factor that affects their productivity and adaptability. Yet, it is still unclear what factors at the genetic level make soybeans able to thrive in a wide range of latitudes. It's a fundamental biological process that guarantees successful plant reproduction. For flowers to bloom at the right time of year, a complex regulatory network of genes must be tightly regulated. Soybeans, an important legume, are typical short-day dicotyledons that only flower when the day length drops below a certain threshold. Her growth is limited by an inherent sensitivity to day length. Therefore, increasing the area under cultivation of this plant to low and/or elevated concentrations necessitates the creation of soybean genotypes that are not impacted by day length [23]**.** Exploring the various roles that CRISPR-Cas (a form of genome editing) plays in flowering and flower advancement, as well as its potential biotechnological applications in this area, is the primary goal of this research. To thrive in ever-shifting environments, plants must undergo a wide range of physiological and morphological transformations. A plant's normal flowering time is largely determined by environmental cues like low temperature and light. When deciding on a blooming period, the temperature is also an important factor to think about. Therefore, plant reactions to seasonal temperature changes are not universal. Some plants, known as vernalizes, need to be exposed to cold temperatures for an extended period before they will flower when the weather warms up [24]**.** Flowering is an essential productivity feature in soybeans that are affected by seasonal changes in day duration. Several circadian clock-associated genes have been found in recent research that have a role in controlling flowering time in response to changing light phases. In soybean, twelve maturation sites (E1 through E11 and J) that influence blooming and maturity time have been found and described phenotypically and genetically. [25-34]**.** Dominant alleles for genes E1, E2, E3, E4, E7, E8, and E10 suppress flowering, while dominant alleles for genes E6, E9, E11, and J stimulate it. E1, E3, E4, E7, and E8 mediate photoperiod sensitivity to varying light quality, especially in artificially generated LDs [35]. The E1 locus is responsible for the greatest variation in flowering time, and its associated protein contains a B3-like domain [36]**.** E2 is the GIGANTEA (GI) homolog in the plant species Arabidopsis thaliana [37]**,** , Her PHYTOCHROME A (PHYA2) and PHYA3 genetic traits have counterparts in *Arabidopsis thaliana*, designated as E3 and E4, respectively [38, 39]. Soybean FT2a and FT5a, her two Flower initiation LOCUS T (FT) homogeneity, are florigen coding variables that regulate flowering time [12, 40]**.** Soybeans' genomes can be edited in a straightforward, effective, and highly specific way using the CRISPR/Cas9 (clustered regularly interspaced short palindromic repetition) system [41, 42]**.** Recent effective gene manipulation has led to the discovery of genes involved and germplasm in soybean [13, 18, 40, 43, 44]**.** He used his CRISPR/Cas9 technique to create homozygous quadruple mutations of the LNK2 gene in soybean, which have functional homologs of AtLNK2. The triple mutant blossomed quickly in the bright sunlight. The results raise the possibility that fresh genes in the LNK2 family influence soybean blooming time, which could lead to improved soybean breeding.[45]. The flowering roles of GmFT2a and GmFT5a, two FT homogeneity, are similar. Early flowering in soybean was induced by the upregulation of these two genes in reaction to prolonged daylight [46]**.** CRISPR/Cas9-mediated T2 soybean mutant ft2a was CRISPR/Cas9-mediated and bloomed under both long-day and short-day conditions [40]**.** Extensive examination of ft2a, ft5a, and ft2a-ft5a mutants under short- and long-day circumstances. [47] In short-day settings, GmFT2a was found to be superior to GmFT5a, while in long-

day settings, GmFT5a proved superior. Soybean acclimatization to high temperatures also depends on GmFT5a. Base-edited mutants showed the ability to fine-tune flowering time using a variety of CRISPR/Cas9-based methods, despite the fact that they flowered later than knockout mutants.

3.6 Improving Oil Contents:

Scientists are investigating methods of continuing to increase the seed oil yield of oilseeds to meet the rising demand for vegetable oils. To achieve these results, scientists have used both conventional breeding and transgenic methods. However, the polyploid essence of many soybeans and the associated time constraints significantly reduce the success of conventional breeding methods. Because of this, CRISPR/Cas genetic modification has emerged as a potentially revolutionary breeding technique that could lay the groundwork for meeting rising demand and reducing regulatory burden. Our study uncovered several candidate genes for genome editing in this context and uncovered novel CRISPR-related technologies with the potential for future use in oilseed crops [48]**.** Genome editing strategies based on clustered regularly interspaced short palindromic repeats [CRISPR/Cas] have been developed to lower the bar without sacrificing efficiency. Because of its high mutagenicity, it has garnered a great deal of attention over the past decade. Specific DNA regions have been identified. [49]**.** Genetic engineering techniques include the over-expression of native or foreign genes and the back of intrinsic gene expression to speed up advancements in seed oil production and quality [50]**.** Initial reports on oilseed living things like canola, camel, and soybean reveal a wide range of CRISPR/Cas-mediated editorial rates, from 0% to 100% [51]**.** This wide range of variation could be the result of alterations to factors such as target sites, sgRNA type, transformation processes, organizers used to enhance Cas and sgRNA production, and background vectors [52, 53]**.** Evidence from previous work on improving seed oil through gene hit utilizing CRISPR/Cas-mediated genetic modifications indicates that altering fatty acid content is feasible due to the fundamental genetically based of these features. However, due to the intricate nature of lipid accumulation and our limited knowledge of the biochemical pathways involved, increasing seed oil content by this method is expected to be difficult [54, 55]**.** Only slight gains in seed oil content have been achieved despite the application of transgenic methods that involve genetic variants and/or concentrate on specific tissue. Additional intriguing gene objectives that have been shown to increase seed oil yield when the bottom or changed through genome editing will almost certainly be studied in future advancements in this field. Furthermore, there are a variety of small and unique mutations that lead to increased enzymatic activity that are being uncovered by studies employing directed progression to alter numerous genes associated in lipid biosynthesis [56]([57] **,** In this way, researchers may be able to use base- or prime-editing to improve the function of a gene of interest instead of just knocking it out entirely. As in tomato, CRISPR/Cas has been shown to control gene expression by eliminating a repressor factor from the promoter of a specific gene [58]. Since genome editing does not allow us to restrict genetic changes to specific tissue types, we must exercise caution when silencing or repairing genes; doing so could have unintended consequences that have a profound effect on a crop's agronomic performance. By targeting cis factors, researchers in other plant species have been able to fine-tune gene dosage and mitigate these effects by inducing a wide range of alleles with varying expression levels. using CRISPR/Case, which is a technique with great potential for improving oil-related characteristics [59]**.**

3.7 Regulation of nodules formation

The symbiotic relationship between legumes and rhizobia is extremely successful and plays an important role in sustainable agriculture. It can help to increase crop productivity, resulting in high-yielding fertilizers, and it can help to reduce the usage of nitrogenous fertilizer since this collaboration can fix nitrogen from the air [60]**.** Soybean approximately fixed about 16.4 (Teragram) of Nitrogen which is almost 77% of the Nitrogen fixed by all legumes with the help of symbiotic relationship between legumes and Rhizomes in an entire year [61]. Many genes participate as a host in legumes and perform their roles in the SNF (a

symbiotic nitrogen Fixation) process for example identification of rhizobia, and determining the nature of Positive or negative regulation based on the number of nodules, signal transmission in nodules, and development [62]**.** CRISPR/Cas9 technology was used to investigate the gene that controls the production of nodules in soybean. Unfortunately, a significant number of nodule formations cause plant development to be slowed due to excessive photosynthesis during the nodule formation process. The soybean (Glycine max) Rhizobia-Induced Nodule Number Control 1 and 2 (GmRIC1 and GmRIC2) paralogous genes contribute to auto-regulation for the signal of nodule development, number of nodules, and size of nodules formation. [62]**.** CRISPR/Cas9 targeted and selectively edited GmRIC1 and GmRIC2 to increase nodule formation and size in soybean (Bai et al., 2020). In addition, the root-determined nodulation1 (GmRDN1) triple mutants GmRDN1-1, GmRDN1-2, and GmRDN1-3 have fewer nodules and abnormal vegetative growth which illustrates the need for *GmRDN* for normal vegetative growth and nodule formation [63]**.** Another research showed that the genes that direct Root Hair like *(GmRDH 3a/GmRDH3b)* along with hairy genes of the meristem (*GmHAM4a*/*GmHAM4b*) and leucine-rich add which repeats in soybean (*GmLRX5*) has a negative correlation with nodule formation [64]**.** Rhizobia produces (tRNAs) derived components, which are employed as a positive regulator of nodule production and as a suppressor of root nodulation in soybean. The elimination of suppressor genes When compared to controls, CRISPR/Cas9 resulted in the formation of more nodules in soybean [64]**.**

3.8 Abiotic stresses

Abiotic conditions such as salt, drought, temperature, floods, or insufficient water supply have also influenced soybean productivity [65]**.**According to estimates, soybean growers and farmers lose 57 million USD worth of crops each year owing to salt and drought can cause yields to drop by more than 50% [65]**.** As a result, scientists have been striving to uncover essential genes involved in the abiotic stress response system to overcome the existing constraint. To improve drought and salt tolerance, soybean researchers have used CRISPR/Cas9 to analyze the function of stress-related genes.

3.8.1 Drought Tolerance:

Severe abiotic stress and drought has reduced annual global output by half. Soybean yield and quality are significantly impacted by drought conditions, making this a major global food security concern [66]**.** Stomatal closure, total biomass, generate, and other important characteristics all decrease during drought [67]**.** Reactive oxygen species (ROS) trigger oxidation in crops and disrupt many processes [68] which are then accumulated in huge quantities [69] protein degradation, enzyme inactivation, and genetic damage are all possible outcomes [70]**.** High levels of reactive oxygen species (ROS) cause cell damage, which in turn affects a variety of processes, including soybean plant growth and development. [71]. Stress from drought reduces photosynthetic activity and disrupts the absorbed supply chain, resulting in a lower soybean growth ratio during grain filling. Seed germination may be reduced by 9-35% under moderate to severe drought stress, according to previous studies [72] After seven days of drought stress, soybean seed sugar content decreased by 9%[73]. Soybeans are often made drought-resistant through molecular methods. The AtMYB44 gene from Arabidopsis thaliana was transferred directly into a soybean genotype using the Agrobacterium-mediated gene transfer method, leading to increased yield and quality under arid conditions [74]. Soybean engineering for drought stress acceptance will benefit from advances in Agrobacterium-mediated transgenes technology and tissue culture renewal of soybean cultivars [75]. CRISPR/Cas9, a revolutionary gene-editing tool, has produced promising results for improving soybean drought tolerance [76]. The use of CRISPR/Cas9 for gene editing has proven to be an efficient strategy for crop improvement. GmNAC8, a representative of the NAC gene family, functions as a regulatory element in the soybean plant during periods of drought [77]. By making changes to the gene with CRISPR/Cas9 technology in soybean, its function was verified. Tolerance to drought was decreased in GmNAC8 takedown lines versus control plants and increased in GmNAC8 upregulation lines. Soybean

drought resistance is significantly aided by the connection between GmNAC8 and the drought-induced protein GmDi19-3. [77].

3.8.2 Salt tolerance:

Salt tolerance is another abiotic factor that prevents healthy soybean production. One of the most significant environmental stresses affecting agricultural output, soil salinity affects millions of hectares of land around the world and results in enormous annual economic losses. Roughly 62 million acres (about 15 million square kilometers) are currently threatened by high salt concentration. Just 20% of farmland receives water for growing food. It is predicted that by 2050, more than half of all farmable land will have become salt-affected [78, 79]. Soybean's complete genome sequence has aided in our comprehension of the underlying mechanisms of gene expression and control as they pertain to salinity [40] Several genes have been identified in soybeans that increase their tolerance to salt stress [80]. Salt stress tolerance is improved by GmFLD19, which decreases Na ion and malondialdehyde densities, boosts antioxidant enzymatic activity, and decreases chlorophyll content [81]. Soybeans' sensitivity to salt stress is enhanced by GmNARK's promotion of ABA biosynthesis. Utilizing the CRISPR/Cas9 system, they demonstrated that GmNAC06 upregulation in soybean hairy roots functions as a regulatory element of salt tolerance. Soybean salt tolerance is facilitated by the sodium/hydrogen exchanger GmNHX5, the functional mechanism of which has been the subject of another study [81]. Soybean plants' salt tolerance was diminished after being modified using CRISPR/Cas9 technology. Soybean hairy root yield was improved by GmNHX5 overexpression by keeping the K+/Na+ ratio stable to avoid organelle damage [81]. To improve plant abiotic stress tolerance, AITRs are a promising group of candidate genes for CRISPR/Cas9 genome editing [82] [83] Salt tolerance increased in the Gmaitr mutant seedlings, and their roots and stems grew longer. Since this occurs, it is possible that GmAITR gene editing can improve soybean salt tolerance [84].

3.9 Development of Herbicide Resistant plant:

Plant biotechnology relies heavily on herbicide resistance for weed management and as a diagnostic biomarker for transformation-based genetic engineering, which in turn helps farmers boost output. Recent years have seen the use of CRISPR/Cas9 to develop herbicide-resistant crops like rice [85], Arabidopsis [86], watermelon[87], and oilseed rapeseed [88]. Here we discuss the most recent advances in the development of weed killer plants utilizing CRISPR/Cas9-mediated gene editing technology, with a special emphasis on the targeted modification of endogenous genes such as acetoacetate biosynthetic pathway (ALS), possibly resulting synthase (EPSPS), cellulose key enzyme A subunit 3 (CESA3), and splicing factor 3B subunit 1. (SF3B1). More precise and effective genome editing tools are required because CRISPR/Cas-based genome engineering strategies are already being used extensively to develop crops with extra features like herbicide tolerance. This review will focus on the generation of HR plants through CRISPR/Cas9-mediated gene editing, as well as candidate genes for the generation of HR plants through loss-of-function mutations [66].

3.10 Disease Resistance

Soybean production and quality have been severely impacted by plant diseases. [89]**.** Soybean cyst nematode and charcoal rot have caused 33% of economic losses in soybean crops per hectare on average [90]**.** The CRISPR/Cas9 method has provided fresh insights into the development of disease-resistant soybean. This approach has produced several effective outcomes in a variety of crops [91-93]**.** The genome of plants is targeted in this strategy to generate resistance to certain diseases [89]**.** or the host has responsibility for creating plant pathogen defenses [94]**.** Although little is known about pathogen improvements in soybean using CRISPR/Cas9, the technique can modulate pathogen resistance in soybean [95] [96]**.**

3.10.1 Viral resistance

The deadly disease Soybean Mosaic Virus has severely harmed soybean output (SMV) [97]**.** Soybean root hair and plants were treated with CRISPR/Cas9 to concurrently target three isoflavonoids synthesize genes (GmFNSII-1, GmF3H2, and GmF3H1), which resulted in an increase in isoflavone concentration in the seeds and leaves of mutant soybean plants [98]. High isoflavone content soybean plants have shown serious opposition to soybean mosaic virus strain SC7 [98]. After infection with strain SC7, the amount of SMV coat protein was lower in triple mutants.

3.10.2 Nematode resistance

Genes belonging to the nucleotide-binding-site leucine-rich repeat (NBS-LRR) family are responsible for effector-triggered immunity, which occurs in response to extremely specialized pathogenic effectors. In plants, NBDLRR is tandemly duplicated sequences that can undergo recombination to create novel disease resistance [93, 99]**.** They were able to prevent Phakopsora pachyrhizi from infecting soybean [19, 100] and Phytophthora sojae [95, 101] contributes to the spread of soybean rust To identify novel resistance genes, the technique was used to generate rearrangements via targeted chromosomal cleavage [96]**.** Soybean NBS-LRR families Rpp1L and Rps1 were selected, and double-stranded break (DSB) and fixing with CRISPR/Cas9 were used to generate specific adjustments in the Rpp1L and Rps1 clusters. New disease-fighting properties may emerge from this unusual pairing [96]**.**

3.11 Male sterile line development:

Heterosis breeding is a very efficient way for the genetic improvement of crops [102]. Heterosis (hybrid vigor) is a phenomenon in which one or more traits of heterozygous progeny are superior than both of its homozygous parents [21]. For hybrid seed production, male sterility is very important [21] For the first time in the history of soybeans male sterile lines were developed using AMS homologs, and targeted mutagenesis. The GmAMS1 mutation resulted in male sterile soybeans. This gene stimulates soybean pollen production. Researchers employed the CRISPR/Cas9 technology to successfully modify the MS1 gene to create soybean male sterile lines. Out-crossing soybean population is developed by using these male sterile lines [103].

3 FUTURE PROSPECTS

The genome system of soybean is paleopolyploid. The complete sequence of the soybean genome is done with the help of the whole genome shot gun method [104]. Among the major issues regarding the breeding of soybean is the complex structure of the genome and along with that Genome size is 1.1 GB in length while consisting of 46, 430 genes for the coding of protein. 75% of the genome is multiple copies of genes. We are unaware of the function of many known genes. This CRISPR/Cas9 is a reliable tool for estimating gene function because this system provides us the facility of both Single-gene knockout along with multiple gene editing [105, 106]. Effective transformation is considered a keynote for the improvement of advanced breeding lines in the case of soybean.[107]. The commonly used method of gene transformation is Agrobacterium with just 5% efficiency in soybean which is very low as compared to rice which is around about 90%[107]. So viral vectors were used to introduce CRISPR/Cas9 in plants [89] by using particle bombardment [108] Cas9-sgRNA Ribonucleoproteins (RNPs)[109] and nanoparticles also proposed alternative ways. When the two genes which are regulating start to overexpress *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*), has increased the efficiency of transformation in various types of genotypes and species. [110]. Forgoing the need for tissue culture, CRISPR/Cas9 genome-edited dicots plants were produced through de novo induction in the meristem. The CRISPR/Cas9- mediated genome editing system was used successfully in soybean for creating roots with hair and a stable and smooth transgenic plant along with somatic embryos from its first use in 2015 [111]. At this stage the progress of CRISPR/Cas9 with its new modern techniques. At the moment, the

revolution in CRISPR/Cas9 with new emerging techniques and tools and different variations in Cas can update soybean complex gene scenario, which is within the scope of current research. spCas9-NG, peak editing, xCas9, Cpf1 (Cas12a), Cas1,3, and Cas different versions are just some of the cutting-edge CRISPR/Cas tools used in the genome editing system [112]. As per our information, the method which is described above never been utilized efficiently for genome editing in soybean. A has been noted in recent research that the 3-varients of Cas9 enzyme , xCas9, SpCas9-NG, and XNG-Cas9 (an xCas9 and the Cas9-NG hybrid) has introduced the desired mutation and results in soybean crop [113].Even Cas9 is the popular and largely functional variant of CRISPR, yet there is a potential in different alternatives variants for enhancement in targeted selection and genome editing of soybean in efficient way in researches. The tag of (GMO) abbreviated as genetically modified crops is also main concern for the breeders as it is considered by socio-economic concerns along with scientific proofs [114].The rules and regulation for the genetically modified plant has made a standard for the improvement of traits and its sales and marketing but this made it too much expensive and time consuming. If GE crops got exempted from these protocols in regulation process it will boost the improvement pathway of crops [115] In monitoring sense GE crops has a benefit over GMO crop. After completing the editing in a particular gene, it is very easy to remove CRISPR/Cas9 reactants and mutation free transgenic organism is attained [116]. Genetically edited soybean oil with higher concentration of oleic acid and less linolenic acid content are introduced in USA by Calyxt and TALEN on commercial level. However the action required for regulation and evaluation has been dropped on drought resistance soybean by the agriculture department in (USDA) [117]. Globally harmonization of this regulatory instruction for GE crops is very important to eliminate specific restrictions using genome editing technology for the betterment and improvement of crops.

4 Conclusion

CRISPR-based technologies, for example, have emerged to give excellent tools for soybean improvement. Various reports on the effective usage of CRISPR/Cas9 have been given. With the progress of CRISPR/Cas9 and the advent of tools and Cas variations, research on complicated gene activity in soybeans may expand. Soybean quality, yield, and resistance to biotic and abiotic stressors have all been improved using this method. Recent TALENs-based high oleic soybean field trials have proven the crop's potential.

5 References:

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